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World Prematurity Day (WPD) was first observed in 2009, following a 2008 initiative by the European Foundation for the Care of Newborn Infants. Advances in neonatal healthcare have contributed to improved treatment outcomes for premature babies worldwide. However, a large number of them still lose their lives during the first weeks after hospital discharge. Recognizing the importance of this issue, the World Health Organization (WHO) included WPD in the official WHO calendar in 2025. The theme of this year's WPD is *"Small babies, big potential. Let us invest in continuous health monitoring and care to protect their future."*

Svetski dan prevremeno rođenih beba (SDPRB) je prvi put obeležen 2009. na inicijativu Evropske fondacije za negu novorođenčadi iz 2008. Napredak u oblasti neonatalne zdravstvene zaštite doprineo je poboljšanju ishoda lečenja prevremeno rođenih beba širom sveta. Međutim i dalje je veliki broj onih koje izgube život tokom prvih nedelja nakon otpusta iz bolnice. Prepoznajući značaj ovog pitanja, Svetska zdravstvena organizacija (SZO) je 2025. uvrstila SDPRB u zvanični kalendar SZO. Tema ovogodišnjeg SDPRB je *"Male bebe veliki potencijal. Uložimo u kontinuirano zdravstveno praćenje i negu kako bismo zaštitili njihovu budućnost."*

LETTER TO THE EDITOR
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Impact of artificial intelligence on statistical analyses in biomedical research

Uticaj veštačke inteligencije na statističke analize u biomedicinskim istraživanjima

To the Editor

Artificial intelligence (AI) is increasingly being integrated into various scientific fields, particularly medicine and biomedical research [1]. One of the reasons is the precision of neural networks or machine learning that characterizes AI [2]. AI is rapidly transforming modern medicine, precisely with the aforementioned highly precise algorithms [3].

Among the early applications of AI in medicine was the interpretation of radiological images, while its use has since expanded to other fields, including surgery and robotic-assisted surgical procedures [4–6]. As AI advances, the limitations of AI-based decisions are becoming increasingly apparent, leading to ethical dilemmas and conflicting opinions that can be a significant problem in medical practice. The same is true of statistical analysis and interpretation of results. Any misinterpretation of results can have far-reaching consequences.

Statistical data analysis is not outside the scope of AI. AI can be used both for data analysis and statistical results, as well as for analyzing already done statistics and finding statistical errors [7]. Large language models (LLMs), such as Chat Generative Pre-Trained Transformer (ChatGPT) and Gemini, have shown impressive capabilities in text generation and in generating R code [8]. R codes are computer scripts written in the R programming language for statistical analysis. One study demonstrated that, although LLMs are capable of generating correct and interpretable R code in many cases, careful human oversight remains essential when integrating AI into statistical analyses to minimize the risk of misuse [3]. Another study showed that generative AI tools, such as ChatGPT, can facilitate data analysis; however, human oversight and adequate statistical knowledge remain essential [9].

The appropriate selection of statistical tests is crucial for ensuring the validity and reliability of research findings [10]. Errors, such as using the wrong test or misinterpreting data, can easily lead to incorrect conclusions in a study [11]. The most common errors in statistical analyses that lead to inaccurate results and misleading conclusions are errors in statistical design, data presentation, data outlier treatment, the use of parametric and nonparametric tests, the disclosure of post-hoc analyses, and the definition of the hypotheses [7]. Therefore, by identifying statistical errors, AI systems increase the reliability of research. AI helps non-experts analyze data, but it can be unpredictable for experts dealing with complex data analysis. Recent studies show that AI is increasingly helpful in research, assisting in spotting errors in methodology, citations, and statistical analyses. AI can work independently, but it works better if it is managed by someone with statistical knowledge [7]. AI also brings risks like misreading results, ethical issues, and privacy concerns, so researchers must make final decisions.

About two years ago, a paper was published in *Nature* discussing the use of AI in reviewing paper text and detecting errors in methodology, calculations, and inconsistencies in the citation of papers and statistical analysis [12]. Similar concerns arise when AI is used for manuscript drafting and data analysis, where errors generated by AI may require careful human verification [13]. There are also papers that focus exclusively on the analysis of the use of the Granularity-Related Inconsistency of Means – GRIM test as an AI tool for the detection of statistical errors [14].

A 2026 evaluation of an LLM agent across five stages of clinical data analysis found that it performed reliably in generating research questions and statistical analysis plans, but systematic errors emerged during analysis execution. These findings highlight the need for expert verification before AI-generated results are reported [15].

That being said, human oversight is always necessary to review research results and ensure reliability. Therefore, only people can make the final decision regarding the obtained results of the study, either independently or with the use of AI tools, which significant

Correspondence to: Nemanja Rančić, University of Defence, Faculty of Medicine of the Military Medical Academy, Crnotravska 17, 11 000, Belgrade, Serbia. E-mail: nece84@hotmail.com

ly facilitate and accelerate this process. Future comparative studies are needed to evaluate data analyzed by AI programs *versus* data analyzed by humans, in order to identify the advantages and disadvantages of each approach to making final decisions. This would likely significantly improve the work of AI software both for analyzing raw data and for controlling already processed data.

Nemanja Rančić[*†], Filip Pilipović[‡], Milica Djurdjević[*]

[*]University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; [†]Military Medical Academy, Institute of Radiology, Belgrade, Serbia; [‡]Institute for Orthopaedic Surgery "Banjica", Belgrade, Serbia

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Association of low muscle mass with lung function in hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease and its related factors

Povezanost smanjene mišićne mase sa plućnom funkcijom kod hospitalizovanih bolesnika sa akutnim pogoršanjem hronične opstruktivne bolesti pluća i faktori povezani sa njom

Rong Qi*, Mingxia Shen†, Juan Su*, Xiaotong Jia‡, Zhuandi Wang*, Zhiting Li*, Meimei Li*, Ruimei Wu*

***Lanzhou Hospital of Traditional Chinese Medicine, Department of Respiratory and Critical Care Medicine, Lanzhou, Gansu, China; †The Affiliated Hospital of Gansu University of Chinese Medicine, Department of Geriatric Medicine, Lanzhou, Gansu, China; ‡Wuwei Hospital of Traditional Chinese Medicine, Department of Pulmonology, Wuwei, Gansu, China**

Abstract

Background/Aim. Patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and with low muscle mass (LMM) experience higher hospitalization rates, prolonged hospital stays, and a significantly increased risk of adverse events. The aim of this study was to examine the association of LMM with pulmonary function in hospitalized patients with AECOPD, compare lung function between patients with and without LMM, and identify factors independently associated with LMM in this population. **Methods.** A total of 106 hospitalized patients with AECOPD were enrolled between December 2024 and December 2025. Based on the appendicular skeletal muscle mass (SMI) index – SMMI derived from bioelectrical impedance analysis (BIA), the patients were stratified into an LMM group (n = 60) and a non-LMM group (n = 46). Demographics, serological markers, pulmonary function, and body composition were compared. Factors independently associated with LMM were identified using regression models. **Results.** The groups differed significantly in age, SMM, and body mass index (BMI). Regarding pulmonary function, forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1), and peak expiratory flow (PEF) were lower in the LMM group (all $p < 0.05$). Multivariate logistic regression identified older age [odds ratio (OR) = 1.127, 95% confidence interval (CI): 1.045–1.216], lower BMI (OR = 0.631, 95% CI: 0.522–0.763), and lower FEV1 (OR = 0.112, 95% CI: 0.031–0.403) as independently associated factors for LMM in hospitalized patients with AECOPD. **Conclusion.** LMM is significantly associated with impaired pulmonary function in hospitalized patients with AECOPD. Older age, lower BMI, and reduced FEV1 were independently associated with LMM, highlighting the importance of early assessment of muscle status in this population.

Keywords:
aged; body composition; muscular atrophy; pulmonary disease, chronic obstructive; respiratory function tests.

Apstrakt

Uvod/Cilj. Bolesnici sa akutnim pogoršanjem hronične opstruktivne bolesti pluća (*acute exacerbation of chronic obstructive pulmonary disease* – AECOPD) i sa smanjenom mišićnom masom (*low muscle mass* – LMM) imaju veću učestalost hospitalizacije, duže trajanje bolničkog lečenja i značajno povećan rizik od neželjenih događaja. Cilj rada bio je da se ispita povezanost LMM sa plućnom funkcijom kod hospitalizovanih bolesnika sa AECOPD, uporedi plućna funkcija između bolesnika sa i bez LMM, kao i da se identifikuju faktori koji su nezavisno povezani sa LMM u ovoj populaciji. **Metode.** U studiju je bilo uključeno ukupno 106 hospitalizovanih bolesnika sa AECOPD u periodu od decembra 2024. do decembra 2025. godine. Na osnovu indeksa apendikularne skeletne mišićne mase (*skeletal muscle mass index* – SMMI), određenog primenom analize bioelektrične impedanse (*bioelectrical impedance analysis* – BIA), bolesnici su podeljeni u grupu sa LMM (n = 60) i grupu bez LMM (n = 46). Upoređivani su demografski podaci, serološki markeri, parametri plućne funkcije i sastav tela. Faktori koji su nezavisno povezani sa LMM

Correspondence to: Mingxia Shen, The Affiliated Hospital of Gansu University of Chinese Medicine, Department of Geriatric Medicine, No. 732, Jiayuguan West Road, Chengguan District, Lanzhou, Gansu, China. E-mail: 18219816316@163.com

identifikovani su primenom regresionih modela. **Rezultati.** Grupe su se značajno razlikovale u pogledu starosti, skeletne mišićne mase (SMM) i indeksa telesne mase (*body mass index* – BMI). U pogledu plućne funkcije, forsirani vitalni kapacitet (*forced vital capacity* – FVC), forsirani ekspiratorni volumen u 1 sekundi (FEV1) i vršni ekspiratorni protok (*peak expiratory flow* – PEF) bili su niži u LMM grupi (svi $p < 0,05$). Rezulatati multivarijantne logističke regresije pokazali su da su starija životna dob [*odds ratio* (OR) = 1,127, 95%, *confidence interval* (CI): 1,045–1,216], niži BMI (OR = 0,631, 95% CI: 0,522–0,763) i niži FEV1 (OR = 0,112, 95% CI: 0,031–0,403) nezavisno povezani faktori sa LMM kod hospitalizovanih bolesnika sa AECOPD. **Zaključak.** Prisustvo LMM je značajno povezano sa narušenom plućnom funkcijom kod hospitalizovanih bolesnika sa AECOPD. Starija životna dob, niži BMI i snižena vrednost FEV1 bili su nezavisno povezani sa LMM, što ukazuje na značaj rane procene stanja mišića kod ove populacije.

Ključne reči:
stare osobe; telo, sastav; mišići, atrofija; pluća, opstruktivna bolest, hronična; respiratorna funkcija, testovi.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common, progressive respiratory condition characterized by persistent airflow limitation, with dyspnea, cough, and sputum production as its main clinical manifestations. As one of the leading causes of death worldwide, its high prevalence and disability impose a substantial socioeconomic and public health burden [1]. COPD not only causes progressive lung function decline that severely impairs daily activities, work capacity, and quality of life (QoL), but also exerts significant systemic effects, frequently accompanied by extrapulmonary comorbidities [2]. These comorbidities interact with the core pathophysiological processes of COPD and collectively influence disease progression and clinical outcomes [3]. Therefore, the scientific management of comorbidities has become essential for improving the overall prognosis of COPD patients.

Among these comorbidities, low muscle mass (LMM) (a core component of sarcopenia) deserves particular attention, although it remains underrecognized and underdiagnosed in clinical practice. LMM is an age-related condition involving loss of skeletal muscle mass (SMM), which seriously threatens the health and independent living of older adults, making early identification and intervention critical [4]. COPD itself is a major associated factor for LMM, with studies reporting a high and increasing proportion of LMM among COPD patients [5]. A close pathophysiological link exists between the two conditions, involving several shared mechanisms. First, chronic hypoxia in COPD reduces exercise tolerance and physical activity, while respiratory muscles undergo disuse atrophy from insufficient mechanical stimulation. Second, increased ventilatory load raises energy expenditure at rest and during activity, which–frequently coupled with malnutrition–accelerates skeletal muscle catabolism. Third, systemic inflammation and oxidative stress imbalance not only directly damage lung tissue but also promote skeletal muscle protein breakdown through multiple pathways [6]. During acute exacerbations, these processes are amplified, leading to accelerated muscle loss. In turn, declining respiratory muscle function—particularly of the diaphragm–further impairs pulmonary function and airway clearance, increasing infection risk and establishing a vicious cycle. Under the combined influence of chronic inflammation, hypoxia, glucocorticoid use, and anabolic disturbances, COPD patients face a markedly elevated risk of developing LMM.

Previous epidemiological studies, mostly in community-dwelling or stable outpatients, have reported an overall proportion of LMM of approximately 21.6% in COPD patients, significantly higher than in non-COPD populations [7, 8]. However, much higher rates (up to 60–70%) have been observed in hospitalized or acutely exacerbating COPD patients. Moreover, LMM has been identified as an independently associated factor for increased mortality in COPD patients [9]. Nevertheless, the precise pathways through which LMM affects COPD patients and its key influencing factors remain incompletely understood.

Although previous studies have reported associations between LMM and impaired lung function in COPD, most have focused on Western populations or have not simultaneously incorporated multiple bioelectrical impedance analysis (BIA) parameters such as soft lean mass (SLM), SMM, phase angle (PhA), and respiratory impedance [10–12]. Furthermore, data from hospitalized COPD patients in the Loess Plateau region–characterized by high altitude, dry and dusty springs, and large diurnal temperature variations–remain scarce.

The aim of this study was to clarify the correlation between LMM and pulmonary function parameters (including percent-predicted values), to systematically compare lung function and body composition profiles between COPD patients with and without LMM, and to identify independent associated factors for LMM in this population, thereby providing region-specific evidence for early identification of high-risk groups and targeted interventions.

Methods

Study participants

This was a single-center, retrospective, cross-sectional study. A total of 106 patients diagnosed with acute exacerbation of COPD (AECOPD) were consecutively enrolled from the Department of Respiratory and Critical Care Medicine, Lanzhou Hospital of Traditional Chinese Medicine, Lanzhou, Gansu, China, between December 2024 and December 2025. The study was approved by the Ethics Committee of

Lanzhou Hospital of Traditional Chinese Medicine (No. LZ-TCM-EC-2025-036, from March 5, 2025), and informed consent was waived due to the retrospective design.

Inclusion criteria were as follows: diagnosis of COPD confirmed by post-bronchodilator forced expiratory volume in 1 sec (FEV1)/forced vital capacity (FVC) < 0.70 according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2025 guidelines; hospitalization for acute exacerbation; age ≥ 18 years; and ability to undergo BIA and spirometry.

Exclusion criteria were as follows: neuromuscular diseases, malignant tumors, or severe endocrine disorders affecting muscle metabolism; severe heart failure, renal failure, or hepatic insufficiency; severe edema or fluid imbalance; use of systemic corticosteroids within the previous 1 month; inability to cooperate with examinations.

LMM was defined based on the SMM index (SMMI) component of the Asian Working Group for Sarcopenia (AWGS) 2019 consensus criteria [3]. The SMMI was calculated as appendicular SMM divided by height squared (kg/m²) derived from the BIA device. Due to the retrospective nature of data collection, muscle strength (e.g., handgrip strength) and physical performance (e.g., gait speed) were not assessed; therefore, the term "sarcopenia" was not used, and this study strictly focused on LMM. The cutoff values for LMM were SMMI < 7.0 kg/m² for males and SMMI < 5.4 kg/m² for females. Although the AWGS 2019 consensus recommends a general BIA-derived threshold of 5.7 kg/m² for females, the 5.4 kg/m² cutoff adopted here was derived from the manufacturer's validated clinical reference range for the InBody S10 device in Asian populations, as documented in the device's clinical validation report. This device-specific threshold was used to ensure consistency between the classification algorithm and the measurement hardware. Based on these criteria (i.e., based on SMMI determined by BIA), patients were stratified into an LMM group (n = 60) and a non-LMM group (n = 46). It should be emphasized that, due to the retrospective design, muscle strength and physical performance were not assessed; therefore, the classification strictly reflects low SMM by SMMI, rather than a comprehensive diagnosis of sarcopenia. All patients were enrolled at the time of hospital admission for an AECOPD.

Data collection

General clinical data, including age, sex, COPD duration, body mass index (BMI), and SMMI, were extracted from the hospital's electronic medical record system. Fasting venous blood samples were collected on the morning after admission and analyzed using standardized laboratory instruments. The assessed serological parameters included white blood cell (WBC) count, neutrophil (Neut) count, lymphocyte (Lym) count, albumin (Alb), hemoglobin (Hb), serum creatinine (Cr), and interleukin (IL)-6.

Pulmonary function tests were performed using a spirometer (Jaeger MasterScreen, Vyaire Medical) according to American Thoracic Society – ATS and European Respiratory Society – ERS 2019 standards when patients were clinically stable (i.e., resolution of acute symptoms, no need for supplemental oxygen, and stable medication regimen for at least 48 hrs), either before discharge or during a follow-up outpatient visit within two weeks after discharge. Among the enrolled patients, 58% were tested before discharge and 42% during outpatient follow-up. All recorded values were post-bronchodilator measurements, obtained 15 min after inhalation of 400 μg of salbutamol. Predicted values were calculated using the Global Lung Function Initiative – GLI 2012 reference equations. At least three acceptable maneuvers were recorded, and the reproducibility criteria (difference between the best two FEV1 and FVC values) were within 150 mL. The recorded indices comprised FVC, FEV1, the FEV1/FVC ratio, peak expiratory flow (PEF), FEV1% predicted, and respiratory impedance parameters. To account for potential heterogeneity in testing timing, we performed a sensitivity analysis by including the testing timepoint (pre-discharge vs. post-discharge) as a covariate in the multivariate logistic regression model, which showed no significant interaction.

Body composition was measured using a multi-frequency BIA device (InBody S10, Biospace Co., Ltd.). To minimize measurement bias in hospitalized patients with AECOPD, the following strict standardized protocol was followed: all measurements were performed on the morning following admission (within 12–24 hrs) after clinical stabilization; patients were required to fast overnight (≥ 8 hrs) and to empty their bladder immediately before the test; measurements were conducted in the supine position after a 5-min resting period; patients with obvious peripheral edema, pleural effusion, ascites, or those receiving intravenous fluids or high-dose diuretics within the 24 hrs prior to testing were strictly excluded; the device was calibrated daily according to the manufacturer's instructions before any measurements. The following variables were obtained: SLM, SMM, body fat mass (BFM), BFM percentage (BFM%), fat-free mass (FFM), and PhA.

Statistical analysis

All statistical analyses were performed using SPSS version 27.0. Normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables are presented as mean ± standard deviation and were compared using the independent-samples *t*-test. Non-normally distributed variables are presented as median (interquartile range) and were compared using the Mann-Whitney *U* test. Categorical variables are summarized as frequency (percentage) and were analyzed using the chi-square test or Fisher's exact test, as appropriate. For variables with extreme variability, outliers were verified against original medical records. Correlations between pulmonary function and body composition parameters were examined using Spearman's rank correlation coefficient. Multivariable logistic regression was performed to identify independently associated factors for LMM in AECOPD patients. To avoid circularity and multicollinearity arising from the fact that the outcome (LMM group) is defined by SMMI, the collinear body-composition variables (SLM, SMM, and BFM) were excluded from the final re-

gression model; the covariates entered were age, sex, BMI, FVC, FEV1, PEF, and Alb. Variables were entered using the forward stepwise (likelihood ratio) method. Model fit was assessed with the Hosmer–Lemeshow goodness-of-fit test ($p = 0.624$), and multicollinearity was checked *via* the variance inflation factor (VIF) (all VIF < 2.0). The study flow diagram was presented in Figure 1.

Results

Comparison of relevant data between the low muscle mass group and the non-low muscle mass group

Serological parameters, including WBC, Neut, Lym, Hb, Alb, Cr, cystatin C (Cys C), C-reactive protein (CRP), and IL-6, were compared between the two groups. The results showed that the median Alb level in the LMM group was 39.87 g/L, significantly lower than that in the non-LMM group (43.08 g/L), with a statistically significant difference ($Z = -2.89$, $p = 0.004$). No statistically significant differences were observed in the other serological parameters between the two groups ($p > 0.05$) (Table 1).

Comparison of pulmonary function parameters between the two groups revealed that the LMM group exhibited significantly lower absolute and percent-predicted FVC (median percent predicted: 62.4% vs. 75.8%, $p < 0.001$), FEV1 (51.2% vs. 65.4%, $p < 0.001$), and PEF (48.9% vs. 61.7%, $p = 0.002$) than the non-LMM group. No significant differences were observed in FEV1/FVC ratio or respiratory impedance ($p > 0.05$). All between-group comparisons primarily relied on these percent-predicted values, which inherently adjust for age, sex, and height (Table 2).

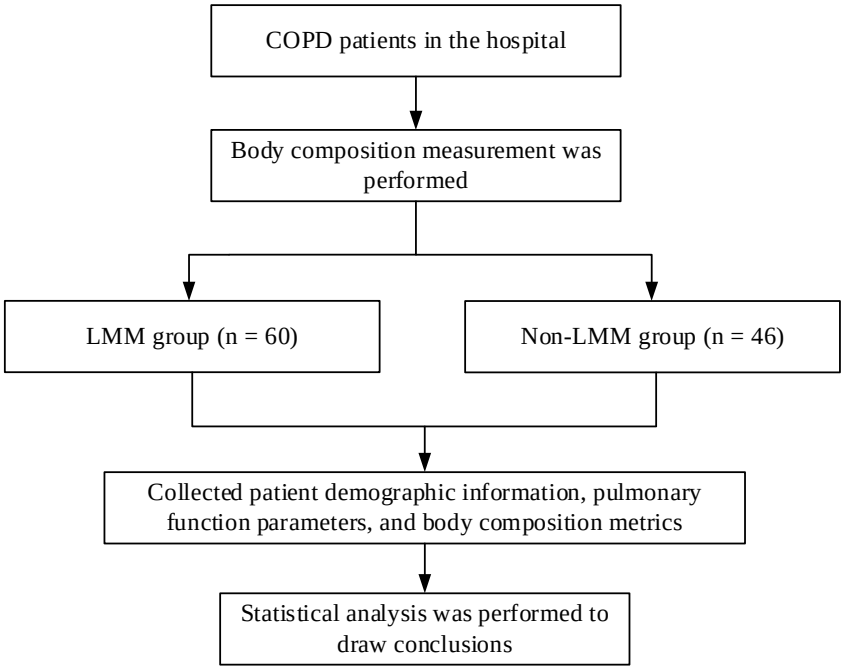

Fig. 1 – Flowchart of the research methodology.
COPD – chronic obstructive pulmonary disease; LMM – low muscle mass; n – number.

Table 1

Comparison of serological parameters between LMM and non-LMM groups

Parameter	LMM (n = 60)	Non-LMM (n = 46)	Test statistic	*p*-value
WBC, $\times 10^9$/L	7.95 (6.12–9.87)	6.89 (5.53–8.42)	$Z = -1.58$	0.114
Neut, $\times 10^9$/L	5.23 (4.01–7.89)	5.01 (3.87–6.95)	$Z = -0.61$	0.542
Lym, $\times 10^9$/L	1.48 (0.95–2.11)	1.22 (0.81–1.65)	$Z = -1.79$	0.073
Hb, g/L	148.97 ± 34.60	147.86 ± 25.09	$t = -0.36$	0.757
Alb, g/L	39.87 (35.92–43.82)	43.08 (38.54–47.61)	$Z = -2.89$	0.004
Cr, umol/L	95.29 ± 31.85	96.43 ± 36.92	$t = 0.12$	0.807
Cys C, mg/L	1.40 ± 0.61	1.48 ± 0.61	$t = -0.72$	0.451
CRP, mg/L	12.45 (4.22–32.80)	10.10 (3.56–29.15)	$Z = -0.67$	0.503
IL-6, pg/mL	13.80 (6.15–29.40)	12.70 (5.90–26.45)	$Z = -0.08$	0.936

LMM – low muscle mass; n – number; WBC – white blood cell; Neut – neutrophil; Lym – lymphocyte; Hb – hemoglobin; Alb – albumin; Cr –creatinine; Cys C – cystatin C; CRP – C-reactive protein; IL – interleukin. All values are given as mean ± standard deviation or median (interquartile range).
***Note*: Reference ranges for laboratory parameters were: WBC 3.5–9.5 × 10^9/L; Neut 1.8–6.3 × 10^9/L; Lym 1.1–3.2 × 10^9/L; Hb 130–175 g/L (male)/115–150 g/L (female); Alb 35–55 g/L; Cr 44–104 μmol/L (male)/44–88 μmol/L (female); Cys C < 1.03 mg/L; CRP < 10 mg/L; and IL-6 < 7 pg/mL.**

Table 2

Comparison of pulmonary function parameters between LMM and non-LMM groups

Parameter	LMM (n = 60)	Non-LMM (n = 46)	Test statistic	*p*-value
FVC, L	2.44 (2.10–2.82)	2.98 (2.58–3.56)	Z = -4.68	< 0.001
FVC, % predicted	62.4 (54.1–71.3)	75.8 (66.2–85.5)	Z = -4.92	< 0.001
FEV1, L	1.40 (0.97–1.70)	1.76 (1.52–1.99)	Z = -4.37	< 0.001
FEV1, % predicted	51.2 (38.5–63.8)	65.4 (54.0–76.9)	Z = -4.81	< 0.001
FEV1/FVC, %	54.19 ± 14.70	54.36 ± 14.58	t = 0.10	0.923
PEF, L/s	3.70 (2.61–4.78)	4.68 (3.56–5.79)	Z = -2.85	0.004
PEF, % predicted	48.9 (36.2–63.5)	61.7 (47.8–75.6)	Z = -3.12	0.002
Respiratory impedance, $cmH_2O/L/s$	23.95 ± 8.72	24.89 ± 10.21	t = 0.51	0.601

LMM – low muscle mass; n – number; FVC – forced vital capacity; FEV1 – forced expiratory volume in 1 sec; PEF – peak expiratory flow.
All values are given as mean ± standard deviation or median (interquartile range).
***Note*: Reference ranges for pulmonary function parameters: FVC ≥ 80% of predicted; FEV1 ≥ 80% of predicted; FEV1/FVC ≥ 0.70; and PEF ≥ 80% of predicted.**

Table 3

Body composition parameters in LMM and non-LMM groups

Parameter	LMM (n = 60)	Non-LMM (n = 46)	Test statistic	*p*-value
SLM, kg	40.54 ± 6.71	44.96 ± 4.21	t = 3.40	< 0.001
SMM, kg	23.26 ± 3.89	25.13 ± 2.58	t = 2.77	0.006
BFM, kg	16.18 ± 6.34	18.88 ± 4.85	t = 2.46	0.014
FFM, kg	42.23 (38.50–46.10)	49.04 (45.12–52.78)	Z = -1.79	0.073
BFM, %	26.95 (21.16–32.74)	24.93 (19.20–30.65)	Z = -0.72	0.458
PhA, °	4.31 (3.90–4.72)	4.40 (3.87–4.92)	Z = -0.70	0.485

LMM – low muscle mass; n – number; SLM – soft lean mass; SMM – skeletal muscle mass; BFM – body fat mass; FFM – fat-free mass; PhA – phase angle.
All values are given as mean ± standard deviation or median (interquartile range).

Table 4

Correlation analysis of SMMI and BMI with pulmonary function parameters

Parameter	BMI		SMMI	
	r_s	*p*-value	r_s	*p*-value
FVC, L	0.283	0.002	0.310	0.001
FEV1, L	0.228	0.012	0.226	0.013
FEV1/FVC, %	-0.002	0.970	-0.045	0.609
PEF, L	0.136	0.118	0.165	0.062
Respiratory impedance, $cmH_2O/L/s$	0.173	0.058	0.114	0.217

SMMI – skeletal muscle mass index; BMI – body mass index; FVC – forced vital capacity; FEV1 – forced expiratory volume in 1 sec; PEF – peak expiratory flow.
***Note*: Spearman's rank correlation coefficient (r_s) was used.**

Comparison of body composition between the two groups revealed that AECOPD patients with comorbid LMM exhibited significantly lower levels of SLM (t = 3.40, p < 0.001), SMM (t = 2.77, p = 0.006), and BFM (t = 2.46, p = 0.014) compared to those without LMM (Table 3).

Correlation analysis was performed between SMMI, BMI, and pulmonary function parameters. The results showed that FVC (r_s = 0.310, p = 0.001) and FEV1 (r_s = 0.226, p = 0.013) were positively correlated with SMMI. Similarly, FVC (r_s = 0.283, p = 0.002) and FEV1 (r_s = 0.228, p = 0.012) were also positively correlated with BMI. No significant correlations were observed between the remaining pulmonary function indicators and SMMI or BMI (Table 4).

Correlation analysis was conducted between SMMI, BMI, and body composition parameters. The results showed that SLM (r_s = 0.235, p = 0.010) and SMM (r_s = 0.276, p = 0.002) were positively correlated with SMMI. Similarly, SLM (r_s = 0.235, p = 0.011) and SMM (r_s = 0.221, p = 0.015) were also positively correlated with BMI. Additionally, SMMI showed a positive correlation with BFM (r_s = 0.192, p = 0.039). No significant correlations were observed between the remaining body composition parameters (FFM, BFM%, and PhA) and either SMMI or BMI (Table 5).

Correlation analysis between pulmonary function and body composition parameters in the two groups revealed

that FVC (r_s = 0.316, p = 0.001), FEV1 (r_s = 0.262, p = 0.003), and PEF (r_s = 0.294, p = 0.001) were positively correlated with SLM. Similarly, FVC (r_s = 0.317, p = 0.001), FEV1 (r_s = 0.272, p = 0.002), and PEF (r_s = 0.292, p = 0.001) were positively correlated with SMM. In addition, PEF (r_s = 0.235, p = 0.010) was positively correlated with BFM (Table 6).

Correlation analysis was performed between body composition parameters and the FEV1/FVC ratio as well as respiratory impedance. The results showed that FEV1/FVC ratio was positively correlated with BFM (r_s = 0.239, p = 0.009) and BFM% (r_s = 0.272, p = 0.002). In addition, respiratory impedance was negatively correlated with PhA (r_s = -0.195, p = 0.031). No significant correlations were found between FEV1/FVC ratio or respiratory impedance and the remaining body composition parameters (Table 7).

Associated factors for low muscle mass in patients with acute exacerbation of chronic obstructive pulmonary disease

To identify factors associated with LMM in patients with AECOPD, multivariate logistic regression analysis was performed with the presence of LMM as the dependent variable. After excluding the collinear body-composition variables (SLM, SMM, and BFM) to avoid circularity and multicollinearity, the following covariates were entered: age, sex, BMI, FVC, FEV1, PEF, and Alb. The results showed that older age [odds ratio (OR) = 1.127, 95% confidence interval (CI): 1.045–1.216, p = 0.002], lower BMI (OR = 0.631, 95% CI: 0.522–0.763, p < 0.001), and lower FEV1 (OR = 0.112, 95% CI: 0.031–0.403, p < 0.001) were significantly associated with the occurrence of LMM (Table 8).

Table 5

Correlation analysis of SMMI and BMI with body composition parameters

Parameter	BMI		SMMI	
	r_s	p-value	r_s	p-value
SLM, kg	0.235	0.011	0.235	0.010
SMM, kg	0.221	0.015	0.276	0.002
FFM, kg	-0.116	0.207	0.045	0.672
BFM, kg	0.108	0.243	0.192	0.039
BFM, %	-0.090	0.370	-0.072	0.458
PhA, °	-0.054	0.574	-0.018	0.845

SMMI – skeletal muscle mass index; BMI – body mass index; SLM – soft lean mass; SMM – skeletal muscle mass; FFM – fat-free mass; BFM – body fat mass; PhA – phase angle.
***Note*: Spearman's rank correlation coefficient (r_s) was used.**

Table 6

Correlation analysis of body composition parameters with pulmonary function indicators (FVC, FEV1, and PEF)

Parameter	FVC		FEV1		PEF	
	r_s	p-value	r_s	p-value	r_s	p-value
SLM, kg	0.316	0.001	0.262	0.003	0.294	0.001
SMM, kg	0.317	0.001	0.272	0.002	0.292	0.001
FFM, kg	0.084	0.339	0.061	0.514	0.167	0.072
BFM, kg	0.046	0.615	0.151	0.114	0.235	0.010
BFM, %	-0.117	0.174	0.031	0.709	0.106	0.256
PhA, °	0.096	0.316	0.131	0.155	0.131	0.161

FVC – forced vital capacity; FEV1 – forced expiratory volume in 1 sec; PEF – peak expiratory flow; SLM – soft lean mass; SMM – skeletal muscle mass; FFM – fat-free mass; BFM – body fat mass; PhA – phase angle.
***Note*: Spearman's rank correlation coefficient (r_s) was used.**

Table 7

Correlation analysis of body composition parameters with FEV1/FVC ratio and respiratory impedance

Parameter	FEV1/FVC ratio		Respiratory impedance	
	r_s	p-value	r_s	p-value
SLM, kg	0.003	0.963	-0.161	0.081
SMM, kg	0.045	0.620	-0.144	0.121
FFM, kg	0.019	0.841	-0.163	0.079
BFM, kg	0.239	0.009	0.019	0.836
BFM, %	0.272	0.002	-0.041	0.665
PhA, °	0.072	0.437	-0.195	0.031

FEV1 – forced expiratory volume in 1 sec; FVC – forced vital capacity; SLM – soft lean mass; SMM – skeletal muscle mass; FFM – fat-free mass; BFM – body fat mass; PhA – phase angle.
***Note*: Spearman's rank correlation coefficient (r_s) was used.**

Table 8

Multivariate logistic regression analysis of factors associated with low muscle mass in AECOPD patients

Parameter	*β*	SE	Wald	*p*-value	OR	95% CI for OR
Age	0.122	0.039	9.822	0.002	1.127	1.045–1.216
BMI	-0.460	0.097	22.576	< 0.001	0.631	0.522–0.763
FEV1	-2.187	0.656	11.133	< 0.001	0.112	0.031–0.403
Constant	5.003	2.993	2.789	/	/	/

AECOPD – acute exacerbation of chronic obstructive pulmonary disease; SE – standard error; OR – odds ratio; CI – confidence interval; BMI – body mass index; FEV1 – forced expiratory volume in 1 sec.

Discussion

Low SMM is an age-related condition characterized by muscle atrophy, leading to adverse outcomes such as reduced QoL, impaired mobility, and exercise intolerance [5, 6]. As one of the complications of COPD, the development of muscle wasting is associated not only with reduced physical activity but also with mechanisms involving chronic inflammation and nutritional imbalances [9, 13]. AECOPD patients with comorbid LMM experience higher hospitalization rates, prolonged hospital stays, and a significantly increased risk of adverse events such as osteoporosis [14]. Ultimately, a synergistic interaction may exist between muscle loss and declining lung function, potentially contributing to adverse clinical outcomes; however, the causal nature of this relationship requires further investigation.

Regarding studies on the relationship between COPD and muscle wasting, current evidence suggests that factors such as age, sex, smoking, BMI, disease duration, and inflammatory markers influence the presence of LMM in COPD patients [15–17]. The present analysis identified age, BMI, and FEV1 as factors associated with LMM in this study population. A total of 106 AECOPD patients were enrolled and divided into an LMM group and a non-LMM group based on the presence of LMM. Among them, 60 patients (approximately 56.6%) were identified with LMM, while 46 were not. This proportion is consistent with a previous international study reporting a high prevalence of muscle wasting in AECOPD patients, and aligns with the findings of Trajanoska et al. [14]. Among patients with LMM, 91.7% (55/60) were male and 8.3% (5/60) were female; however, statistical analysis revealed no significant difference between sexes. This is in line with the ongoing debate regarding sex differences in muscle mass. Some studies have reported a higher prevalence of low muscle mass in males than in females [18]. The lack of statistical significance in this study may be attributable to the relatively small sample size and the low proportion of female participants.

Smoking is a well-established associated factor for COPD and is also associated with increased mortality [16]. Smokers are more prone to respiratory symptoms, pulmonary function abnormalities, systemic inflammation, and higher mortality rates [17]. Evidence suggests that smoking enhances pulmonary oxidative stress, reduces body weight and muscle mass, and induces muscle fiber atrophy [19]. Chronic smoking may suppress muscle protein synthesis, elevate inflammatory cytokine levels, impair energy metabolism, and reduce oxidative phosphorylation capacity, leading to skeletal muscle damage [20, 21]. Over time, these effects contribute to muscle wasting. This study found a significant difference in BMI between the LMM and non-LMM groups, with patients in the LMM group exhibiting significantly lower BMI values. As BMI is commonly used to assess nutritional status, this finding suggests that patients in the LMM group may be at higher risk of malnutrition. A cross-sectional study involving 263 COPD patients reported that approximately 24% had LMM, of whom about 14% were malnourished. Moreover, patients with LMM and malnutrition showed significantly lower BMI, handgrip strength, and FFM compared to those without [22]. Costa et al. [23] also demonstrated that AECOPD patients with LMM had significantly lower BMI than those without. These findings are consistent with the results of the present study regarding BMI alterations.

The proportion of LMM in our hospitalized cohort (~60%) was considerably higher than the previously reported 21.6%. This discrepancy is likely explained by differences in study populations: the 21.6% estimate came predominantly from community-dwelling COPD patients with stable disease [7], whereas our patients were enrolled during acute exacerbations requiring hospitalization. Acute exacerbations amplify systemic inflammation, reduce dietary intake, increase catabolic stress, and often involve corticosteroid use, all of which accelerate muscle wasting [4]. Additionally, our cohort had a higher median age and a predominance of males, both well-recognized associated factors for muscle loss. Therefore, the higher proportion in our study reflects the more severe clinical spectrum of COPD in hospitalized settings.

A meta-analysis has shown that patients with COPD have significantly elevated inflammatory markers [e.g., leukocytes, CRP, IL-6, fibrinogen, and tumor necrosis factor (TNF)-α], indicating persistent systemic inflammation [24]. Notably, even former smokers maintain a state of low-grade inflammation. Dahl et al. [25] further reported that elevated levels of systemic inflammatory markers such as fibrinogen accelerate the decline in lung function among COPD patients. As previously discussed, inflammatory mediators and oxidative stress play critical roles in both acute exacerbations of COPD and the pathogenesis of muscle wasting. An imbalance between oxidation and antioxidant defenses can damage alveolar epithelium and drive airway remodeling, increasing the work of breathing, elevating energy expenditure, and promoting subsequent skeletal muscle breakdown. Studies have shown that patients with LMM have significantly higher levels of CRP and IL-6 compared to those without, and

similar findings have been reported in COPD patients with comorbid muscle wasting, who exhibit elevated IL-6 and TNF-α levels [26]. In the present study, however, no significant differences were observed between the two groups in terms of CRP or IL-6 levels. This lack of association may be attributable to differences in assay methods or prior antibiotic use before hospital admission. Serum Cr, a metabolite produced by muscle tissue, is primarily influenced by SMM and serves as an indicator of overall muscle mass [27]. Cys C, a small-molecular-weight protein, is expressed on the surface of all nucleated cells. Some studies have suggested that the Cr-to-Cys C ratio may serve as a surrogate marker for muscle wasting [28]. However, the present study did not identify significant differences between the LMM and non-LMM groups in terms of serum Cr, Cys C, or their ratio, possibly due to variations in study populations or laboratory methods.

It has been reported that patients with LMM exhibit higher leukocyte and Neut counts than those without. With advancing age, Neut functions such as chemotaxis decline, contributing to systemic inflammation and subsequent muscle loss [29]. Nevertheless, the current study found no significant differences in leukocyte, Neut, or Lym counts between the two groups, which may be related to prior medication use or assay sensitivity. Among all serological markers assessed, only Alb levels differed significantly between the groups. Alb also indicates nutritional status, and the lower Alb levels observed in the LMM group suggest poorer nutritional status, which may reduce muscle protein synthesis and ultimately affect muscle strength and mass.

Pulmonary function testing, a key measure of respiratory muscle strength and function, is essential for the diagnosis of COPD. While muscle loss research has focused primarily on appendicular skeletal muscle loss, the decline in respiratory muscle function due to muscle wasting should not be underestimated. The respiratory muscles, including the diaphragm and intercostal muscles, work synergistically to facilitate breathing [30]. Studies have shown that diaphragm and respiratory muscle strength decline with age [31], with the diaphragm–accounting for 60% to 80% of respiratory function–being particularly susceptible to muscle wasting [32]. This decline in respiratory muscle function results from reduced lung compliance and elasticity, reflected in altered pulmonary function parameters such as FVC, FEV1, and PEF [33, 34]. Another study suggested that rapid declines in lung function are associated with reductions in muscle mass and strength [35].

The present study found that FVC, FEV1, and PEF were lower in the LMM group compared to the non-LMM group. Among these, FEV1 is a critical pulmonary function indicator. An FEV1/FVC ratio < 70% indicates airflow limitation, whereas a reduced FVC with preserved or increased FEV1/FVC ratio suggests restrictive impairment. A low FEV1 (< 80% predicted) generally reflects obstructive or mixed ventilatory defects, rather than an isolated restrictive pattern. Regardless of the pattern–restrictive, obstructive, or mixed–FEV1 serves as a valuable parameter for assessing ventilatory function [36]. Jeon et al. [10] examined the relationship between lung function and muscle wasting in 463 older adults in Korea and found that lower FVC and FEV1 values were associated with a higher risk of LMM, with this association being more pronounced in males. After adjusting for age, smoking, and physical activity, FEV1 remained correlated with LMM in both sexes, leading to the conclusion that reduced lung function is associated with LMM in community-dwelling older adults. Park et al. [11] reported similar findings in healthy individuals without respiratory disease, showing that LMM was significantly associated with an increased risk of FVC < 80% and FEV1 < 80%. PEF has also been shown to be lower in patients with LMM, as the decline in muscle mass and strength affects respiratory muscles. Ridwan et al. [37] suggested that PEF is independently associated with muscle wasting. In line with Mansour et al. [38], the present study found no significant difference in FEV1/FVC ratio between the two groups.

This study focused on the association of LMM with pulmonary function in AECOPD patients. The SMMI, a diagnostic indicator of LMM, is derived from appendicular SMM normalized by height squared. SMMI has been reported not only to influence muscle mass negatively but also to have prognostic implications [39]. As previously noted, FVC, FEV1, and PEF were lower in the LMM group. Correlation analyses revealed that both SMMI and BMI were positively correlated with FEV1 and FVC. Won et al. [40] similarly found positive correlations between SMMI and FVC and FEV1. Martínez-Luna et al. [12] also provided data supporting a positive correlation between SMMI and FEV1. The relationship between BMI and lung function in COPD is complex and involves the so-called “obesity paradox”. In the general population, higher BMI is often associated with reduced lung function due to mechanical restriction. However, in COPD, some studies have shown that overweight or mildly obese patients may have better preserved FEV1 and lower mortality, possibly due to greater nutritional reserves and reduced systemic inflammation [39]. Nevertheless, this association is not linear; Ochs-Balcom et al. [41] reported that when BMI exceeds 25 kg/m², it may be negatively correlated with FEV1 and FVC. Our finding that lower BMI is associated with LMM in COPD likely reflects the protective role of nutritional status rather than a direct beneficial effect of adiposity on lung mechanics. Consistent with other studies, the present study found no association between SMMI or BMI and FEV1/FVC ratio. Skeletal muscle accounts for approximately 40–50% of body weight [42, 43]. LMM has been identified as a predictor of mortality in certain populations, including COPD patients and the elderly [39]. The present study revealed significant differences in SLM, SMM, and BFM between the LMM and non-LMM groups. Furthermore, correlation analyses indicated that SMM and SLM were positively correlated with FEV1, FVC, and PEF. These findings may explain the LMM and poorer pulmonary function observed in the LMM group, potentially indicating a worse prognosis.

PhA, which reflects cellular integrity and function, serves as an indicator of nutritional status and prognosis in various diseases. Low PhA has been associated with reduced FEV1, QoL, muscle strength, and exercise tolerance [44], as well as with impaired pulmonary function [45]. In COPD patients, PhA is an independent predictor of mortality [46]. How-

ever, the present study found no significant difference in PhA between groups, possibly due to variations in patient status during BIA or individual differences. Multivariate analysis identified age, BMI, and FEV1 as factors associated with the presence of LMM in this study population. Age emerged as a factor with positive association, while BMI and FEV1 showed inverse associations, implying potential nutritional and disease-severity pathways.

Limitations of the study

Several limitations of this study should be acknowledged. First, the sample size was relatively small (n = 106), which may limit the statistical power for detecting certain associations and precludes subgroup analyses. Second, this was a single-center study conducted in Lanzhou, Northwest China; the findings may not be generalizable to other populations or regions. Third, the cross-sectional design prevents any causal or temporal conclusions regarding the relationships between LMM, lung function, and other parameters; we can only report associations, not causality. Fourth, body composition was assessed using BIA, which can be influenced by hydration status and does not directly measure muscle quality or infiltration by fat. Fifth, and most importantly, we did not assess muscle strength (e.g., handgrip) or physical performance (e.g., gait speed, chair stand test) because of the retrospective nature of data collection, so we defined LMM solely by low SMMI according to the AWGS 2019 criteria. Consequently, these findings cannot be extrapolated to a diagnosis of sarcopenia; they strictly apply to LMM. It is important to note that these findings are strictly applicable to the assessment of LMM based on SMMI, rather than a comprehensive diagnosis of sarcopenia, which would require evaluation of muscle strength and physical performance. Sixth, important potential confounders–including sex, cumulative smoking exposure, GOLD stage, exacerbation history, COPD duration and severity, systemic and inhaled corticosteroid use, comorbidities, level of physical activity, and nutritional status–were not available in the medical records and therefore could not be adjusted for in the multivariate analysis. Consequently, the terms “risk factors” and “protective factors” are inappropriate in this context. These findings strictly represent observational associations. Seventh, all patients were enrolled during acute exacerbations, which may limit the applicability of these results to stable COPD patients. Additionally, heterogeneity in the timing of spirometry (pre-discharge vs. post-discharge) was evaluated *via* sensitivity analysis, which showed no significant interaction; however, we acknowledge this as a potential source of residual bias. Future prospective studies with larger sample sizes, multicenter designs, comprehensive muscle assessments (including strength and function), and detailed recording of confounders are needed to confirm and extend these findings.

Conclusion

This study examined the association between LMM, body composition, pulmonary function, and clinical parameters in hospitalized AECOPD patients. Patients with comorbid LMM exhibited significantly worse pulmonary function–particularly FVC, FEV1, and PEF–indicating a significant statistical association between LMM and reduced lung function. Significant positive correlations were found between body composition (SMMI, SMM, SLM) and pulmonary function (FVC, FEV1) in patients with LMM, underscoring the link between peripheral and respiratory muscle status. Multivariate logistic regression identified older age, lower BMI, and lower FEV1 as independently associated factors for LMM in AECOPD. Advanced age showed a positive association, likely reflecting cumulative effects of anabolic resistance, chronic inflammation, and inactivity. Higher BMI and preserved FEV1 showed inverse associations with LMM, which may reflect more favorable nutritional reserves and milder disease severity; however, these relationships should be regarded strictly as observational associations. Early identification of at-risk individuals, especially older adults with low BMI and advanced airflow limitation, may be a potential target for interventions to mitigate muscle wasting; however, these findings should be interpreted as observational associations only, and future prospective studies are warranted to confirm causality.

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Effects of simulated gastric acid on the optical properties of CAD/CAM glass-ceramics

Uticaj simulirane želudačne kiseline na optička svojstva CAD/CAM staklokeramike

Isil Kecik Buyukhatipoglu, Derya Dogan Evlice, Fatih Sari, Asli Secilmis

Gaziantep University, Faculty of Dentistry, Department of Prosthodontics, Gaziantep, Türkiye

Abstract

Background/Aim. The influence of glazing and polishing procedures on surface changes in contemporary computer-aided design (CAD) and computer-aided manufacturing (CAM) – CAD/CAM glass-ceramics during exposure to simulated gastric acid remains unclear. The aim of this study was to investigate the effects of simulated gastric acid on the optical properties of glazed or polished glass-ceramic materials based on lithium disilicate, advanced lithium disilicate, and zirconia-reinforced lithium silicate. **Methods.** The specimens were divided into six experimental groups according to the ceramic material and surface-finishing procedure recommended by the manufacturers: CEREC Tessera™-glazed (CTG), CEREC Tessera™-polished (CTP), IPS e.max® CAD-glazed (IPSG), IPS e.max® CAD-polished (IPSP), VITA Suprinity®-glazed (VSG), and VITA Suprinity®-polished (VSP), with 10 specimens allocated to each group (n = 10). To simulate gastric conditions, the samples were immersed in hydrochloric acid (HCl, 0.06 M; pH 1.2) and incubated at 37 °C for 96 hrs. Before and after acid immersion, perceptual lightness (L*), red-green (a*), and yellow-blue (b*) coordinate values were recorded using a calibrated digital spectrophotometer. Color difference (ΔE_{00}) and translucency parameter (TP_{00}) were subsequently calculated using the CIEDE2000 formula. The data were analyzed using the Kruskal-Wallis and Wilcoxon signed-rank tests at a significance level of $p < 0.05$. **Results.** Acid immersion did not significantly affect the ΔE_{00} of the tested CAD/CAM glass-ceramics ($p > 0.05$). In contrast, TP_{00} was significantly influenced by both ceramic material and surface-finishing protocol. Significant reductions in TP_{00} values were observed in the CTG, CTP, and IPSG groups after acid immersion, whereas IPSP, VSG, and VSP showed no significant changes. Analysis of ΔTP_{00} revealed significant intergroup differences, with the highest median values observed in the IPSG, CTP, and VSP groups and the lowest in IPSP. **Conclusion.** Within the limitations of this *in vitro* study, no significant differences in ΔE_{00} were observed among the tested experimental groups following exposure to simulated gastric acid. However, TP_{00} appeared to be more sensitive to the acid challenge than color, and the changes varied according to the tested ceramic material and surface-finishing protocol.

Keywords:
ceramics; color; dental materials; fluorescence; gastric acid; materials testing.

Apstrakt

Uvod/Cilj. Uticaj postupaka glaziranja i poliranja na promene površinskih osobina savremenih staklokeramičkih materijala izrađenih primenom kompjuterski potpomognutog dizajniranja (*computer-aided design* – CAD) i kompjuterski potpomognute izrade (*computer-aided manufacturing* – CAM) – CAD/CAM tokom izlaganja simuliranoj želudačnoj kiselini još uvek nije dovoljno razjašnjen. Cilj rada bio je da se ispita uticaj simulirane želudačne kiseline na optička svojstva glaziranih ili poliranih staklokeramičkih materijala na bazi litijum-disilikata, naprednog litijum-disilikata i litijum-silikata ojačanog cirkonijom. **Metode.** Uzorci su podeljeni u šest eksperimentalnih grupa prema vrsti keramičkog materijala i postupku završne obrade površine koji preporučuju proizvođači: CEREC Tessera™-glazirana (CTG), CEREC Tessera™-polirana (CTP), IPS e.max® CAD-glazirana (IPSG), IPS e.max® CAD-polirana (IPSP), VITA Suprinity®-glazirana (VSG) i VITA Suprinity®-polirana (VSP), sa po 10 uzoraka u svakoj grupi (n = 10). Radi simulacije uslova u želucu, uzorci su potopljeni u hlorovodoničnu kiselinu (HCl, 0,06 M; pH 1,2) i inkubirani na temperaturi od 37 °C tokom 96 sati. Pre i nakon potapanja u kiselinu, početne vrednosti koordinata perceptivne svetline (L*), žutocrvene (a*) i žutoplave (b*) izmerene su pomoću kalibrisanog digitalnog spektrofotometra. Razlika u boji (ΔE_{00}) i vrednosti

Correspondence to: Fatih Sari, Gaziantep University, Faculty of Dentistry, Department of Prosthodontics, Şehitkamil, 27 310 Gaziantep, Türkiye. E-mail: fatihsa00@hotmail.com

parametra translucencije (TP‰) naknadno su izračunate pomoću formule CIEDE2000. Podaci su analizirani primenom Kruskal-Wallis-ovog i Wilcoxon-ovog *signed-rank* testa, uz nivo značajnosti od $p < 0,05$. **Rezultati.** Potapanje u kiselinu nije značajno uticalo na ΔE‰ ispitivanih CAD/CAM staklokeramika ($p > 0,05$). Nasuprot tome, na TP‰ značajno su uticali i vrsta keramičkog materijala i postupak završne obrade površine. Značajno smanjenje vrednosti TP‰ uočeno je u grupama CTG, CTP i IPSG nakon potapanja u kiselinu, dok u grupama IPSP, VSG i VSP nisu utvrđene značajne promene. Analiza promene parametra ΔTP‰ pokazala je značajne razlike između grupa, pri čemu su najviše vrednosti medijane zabeležene u grupama IPSG, CTP i VSP, a najniže u grupi IPSP. **Zaključak.** Uz uvažavanje ograničenja ove *in vitro* studije, nisu utvrđene značajne razlike u vrednostima ΔE‰ između ispitivanih eksperimentalnih grupa nakon izlaganja simuliranoj želudačnoj kiselini. Međutim, TP‰ se pokazao osetljivijim na delovanje kiseline u poređenju sa bojom, a promene su varirale u zavisnosti od ispitanog keramičkog materijala i postupka završne obrade.

Ključne reči:
keramika; boja; stomatološki materijali; fluorescencija; želudačna kiselina; materijali, testiranje.

Introduction

Dental erosion is the chemical dissolution of tooth structure by acids without bacterial involvement [1]. An example of an external risk factor includes beverages like soft drinks, which increase dietary acidity, while gastric acid is an internal risk factor [2–5].

The etiology of acid in the intraoral region is multifactorial, including, but not limited to, gastroesophageal reflux disease (GERD), eating disorders such as bulimia nervosa, and nausea during pregnancy. GERD is a prevalent medical condition with a global prevalence ranging from 2.5% to 28.8% worldwide, with a higher prevalence observed in Europe and the United States [6, 7]. GERD can develop when the upper esophageal sphincter involuntarily relaxes, allowing acid from the stomach to pass into the mouth [8]. Bulimia nervosa is a clinical condition that includes eating disorders such as excessive concern about body weight and shape, self-induced purging, and preventing weight gain [9]. In addition to erosive tooth wear, high levels of intraoral acid can also affect the surface properties of restorative materials [10–12].

Advancements in ceramics, bonding protocols, and fabrication methods have facilitated the production of aesthetically pleasing, biocompatible, and durable fixed prosthetic restorations. Translucency parameter (TP‰) and optical properties that affect the aesthetic outcomes of ceramic restorations have been linked to patient satisfaction [13, 14]. Finishing and polishing enhance the restoration's surface quality, protect opposing teeth from abrasion, and prevent bacteria from adhering to the restoration surface. In the literature, there are studies reporting that polishing and finishing of the restoration improve color stability [15, 16].

IPS e.max® CAD (IPS) was introduced in 2006 as a lithium disilicate glass-ceramic material prepared for computer-aided design (CAD) and computer-aided manufacturing (CAM) – CAD/CAM systems. Its use has increased over time for chairside restorations, due to its aesthetic nature, impressive strength, durability, and ease of use. VITA Suprinity® (VS) is a zirconia-reinforced lithium silicate (ZLS) glass-ceramic containing 8–12% by weight (wt%) zirconium dioxide (ZrO_2) [17–19].

CEREC Tessera™ (CT) is an advanced lithium disilicate glass-ceramic introduced in 2021. It consists of lithium disilicate and lithium aluminum silicate (virgilite) crystals embedded in a zirconia-enriched glassy matrix [20]. To the best of our knowledge, no previous study has evaluated the effect of simulated gastric acid exposure on the optical properties of this recently introduced material. The manufacturer also offers a rapid glazing/crystallization protocol that shortens the overall fabrication time of CT restorations [20]. Contemporary CAD/CAM glass-ceramics differ considerably in crystalline composition, crystal morphology, crystal volume fraction, and glass matrix characteristics. These microstructural differences may influence their optical behavior, chemical durability, and resistance to acid-induced degradation [21, 22].

Restoration surfaces encountered clinically may be either factory-glazed or adjusted and subsequently polished [23]. Despite the widespread clinical use of glazing and mechanical polishing, their effects on the mechanical and optical properties of glass-ceramic materials remain incompletely understood, and no standardized protocol has yet been established for the final finishing of milled ceramic surfaces [13, 23, 24]. Previous investigations have demonstrated that surface-finishing procedures significantly influence the optical properties, surface integrity, and resistance of ceramic materials to environmental degradation [25, 26]. Therefore, evaluating three contemporary CAD/CAM glass-ceramic systems under identical simulated gastric acid conditions may improve understanding of how material composition and surface-finishing influence their optical performance.

The aim of this study was to examine the effects of simulated gastric acid on the optical properties of glazed and polished CAD/CAM glass-ceramics under standardized experimental conditions.

The first null hypothesis was that simulated gastric acid immersion would not cause significant differences in color change (ΔE‰) among the tested ceramic materials and surface-finishing procedures. The second null hypothesis was that no significant differences in TP‰ would be observed among the tested ceramic materials and surface-finishing procedures following simulated gastric acid immersion.

Methods

As this was an *in vitro* study performed exclusively on commercially available CAD/CAM ceramic materials, ethical approval was not required. An *a priori* sample size calculation was performed using G*Power software (version 3.1.9.7; Heinrich Heine University Düsseldorf, Düsseldorf, Germany). The calculation was based on a two-way fixed-effects analysis of variance – ANOVA for evaluating the material × surface-finishing interaction in a 3 × 2 factorial design. Assuming an effect size of $f = 0.60$, a significance level of $\alpha = 0.05$, and a statistical power of 80% ($1-\beta = 0.80$), the minimum required sample size was calculated to be 31 specimens. To provide equal allocation across the six experimental groups, at least 36 specimens were required (n = 6 *per* group). Considering previous *in vitro* research evaluating the color stability and TP‰ of CAD/CAM ceramics [26], the sample size was increased to 10 specimens *per* group, resulting in a total of 60 specimens. Under these assumptions, the final sample size provided approximately 98.7% statistical power for detecting the specified interaction effect.

Chemical compositions, crystal structures, and firing temperatures of the three different tested materials are presented in Table 1. A total of 60 ceramic specimens with dimensions of 10 × 10 mm and a thickness of 1.0 ± 0.05 mm were prepared from A2 HT color blocks using a precision cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) (n = 20 *per* material). The specimens' surfaces were then polished with 400, 600, 800, and 1200-grit silicon carbide papers [27, 28] (3M ESPE, St. Paul, MN, USA) under water cooling for 15 s prior to the application of the polishing or glazing procedures. The thickness of each specimen was subsequently measured with a digital micrometer (Minitech 233 Press, Grenoble, France) with an accuracy of 0.01 mm.

Subsequently, the specimens were divided into two subgroups according to the surface-finishing treatment to be applied (n = 10). To minimize inter-operator variability, all polishing, glazing, and color measurement procedures were performed by a single prosthodontist (D.D.E.). Before the study, the operator was calibrated according to the experimental protocol and standardized for all experimental procedures.

The specimens were allocated into six experimental groups (n = 10 *per* group) according to the ceramic material and surface-finishing protocol: CT-glazed (CTG), CT-polished (CTP), IPS-glazed (IPSG), IPS-polished (IPSP), VS-glazed (VSG), and VS-polished (VSP).

All specimens were fabricated under highly standardized laboratory conditions and possessed identical dimensions, thicknesses, shades, and surface preparation procedures. The glazing and polishing procedures were carried out using the manufacturer-recommended systems specific to each ceramic material to reflect routine clinical practice, in accordance with the methodology described by Demirel et al. [29]. To minimize operator-related variability, all glazing, polishing, and optical measurements were performed by a single operator under standardized laboratory conditions. All polishing procedures were performed using low-speed handpieces operated by the same operator at a standardized rotational speed to ensure procedural consistency.

The appropriate glaze material was applied to one surface of the specimens in the CTG, IPSG, and VSG groups and fired in a porcelain furnace (Programat P300, Ivoclar Vivadent). Likewise, one surface of the specimens in the CTP, IPSP, and VSP groups was polished using the manufacturer-recommended polishing system with rubber polishers mounted on a low-speed handpiece (NSK, Nakanishi Inc., Tochigi, Japan). The groups, materials, and surface-finishing protocols are summarized in Table 2.

Table 1

The chemical compositions, crystallization, and firing temperatures of the used materials

Material	CEREC Tessera™	IPS e-max® CAD	VITA Suprinity®
Manufacturer	Dentsply Sirona, York, PA, USA	Ivoclar Vivadent, Schaan, Liechtenstein	Vita Zahnfabrik, Bad Säckingen, Germany
Chemical composition, wt%	$Li_2Si_2O_5$: 90%, Li_3PO_4: 5%, $Li_{0.5}Al_{0.5}Si_2.5O_6$: 5%, (the lithium disilicate and virgilite crystals are embedded in a zirconia-enriched glassy matrix)	SiO_2: 57–80%, Li_2O: 11–19%, K_2O: 0–13%, other oxides: 0–8%	SiO_2: 56–64%, Li_2O: 15–21%, ZrO_2: 8–12%, P_2O_5: 3–8%, K_2O: 1–4%, Al_2O_3: 1–4%, CeO_2: 0–4%, pigments: 0–4%
Crystallization	supplied fully crystallized; final matrix firing (glaze firing) performed at 760 °C (≈ 2 min; approximately 4.5 min in CEREC SpeedFire), producing surface healing and final mechanical properties	standing-by: 403 °C, heating cycle: 90 °C/min, firing cycle: 650 °C, heating cycle 20 °C/min, firing cycle: 730 °C, holding cycle: 2 min	standby: 400 °C heating cycle: 55 °C /min firing cycle: 840 °C vacuum on/off: 410/840 °C cooling cycle: 680 °C

***Note*: CEREC Tessera™ composition is expressed as crystalline phases (wt%) as reported by the manufacturer [20], whereas IPS e.max® CAD and VITA Suprinity® are expressed as oxide content [27]. Data were compiled from the manufacturers' technical documentation [20] and previous studies [17–20, 27].**

Table 2

Materials and finishing protocols used in the groups

Groups	Materials used (manufacturer)	Glazing/polishing protocol
CEREC Tessera™		
CTG	Universal Spray Glaze (DeguDent GmbH, Hanau-Wolfgang, Germany)	starting application: 400 °C closing time: 1 min pre-heating: 1 min heating cycle: 55 °C/min final cycle: 760 °C waiting time: 2 min no vacuum
CTP	OptraGloss Ceramic Kit (Ivoclar Vivadent AG, Schaan, Liechtenstein)	pre-polishing with dark blue (Flame VP), high brightness polisher (Light Blue/Flame HP) high gloss polisher (Light Blue/Wheel HP)
IPS e.max CAD®		
IPSG	IPS e.max® Ceram Glaze Spray (Ivoclar Vivadent AG, Schaan, Liechtenstein)	standing-by application: 403 °C heating cycle: 60 °C/min firing cycle: 710 °C
IPSP	OptraGloss Ceramic Kit (Ivoclar Vivadent AG, Schaan, Liechtenstein)	pre-polishing with dark blue (Flame VP) high brightness polisher (Light Blue/Flame HP) high gloss polisher (Light Blue/Wheel HP)
VITA Suprinity®		
VSG	Vita Akzent Plus Glaze (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany)	standing-by application: 400 °C closing time: 4 min heating increase rate: 80 °C /min holding cycle: 800 °C holding cycle: 1 min
VSP	Vita Suprinity® Polishing Set (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany)	pre-polishing using pink diamond polishing using white diamond

CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP – IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.
***Note*: Data were compiled from the manufacturers' technical documentation [20] and previous studies [17–20, 27].**

Color measurements were performed before and after simulated gastric acid aging using a digital spectrophotometer (VITA EasyShade Advance 4.0, VITA Zahnfabrik GmbH, Bad Säckingen, Germany). The instrument was calibrated according to the manufacturer's instructions before each measurement session [30]. All measurements were conducted in the same laboratory under standardized ambient lighting conditions to ensure consistency throughout the study. Because the spectrophotometer incorporates a standardized internal light source, the influence of ambient lighting on color measurements was minimized.

Measurements were performed against standardized white, black, and neutral gray backgrounds. The neutral gray background was used exclusively for ΔE_{00} measurements obtained before and after simulated gastric acid immersion to ensure identical optical conditions for repeated color assessment, whereas the white and black backgrounds were used for TP_{00} calculations [31]. During each measurement, the spectrophotometer tip was positioned perpendicular to the specimen surface using a standardized measurement geometry in accordance with the manufacturer's operating instructions [30]. Three consecutive measurements were obtained for each specimen, and the mean L*, a*, and b* values were used to calculate the ΔE_{00} and TP_{00} values for statistical analysis. The L* coordinate represents perceptual lightness (0 = black, 100 = white), whereas the a* and b* coordinates represent the red-green and yellow-blue chromatic axes, respectively, with positive and negative values indicating the direction of color change.

ΔE_{00} were calculated using the CIEDE2000 formula [32, 33] from measurements obtained over the neutral gray background at baseline and after simulated gastric acid aging:

$$\Delta E_{00} = [(\Delta L'/(kLSL))^2 + (\Delta C'/(kCSC))^2 + (\Delta H'/(kHSH))^2 + RT(\Delta C'/(kCSC))(\Delta H'/(kHSH))]^{1/2}$$

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ represent the differences in lightness, chroma, and hue, respectively; SL, SC, and SH are the corresponding weighting functions; kL, kC, and kH are the parametric correction factors; and RT is the rotation term accounting for the interaction between chroma and hue differences, as described by Sharma et al. [32].

The TP_{00} was determined from the color coordinates measured over the white and black backgrounds using the CIEDE2000-based translucency approach. For all calculations, the parametric factors for lightness, chroma, and hue were set to 1 under reference conditions [33, 34]. Three consecutive measurements were performed for each specimen. The mean L*, a*, and b* values obtained from these measurements were calculated and subsequently used to determine the ΔE_{00} and TP_{00} values for statistical analysis. The greater the TP_{00} value, the higher the material's

translucency. A value of 0 reveals the presence of opacity, while high TP_{00} values indicate translucent materials:

$$TP_{00} = \{[(L'B - L'W)/SL]^2 + [(C'B - C'W)/SC]^2 + [(H'B - H'W)/SH]^2 + RT[(C'B - C'W)/SC][(H'B - H'W)/SH]\}^{1/2}$$

TP_{00} values were calculated according to Paravina et al. [33]. Subsequently, the absolute change in translucency parameter (ΔTP_{00}) was calculated as the absolute difference between the baseline and post-immersion TP_{00} values and used for statistical analysis. ΔTP_{00} was defined as the absolute difference between the TP_{00} values measured before and after simulated gastric acid immersion ($|TP_{00baseline} - TP_{00post}|$).

The specimens were immersed in hydrochloric acid (HCl) 0.06 M, pH 1.2 to simulate gastric acid exposure according to a previously published protocol [22, 34, 35]. The specimens were stored in the acid for 96 hrs at 37 °C in an incubator, and the pH was monitored every 24 hrs [22, 35]. After acid immersion, the specimens were rinsed with distilled water. Color measurements were then repeated, and the ΔE_{00} was calculated according to the CIEDE2000 formula [32]. Subsequently, TP_{00} values were recalculated [34].

Representative specimens from each group were examined before and after simulated gastric acid immersion using a field-emission scanning electron microscope (FESEM) (Gemini SEM 300, ZEISS, Oberkochen, Germany). Prior to analysis, all specimens were sputter-coated with a thin gold–palladium (Au–Pd) layer using a sputter coater to improve electrical conductivity and minimize charging artifacts. Surface morphology and microstructural alterations were then evaluated under standardized imaging conditions at ×10,000 magnification.

Statistical analysis

Descriptive statistics were presented as median and interquartile range for numerical variables. The normality of data distribution was assessed using the Shapiro-Wilk test. Intergroup comparisons were performed using the Kruskal-Wallis test, followed by the Dunn multiple comparison test with Bonferroni correction when significant differences were detected. Within-group comparisons between baseline and post-immersion measurements were performed using the Wilcoxon signed-rank test. Effect sizes were calculated and reported as η^2H for the Kruskal-Wallis test and r for the Wilcoxon signed-rank test, together with 95% confidence intervals where appropriate. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA).

Results

The results of the statistical analyses evaluating the effect of simulated gastric acid immersion on the ΔE_{00} of the glass-ceramic materials are presented in Table 3. No significant differences in ΔE_{00} values were observed among the experimental groups ($p = 0.152$). The distribution of ΔE_{00} values among the study groups is illustrated in Figure 1.

The results of the statistical analyses of TP_{00} values are presented in Table 4, and Figure 2 illustrates the TP_{00} values of the experimental groups. Significant differences in TP_{00} values were observed among the groups both before and after simulated gastric acid immersion ($p < 0.001$). Before simulated gastric acid immersion, the VSG and VSP groups exhibited significantly higher TP_{00} values than the CTG group. After simulated gastric acid immersion, the CTG, CTP, and IPSG groups demonstrated significantly lower TP_{00} values than the IPSP, VSG, and VSP groups.

According to the Wilcoxon signed-rank test, simulated gastric acid immersion resulted in significant reductions in TP_{00} values in the CTG ($p = 0.005$), CTP ($p = 0.028$), and IPSG ($p = 0.005$) groups, whereas no significant changes were observed in the IPSP ($p = 0.169$), VSG ($p = 0.059$), or VSP ($p = 0.445$) groups (Table 4).

The absolute ΔTP_{00} after simulated gastric acid immersion differed significantly between the experimental groups ($p = 0.003$) (Table 5).

Field-emission scanning electron microscope results

Representative FESEM micrographs of the tested glass-ceramics before and after simulated gastric acid immersion were presented in Figure 3. The CTG before acid immersion exhibited a relatively smooth and homogeneous surface (Figure 3a). After acid immersion, the surface displayed

Table 3

Color difference (ΔE_{00}) of the groups

Groups	Median (Q1–Q3)	95% CI	*H*-test	*p*-value	η^2H
CTG	1.5 (1.22–1.74)	1.09–1.86	8.077	0.152	0.057
CTP	1.83 (1.65–1.95)	1.65–1.99			
IPSG	1.63 (1.6–1.71)	1.57–1.93			
IPSP	1.71 (1.25–2.00)	1.25–2.00			
VSG	1.5 (1.5–2.3)	1.50–2.30			
VSP	2.09 (2.04–2.09)	1.85–2.25			

Q1-Q3 – first quartile-third quartile; CI – confidence interval; CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP – IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.

***Note*: Descriptive and inferential statistics for ΔE_{00} values of the six experimental groups after simulated gastric acid immersion. Group comparisons were performed using the Kruskal-Wallis test**.

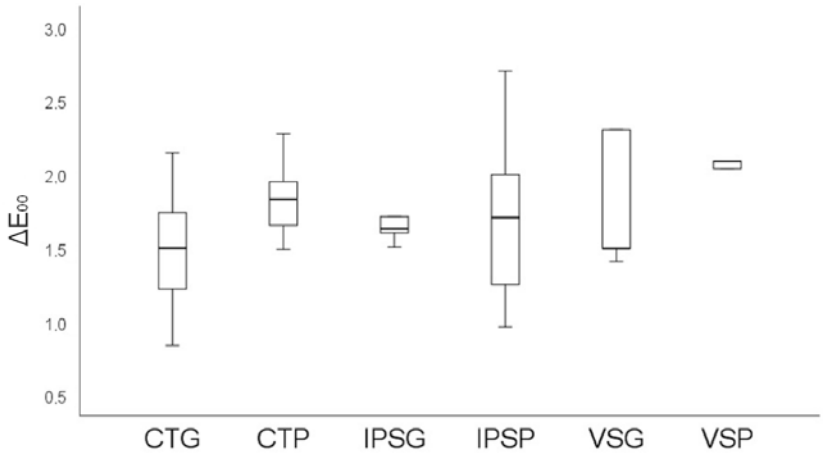

Fig. 1 – Box-and-whisker plots illustrating the distribution of color difference (ΔE_{00}) values for the six experimental groups following simulated gastric acid immersion. The boxes represent the interquartile range, the horizontal line within each box indicates the median, and the whiskers denote the minimum and maximum values. CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP –IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.

Table 4

Translucency parameter (TP_{00}) values before and after simulated gastric acid immersion for each experimental group

Groups	TP_{00} before		TP_{00} after		*Z*	*p*-value	*r*
	median (Q1–Q3)	95% CI	median (Q1–Q3)	95% CI			
CTG	11.68 (10.32–15.98)[a]	10.24–15.98	9.68 (8.29–10.16)[a]	7.13–10.42	-2.803	0.005*	0.886
CTP	14.17 (12.71–19.19)[ab]	12.56–19.19	10.53 (6.77–12.65)[a]	6.77–13.21	-2.191	0.028*	0.693
IPSG	17.09 (16.11–17.52)[ab]	15.90–17.59	10.84 (10.08–11.4)[a]	10.08–11.40	-2.803	0.005*	0.886
IPSP	18.07 (16.15–19.4)[b]	15.92–19.40	18.00 (16.3–18.43)[b]	13.79–18.43	-1.376	0.169	0.435
VSG	21.99 (21.41–22.24)[c]	21.41–22.24	18.21(18.21–23.14)[b]	18.21–23.14	-1.886	0.059	0.596
VSP	21.54 (17.09–24.45)[c]	17.09–24.88	20.48 (17.14–22.74)[b]	17.14–22.74	-0.764	0.445	0.242
H-test	42.035		27.778				
p-value	0.001**		0.001**				
η^2H	0.686		0.422				

Q1–Q3 – first quartile-third quartile; CI – confidence interval; CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP – IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.

***Note*: *$p < 0.05$; **$p < 0.001$. Different superscript lowercase letters within the same column indicate statistically significant differences between groups (Dunn *post hoc* test with Bonferroni correction, $p < 0.05$), whereas groups sharing at least one superscript letter do not differ significantly. *p*-values represent within-group comparisons between baseline and post-immersion measurements using the Wilcoxon signed-rank test. η^2H denotes the effect size for the Kruskal-Wallis test.**

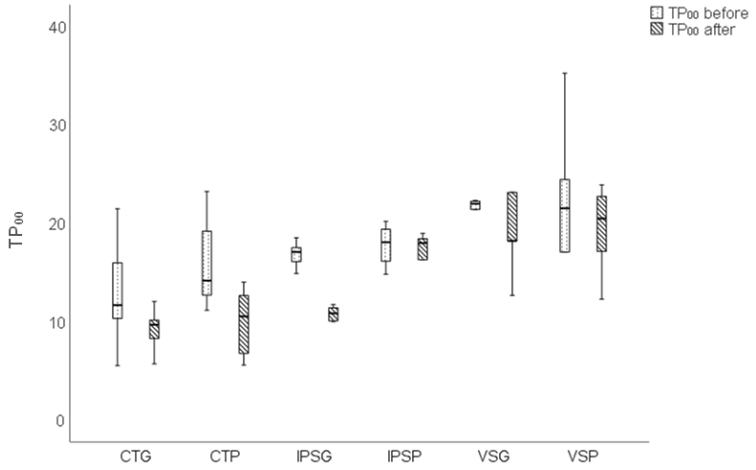

Fig. 2 – Translucency parameter (TP_{00}) values before and after simulated gastric acid immersion for the six experimental groups. The boxes represent the interquartile range, the horizontal line within each box indicates the median, and the whiskers denote the minimum and maximum values. CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP – IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.

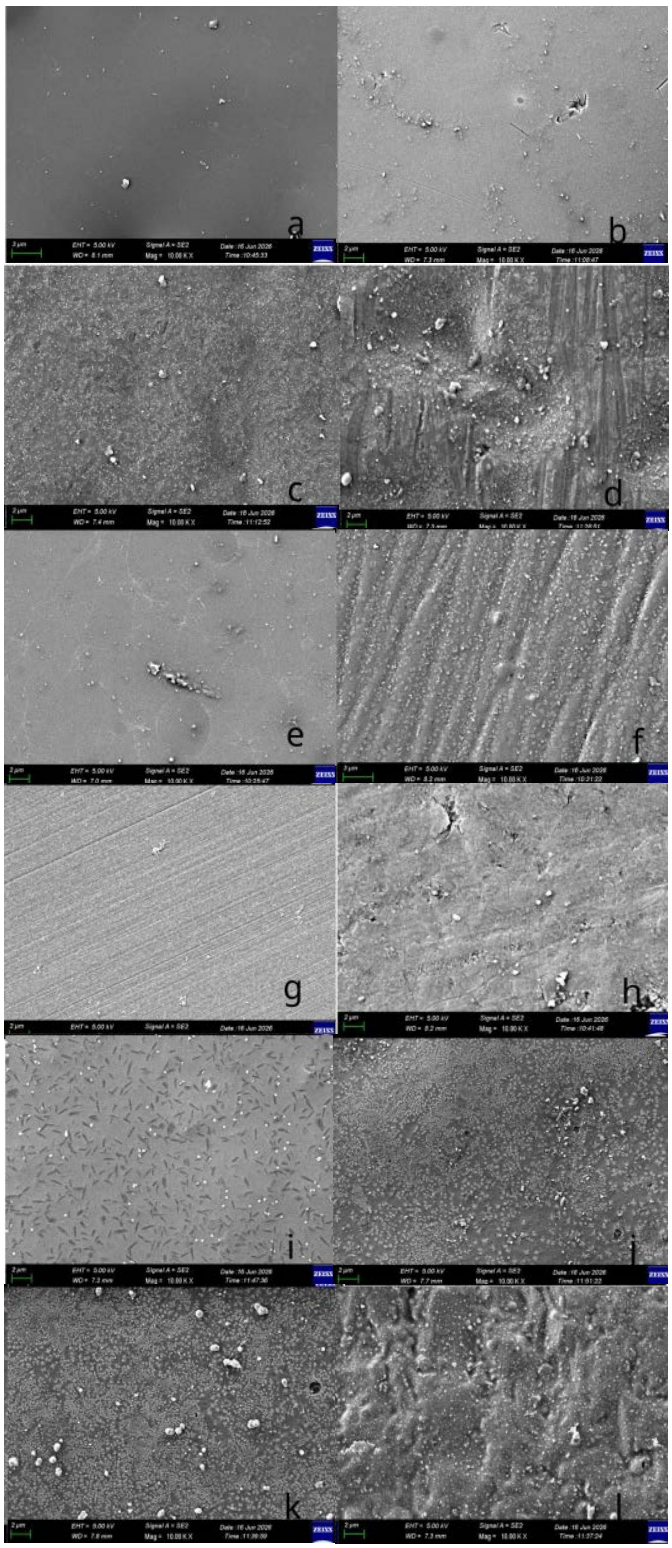

Fig. 3 – Representative FESEM micrographs of the tested glass-ceramics before and after simulated gastric acid immersion (magnification × 10,000): CTG before (a) and after acid immersion (b); CTP before (c) and after acid immersion (d); IPSG before (e) and after acid immersion (f); IPSP before (g) and after acid immersion (h); VSG before (i) and after acid immersion (j); VSP before (k) and after acid immersion (l). FESEM – field-emission scanning electron microscope; CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP – IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.

Table 5

Absolute change in translucency parameter (ΔTP_{00}) of the experimental groups after simulated gastric acid immersion

Groups	Median (Q1–Q3)	95% CI (median)	*H*-test	*p*-value	η^2H
CTG	2.23 (1.02–7.31)[ab]	0.87–7.31	17.604	0.003*	0.233
CTP	5.13 (1.31–11.89)[a]	1.31–11.89			
IPSG	6.02 (5.03–7.00)[a]	5.01–7.03			
IPSP	0.96 (0.35–1.54)[b]	0.35–3.14			
VSG	2.40 (0.90–3.78)[ab]	0.90– 3.78			
VSP	4.64 (3.75–6.83)[a]	2.91–6.84			

Q1-Q3 – first quartile-third quartile; CI – confidence interval; CTG – CEREC Tessera™-glazed; CTP – CEREC Tessera™-polished; IPSG – IPS e.max® CAD-glazed; IPSP – IPS e.max® CAD-polished; VSG – VITA Suprinity®-glazed; VSP – VITA Suprinity®-polished.
***Note*: *$p < 0.05$. Different superscript letters indicate statistically significant differences between groups. Kruskal-Wallis test was used for comparisons between groups.**

increased granular irregularities and scattered superficial deposits (Figure 3b). Before acid immersion, the polished CTP exhibited a relatively granular surface with distinct polishing grooves (Figure 3c). After simulated gastric acid immersion, these polishing grooves remained evident, and the surface morphology appeared largely preserved, although slight accentuation of the surface irregularities was observed (Figure 3d). IPSG presented a relatively smooth and homogeneous surface before acid immersion (Figure 3e), while after immersion, the overall surface morphology was largely preserved with the presence of superficial particles (Figure 3f). The IPSP exhibited characteristic polishing grooves before acid immersion (Figure 3g), whereas localized surface defects and increased surface irregularity were observed after immersion (Figure 3h). The VSG exhibited numerous uniformly distributed elongated crystalline structures before acid immersion (Figure 3i), and these crystalline features remained distinguishable after immersion with only minor superficial deposits (Figure 3j). Similarly, the VSP specimen displayed a fine granular surface before acid immersion (Figure 3k), while a slightly more irregular surface morphology with scattered deposits was observed after immersion (Figure 3l). Overall, FESEM examination demonstrated that the extent of surface morphological alterations following simulated gastric acid immersion differed among the tested glass-ceramic materials and according to the surface-finishing protocol.

Discussion

In the present study, the effects of simulated gastric acid immersion on the optical properties of glazed and polished CAD/CAM glass-ceramics were investigated. The first null hypothesis was accepted because no significant differences were observed in the ΔE_{00} values among the investigated groups. However, significant changes in TP_{00} values were observed in the CTG, CTP, and IPSG groups following simulated gastric acid immersion. Therefore, the second null hypothesis was rejected.

All specimens were standardized using sequential silicon carbide abrasive papers up to 1200-grit prior to the application of glazing or polishing procedures [27, 28]. Although the same surface preparation protocol was applied to all groups, differences in crystalline structure, crystal size, and glass matrix composition among the investigated ceramics may have resulted in material-specific surface characteristics following abrasion. Since the optical behavior of ceramic materials is influenced not only by their intrinsic microstructure but also by surface characteristics, the interaction between the ceramic substrate and the corresponding finishing protocol may have contributed to the observed outcomes. Therefore, the present findings should be interpreted within the context of the specific glazing and polishing systems used for each material, as these protocols represent the clinically intended finishing procedures for the investigated CAD/CAM ceramics. This methodological approach is consistent with that adopted by Demirel et al. [29], who evaluated each material using manufacturer-recommended finishing protocols to better reflect clinical practice. Consequently, the influence of microstructural differences and material-specific finishing procedures on the observed results cannot be completely excluded.

Studies evaluating the effects of simulated gastric acid on the optical properties of contemporary CAD/CAM glass-ceramics, particularly ZLS and advanced lithium disilicate materials, remain limited [36–38].

Previous studies have demonstrated that the corrosive effects of acidic media on dental ceramics depend on factors such as acid concentration, pH, temperature, and exposure duration [22, 25, 35–38]. Although ISO 6872 [39] recommends a solubility test using 4% acetic acid for 16 hrs at 80 °C, accelerated ageing protocols employing stronger acidic environments have been widely adopted to simulate long-term clinical exposure. In the present study, HCl (pH 1.2) was selected according to the method described by Hunt and McIntyre [40], and specimens were continuously immersed for 96 hrs at 37 °C. This protocol was chosen to facilitate comparison with previous studies under standardized laboratory conditions. Previous reports have suggested that such accelerated immersion protocols may correspond to more than 10 years of clinical exposure [41], although the original Hunt and McIntyre [40] protocol has been estimated to represent approximately 2–3 years of *in vivo* conditions.

While continuous immersion does not reproduce the protective effects of saliva or the intermittent nature of gastroesophageal reflux, no standardized *in vitro* gastric acid ageing protocol has yet been established [42]. Therefore, continuous immersion remains a valid and widely accepted approach for evaluating acid-induced changes in dental ceramics and facilitating comparisons among different materials.

Several studies have reported perceptibility and acceptability thresholds for ΔE_{00} in dentistry [33, 43]. Paravina et al. [33] proposed ΔE_{00} values of 0.8 and 1.8 as the perceptibility and acceptability thresholds for dentists. Ghinea et al. [43] further reported 50 : 50% perceptibility and acceptability thresholds of $\Delta E_{00} = 1.25$ and $\Delta E_{00} = 2.23$, respectively.

Consistent with these thresholds, Alhotan et al. [37] evaluated the color stability of pressable and machinable ZLS glass-ceramics following simulated gastric acid immersion. After 96 hrs, ΔE_{00} values remained below the perceptibility threshold, whereas after 192 hrs, all groups exceeded the perceptibility threshold but remained below the acceptability threshold. Likewise, Cruz et al. [36] reported no statistically significant differences in ΔE_{00} between crystallized IPS and VS after 96 hrs of simulated gastric acid immersion, and both materials exhibited clinically imperceptible color changes. Similarly, no statistically significant differences in ΔE_{00} values were observed among the investigated materials after 96 hrs of simulated gastric acid immersion in the present study. Although the median ΔE_{00} values of some groups approached or were above the thresholds proposed in previous studies, simulated gastric acid immersion did not produce statistically significant ΔE_{00} among the investigated materials.

The present findings are partially consistent with those of Kulkarni et al. [38], who investigated the influence of artificial gastric acid combined with toothbrush abrasion on feldspathic porcelain, lithium disilicate glass-ceramic, and monolithic zirconia. Significant alterations in surface roughness, color, and gloss were reported for feldspathic porcelain and lithium disilicate, whereas monolithic zirconia remained largely unaffected. Likewise, no statistically significant color changes were detected in the CAD/CAM glass-ceramics evaluated in the present study, indicating that simulated gastric acid exerted only a limited effect on color stability. Similarly, Alnasser et al. [44] reported no statistically significant changes in the surface roughness of lithium disilicate ceramics polished with silicon carbide papers after 45 and 91 hrs of exposure to simulated gastric acid (5% HCl), further supporting the limited influence of simulated gastric acid on the surface characteristics of lithium disilicate ceramics.

Several studies have investigated the TP_{00} of contemporary CAD/CAM glass-ceramics [38, 44–46]. Kulkarni et al. [38] evaluated the optical changes of lithium disilicate glass-ceramic following artificial gastric acid exposure combined with toothbrush abrasion. Although the IPS specimens exhibited significant changes in color, gloss, and surface roughness, TP_{00} did not change significantly in any of the tested materials. Freitas et al. [45] examined crystallized specimens and reported that CT exhibited TP_{00} comparable to IPS and higher than VS.

Dal Piva et al. [46] evaluated the optical properties of IPS and CT following different firing and glazing protocols. They reported that firing procedures did not significantly affect the ΔE_{00} or TP_{00} values of IPS, whereas CT exhibited clinically unacceptable ΔE_{00} compared with the manufacturer-recommended firing protocol. Furthermore, IPS exhibited greater TP_{00} and brightness than CT irrespective of the firing protocol, suggesting that CT is more sensitive to variations in thermal processing. In the present study, all firing and glazing procedures were performed according to the manufacturers' recommendations. The only exception was the CTP group, in which glazing and the associated firing cycle were intentionally omitted to represent the polished clinical condition. While these findings emphasize the influence of manufacturing and surface-finishing procedures on the initial optical properties of CAD/CAM ceramics, the long-term preservation of TP_{00} under adverse oral conditions is equally important for clinical performance. Accordingly, the present study evaluated the effect of simulated gastric acid exposure on the ΔTP_{00} of CAD/CAM glass-ceramics with different surface-finishing protocols. Although all tested materials exhibited some degree of TP_{00} alteration following acid immersion, the magnitude of the change varied according to the ceramic type. Similarly, Tango et al. [14] demonstrated that the retention of TP_{00} differed significantly among CAD/CAM restorative materials after exposure to staining solutions and artificial aging, indicating that the ability to preserve TP_{00} is material dependent. Consistent with this concept, the present study also demonstrated material-dependent differences in ΔTP_{00} following simulated gastric acid immersion.

Previous studies investigating virgilite-containing lithium disilicate ceramics have demonstrated that microstructural alterations can influence optical properties. Rizk et al. [47] reported that repeated firing cycles significantly reduced TP_{00} values while increasing ΔE_{00} values, indicating that changes within the crystalline and glass phases can modify light transmission and scattering behavior. Although the aging protocol differed from that used in the present study, these findings suggest that alterations in the crystal-glass matrix may affect the optical performance of advanced lithium disilicate ceramics. Similarly, the significant reduction in TP_{00} observed in the CT groups may be associated with acid-induced microstructural changes. However, unlike the findings of Rizk et al. [47], no significant differences in ΔE_{00} were detected among the investigated materials, suggesting that simulated gastric acid exposure had a greater influence on TP_{00} than on overall color stability under the present experimental conditions.

Elsaka and Elnaghy [21] demonstrated that VS possesses a homogeneous fine crystalline structure, whereas conventional lithium disilicate ceramics, such as IPS, are characterized by needle-shaped lithium disilicate crystals embedded within a glassy matrix. A finer and more homogeneous crystal distribution may promote more

uniform light transmission and enhance resistance to optical changes following acid exposure. Conversely, degradation of the crystal-glass matrix interface may increase optical heterogeneity and adversely affect TP‰. The previous study has also reported that acidic media can dissolve the glassy matrix and expose crystalline phases in both lithium disilicate and ZLS ceramics, thereby compromising their optical properties [21].

When interpreted in relation to the proposed TP‰ perceptibility (0.62) and acceptability (2.62) thresholds for ΔTP‰ [34], the median ΔTP‰ values of all groups were above the proposed perceptibility threshold. The median values of the IPSP, CTG, and VSG groups remained below the proposed acceptability threshold, whereas those of the CTP, IPSG, and VSP groups were above this reference value. These findings indicate that the magnitude of TP‰ change at the group level was above the proposed acceptability threshold in the latter groups. However, threshold comparisons based on group medians should not, by themselves, be interpreted as evidence of a statistically significant directional change or as indicating that every individual specimen underwent a clinically unacceptable change.

The observed differences in TP‰ and ΔTP‰ may be related to variations in material composition, crystalline structure, crystal size distribution, and the refractive index relationship between the crystalline and glassy phases. Based on previously reported microstructural characteristics, the consistently higher TP‰ values observed for VS may be partly associated with its relatively fine and homogeneous ZLS crystal structure and the proposed refractive-index relationship between its crystalline and glassy phases, which could potentially facilitate light transmission and reduce light scattering. However, these explanations remain hypothetical because crystal size distribution, refractive indices, and phase composition after acid exposure were not directly characterized in the present study. Although the VSP group exhibited a relatively high median absolute ΔTP‰, no significant within-group difference was detected by the Wilcoxon signed-rank test. This apparent discrepancy reflects the different statistical concepts represented by the two analyses. Whereas the Wilcoxon test evaluates paired changes while preserving the direction of change, ΔTP‰ represents only the absolute magnitude of the change irrespective of whether TP‰ increased or decreased. Consequently, opposite directions of change among individual specimens may result in a relatively high median ΔTP‰ despite the absence of a statistically significant directional change. Therefore, the VSP findings most likely reflect inter-specimen variability rather than a uniform material response to acid exposure, and their clinical significance should be interpreted with caution. Overall, these findings suggest that the optical response to simulated gastric acid may have been influenced by both the reported microstructural characteristics of the ceramic and the applied surface-finishing protocol.

The observed differences in TP‰ and ΔTP‰ among the tested materials may be attributed not only to their intrinsic microstructural characteristics but also to the surface-finishing protocol applied. In agreement with the findings of Al-Johani et al. [27], surface treatment has a significant influence on the optical and topographical properties of CAD/CAM lithium silicate-based glass-ceramics. The authors demonstrated that surface-finishing procedures modify surface morphology and roughness, thereby affecting light transmission and optical behavior. These observations suggest that the optical response of the tested ceramics may have been influenced by the combined effects of their reported microstructural characteristics and the applied surface-finishing protocols, rather than by material composition alone.

FESEM observations revealed more pronounced surface irregularities in CT, comparatively limited alterations in IPS, and largely preserved surface morphology in VS after acid exposure. These observations were generally consistent with the optical findings; however, they do not provide direct evidence regarding crystal size distribution, refractive indices, or phase composition.

Limitations of the study

Despite these findings, several limitations of the present study should be acknowledged. The limitation of this study is that the power analysis was based on a large effect size; therefore, smaller material × surface-finishing interaction effects may not have been detected.

Although the specimens were fabricated under standardized laboratory conditions with uniform dimensions, thicknesses, and shades, formal randomization was not performed during allocation to the surface-finishing groups. Therefore, the potential for allocation-related bias cannot be completely excluded and should be acknowledged as a methodological limitation of the present study. In addition, each ceramic material was finished using the glazing and polishing system recommended by its manufacturer. Although this approach reflects routine clinical practice, differences in the glazing materials and polishing systems may have influenced the optical outcomes. Furthermore, as with all *in vitro* studies, the experimental conditions could not fully reproduce the complexity of the oral environment, including saliva, temperature fluctuations, pH changes, and the intermittent nature of gastric reflux. Finally, surface characterization was limited to qualitative FESEM observations. Although FESEM supported the interpretation of the optical findings by demonstrating acid-induced surface alterations, additional quantitative analyses, including surface roughness, microhardness, and three-dimensional surface topography, would provide a more comprehensive understanding of the relationship between surface degradation and the optical behavior of CAD/CAM glass-ceramics. Furthermore, crystal size distribution, refractive indices, and phase composition after acid exposure were not directly characterized. Therefore, the proposed microstructural explanations for the observed optical differences should be regarded as hypotheses based on

previously published evidence. Moreover, only a single ceramic shade (A2 HT), specimen thickness (1 mm), and simulated gastric acid exposure protocol were evaluated. Therefore, the present findings should be interpreted with caution and cannot be generalized to all clinical situations. Further *in vitro* and clinical studies incorporating different material configurations, restoration thicknesses, shades, and more clinically relevant aging and exposure protocols are warranted.

Conclusion

Within the limitations of this *in vitro* study, simulated gastric acid exposure did not result in significant differences in color difference among the tested experimental groups. However, translucency appeared to be more sensitive to the acid challenge than color. The changes in translucency parameter and in the extent of change in the translucency parameter indicated that the susceptibility of translucency to acidic challenge varied among the tested ceramic materials and surface-finishing protocols. These findings are limited to the investigated ceramic materials, shade, specimen thickness, and simulated gastric acid exposure protocol and should not be directly generalized to other computer-aided design/computer-aided manufacturing ceramics or clinical reflux conditions.

Conflict of interest

The authors declare no conflict of interest.

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Cystic echinococcosis of the liver in the Autonomous Province of Vojvodina, Serbia – challenges in clinical practice

Cistična ehinokokoza jetre u Autonomnoj pokrajini Vojvodini, Srbija – izazovi u kliničkoj praksi

Maja Drljača*†, Jelena Glogovac*†, Maria Pete*†, Miloš Milošević‡, Dušan Lalošević*§, Slobodan Torbica*‖, Maja Ružić*†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; University Clinical Center of Vojvodina, †Clinic for Infectious Diseases, ‖Center for Radiology, Novi Sad, Serbia; ‡Oncology Institute of Vojvodina, Clinic for Surgical Oncology, Sremska Kamenica, Serbia; §Pasteur Institute, Institute for Antirabies Protection, Novi Sad, Serbia**

Abstract

Background/Aim. Cystic echinococcosis (CE) of the liver represents a significant health and economic burden in endemic regions, with a rising incidence in the Autonomous Province of Vojvodina, Serbia. The aim of the study was to investigate the therapeutic modalities applied in patients with hepatic CE treated at the University Clinical Center of Vojvodina over an 11-year period, compare their outcomes, and analyze indirect indicators of economic burden. **Methods.** A retrospective analysis was conducted, including 90 patients with hepatic CE diagnosed and/or treated at the University Clinical Center of Vojvodina between 2013 and 2024. Diagnosis was based on epidemiological data, serological testing, and imaging methods, with liver cysts classified according to World Health Organization criteria. Patients were stratified into five groups according to the therapeutic approach, and treatment modalities were analyzed in relation to the number and length of hospitalizations, cyst stage, antibody titer, and the number of albendazole courses. **Results**. Women comprised the majority of patients (n = 53; 59%; $p < 0.001$), with a mean age of 55 years (range: 16–86 years). The mean length of hospital stay was 32.82 days and did not differ significantly according to the treatment modality ($p = 0.071$). The number of hospitalizations was significantly higher in patients treated with invasive methods (surgery/ puncture, aspiration, injection, and re-aspiration – PAIR; $p < 0.001$). The majority of patients was treated with combination therapy: surgery with albendazole (22/90) or albendazole combined with PAIR (19/90). The highest percentage of patients (25.6%) received a single course of albendazole, whereas the number of albendazole courses differed significantly according to therapeutic approach ($p < 0.01$). Cyst stage and antibody titer showed no significant association with treatment choice ($p = 0.087$; $p = 0.891$). **Conclusion.** A national protocol incorporating established international criteria, with routine serological screening and a structured two-year follow-up, could be useful in the future care of patients with hepatic CE.

Keywords:
albendazole; echinococcosis, hepatic; serbia; surgical procedures, operative; treatment outcome.

Apstrakt

Uvod/Cilj. Cistična ehinokokoza (CE) jetre predstavlja značajan zdravstveni i ekonomski problem u endemskim sredinama, sa porastom incidencije u Autonomnoj pokrajini Vojvodini, Srbija. Cilj rada bio je da se ispitaju modaliteti lečenja primenjeni kod obolelih od CE jetre, lečenih u Univerzitetskom kliničkom centru Vojvodine tokom 11-godišnjeg perioda, uporede njihovi ishodi i analiziraju indirektni pokazatelji ekonomskog opterećenja. **Metode.** Sprovedena je retrospektivna analiza 90 bolesnika koji su imali CE jetre, dijagnostikovanih i/ili lečenih u Univerzitetskom kliničkom centru Vojvodine od 2013. do 2024. godine. Dijagnoza je bila zasnovana na epidemiološkim podacima, serološkom testiranju i radiološkim metodama, uz klasifikovanje cista jetre prema kriterijumima Svetske zdravstene organizacije. Bolesnici su prema terapijskom pristupu bili podeljeni u pet grupa, a potom su analizirani modaliteti lečenja u odnosu na broj i dužinu hospitalizacije, stadijum ciste, titar antitela i broj sprovedenih ciklusa terapije albendazolom. **Rezultati.** Žene su činile većinu obolelih (n = 53; 59%; $p < 0,001$), prosečnog životnog doba od 55 godina (raspon: 16–86 godina). Prosečna dužina hospitalizacije iznosila je 32,82

Correspondence to: Maja Drljača, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: maja.drljaca@mf.uns.ac.rs

dana i nije se značajno razlikovala prema modalitetu lečenja (p = 0,071). Broj hospitalizacija bio je značajno veći kod invazivnih metoda lečenja (hirurgija/ *puncture, aspiration, injection, and re-aspiration* – PAIR; p < 0,001). Većina bolesnika lečena je kombinovanom terapijom: hirurški zahvat uz albendazol (22/90) ili albendazol u kombinaciji sa PAIR (19/90). Najviši procenat bolesnika (25,6%) primio je jedan ciklus terapije albendazolom, dok se broj primenjenih ciklusa albendazola značajno razlikovao u odnosu na terapijski pristup (p < 0,01). Nije utvrđena značajna povezanost stadijuma ciste i titra antitela sa izborom terapijskog pristupa (p = 0,087; p = 0,891). **Zaključak.** Nacionalni protokol koji bi uključivao ustanovljene međunarodne kriterijume, sa rutinskim serološkim skriningom i strukturiranim dvogodišnjim praćenjem, mogao bi biti koristan u budućem lečenju obolelih od CE jetre.

Ključne reči:
albendazol; ehinokokoza jetre; srbija; hirurgija, operativne procedure; lečenje, ishod.

Introduction

Human echinococcosis (HE) is a zoonosis caused by metacestodes of tapeworms belonging to the genus *Echinococcus* (Figure 1). To date, four species of HE causative agents have been described: *Echinococcus (E.) granulosus* – the causative agent of cystic echinococcosis (CE), also known as hydatidosis, and the most common cause of human infection; *E. multilocularis* – the causative agent of alveolar echinococcosis; and *E. vogeli* and *E. oligarthus* – the causative agents of several reported cases of polycystic echinococcosis in Central and South America [1]. The scientific literature also describes two relatively new species, *E. shiquicus* and *E. felidis*, but their significance in human morbidity remains uncertain [2, 3]. Humans serve as intermediate hosts, with transmission occurring accidentally through ingestion of parasite eggs *via* direct contact with definitive hosts (e.g., dogs, foxes, other members of the Canidae family), or indirectly through contaminated food or water. HE is a widely distributed disease, predominating in Europe, Asia, South and Central America, Australia, the European and African parts of the Mediterranean region, the Middle East, sub-Saharan countries, Russia, and China [4]. High endemicity is typically observed in pastoral and livestock-raising communities, particularly in resource-limited rural settings where close contact between dogs and intermediate hosts sustains the parasite life cycle [5, 6]. Increased transmission is consistently associated with home slaughter of livestock, inadequate disposal of infected viscera, and the practice of feeding raw offal to dogs [7–9]. In addition, poor sanitation, limited veterinary supervision, and uncontrolled dog populations further facilitate environmental contamination with *Echinococcus* eggs and endemic transmission [10–12].

The western Balkans region (former Socialist Federal Republic of Yugoslavia – SFRY) is classified as an endemic area for hydatidosis, with an incidence of 4.8 cases *per* 100,000 inhabitants [13]. Hyperendemic areas with a disease incidence of over 12 *per* 100,000 inhabitants are defined as: Dalmatia, Herzegovina, northern Montenegro, eastern Serbia, the Raška region, Autonomous Province of Kosovo and Metohija, and North Macedonia [14, 15]. The cumulative incidence of officially reported cases in the Republic of Serbia over ten years (2001–2010) ranges from 0.46/100,000 in the central part to 39.0/100,000 in the southern part of the country. Traditionally, in the territory of the northern Autonomous Province (AP) of Vojvodina, Serbia, 118 cases of CE of the liver have been recorded over the last 42 years [16]. Over the past three years, a major concern has been the emergence of alveolar echinococcosis caused by *E. multilocularis*, with four cases reported between 2021 and 2024 [17]. The southward shift of the endemic zone has made the Mediterranean one of the main areas for researching the impact of climate change on zoonoses today. The question remains open as to why this disease is moving from the traditionally cold and humid areas of northern Europe to the warm and dry climatic

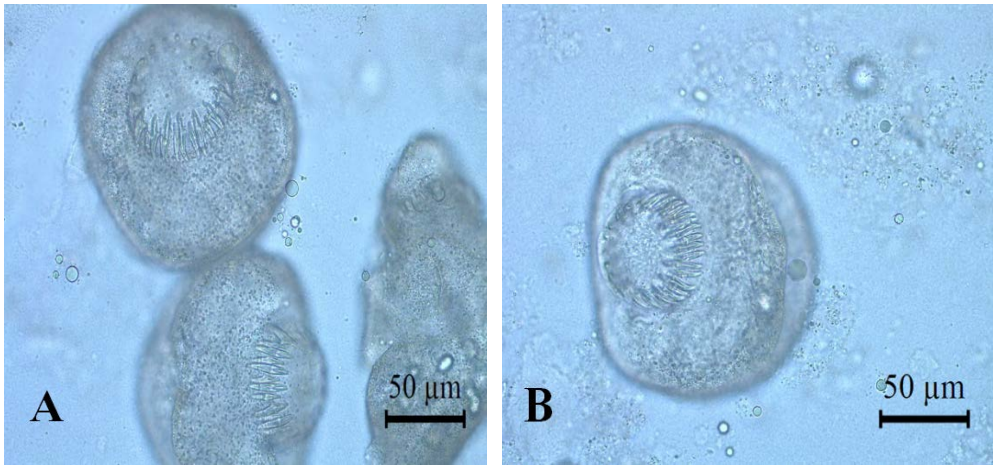

Fig. 1 – Morphologically preserved protoscoleces obtained from a hepatic echinococcal cyst (A, B).
Image courtesy of Professor Lalošević.

zones of the Mediterranean. It has been proven that the expansion of *E. granulosus* in the fox population is a consequence of successful rabies eradication campaigns and colonization of urban environments, representing a paradigm for the rise in prevalence within the human population [18]. Despite activities within the World Health Organization (WHO) "One Health" campaign, awareness regarding the significance of HE has not reached clinicians [19].

The aim of the study was to investigate the therapeutic approaches to liver CE provided in the University Clinical Center of Vojvodina (UCCV), Serbia, encompassing the last decade, as well as to compare treatment modalities and examine indirect indicators of healthcare burden, represented by hospitalization length and hospitalization frequency, in the absence of direct cost data.

Methods

This retrospective study analyzed medical records of patients with hepatic CE who were examined and/or treated at the UCCV. The study encompassed the time period from 2013 to 2024 and included a total of 90 patients who originated from regions of Bačka, Banat, Srem, or the Mačva district. This study was approved by the Ethics Committee of UCCV (No. 00-62, from February 29, 2024). This study was conducted in accordance with the ethical principles for research involving humans and/or human material.

The diagnosis of hepatic CE was established by abdominal ultrasonography (Figure 2) according to the cyst characteristics classified by the WHO Informal Working Group on Echinococcosis (WHO-IWGE) [20]. All of the observed liver cysts were solitary; cysts in other organs were not observed. Serological confirmation was obtained *via* an indirect hemagglutination test. *E. granulosus* antibody titers were classified as non-significant (1/80 or lower), mildly positive (up to 1/320 but above 1/80), or positive (greater than 1/320).

Based on treatment modalities applied, patients were divided into five groups: untreated patients, patients treated with albendazole alone, patients treated with surgery alone, patients treated with surgery combined with albendazole, and patients treated with puncture, aspiration, injection, and re-aspiration (PAIR) combined with albendazole (Figures 3–5). Surgical treatment was performed at the Clinic for Abdominal and Endocrine Surgery of UCCV, while the PAIR procedure was carried out at the Center for Radiology of UCCV. Patients received antihelminthic therapy with albendazole administered at a dose of 400 mg twice daily in the outpatient setting. Treatment was given in 28-day courses in accordance with the protocols available at the time [21]. None of the patients received continuous albendazole therapy. Follow-up examinations were performed at the end of each course by an infectious disease specialist at the Clinic for Infectious Diseases of UCCV. We analyzed the gender and age structure of the patients, length of hospitalization expressed in hospital days, number of hospitalizations, and number of hospitalizations before and after the selected treatment method. The length and number of hospitalizations were compared across treatment modalities. Additionally, cyst stage according to WHO-IWGE was compared across therapeutic modalities, and antibody titer values were also analyzed in relation to the treatment options and WHO cyst development stages.



Fig. 2 – Ultrasonographic appearance of a hepatic echinococcal cyst showing the water lily sign (CE3a stage). The arrow shows the detached, collapsed endocystic membrane floating within the cyst fluid, producing the characteristic water lily appearance. Image courtesy of Assist. Dr. Torbica.



Fig. 3 – Fluoroscopic appearance of a hepatic hydatid cyst during the initial phase of the PAIR procedure. The contrast-opacified cyst shows irregular intraluminal filling defects corresponding to collapsed parasitic membranes. The image depicts the puncture and aspiration phase, which precedes the injection of a scolicidal solution and subsequent re-aspiration.
Image courtesy of Assist. Dr. Torbica.
PAIR – puncture, aspiration, injection, and re-aspiration.



Fig. 4 – Gross specimen obtained after aspiration of a hepatic echinococcal cyst, showing abundant folded membranous material within the aspirated fluid, suggestive of detached hydatid membranes and cyst contents.
Image courtesy of Assist. Dr. Torbica.

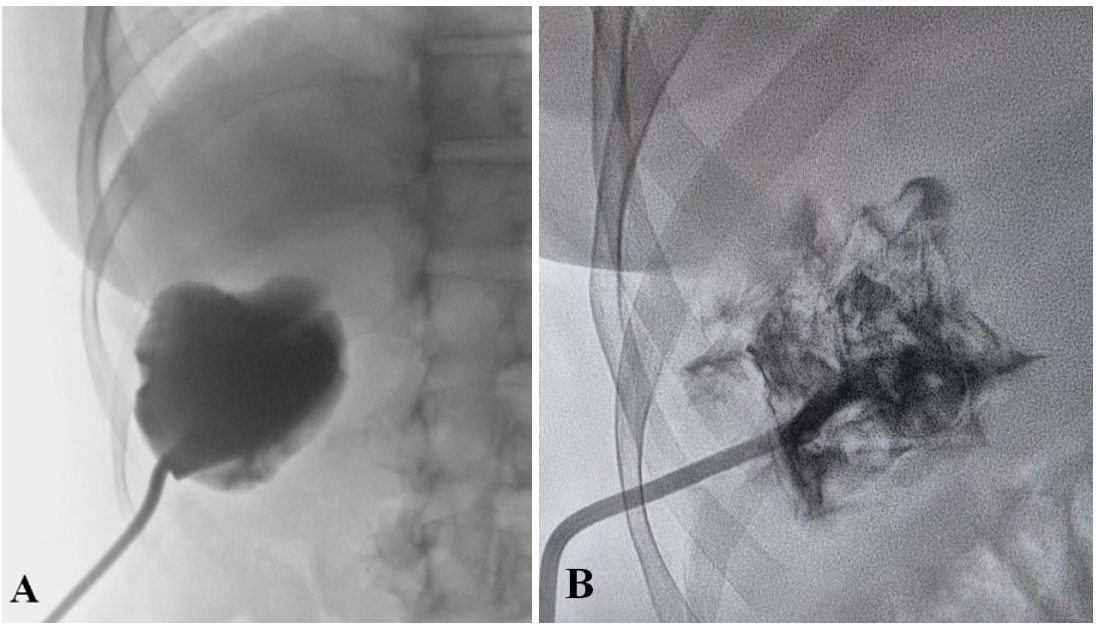

Fig. 5 – PAIR procedure: fluoroscopic images showing (A) instillation of a scolicidal agent and (B) re-aspiration of the scolicidal agent from a previously drained hepatic echinococcal cyst. Image courtesy of Assist. Dr. Torbica.
PAIR – puncture, aspiration, injection, and re-aspiration.

Table 1

Relationship between treatment modality and length of hospitalization

Variable	Albendazole	Surgery	Untreated	Albendazole + surgery	Albendazole + PAIR	*p*-value
Length of stay, days						
mean	26.68	10.25	10.8	42.71	37.88	0.071
range	1.0–79.0	6.0–20.0	1.0–27.0	12.0–143.0	4.0–102.0	

PAIR – puncture, aspiration, injection, and re-aspiration.
***Note*: Differences in length of hospital stay between treatment modalities were assessed using the analysis of variance – ANOVA test.**

Cure was defined as the absence of disease recurrence during the two-year follow-up period after the selected treatment modality. Recurrence was defined as radiologically confirmed cyst reactivation, significant antibody titer changes, or both.

Statistical analysis

Data were processed using the JASP software (JASP 0.19). Categorical variables are presented as numbers (percentages), and differences between groups were compared using the chi-square (χ^2) test, or the *t*-test, as appropriate. Continuous variables are expressed in percentiles, and differences between groups were compared using the analysis of variance – ANOVA test, considering that the distribution for most variables significantly deviated from normal. Data are presented in tabular form. Values of $p < 0.05$ were considered statistically significant.

Results

Of the 90 patients, 53 (59%) were female, and 37 (41%) were male, with a significant female predominance ($p < 0.001$). The mean age of the cohort was 55 years (range: 16–86 years), which was comparable between men (mean: 58 years, range: 25–81 years) and women (mean: 53 years, range: 16–86 years); age did not differ significantly by gender ($p = 0.141$). Notably, the youngest (16 years) and the oldest (86 years) patients in the cohort were both women.

Hospitalization burden

Mean length of hospital stay across the cohort was 32.82 days (range: 1–143 days). This burden varied by treatment modality, with combined albendazole therapy – administered alongside either surgery or PAIR – associated with the longest average hospital stays, although this difference did not reach statistical significance ($p = 0.071$) (Table 1).

While the total duration of hospitalization did not vary significantly by treatment, the number of hospitalizations *per* patient did, ranging from 0 to 7 and skewing higher among patients treated with invasive methods ($p < 0.001$) (Table 2). The single highest number of hospitalizations was seven, in a patient who was treated surgically and with albendazole.

Splitting this hospitalization count into periods before and after the selected treatment clarifies where the burden

falls. Patients who went on to receive combination therapy, required significantly more hospitalizations before the beginning of the treatment than those managed exclusively with pharmacological therapy or surgery alone ($p < 0.001$), consistent with the additional diagnostic workup needed to individualize their therapy. No such association was observed for hospitalizations after treatment ($p = 0.335$) (Table 3). The majority of patients (49/66) required no further hospitalization once their therapeutic protocol had concluded, and only one patient was treated with combined albendazole and surgery and was hospitalized as many as four times afterwards.

Of the 90 patients, 26 (28.9%) remained untreated, 19 (21.1%) received albendazole alone, 4 (4.4%) underwent surgery alone, 22 (24.4%) received combined albendazole and surgical treatment, and 19 (21.2%) were treated with albendazole and PAIR (Table 4). PAIR was never performed without concomitant pharmacological therapy. Among the 60 patients who received albendazole, the largest group, 23 (25.6%), completed a single 28-day course, while one patient (1.1%) completed six courses. The number of courses differed significantly by treatment modality ($p < 0.01$): patients managed with combination therapy were more likely to receive multiple courses, reflecting the standard practice of prolonged adjuvant albendazole alongside surgical or PAIR intervention (Tables 4 and 5).

Treatment modality showed no significant association with cyst development stage ($p = 0.087$), although invasive and combined chemotherapeutic protocols were preferentially applied to advanced CE4 and CE5 cysts, most likely reflecting symptomatic mass effect rather than a formal stage-based algorithm (Table 6).

No data were collected regarding antibody titers for 21 patients, and two patients with positive antibody titers did not undergo radiological evaluation. Neither antibody titer by treatment modality ($p = 0.891$) nor antibody titer by WHO

Table 2

Relationship between treatment modality and hospitalization count

Variable	Albendazole	Surgery	Untreated	Albendazole + surgery	Albendazole + PAIR	Total	*p*-value
Hospitalization count							
0	3	0	21	0	0	24	
1	8	4	4	8	7	31	
2	5	0	0	4	4	13	
3	2	0	0	5	2	9	< 0.001
4	1	0	0	3	5	9	
5	0	0	1	1	1	3	
6	0	0	0	0	0	0	
7	0	0	0	1	0	1	
total	19	4	26	22	19	90	

PAIR – puncture, aspiration, injection, and re-aspiration.
All values are given as numbers.
***Note*: The association was assessed using the Chi-square test (χ^2).**

Table 3

Relationship between treatment modality and hospitalization count before and after treatment

Variable	Albendazole	Surgery	Untreated	Albendazole + surgery	Albendazole + PAIR	Total	*p*-value
Hospitalization count before treatment							
0	0	4	0	8	4	16	
1	10	0	4	10	10	34	
2	3	0	0	3	5	11	
3	3	0	0	1	0	4	< 0.001
4	0	0	0	0	0	0	
5	0	0	1	0	0	1	
total	16	4	5	22	19	66	
Hospitalization count after treatment							
0	16	4	5	12	12	49	
1	0	0	0	7	5	12	
2	0	0	0	1	0	1	0.335
3	0	0	0	1	2	3	
4	0	0	0	1	0	1	
total	16	4	5	22	19	66	

PAIR – puncture, aspiration, injection, and re-aspiration.
All values are given as numbers.
***Note*: The association was assessed using the Chi-square test (χ^2).**

Table 4

The association between the number of patients and the number of albendazole courses

Variable	Values
Treatment modality	
untreated	26 (28.9)
albendazole	19 (21.1)
surgery	4 (4.4)
albendazole + surgery	22 (24.4)
albendazole +PAIR	19 (21.2)
total	90 (100)
Albendazole courses, count	
0	30 (33.3)
1	23 (25.6)
2	4 (4.4)
3	12 (13.3)
4	15 (16.7)
5	5 (5.6)
6	1 (1.1)
total	90 (100)

PAIR – puncture, aspiration, injection, and re-aspiration.
All values are given as numbers (percentages).

Table 5

The association between the treatment modality and number of albendazole courses

Variable	Albendazole	Albendazole + surgery	Albendazole + PAIR	Total	*p*-value
Albendazole courses					
1	6	9	8	23	< 0.01
2	2	1	1	4	
3	6	2	4	12	
4	5	8	2	15	
5	0	1	4	5	
6	0	1	0	1	
total	19	22	19	60	

PAIR – puncture, aspiration, injection, and re-aspiration.
All values are given as numbers.
***Note*: The association between treatment modality and the number of albendazole courses was determined using the Chi-square test (χ^2).**

Table 6

Cyst development stage and treatment modalities

Variable	WHO classification of CE							*p*-value
	CL	CE1	CE2	CE3a	CE3b	CE4	CE5	
Treatment modalities								0.087
pharmacological	1	0	3	5	4	2	4	
surgical	0	0	1	0	0	0	1	
untreated	1	3	1	3	1	3	13	
pharmacological + surgical	0	4	6	2	1	3	5	
pharmacological + PAIR	0	0	5	8	2	2	2	
total	2	7	16	18	8	10	25	

WHO – World Health Organization; CL – cystic lesion; CE – cystic echinococcosis; PAIR – puncture, aspiration, injection, and re-aspiration.
All values are given as numbers.
***Note*: The association between treatment modalities and the WHO classification of CE was assessed using the Chi-square test (χ^2).**

cyst stage ($p = 0.649$) reached statistical significance, indicating that seropositivity did not track cleanly with either the therapeutic decision or the radiological stage of the cyst (Table 7).

Overall, 55 of 90 patients (61.1%) achieved cure and 35 (38.9%) were lost to follow-up, with no confirmed recurrences. Surgery alone had the highest cure rate (4/4, 100%), followed closely by albendazole alone and albendazole with PAIR (each 15/19, 78.9%). Untreated patients had the lowest cure rate (8/26, 30.8%) and the highest rate of loss to follow-up (18/26, 69.2%) (Table 8).

Table 7

Serological and radiological analyses

Variable	Antibody titer				*p*-value
	non-significant	mildly positive	positive	total	
Treatment modality					
albendazole	6	2	9	17	0.891
surgical	0	0	1	1	
untreated	4	3	5	12	
albendazole + surgical	5	3	12	20	
albendazole + PAIR	4	5	10	19	
total	19	13	37	69	
WHO classification of CE					
CL	0	1	1	2	0.649
CE1	4	1	1	6	
CE2	4	3	7	14	
CE3a	3	3	8	14	
CE3b	3	0	4	7	
CE4	2	1	5	7	
CE5	3	4	9	16	
total	19	13	35	67	

PAIR – puncture, aspiration, injection, and re-aspiration; WHO – World Health Organization; CE – cystic echinococcosis; CL – cystic lesion.
All values are given as numbers.
***Note*: The association between antibody titer and treatment modalities, and between antibody titer and WHO cyst stage, was assessed using the Chi-square test (χ^2). Antibody titer was categorized as non-significant (1/80 or less), mildly positive (1/320 or less, but greater than 1/80), and positive (over 1/320).**

Table 8

Treatment outcomes by modality

Variable	Cured	Recurrence	Lost to follow-up	Total
Albendazole	15 (78.9)	0 (0)	4 (21.1)	19 (100)
Surgery	4 (100)	0 (0)	0 (0)	4 (100)
Untreated	8 (30.8)	0 (0)	18 (69.2)	26 (100)
Albendazole + surgery	13 (59.1)	0 (0)	9 (40.9)	22 (100)
Albendazole + PAIR	15 (78.9)	0 (0)	4 (21.1)	19 (100)
Total	55 (61.1)	0 (0)	35 (38.9)	90 (100)

PAIR – puncture, aspiration, injection, and re-aspiration.
All values are given as numbers (percentages).

Discussion

In endemic areas, the wide distribution of pastures and forests, as well as the abundance of animal resources, provides a good environment for the growth and successful reproduction of *E. granulosus* [22, 23]. The infection rate of wild and domestic animals is high, and in the context of specific cultural and socioeconomic factors, all prerequisites for transmission to the human population exist [24, 25]. Over the past 42 years, during which echinococcal disease has been monitored in the AP of Vojvodina, 118 cases have been recorded [16]. However, in the last 11 years, a total of 90 cases of CE have been treated at the UCCV. The diagnosis of echinococcosis is often established late due to the slow and asymptomatic development of the disease in the early phases, as cysts can grow for years before causing clinical symptoms. Additionally, symptoms are often non-specific and can mimic other diseases, which hinders timely recognition and requires detailed radiological and serological processing [26]. People in rural areas discover echinococcosis later due to limited access to healthcare institutions, insufficient information about the disease and its symptoms, as well as due to more frequent contact with domestic animals and dogs, which are the main carriers of the parasite [27]. Preventive measures, such as proper hygienic practices and veterinary surveillance, are often insufficiently implemented in these environments.

Our study shows a significant predominance of female patients ($p < 0.001$). Similar findings have been documented in multiple studies, attributing the observed predisposition to sex hormone-mediated modulation of the immune response [28–30]. Early marriage and frequent pregnancies in rural communities may reduce immunity through the effects of repeated childbirths, thereby increasing the risk of echinococcosis in adult women [25, 31, 32]. The larger share of the female population in our cohort could certainly be explained by the cultural characteristics of the climate, where women usually care for dogs and are involved in gardening tasks. However, the higher prevalence observed among females may also reflect improved health surveillance in women, leading to more frequent detection of this asymptomatic infection; thus, the gender disparity cannot be generalized to the entire popu-

lation. Interestingly, gender bias was not recorded in children who participated in screening programs in some provinces in China [31].

In the observed group of patients, the average age of patients was 55 years, with the youngest being 16 and the oldest 86 years old. These results are expected because echinococcus cysts grow slowly and remain asymptomatic for months, even years, after infection; although most often acquired in childhood, they are often diagnosed later. According to data from the European Register for CE, the disease was diagnosed at a mean age of 46.20 years, while in China, the average age at diagnosis was 44.5 years – in both examples, a significantly younger age of detected patients than in our cohort [33, 34]. Therefore, the average age of the afflicted is conditioned by the degree of development of mass screening programs and surveillance activity over HE, rather than the natural course of the disease itself [35]. The Centers for Disease Control and Prevention advocated the "One Health" approach for controlling numerous zoonoses, including echinococcosis. This approach recognizes that the health of people, animals, and the environment is interconnected, so it requires multidisciplinary cooperation for the effective management of this zoonotic disease. "One Health" implies veterinary interventions, medical staff efforts to educate communities about disease transmission, and multidisciplinary cooperation among doctors, ecologists, and local authorities in raising awareness of environmental factors that support the parasite cycle. By implementing "One Health principles", it is possible to significantly reduce the frequency of echinococcosis, especially in endemic areas, through the synergy of health and environmental measures [36].

Treatment of human CE consumes significant health resources. An extensive study conducted in Spain on 1,004 patients showed an average of 25.5 hospital days required *per* patient [37]. The average number of days spent in hospital in our cohort was considerably higher (32.82 days). Understandably, the treatment modality significantly affects the length of hospitalization, so patients in our cohort treated surgically and then with albendazole were hospitalized the longest on average, spending 42.71 days in hospital. Mean hospital stay was shorter for albendazole monotherapy (26.68 days) vs. combined PAIR-albendazole therapy (37.88 days). Data from contemporary literature support our research, consistently indicating a shorter length of hospital stay for patients treated with the PAIR method compared to open laparotomy [38]. Invasive interventions for the treatment of CE are anticipated to entail a higher complication rate, and thus require prolonged hospitalization [39–42]. In our study, this observation was not substantiated, as no increase in the number of hospitalization days was observed among patients treated with PAIR or surgery ($p = 0.071$). Our study showed statistical significance when observing the number of hospitalizations, indicating the need for a higher number of hospitalizations in patients treated with invasive methods ($p < 0.001$). Although patients treated with invasive methods required a greater number of hospitalizations, no statistically significant difference was observed regarding the total length of hospital stay, as previously stated. These findings suggest that no significant complications were encountered that would have prolonged hospital stays. Our study revealed a statistically significant association between the number of prior hospitalizations and the selected treatment modality, whereas no such association was evident for post-treatment hospitalizations. This disparity likely stems from comprehensive laboratory, radiological, and clinical evaluations undertaken to optimize and individualize therapy for each patient. As noted previously, hospitalization duration varies with cyst location, size, complications, and treatment approach, encompassing costs for examinations, laboratory analyses, perioperative care, surgical procedures, and inpatient days [37, 41, 42]. These thorough assessments are thus essential in managing echinococcal disease, enabling accurate diagnosis, tailored interventions, and reduced healthcare resource consumption.

When discussing CE therapy, there are several treatment modalities, ranging from pharmacological treatment to more invasive procedures. Indications for surgery should be carefully evaluated against alternative treatment options. Surgery remains the primary treatment choice for complex cysts, removal of large CE2–CE3 cysts with a greater number of daughter cysts, superficially located cysts that may rupture spontaneously or as a result of trauma, and cases where PAIR is unavailable. It is likewise indicated for infected cysts, cysts communicating with the biliary tree, or those exerting pressure on adjacent vital organs [38, 43, 44]. PAIR is indicated for inoperable patients, those who refuse surgery, or in cases of recurrence after surgery. Certain studies favor the PAIR method, highlighting lower invasiveness, a lower rate of CE recurrence, a shorter recovery and hospitalization period, and consequently lower costs to the healthcare system [38, 45, 46]. The best results arise from a combination of PAIR with anthelmintics for CE1 and CE3a cysts larger than 5 cm, which can be a first-line treatment, as well as in the therapy of pregnant women with symptomatic cysts or children < 3 years of age [45, 47]. Medications should be used to prevent recurrence following surgery or PAIR [15, 48, 49].

The selection of therapeutic modality is determined by the WHO-IWGE cyst classification [50]. In our study, treatment choice for hepatic CE showed no statistically significant association with WHO-IWGE cyst stage ($p = 0.087$). Analysis of results indicated that invasive-chemotherapeutic protocols were applied to select CE4 and CE5 cysts due to symptomatic mass effect.

Selection of the initial anthelmintic agent for CE does not present a substantial clinical challenge, as albendazole is the sole pharmacotherapeutic option available. The principal considerations, therefore, pertain to its dosage regimen, treatment duration, and vigilant monitoring for adverse effects [51–54]. Patients with larger cysts, multiple or disseminated echinococcosis, and especially with echinococcosis of the liver, central nervous system, and bones, should receive higher doses of albendazole over a longer period of time [15, 48]. In our research, 60 out of 90 patients received albendazole in courses, i.e., cycles of 28 days. Of the 60 albendazole-treated patients, 23 (25.6%) received one course, the most frequent regimen, while a single patient (1.1%) un-

derwent six courses, the highest number observed. The choice of therapeutic modality showed a statistically significant association with the number of albendazole cycles administered ($p < 0.01$), with the highest number of courses (5 to 6) being received by patients undergoing invasive procedures. This association reflects the standard clinical practice of administering prolonged albendazole regimens as adjuvant therapy in conjunction with surgical and PAIR interventions, thereby minimizing the risk of secondary dissemination consequent to cyst rupture or leakage, as well as mitigating the likelihood of disease recurrence.

Various studies show that immune tolerance to *Echinococcus* antigens leaves approximately 30% of patients immunologically silent [55, 56]. The cause of seronegativity may also be the formation of immune complexes. Craig [57] proved back in 1986 that circulating parasitic antigens can "trap" antibodies, which in that case cannot be detected by serological testing, and subsequently, Rogan and Craig [58] proved that seroreactivity is influenced by the ultrasonic morphological type of the cyst. Some authors believe these morphological types represent advanced developmental stages of the hydatid cyst, progressing from highly viable cysts (types CE1 and CE2) to septated and heteroge-neous forms (types CE3 and CE4), and ultimately calcified cysts (type CE5). It has been established that seropositivity increases as the cyst develops and drops sharply with type CE5 [15]. A variety of serological tests are currently used to support the diagnosis of CEs, most commonly the indirect hemagglutination assay, indirect immunofluorescence assay, and enzyme-linked immunosorbent assay (ELISA). Given the varying specificity and sensitivity of these methods, some papers from contemporary literature advise the application of two different serological tests for each tested serum as a way of increasing diagnostic sensitivity [59]. In our center, all sera were tested using the indirect hemagglutination method, and a percentage of tested sera showed mildly positive, i.e., borderline antibody titer values. It is assumed that borderline values occur at the beginning of infection, when the amount of antibodies is small, or because the level of specific antibodies is variable depending on the localization and developmental stage of the cyst. In patients with a borderline antibody titer, repeat analysis of a newly obtained serum sample after 2–4 weeks is recommended, as specific antibody levels may increase during this period to a level detectable by ELISA if the disease is present [4]. In our study, the highest percentage of patients with a positive antibody titer was among patients who were treated with combined surgery and pharmacological therapy, but the results indicate that the choice of treatment method did not show a statistically significant association with the finding of antibodies in the patients' serum ($p = 0.891$). The degree of seropositivity is not the best indicator of cyst avidity, which, in our research, also did not show a statistically significant association with the choice of therapy, as antibody detectability may depend on the location, integrity, or stage of the CE.

Surgery and albendazole-based interventional treatment were associated with the most favorable outcomes in our cohort, while untreated patients had the lowest cure rate and the highest loss to follow-up. This pattern is in agreement with the literature, which identifies surgery as an effective curative modality in selected cases and supports PAIR combined with albendazole as a well-established treatment option for appropriately selected patients with CE [60, 61]. Nevertheless, the lack of observed recurrence should be interpreted cautiously because the relatively high loss to follow-up may have affected outcome assessment.

Echinococcosis has been documented in the AP of Vojvodina since 1982, and numerous studies have established its medical and public health importance as the most common parasitic disease in the region [62]. Established international criteria for cyst classification and treatment selection already exist and are well described in the literature, including the treatment-algorithm studies cited above [20, 38, 43–45, 47, 50, 60, 61]. Despite established diagnostic criteria and ongoing initiatives under the "One Health" framework, diagnosis of infiltrative cystic focal liver lesions locally does not routinely incorporate serological testing for echinococcal disease – reflecting, as previously noted, that awareness of HE has not consistently reached clinicians [19]. Early screening is critical given the asymptomatic progression of infection: undetected cysts can attain considerable size, resulting in compression of adjacent structures and severe, potentially lethal sequelae, whereas timely diagnosis enables a precise therapeutic approach and reduces the need for invasive and costly interventions. Nearly 39% of patients in our cohort were lost to follow-up, which further limits firm conclusions about long-term recurrence. We therefore recommend a national protocol that operationalizes these existing international criteria within local clinical practice, rather than a new set of guidelines. Two steps would close the implementation gap identified in this study. First, serological testing for echinococcosis (indirect haemagglutination assay, repeated after 2–4 weeks if borderline) should become a routine, protocolized step in the diagnostic work-up of any solitary cystic focal liver lesion in patients originating from, or with epidemiological exposure to, the endemic AP of Vojvodina districts of Bačka, Banat, Srem, and Mačva – rather than an *ad hoc* addition left to individual clinician judgement. Second, given the high loss to follow-up observed here, structured two-year serological and radiological follow-up should be built into post-treatment protocols, particularly for patients managed with PAIR or surgery, to allow reliable detection of recurrence. Standardizing these two steps within the existing WHO-IWGE and WHO frameworks would improve care across centers and raise clinical awareness locally, without duplicating criteria that are already well established.

Conclusion

This study demonstrates that the implementation of established international criteria for cyst classification and treatment selection remains limited in routine clinical practice, as serological testing is not routinely performed in patients with focal liver lesions. A national protocol incorporating these criteria, together with routine serological screening and structured two-year follow-up, could help address this implementation gap.

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Identification of a plant-derived terpenoid as a potential TET1 modulator in breast cancer

Identifikacija terpenoida biljnog porekla kao potencijalnog modulatora TET1 u karcinomu dojke

[1]Ahmed M. Alharbi*, [1]Pratibha Pandey[†], Asmatanzeem Bepari[‡], Rasha Assad Assiri[§], Danah A. Alturbak[‖], Mohd Saeed[¶], Meenakshi Verma[†], Shivani Sharma, Fahad Khan[††]**

University of Hail, *College of Applied Medical Sciences, Department of Medical Laboratory Science, [¶]College of Science, Department of Biology, Hail, Saudi Arabia; [†]Chandigarh University, University Center for Research and Development, Mohali, Punjab, India; Princess Nourah bint Abdulrahman University, College of Medicine, [‡]Department of Basic Sciences, [§]Department of Basic Medical Sciences, Riyadh, Saudi Arabia; [‖]King Saud University, College of Applied Medical Sciences, Department of Clinical Laboratory Sciences, Riyadh, Saudi Arabia; **Uttaranchal University, Uttaranchal Institute of Technology, Dehradun, Uttarakhand, India; [††]Saveetha Institute of Medical and Technical Sciences, Saveetha Medical College and Hospitals, Department of Community Medicine, Chennai, India

[1]**equal contribution of both authors**

Abstract

Background/Aim. Breast cancer progression is driven by dysregulated signaling pathways and epigenetic alterations. Plant-derived terpenoids have attracted significant attention as promising anticancer agents due to their ability to modulate key molecular and cellular targets. Ten-eleven translocation 1 (TET1) has been identified as an oncogenic factor in aggressive breast cancer subtypes, promoting tumor-supporting transcriptional activities at specific DNA sites and contributing to resistance against oxidative stress-mediated cell death. The aim of this study was to examine the potential of plant-derived terpenoids to target TET1 and to explore the correlation between TET1 modulation, oxidative stress, and apoptosis-mediated cell death in breast carcinoma. **Methods.** Molecular docking using a curvature-based docking approach and *in vitro* assessment of the effects of cryptotanshinone on cell viability, reactive oxygen species (ROS) generation, apoptosis, and the mRNA expression levels of *TET1, SLC7A11*, and *GPX4* in MCF-7 cells were performed to investigate the anticancer potential of plant-derived terpenoids mediated by modulation of TET1 activity in breast cancer. **Results.** A high binding affinity of cryptotanshinone for the CXXC domain of TET1 was found, along with favorable drug-like properties, and cryptotanshinone was selected as the potent terpenoid for further analysis. *In vitro* evaluation revealed a dose-dependent reduction in cell viability of cryptotanshinone-treated MCF-7 cells and apoptotic induction. Additionally, gene expression analysis showed decrease in the mRNA levels of *TET1*, *SLC7A11*, and *GPX4* in a dose-dependent manner, suggesting alterations in antioxidant defense pathway signaling. **Conclusion.** Cryptotanshinone may exert anticancer effects associated with apoptosis induction and oxidative stress modulation, potentially involving the epigenetic regulator TET1, in breast cancer cells. Further studies using animal models and clinical samples are necessary to confirm the therapeutic potential of cryptotanshinone in the treatment of breast cancer.

Keywords:
apoptosis; breast neoplasms; computer simulation; gene expression; in vitro; plant extracts; terpenes.

Apstrakt

Uvod/Cilj. Progresija karcinoma dojke podstaknuta je disregulacijom signalnih puteva i epigenetskim promenama. Terpenoidi biljnog porekla privukli su značajnu pažnju kao potencijalni antikancerogeni agensi zbog svoje sposobnosti da modulišu ključne molekularne i ćelijske mete. *Ten-eleven translocation 1* (TET1) identifikovan je kao onkogeni faktor u agresivnim podtipovima karcinoma dojke, koji podstiče transkripcione aktivnosti na

Correspondence to: Fahad Khan, Saveetha Institute of Medical and Technical Sciences, Saveetha Medical College and Hospitals, Department of Community Medicine, Chennai, India. E-mail: fahadintegralian@gmail.com

specifičnim mestima DNK koje doprinose razvoju tumora i otpornosti na ćelijsku smrt posredovanu oksidativnim stresom. Cilj rada bio je da se ispita potencijal terpenoida biljnog porekla da ciljano deluje na TET1, kao i da se ispita povezanost između modulacije TET1, oksidativnog stresa i apoptozom-indukovane ćelijske smrti u karcinomu dojke. **Metode.** Za ispitivanje antitumorskog potencijala terpenoida biljnog porekla posredovanog modulacijom aktivnosti TET1 u karcinomu dojke primenjeni su molekulsko dokovanje metodom *curvature-based docking* i *in vitro* procena efekata kriptotanšinona na vijabilnost ćelija, stvaranje reaktivnih kiseoničnih vrsta *(reactive oxygen species* – ROS), apoptozu i nivoe ekspresije iRNK gena *TET1, SLC7A11* i *GPX4* u ćelijama MCF-7. **Rezultati.** Utvrđen je visok afinitet vezivanja kriptotanšinona za CXXC domen TET1, uz povoljne osobine slične svojstvima lekova, pa je kriptotanšinon odabran kao potentni terpenoid za dalju analizu. *In vitro* procena pokazala je dozno-zavisno smanjenje vijabilnosti MCF-7 ćelija tretiranih kriptotanšinonom, kao i indukciju apoptoze. Pored toga, analiza ekspresije gena pokazala je dozno-zavisno smanjenje nivoa iRNK gena *TET1*, *SLC7A11* i *GPX4*, što ukazuje na promene u signalnim putevima antioksidativne odbrane. **Zaključak.** Kriptotanšinon može ispoljiti antikancerogeno delovanje povezano sa indukcijom apoptoze i modulacijom oksidativnog stresa, uz moguću uključenost epigenetskog regulatora TET1, u ćelijama karcinoma dojke. Neophodna su dalja istraživanja na životinjskim modelima i kliničkim uzorcima kako bi se potvrdio terapijski potencijal kriptotanšinona u lečenju karcinoma dojke.

Ključne reči:
ćelija, smrt; dojka, neoplazme; simulacija, kompjuterska; geni, ekspresija; in vitro; ekstrakti, biljni; terpeni.

Introduction

Breast cancer is still one of the most frequently diagnosed cancers and ranks as a leading cause of cancer-related mortality among women across the globe. Although there are significant advancements in targeted therapeutic approaches, including early detection, several issues, such as recurrence leading to treatment failure, pose major challenges in triple-negative breast cancer [1]. Several factors are largely responsible for therapy resistance, metastasis, and the capability of tumor cells to adapt to oxidative and metabolic stress [2]. Thus, identifying new molecular targets that can circumvent resistance mechanisms and trigger non-apoptotic tumor cell death is essential [3]. Epigenetic dysregulation has been recognized as a fundamental feature of breast carcinoma, promoting abnormal gene expression patterns that lead to cell proliferation, invasion, and immune evasion. Among all potent epigenetic regulators, ten-eleven translocation 1 (TET1) is known to influence transcriptional activation at cytosine-phosphate-guanine (CpG)-rich genomic regions by oxidizing 5-methylcytosine [4]. Increasing evidence suggests that TET1 may function as an oncogenic driver in breast carcinoma by enhancing metastatic, stemness, and epithelial-mesenchymal transition efficacy through locus-specific epigenetic modifications; however, these effects are context- and isoform-dependent [5, 6]. Targeting the catalytic domain of TET1 remains challenging because of limited structural data and potential inadvertent effects on global deoxyribonucleic acid (DNA) methylation dynamics. TET1 has a conserved CXXC zinc-finger DNA-binding domain that facilitates its interaction with CpG islands and regulatory promoters, thereby enabling transcriptional regulation without enzymatic activity. Therefore, targeting TET1-DNA interactions offers strong potential that further adds to the field of structure-based drug design. In contrast to traditional catalytic inhibitors, focusing on the DNA-binding interface may enhance locus specificity, hence obstructing TET1-mediated activation of oncogenic transcriptional networks at the chromatin level [7, 8].

In apoptosis-resistant breast carcinoma, ferroptosis has attracted increasing attention by inducing reactive oxygen species (ROS) production and lipid accumulation [9]. Two upregulated antioxidant defense systems–nuclear factor erythroid 2-related factor 2 (NRF2)-mediated redox homeostasis and the solute carrier family 7 member 11 (SLC7A11)/glutathione peroxidase 4 (GPX4) axis–mainly help tumor cells to avoid ferroptosis [10]. According to recent research, TET1 (an epigenetic regulator) may be associated with cancer-linked signaling pathways and redox homeostasis through maintaining transcriptional activity [11]. Therefore, molecular docking-based targeting of the TET1 CXXC DNA-binding domain could be a novel epigenetic strategy to explore the TET1-mediated anticancer mechanisms in breast cancer. We focus on the CXXC domain of full-length TET1 ($TET1^{FL}$) not because it drives the phosphoinositide 3-kinase (PI3K)-associated oncogenic activity attributed to the CXXC-lacking isoform of TET1 ($TET1^{ALT}$) [6], but because $TET1^{FL}$-CXXC-mediated CpG binding governs a distinct transcriptional axis relevant to tumor cell survival: locus-specific demethylation that may sustain expression of antioxidant defense genes such as *SLC7A11* and GPX4. We hypothesize that disrupting this CXXC-DNA interaction could reduce antioxidant gene expression and increase oxidative-stress vulnerability in breast cancer cells, representing a mechanism of therapeutic relevance distinct from, and narrower than, $TET1^{ALT}$-driven proliferative oncogenesis. Small compounds found through docking may interact with the TET1 CXXC DNA-binding domain and influence TET1-linked transcriptional programs of antioxidant networks and redox homeostasis [12].

Therefore, the aim of this study was to utilize a molecular docking-based screening of terpenoids against the TET1 CXXC DNA-binding domain, followed by *in vitro* evaluation of the lead compound for its anticancer activity and TET1-associated molecular responses in breast cancer Michigan Cancer Foundation-7 (MCF-7) cells.

Methods

Software used for in silico investigation

Structural retrieval of the target protein was done using the Protein Data Bank (PDB), and ligand acquisition was done using the PubChem server available online. Additionally, the Curvature-based dock (CB-Dock) server was utilized for performing blind docking and cavity prediction. It uses the AutoDock Vina engine to estimate binding affinity and PyMOL visualizer to visualize the binding interactions.

Chemicals and Reagents

Dulbecco's Modified Eagle's Medium (DMEM) (AT151), fetal bovine serum (FBS) (RM10434), and antibiotic-antimycotic solution (A002) were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All supplementary analytical-grade chemicals and reagents used in this research were acquired from reputable commercial vendors and possessed the highest purity. Cryptotanshinone [C5624-5mg, ≥ 98% high-performance liquid chromatography (HPLC) grade], methyl thiazolyl tetrazolium (MTT) (M2128, 98%, powder), and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (D6883, ≥ 97% HPLC) were procured from Sigma-Aldrich, St. Louis, MO, USA, and stock solutions were developed in dimethyl sulfoxide (DMSO) and stored at -20 °C for future usage. The final DMSO concentration was kept below 0.1% (v/v) through meticulous dilution of the working concentrations in the growth medium. Invitrogen SuperScript™ III One-Step reverse transcription PCR (RT-PCR) System (Cat. No. 12574018) was purchased from Thermo Fisher Scientific, while Annexin V fluorescein isothiocyanate (FITC)-conjugated Apoptosis Detection Kit I BD Pharmingen™ (Cat. No. 556547) was purchased from BD Biosciences, San Jose, CA, USA.

Protein and ligand preparation for molecular docking

Three-dimensional (3D) crystal structure of the human TET1 CXXC DNA-binding domain (PDB ID: 6ASD) was selected due to its significance in CpG recognition and experimental resolution. CXXC domain of $TET1^{FL}$ was targeted not only because it drives the PI3K-associated oncogenic activity attributed to the CXXC-lacking $TET1^{ALT}$ isoform, but also because $TET1^{FL}$-CXXC-mediated CpG binding governs a distinct transcriptional axis critical to tumor cell survival: locus-specific demethylation that may sustain expression of antioxidant defense genes such as *SLC7A11* and *GPX4*. Before docking, the protein was stripped of co-crystallized DNA, water molecules, and non-ligand-binding heteroatoms. Missing residues were examined, and hydrogen atoms were added to ensure structural integrity. The protein structure was saved as a PDB file for docking analysis. The PubChem database provided the chemical structures of the selected ligands. Ligands were energy-minimized to obtain stable conformations and were preserved in SDF or MOL2 format. The improved ligand files were then translated to the docking submission format.

Molecular docking using CB-Dock

CB-Dock integrates curvature-based cavity detection with AutoDock Vina-based blind docking to automatically identify potential binding cavities and predict protein–ligand binding poses [13]. The CB-Dock server received the receptor protein (TET1 CXXC domain, PDB: 6ASD). Ligand structures were uploaded individually in the SDF/MOL2 format. Due to curvature-based pocket recognition, CB-Dock found protein binding cavities automatically. The server generated numerous binding cavities sorted by cavity volume and docking capability for each ligand. AutoDock Vina docking simulations produced binding poses and affinity scores (kcal/mol) [14].

The docking output comprised top-ranked binding cavities, projected binding energies, ligand-protein interaction poses, 3D and 2D interaction representations, docking scores, and interaction analyses. Docking results were assessed by binding affinity scores and ligand orientation stability in the anticipated binding pocket. Low binding energy and favorable molecular interactions determined the optimal docking conformations. PyMOL and Discovery Studio Visualizer were used to study protein-ligand interactions, such as hydrogen bonding, hydrophobic contacts, and electrostatic interactions. Residues important for CpG recognition within the CXXC DNA-binding interface were prioritized, as their disruption may prevent TET1 recruitment to genomic targets. Thus, docking to the TET1 CXXC domain provides structural insights into small molecules that modulate TET1-DNA interactions. Modulation of epigenetically regulated antioxidant pathways may increase oxidative stress sensitivity in breast cancer cells, informing the development of novel oxidative stress-inducing therapies.

Pharmacokinetic suitability of the selected ligands using SwissADME

Within the context of the current in silico investigation of antioxidant-pathway-associated proteins in breast cancer, the pharmacokinetic and drug-likeness profiles of the selected ligands were further evaluated using the SwissADME web tool [15]. Particular attention was paid to the gastrointestinal (GI) absorption, bioavailability score [16], and drug-likeness properties. These attributes reflect the ability of the ligand to enter systemic circulation and cross the intestinal barrier and help determine the efficacy of oral drugs. In addition, the bioavailability score is a crucial prognostic indicator of the likelihood that a molecule will display acceptable oral exposure. It does this by incorporating critical physicochemical characteristics such as molecular size, polarity, and lipophilicity of the molecule. Furthermore, the evaluation of drug-likeness through the use of known filters, such as Lipinski's rule of five [17], guarantees that promising candidates for oxidative stress-modulating properties contain advantageous structural and pharmacological qualities that go

beyond docking affinity alone. Collectively, these factors increase the translational relevance of the compounds identified by supporting their potential as orally viable therapeutic agents for managing breast cancer through modulation of oxidative stress pathways.

Assessment of cell viability and half-maximal inhibitory concentration

We obtained the MCF-7 breast cancer cell line from the National Center for Cell Science, Pune, India. The cancer cells were cultured in DMEM containing 10% FBS (Himedia India, Mumbai, India) and 1% antibiotic-antimycotic solution (Himedia India, Mumbai, India). We maintained optimal cell growth conditions in an incubator at 37 °C with 5% carbon dioxide – CO_2. The effect of cryptotanshinone on the cell viability of breast carcinoma MCF-7 cells was assessed using the MTT assay. MCF-7 cells (6,000 cells/well) were plated in 96-well plates and then exposed to different doses of cryptotanshinone (0–8 μM) for 24 hrs. After 24 hrs, 10 μL of MTT dye was transferred to the treated wells, which were then left for 2 hrs at 37 °C. The formazan crystal was then dissolved with 100 μL DMSO, and the microplate reader (Bio-Rad, USA) was used to calculate the optical density.

Furthermore, the half-maximal inhibitory concentration (IC_{50}) value of cryptotanshinone was calculated using GraphPad Prism software (GraphPad Prism Software Inc., San Diego, CA, USA). A dose-response parameter with nonlinear regression was selected to obtain the response curve. The percentage of cell viability was plotted against the logarithm of the cryptotanshinone dose and IC_{50} values were derived from the fitted sigmoidal curve. This method facilitates the selection of concentrations from sub-cytotoxic to cytotoxic levels, thereby minimizing non-specific cell death. The selection of lower concentrations maintains sufficient cell viability to evaluate early molecular and biochemical changes, such as ROS generation and apoptosis induction. Medium-range concentrations facilitate the identification of gradual signaling changes and initiation of apoptotic pathways. At the same time, doses above IC_{50} create an environment favorable for observing significant apoptotic responses without total reduction in viable cells.

Assessment of reactive oxygen species generation

Effects of cryptotanshinone on ROS-mediated oxidative stress were determined using DCFH-DA labelling, as previously outlined by Alshammari et al. [18]. In short, 1×10^4 MCF-7 cells were treated with cryptotanshinone at three different concentrations (2, 4, and 6 μM) for 12 hrs. Cells were later treated with DCFH-DA (10 mM) at 37 °C for 30 min. Then, the intensity of the fluorescence was measured using a multiwell microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, USA). To further confirm the cryptotanshinone's efficacy in ROS generation, cells were previously treated with a ROS inhibitor, 10 mM N-acetyl-L-cysteine (NAC), for 2 hrs. The same methods of ROS analysis were then carried out as previously described.

Quantitative real-time polymerase chain reaction analysis

The total ribonucleic acid (RNA) of cryptotanshinone-treated MCF-7 cells was collected and used to convert into complementary DNA – cDNA using a reverse transcription kit. Relative messenger RNA (mRNA) expression of the TET1 transcript region and antioxidant defense-related molecular targets following cryptotanshinone exposure were evaluated by quantitative real-time polymerase chain reaction (PCR) – qPCR. Relative mRNA expression levels were normalized to β-actin as the internal reference gene. For each sample, the difference between the cycle threshold (Ct) value of the target gene and that of β-actin was calculated as ΔCt, where: [ΔCt = Ct(target gene) - Ct(β-actin)]. The normalized expression level was calculated as ($2^{-\Delta Ct}$). Relative changes in target gene expression between treated and control samples were subsequently determined using the ($2^{-\Delta\Delta Ct}$) method, where: [ΔΔCt= ΔCt(treated)-ΔCt(control)], and relative fold expression was calculated as ($2^{-\Delta\Delta Ct}$). The control group was used as the calibrator and assigned a relative expression value of 1.0. Ct denotes the cycle threshold at which the fluorescence signal crosses the defined detection threshold. Primer sequences are listed in Table 1.

Flow cytometric analysis of apoptosis

To assess apoptosis, Annexin V Apoptosis Detection Kit I (BD Pharmingen™, BD Biosciences, San Jose, CA, USA; Cat. No. 556547) was used according to the manufacturer's instructions. MCF-7 cells (1 × 106 cells/well) were seeded and exposed to different concentrations of cryptotanshinone (2, 4, and 6 μM) after 24 hrs, whereas control cells were maintained under identical conditions without treatment. Breast cancer cells were mixed with 100 μL of binding buffer containing propidium iodide (PI) (5 μg/mL) and annexin V-FITC (0.125 μg/mL). They were then

Table 1

Primer sequences used for quantitative real-time PCR analysis

Gene	Forward primer	Reverse primer
TET1	5'-TCTGTTGTTGTGCCTCTGGA-3'	5'-GCCTTTAAAACTTTGGGCTTC-3'
GPX4	5'-GCCTTTGCCGCCTACTGAAG-3'	5'-ACGAAGCCCCGGTACTTGTC-3'
SLC7A11	5'-GCTCCATGAACGGTGGTGTG-3'	5'-AGAGGAGTGTGCTTGCGGAC-3'
β-actin	5'-GTCTGTGATGCCCTTAGATG-3'	5'-AGCTTATGACCCGCACTTAC-3'

PCR – polymerase chain reaction; *TET1* – ten-eleven translocation 1; *GPX4* – glutathione peroxidase 4; *SLC7A11* – solute carrier family 7 member 11.

incubated at room temperature in the dark for 15 min. We analyzed the samples using a flow cytometer (BD FACSCaliburTM Flow Cytometer; BD Biosciences, San Diego, CA, USA) to determine the proportion of early and late apoptotic cells relative to the remaining cells.

Statistical analysis

The mean ± standard error of the mean is used to represent all datasets of three independent experiments (n = 3). One-way analysis of variance – ANOVA was selected to calculate the results, and the GraphPad Prism tool was employed to conduct Dunnett's multiple comparison test. Data statistical significance was determined by a probability *p*-value less than 0.05.

Results

Drug-likeness and pharmacokinetic profiles of selected terpenoids

Parameters were selected to assess the potential of plant-based compounds as potent candidates for developing anticancer agents. Although numerous parameters exist, we selected only Lipinski criteria, GI absorption, and Bioavailability score for our study. These parameters are essential pharmacokinetic factors that are used in anticancer drug development to assess the oral bioavailability and therapeutic potential of candidate compounds. Lipinski's parameters are utilized for identifying compounds with favorable permeability, solubility, and overall drug-likeness. In cancer therapeutics, GI tract absorption reflects how much of an orally administered drug reaches systemic circulation and influences therapeutic efficacy, formulation strategy, and toxicity. In addition to GI and Lipinski parameters, the bioavailability score provides an approximate proportion of the selected drug that remains unaltered in systemic circulation, which is required for dose optimization, systemic toxicity minimization, and reduced treatment failure. These selected parameters form a fundamental framework for the rational design, screening, and optimization of orally active plant-based products, thereby increasing the likelihood of anticancer therapeutic approaches. The cryptotanshinone compound has shown better outcomes than all other selected terpenoids (Table 2).

Molecular docking and binding affinity analysis of selected terpenoids

Docking was done to elucidate the potent terpenoid that has displayed the best inhibitory activity against the TET1 target. In Table 3, it can be clearly seen that cryptotanshinone has shown the best binding efficacy (-7.0) in docking analysis in comparison to all other selected compounds. Thus, in further sections, we performed two *in vitro* methods, including MTT and RT-PCR assays, to investigate the efficacy of cryptotanshinone in breast carcinoma.

Effect of cryptotanshinone on MCF-7 cell viability

Treatment of MCF-7 cells with cryptotanshinone at concentrations of 0.5, 1, 2, 4, and 8 μM reduced cell viability to 82.74 ± 4.72% ($p < 0.05$), 64.78 ± 4.12% ($p < 0.05$), 55.45 ± 4.33% ($p < 0.001$), 42.9 ± 3.91% ($p < 0.001$), and 23.68 ± 3.53% ($p < 0.001$), respectively, compared with the control sample. In Figure 1A, it can be seen that cryptotanshinone has shown maximum inhibitory potential at its highest dose (8 μM). Based on the dose-response curve, the IC_{50} was 3.89 ± 0.93 μM (Figure 1B). This IC_{50} value was further used to select the doses needed for ROS generation and RT-PCR analysis.

Table 2

Predicted drug-likeness and pharmacokinetic properties of selected plant-derived terpenoids

Compound	Compound identifier	Chemical formulae	Lipinski's rule of five	Gastrointestinal absorption	Bioavailability score
Arnicolide D	182199	$C_{19}H_{24}O_5$	yes; 0 violation	high	0.55
Cryptotanshinone	160254	$C_{19}H_{20}O_3$	yes; 0 violation	high	0.85
Glycyrrhetinic acid	10114	$C_{30}H_{46}O_4$	yes; 1 violation: MLogP > 4.15	high	0.85
Eupaformosanin	5281453	$C_{22}H_{28}O_8$	yes; 0 violation	high	0.55
Artemisinin	68827	$C_{15}H_{22}O_5$	yes; 0 violation	high	0.55
Dihydroartemisinine	3000518	$C_{15}H_{24}O_5$	yes; 0 violation	high	0.55
Tanshinone IIA	164676	$C_{19}H_{18}O_3$	yes; 0 violation	high	0.55
Oridonin	5321010	$C_{20}H_{28}O_6$	yes; 0 violation	high	0.55

MLogP – Moriguchi Log P.

Table 3

Docking results showing Vina scores, interacting residues, and protein–ligand interactions

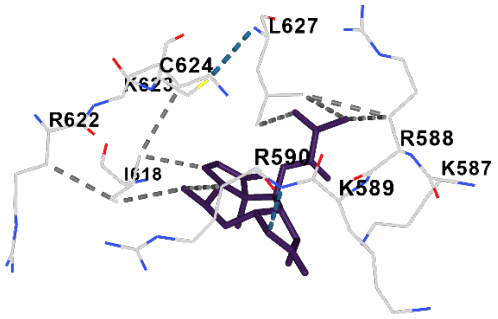
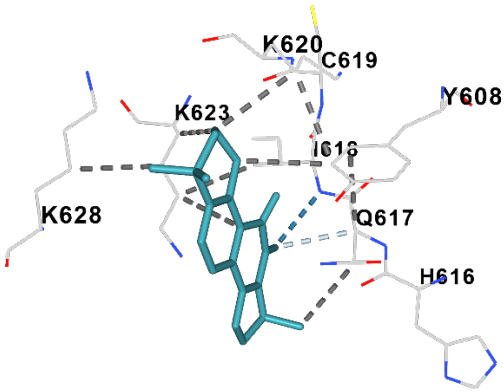
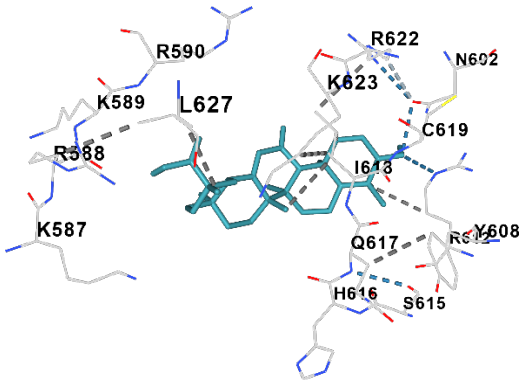
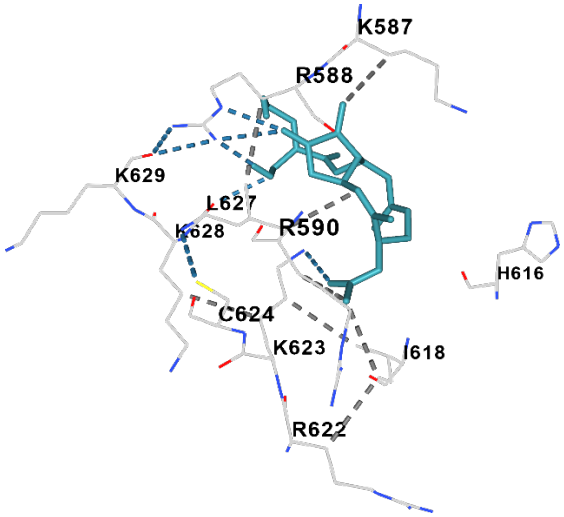
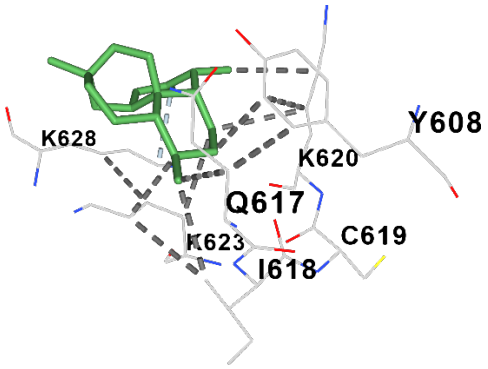
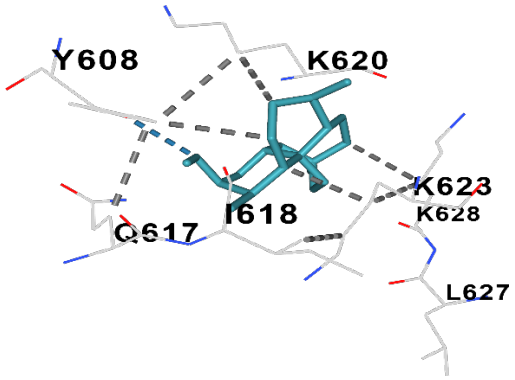
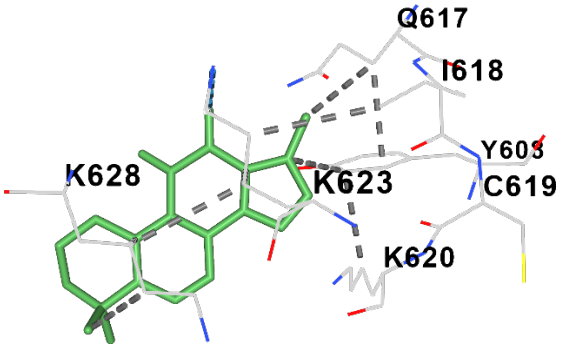
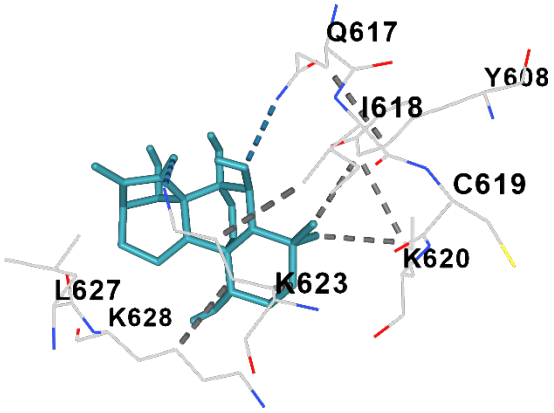
Compound	Vina scores	Contact residues	Protein-ligand interaction
Arnicolide D	-6.0	chain C: LYS587 ARG588 LYS589 ARG590 CYS597 ASN602 TYR608 CYS609 ARG612 SER615 HIS616 GLN617 ILE618 CYS619 ARG622 LYS623 CYS624 LEU627	
Cryptotanshinone	-7.0	chain C: LYS587 ARG588 LYS589 ARG590 THR607 TYR608 ASN611 ASN614 SER615 HIS616 GLN617 ILE618 CYS619 LYS620 LYS623 LEU627 LYS628 LYS629 LYS630	
Glycyrrhetinic acid	-6.6	chain C: LYS587 ARG588 LYS589 ARG590 ASN602 TYR608 CYS609 ARG612 LYS613 SER615 HIS616 GLN617 ILE618 CYS619 ARG622 LYS623 CYS624 LEU627	
Eupaformosanin	-5.7	chain C: GLY586 LYS587 ARG588 LYS589 ARG590 PRO596 CYS597 TYR608 HIS616 GLN617 ILE618 CYS619 LYS620 LYS621 ARG622 LYS623 CYS624 GLU625 LEU627 LYS628 LYS629 LYS630	

Table 3 (continued)

Compound	Vina scores	Contact residues	Protein-ligand interaction
Artemisinin	-6.2	chain C: TYR608 GLN617 ILE618 CYS619 LYS620 LYS623 LEU627 LYS628	
Dihydroartemisinine	-5.9	chain C: TYR608 GLN617 ILE618 CYS619 LYS620 LYS623 LEU627 LYS628	
Tanshinone IIA	-6.7	chain C: GLY586 LYS587 ARG588 TYR608 HIS616 GLN617 ILE618 CYS619 LYS620 LYS623 LEU627 LYS628	
Oridonin	-6.3	chain C: TYR608 GLN617 ILE618 CYS619 LYS620 LYS621 LYS623 LEU627 LYS628	

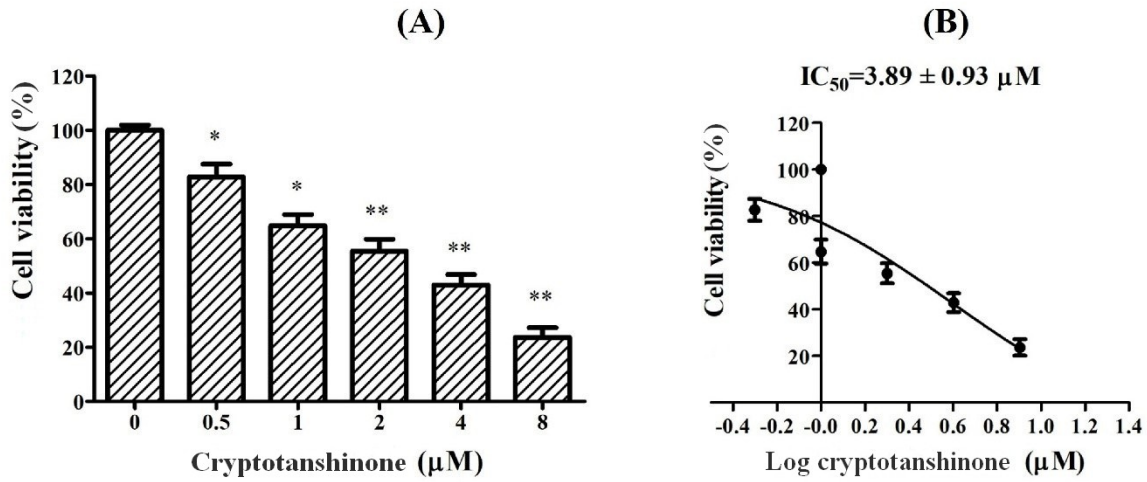


Fig. 1 – A) MTT result showing cell viability inhibition in cryptotanshinone-treated MCF-7 cells; B) dose-dependent IC_{50} curve of cryptotanshinone.
Data are represented as mean ± standard error of the mean of three independent experiments (n = 3).
***Note*: *$p < 0.05$; **$p < 0.001$ compared with the control sample.**
IC_{50} – half-maximal inhibitory concentration; MTT – methyl thiazolyl tetrazolium; MCF-7 – Michigan Cancer Foundation-7; n – number.

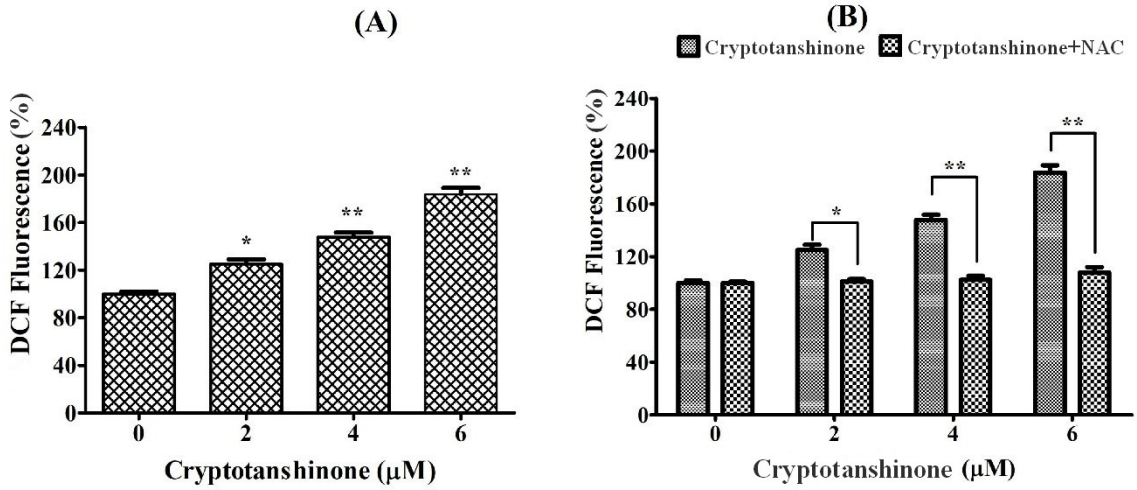


Fig. 2 – Cryptotanshinone induced ROS generation in breast cancer MCF-7 cells: A) effect of cryptotanshinone (0, 2, 4, and 6 μM) on ROS generation in MCF-7 cells; B) quenching of ROS in NAC pre-treated MCF-7 cells.
Data are represented as mean ± standard error of the mean of three independent experiments (n = 3).
***Note*: *$p < 0.05$, **$p < 0.001$ compared with the control sample.**
DCF – dichlorodihydrofluorescein; NAC – N-acetyl-L-cysteine; ROS – reactive oxygen species; MCF-7 – Michigan Cancer Foundation-7; n – number.

Cryptotanshinone-induced reactive oxygen species generation in MCF-7 cells

ROS generation was markedly elevated in a concentration-dependent manner following treatment with 2, 4, and 6 μM doses of cryptotanshinone to approximately 24.96 ± 4.08% ($p < 0.05$), 47.11 ± 3.93% ($p < 0.001$), and 83.78 ± 5.33% ($p < 0.001$), respectively, compared with the control sample (Figure 2A). When the cells were pretreated with the ROS inhibitor NAC and subsequently exposed to cryptotanshinone, the results clearly showed reduced ROS levels in MCF-7 cells (Figure 2B). Based on these results, ROS production might play a role in the cryptotanshinone-induced cell viability inhibition in breast cancer MCF-7 cells.

Effect of cryptotanshinone on TET1, SLC7A11, and GPX4 mRNA expression

To analyze the effects of cryptotanshinone on mRNA expression of *TET1* and associated genes (*SLC7A11* and

GPX4), we performed RT-PCR analysis. As shown in Figure 3, cryptotanshinone significantly downregulated *TET1* mRNA levels to 0.67 ± 0.071 ($p < 0.001$), 0.35 ± 0.049 ($p < 0.001$), and 0.21 ± 0.026 ($p < 0.001$), alongside the *SLC7A11* mRNA expression to 0.82 ± 0.081 ($p < 0.05$), 0.49 ± 0.077 ($p < 0.001$), and 0.24 ± 0.057 ($p < 0.001$), and *GPX4* mRNA expression to 0.77 ± 0.085 ($p < 0.05$), 0.47 ± 0.04 ($p < 0.001$), and 0.29 ± 0.026 ($p < 0.001$) at 2, 4, and 6 μM concentrations, respectively, compared with the control sample.

Cryptotanshinone-induced apoptosis in MCF-7 cells

It was explored whether cryptotanshinone induced apoptosis in MCF-7 cells. Annexin V/PI double staining method showed that the apoptosis rate was markedly increased to 12.6 ± 1.29% ($p < 0.05$), 21.6 ± 2.88% ($p < 0.001$), and 34.2 ± 2.63% ($p < 0.001$), respectively, following a 24 hrs exposure to cryptotanshinone at different concentrations of 2, 4, and 6 μM (Figure 4).

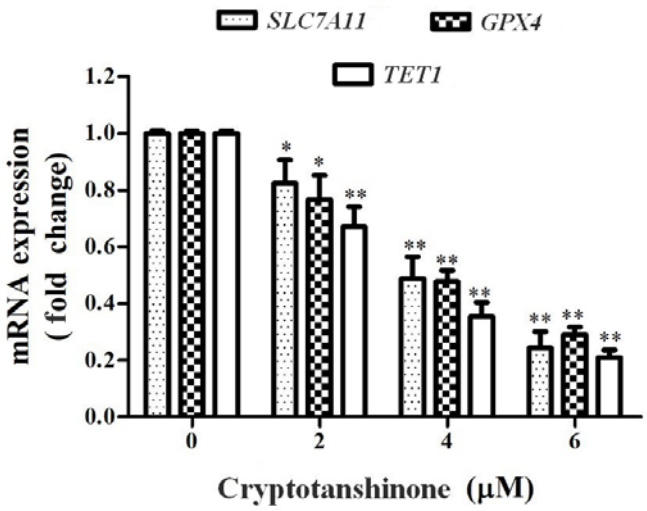


Fig. 3 – RT-PCR analysis for investigating mRNA expression in cryptotanshinone-treated MCF-7 cells.
Data are represented as mean ± standard error of the mean of three independent experiments (n = 3).
***Note*: *$p < 0.05$; **$p < 0.001$ compared with control sample.**
mRNA – messenger ribonucleic acid; *SLC7A11* – solute carrier family 7 member 11; *TET1* – ten-eleven translocation 1; *GPX4* – glutathione peroxidase 4; RT-PCR – reverse transcription polymerase chain reaction; MCF-7 – Michigan Cancer Foundation-7; n – number.

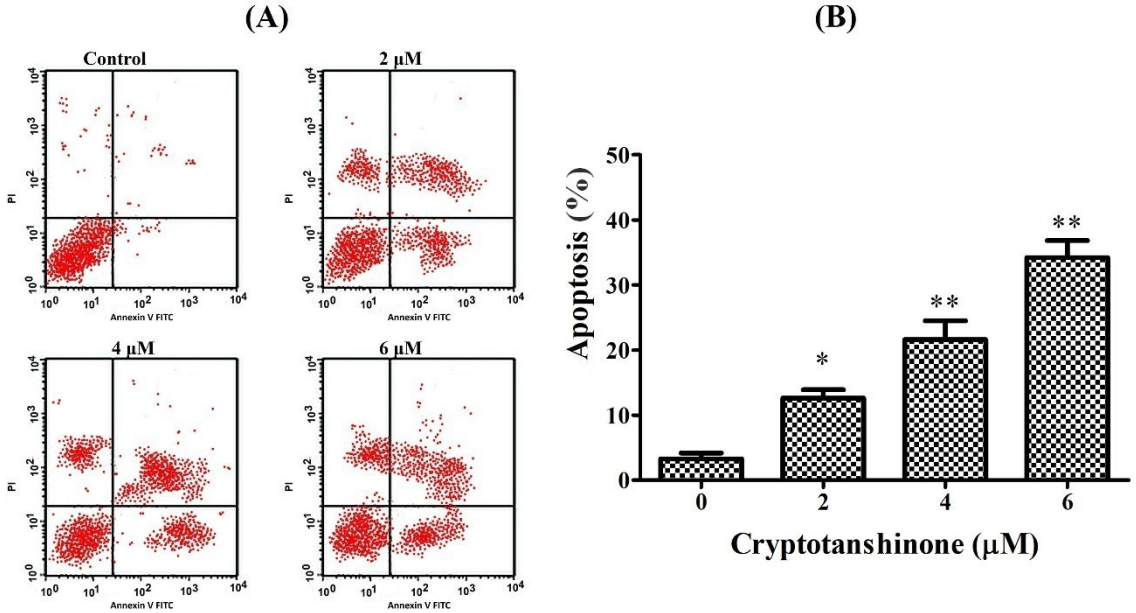


Fig. 4 – Cryptotanshinone-induced apoptosis in breast cancer MCF-7 cells: A) the percent apoptotic cells treated with different concentrations of cryptotanshinone (0, 2, 4, and 6 μM) for 24 hrs analyzed by flow cytometry; B) percentage of apoptotic cells increased following exposure to cryptotanshinone compared with the untreated control.
Data are represented as mean ± standard error of the mean of three independent experiments (n = 3).
***Note*: *$p < 0.05$; **$p < 0.001$ compared with the control sample.**
PI – propidium iodide; FITC – fluorescein isothiocyanate; MCF-7 – Michigan Cancer Foundation-7; n – number.

Discussion

TET1 is an epigenetic regulator often linked to the development of breast cancer because of its involvement in oncogenic pathway transcriptional activation and DNA demethylation [19]. The DNA-binding CXXC domain of TET1 is similarly important because it controls locus-specific recruitment to CpG-rich promoters and enhancers, even though most research focuses on the catalytic dioxygenase activity of TET1 proteins [20]. Abnormal TET1 activity in breast cancer increases tumor formation, metastasis, epithelial-mesenchymal transition, and treatment resistance by activating genes linked to invasion and stemness [21]. Previous studies showed that blocking the DNA-binding domain of TET1 provided a novel means of limiting its function upstream, thereby preventing its localization to oncogenic target regions, even in the absence of direct enzymatic activity inhibition [22–24]. Importantly, TET1-mediated epigenetic reprogramming (genome-wide) leads to disruption of the DNA-binding interface that blocks transcriptional networks associated with aggressive triple-negative breast cancer. Because it offers selective epigenetic intervention and broadens anti-TET1 drug development beyond catalytic inhibitors, targeting the DNA-binding domain is a promising therapeutic approach [25].

Docking with the CXXC DNA-binding domain is crucial since it investigates the disruption of protein-DNA interactions, an emerging technique in anticancer research. TET1 is recognized as an epigenetic regulator that associates with CpG-rich genomic regions *via* its CXXC domain, thereby facilitating the transcriptional activation of genes involved in redox balance, lipid metabolism, and cellular survival [26, 27]. Growing data demonstrate that abnormal TET1-mediated epigenetic reconfiguration may influence cellular redox homeostasis by modulating antioxidant defense pathways, including SLC7A11 and GPX4 [11, 28].

Inhibiting CXXC-mediated DNA binding may prevent locus-specific demethylation processes that sustain tumor cell adaptation to oxidative damage [29]. Thus, docking-based discovery of small compounds that can interfere with the TET1-DNA binding interface may improve oxidative stress-mediated response in aggressive breast cancer subtypes. This epigenetic drug discovery technique targets redox homeostasis, which may help overcome drug resistance and metastasis. Ferroptosis investigations often target GPX4 inhibitors or system xc− inhibition [30], while targeting the epigenetically associated target TET1 may provide a novel strategy for exploring the modulation of these pathways [31].

Among the selected terpenoids, cryptotanshinone showed the best binding interaction with TET1 using CB-Dock analysis. MCF-7 cells treated with cryptotanshinone have shown significant inhibition of cell viability of MCF-7 cells. Moreover, the findings from the Annexin V-FITC assay demonstrated that cryptotanshinone exposure markedly increased apoptotic cell populations in a dose-dependent fashion, consistent with a previous study [32]. Additionally, RT-PCR analysis showed that cryptotanshinone-mediated *TET1* inhibition resulted in enhanced suppression of *SLC7A11* and *GPX4* in breast cancer MCF-7 cells. Our results are in accordance with a study that demonstrated the inhibitory effects of longikaurin A on TET2 expression, accompanied by GPX4 downregulation and ferroptosis induction in glioblastoma [33]. Altogether, this preliminary study suggests that TET1 may be involved in the anticancer effects of cryptotanshinone through mechanisms associated with oxidative stress and apoptosis in MCF-7 breast cancer cells. This potential involvement is supported by molecular docking results and changes in *TET1*, *SLC7A11*, and *GPX4* mRNA expression, together with increased ROS generation and apoptosis following cryptotanshinone treatment. However, further experimental validation, including TET1 protein expression analysis and *in vivo* studies, is required to clarify the role of TET1 in the anticancer effects of cryptotanshinone. Additionally, isoform-specific expression profiling and locus-specific chromatin analyses are warranted to further investigate the proposed molecular mechanisms.

Conclusion

This study showed that cryptotanshinone has the strongest binding affinity for TET1 CXXC DNA-binding domain. *In vitro* experiments demonstrated that cryptotanshinone reduced MCF-7 cell viability, increased reactive oxygen species generation, and induced apoptosis in a concentration-dependent manner. Furthermore, cryptotanshinone treatment downregulated the mRNA expression of *TET1*, *SLC7A11*, and *GPX4*, suggesting a potential association between its cytotoxic effects and alterations in oxidative stress-related pathways. Although these findings support the potential anticancer activity of cryptotanshinone in breast cancer cells, the underlying molecular mechanisms, particularly the involvement of TET1, require further investigation. Additional studies using animal models and clinical samples are needed to validate these findings and assess the therapeutic potential of cryptotanshinone in breast cancer.

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Conflict of interest

The authors declare no conflict of interest.

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Work ability of employees with hypertension and diabetes mellitus: the role of working conditions and psychosocial risks

Radna sposobnost zaposlenih obolelih od hipertenzije i dijabetes melitusa: uloga uslova rada i psihosocijalnih rizika

Nada Stanković*, Jelena Momčilović Vasilov†, Dušan Maksimović‡

***Institute of Occupational Health Niš, Niš, Serbia; †Ministry of Defence, Military Healthcare Department, Belgrade, Serbia; ‡Military Hospital Niš, Department of Internal Medicine, Niš, Serbia**

Abstract

Introduction/Aim. Arterial hypertension (HTN) and diabetes mellitus (DM) represent the most common chronic non-communicable diseases in the working population and are an important cause of reduced work ability. The aim of this study was to examine the impact of working conditions, psychosocial risks, and work organization on the work ability of employees with HTN and/or DM compared with employees without these chronic diseases. **Methods.** A cross-sectional study was conducted among 100 employed participants diagnosed with HTN and/or DM (cases group) and 100 employees without these diseases (control group) in two state-owned public sector companies in Niš, Serbia. The control group was frequency-matched to the cases group by sex, age group, company, and shift/night work schedule. Work ability was assessed using the Work Ability Index (WAI), stress level *via* the 10-item Perceived Stress Scale (PSS-10), and psychosocial factors using a questionnaire on job demands, autonomy, support, and satisfaction. Descriptive statistical methods, Pearson correlation, *t*-test, multiple linear regression, and logistic regression were applied. **Results.** The mean WAI score in the cases and control groups was 28.34 ± 3.18 and 41.76 ± 2.27, respectively, with a statistically significant difference (t = -34.32; $p < 0.001$; Cohen d = 4.85). Impaired work ability (WAI ≤ 27) was recorded in 44% of diagnosed cases, compared with 0% in the control group (χ^2 = 53.88; $p < 0.001$). The strongest correlates of WAI in the cases group were job autonomy (r = 0.549; $p < 0.001$) and supervisor support (r = 0.516; $p < 0.001$), whereas no psychosocial factor reached statistical significance in relation to WAI within the control group. Fear of job loss was reported by 86% of cases and 32% of controls (4.28 vs. 3.03; $p < 0.001$), and negatively correlated with WAI in the cases group (r = -0.317; p = 0.001). The regression model with organizational factors in the cases group explained 56.5% of the variance in WAI (F = 9.43; $p < 0.001$). In logistic regression, the diagnosis of HTN and/or DM independently increased the risk of impaired work ability [odds ratio (OR) = 156.36; 95% confidence interval (CI): 9.44–2,588.59; $p < 0.001$], with pronounced fear of job loss (OR = 3.62) and low autonomy (OR = 2.74) also identifying as independent predictors of impaired WAI. **Conclusion.** Work ability of employees with HTN and DM is significantly lower than that of healthy employees and is influenced by organizational workplace factors. Job autonomy, supervisor support, and job satisfaction are key protective factors, whereas fear of job loss has a negative impact. Comparison with the control group shows that the same psychosocial conditions do not affect work ability equally in healthy and diseased employees–their influence is considerably more pronounced in individuals with chronic illness. The results highlight the need for organizational interventions aimed at preserving work ability.

Keywords:
diabetes mellitus; hypertension; occupational medicine; surveys and questionnaires; work capacity evaluation.

Apstrakt

Uvod/Cilj. Arterijska hipertenzija (*arterial hypertension* – HTN) i dijabetes melitus (DM) predstavljaju najčešće hronične nezarazne bolesti u radno-aktivnoj populaciji i značajan su uzrok smanjenja radne sposobnosti. Cilj rada bio je da se ispita uticaj radnih uslova, psihosocijalnih rizika i organizacije rada na radnu sposobnost zaposlenih obolelih od HTN i/ili DM u poređenju sa zaposlenima koji ne boluju od ovih hroničnih bolesti. **Metode.** Sprovedena je studija preseka kod 100 zaposlenih ispitanika sa dijagnostikovanom HTN i/ili DM (grupa obolelih) i 100

Correspondence to: Nada Stanković, Institute of Occupational Health Niš, Drugog Srpskog Ustanka 19, 18 108 Niš, Serbia. E-mail: stankovic.nada80@gmail.com

zaposlenih bez ovih bolesti (kontrolna grupa) u dve državne firme javnog sektora u Nišu, Srbija. Kontrolna grupa je usklađena sa grupom obolelih po polu, starosnoj kategoriji, firmi i smenskom/noćnom rasporedu rada. Radna sposobnost procenjivana je pomoću *Work Ability Index* (WAI), nivo stresa pomoću *10-item Perceived Stress Scale* (PSS-10), a psihosocijalni faktori putem upitnika o zahtevima posla, autonomiji, podršci i zadovoljstvu. Primenjene su deskriptivne statističke metode, Pearson-ova korelacija, *t*-test, višestruka linearna regresija i logistička regresija. **Rezultati.** Srednja vrednost skora WAI kod obolelih i kontrolne grupe bila je 28,34 ± 3,18 i 41,76 ± 2,27, redom, sa statistički značajnom razlikom (t = -34,32; $p < 0,001$; Cohen-ov d = -4,85). Narušena radna sposobnost (WAI ≤ 27) zabeležena je u 44% slučajeva u grupi obolelih u poređenju sa 0% slučajeva u kontrolnoj grupi (χ^2 = 53,88; $p < 0,001$). U grupi obolelih najjača povezanost sa WAI skorom utvrđena je za autonomiju na poslu (r = 0,549; $p < 0,001$) i podršku nadređenih (r = 0,516; $p < 0,001$), dok u kontrolnoj grupi ni jedan psihosocijalni faktor nije dostigao statističku značajnost u odnosu na WAI. Strah od gubitka posla bio je prisutan kod 86% ispitanika u grupi obolelih i 32% ispitanika u kontrolnoj grupi (4,28 vs. 3,03; $p < 0,001$), i u negativnoj korelaciji sa WAI u grupi obolelih (r = -0,317; p = 0,001). Regresionim modelom koji je uključivao organizacione faktore u grupi obolelih objašnjeno je 56,5% varijanse WAI skora (F = 9,43; $p < 0,001$). U logističkoj regresiji, dijagnoza HTN i/ili DM bila je nezavisno povezana sa rizikom od narušene radne sposobnosti [*odds ratio* (OR) = 156,36; 95% *confidence interval* (CI): 9,44–2 588,59; $p < 0,001$], dok su se izražen strah od gubitka posla (OR = 3,62) i niska autonomija (OR = 2,74) izdvojili kao nezavisni prediktori narušenog WAI. **Zaključak.** Radna sposobnost zaposlenih obolelih od HTN i DM značajno je niža u poređenju sa zdravim zaposlenima i uslovljena je organizacionim faktorima radne sredine. Autonomija na poslu, podrška nadređenih i zadovoljstvo poslom su ključni protektivni faktori, dok strah od gubitka posla deluje nepovoljno. Poređenje sa kontrolnom grupom pokazuje da isti psihosocijalni uslovi ne deluju jednako na radnu sposobnost zdravih i obolelih zaposlenih–njihov uticaj je znatno izraženiji kod osoba sa hroničnim oboljenjima. Rezultati naglašavaju potrebu za organizacionim intervencijama usmerenim na očuvanja radne sposobnosti.

Ključne reči:
dijabetes melitus; hipertenzija; medicina rada; ankete i upitnici; sposobnost, radna, ocena.

Introduction

Arterial hypertension (HTN) and diabetes mellitus (DM) are among the most common chronic non-communicable diseases in the working-age population and are important contributors to reduced work ability, increased sick leave, and premature exit from the workforce [1–4]. According to the World Health Organization – WHO, HTN affects approximately 1.28 billion adults globally [2], while DM prevalence among adults exceeds 10% [3]. In the Republic of Serbia, cardiovascular diseases remain the leading cause of mortality, while diabetes represents an increasing public health burden [5–7].

Work ability is defined as a dynamic relationship between the demands of the workplace and the individual capacities of the employee, including health status, functional abilities, and mental resources [8]. The Work Ability Index (WAI) is the most commonly used assessment tool in occupational medicine and is considered the standard instrument for assessing work ability [9].

In addition to medical determinants, numerous studies indicate that working conditions, particularly shift and night work, may influence blood pressure regulation, glucose metabolism, and overall cardiovascular risk [10–13]. Long-term disruption of the circadian rhythm has been associated with an increased risk of HTN, DM type 2, and metabolic disturbances, including metabolic syndrome [13–15].

Psychosocial risks at work, such as high work intensity, chronic stress, emotional exhaustion, and insufficient social support, may adversely affect employee health [16, 17]. According to the Karasek demand-control model, the combination of high job demands and low control over work defines "job strain", which has been associated with an increased risk of cardiovascular diseases [17, 18].

The Siegrist effort-reward imbalance model emphasizes that stress arises when an employee perceives an imbalance between invested effort and received reward [19].

Social and occupational conditions, including job insecurity, are recognized determinants of employee health [20–22]. The contemporary biopsychosocial approach in occupational medicine emphasizes that work ability depends not only on medical status but also on psychological and social factors of the work environment [8, 20].

Despite the significance of chronic cardiometabolic diseases in the working-age population, studies specifically examining the interaction between psychosocial work factors, job insecurity, chronic disease, and work ability remain limited [20–22].

Furthermore, previous studies investigating work-related psychosocial risks have often lacked an adequate comparison group of healthy employees from the same work environment, making it difficult to distinguish disease-specific effects from those affecting employees more generally.

Therefore, the aim of this study was to assess the work ability of employees with HTN and DM and to examine the impact of working conditions and psychosocial factors on work ability. A particular strength of this study lies in the inclusion of a control group of employees without chronic diseases from the same work environment, enabling differentiation of the effects of psychosocial and organizational factors on work ability according to participants' health status.

Methods

Study design and participants

An analytical cross-sectional study was conducted among 100 employed individuals diagnosed with HTN and/or DM (cases group) in two public sector companies in Niš, Serbia. Concurrently, a control group comprising 100 employees without HTN and/or DM was recruited from the same two companies. Controls were frequency-matched to the cases group by sex (72% men, 28% women), age group (26–35, 36–45, 46–55, and 56–65 years), company (50 *per* company), and shift/night work schedule. Participants in both groups were recruited from the same two public-sector companies and represented comparable occupational categories, reducing the potential influence of workplace-related differences. Data were collected during regular periodic examinations at the Institute of Occupational Health Niš.

The study was approved by the Ethics Committee of the Institute of Occupational Health Niš (No. 2080, from August 29, 2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent. Data were processed anonymously and confidentially.

Inclusion criteria for the cases group were as follows: diagnosed HTN and/or DM confirmed by medical documentation, active employment status, work tenure of at least 1 year, and voluntary written informed consent. Exclusion criteria were the following: severe acute illness, psychiatric disorders preventing questionnaire completion, and refusal to participate.

Inclusion criteria for the control group were: employment in one of the two public sector companies, work tenure of at least 1 year, age 18–65 years, absence of a diagnosis of HTN and/or DM confirmed by medical documentation, no use of antihypertensive or antidiabetic medication, and written informed consent. Exclusion criteria for the control group were: newly detected HTN and/or DM or borderline values (blood pressure > 130/85 mmHg or fasting glycemia > 6.1 mmol/L) during the periodic examination, other severe chronic non-communicable diseases (malignant disease, severe cardiovascular disease, chronic kidney disease, chronic pulmonary disease Global Initiative for Chronic Obstructive Lung Disease – GOLD stage II or higher), psychiatric disorders, pregnancy, and refusal to participate.

Participants were recruited using convenience sampling during regular periodic examinations.

Instruments

Work ability was assessed using the WAI [23], which encompasses seven dimensions: current work ability compared with lifetime best (0–10); work ability in relation to job demands (2–10); number of current diseases diagnosed by a physician (1–7); estimated work impairment due to diseases (1–6); sick leave taken during the past 12 months (1–5); self-prognosis of work ability over the next two years (1–7); and mental resources (1–4). The total WAI score ranges from 7 to 49, categorized as: poor (7–27), moderate (28–36), good (37–43), and excellent (44–49).

In this study, WAI was applied in the Serbian version. Cultural adaptation was performed using a standardized process that included forward translation, reconciliation of translated versions, independent back-translation, comparison with the original questionnaire, cognitive interviewing on a pilot sample of 20 employees to assess comprehensibility, and final linguistic review. Psychometric properties of the Serbian version were examined in the pooled sample (n = 200), including internal consistency (Cronbach $\alpha = 0.82$), test-retest reliability on a subsample of 30 participants over a 2-week interval (intraclass correlation coefficient – ICC = 0.89), and convergent validity with self-rated health and number of diagnoses. The obtained findings indicated satisfactory psychometric performance of the instrument in this study sample.

The level of perceived stress was assessed using the 10-item Perceived Stress Scale (PSS-10) [24], which contains 10 items rated on a 5-point Likert scale (0–4). The total score ranges from 0 to 40, with categories of low (0–13), moderate (14–26), and high perceived stress (27–40).

The Serbian version of the PSS-10, previously validated in Serbian clinical and non-clinical samples [25], was applied in this study. In our sample, the Serbian version of the PSS-10 demonstrated satisfactory internal consistency (Cronbach $\alpha = 0.79$), with psychometric characteristics consistent with those reported in the Serbian validation study [25].

For non-validated self-report instruments used in this study, cultural adaptation procedures were conducted according to the standardized methodology for cross-cultural adaptation proposed by Beaton et al. [26], including forward and back translation, expert review, pilot testing, and linguistic and psychometric evaluation.

Psychosocial factors of the work environment were assessed using 10 items rated on a 1–5 scale, based on the demand-control model [27, 28], including job demands (work pace, intensity, sufficient time), control over work/autonomy (influence on work method and pace), social support (from colleagues and supervisors), fear of job loss, exhaustion after work, and job satisfaction. Items were derived from the Job Content Questionnaire (JCQ) [29] and culturally adapted for use in this study through forward/back translation and cognitive interviewing. In the pooled sample, Cronbach α coefficients for the subscales ranged from 0.71 to 0.83, indicating satisfactory internal consistency.

Statistical analysis

In this way, linguistic, semantic, and conceptual equivalence of the instruments was enhanced for use in the study population. However, except for the previously validated PSS-10, the applied instruments underwent only basic psychometric assessment and cultural adaptation, not full formal validation for the Serbian population. Therefore, the findings should be interpreted in light of these methodological considerations.

Associations between continuous variables were examined using Pearson's correlation coefficient, separately for the case and control groups. Differences in categorical variables were analyzed using the chi-square test or Fisher's exact test, where appropriate.

Multiple linear regression analysis was performed to identify independent predictors of the WAI. Logistic regression analysis was conducted with impaired work ability (WAI ≤ 27) as the dependent variable and diagnosis of HTN and/or DM, shift work, night work, fear of job loss, autonomy, sex, and age as independent predictors. Fear of job loss and autonomy were entered as dichotomized variables based on predefined cut-off values. Although frequency matching by age category was performed, the patients in the cases group remained significantly older, reflecting the naturally higher prevalence of cardiometabolic diseases among older employees. Therefore, age was additionally included as a covariate in regression analyses. Due to complete separation of the outcome in the control group (0 controls with WAI ≤ 27), the Haldane-Anscombe correction (+0.5) was applied when estimating odds ratios (ORs).

Statistical significance was set at $p < 0.05$. Effect size for between-group comparisons was calculated using Cohen *d*, with 0.2, 0.5, and 0.8 values interpreted as small, medium, and large effects, respectively. To control for multiple comparisons in correlation analyses, the Benjamini-Hochberg procedure (false discovery rate – FDR < 0.05) was applied. Prior to interpretation of the regression models, assumptions of linear regression were evaluated, including normality, linearity, homoscedasticity, and lack of problematic multicollinearity.

Multicollinearity was assessed using variance inflation factors (VIF) and tolerance values. All VIF values were below 6, and tolerance values exceeded 0.10, indicating no problematic multicollinearity among the predictors included in the regression model.

Results

The study included a total of 200 participants. Among them were 100 employees with HTN and/or DM (cases group) and 100 healthy controls (control group). The two groups were frequency-matched according to sex, age category, company, and shift/night work schedule. Despite frequency matching, participants with HTN and/or DM were significantly older than controls ($p < 0.001$), whereas work tenure did not differ significantly between the groups (Table 1).

Employees with HTN and/or DM had significantly lower work ability and significantly higher perceived stress than controls (Table 2). Impaired work ability (WAI ≤ 27) was observed exclusively in the cases group (44%), whereas none of the controls had impaired work ability ($p < 0.001$).

Compared with controls, employees with HTN and/or DM reported significantly higher job autonomy, lower

Table 1

Basic demographic and occupational characteristics of participants

Variable	Cases (n = 100)	Controls (n = 100)	Test	*p*-value
Age, years	48.42 ± 8.59	43.39 ± 9.04	$t = 4.03$	< 0.001
Work tenure, years	21.80 ± 6.49	20.26 ± 9.53	$t = 1.34$	0.184
Gender				
male	72 (72)	72 (72)	$\chi^2 = 0.00$	1.000
Secondary education	80 (80)	84 (84)	$\chi^2 = 0.31$	0.581
Higher education	20 (20)	16 (16)	–	–
Shift work	74 (74)	74 (74)	$\chi^2 = 0.00$	1.000
Night work	66 (66)	65 (65)	$\chi^2 = 0.00$	1.000
Sick leave, 12 months	35 (35)	18 (18)	$\chi^2 = 6.57$	0.010
Arterial hypertension	40 (40)	0 (0)	–	–
Diabetes mellitus	30 (30)	0 (0)	–	–
Arterial hypertension + diabetes mellitus	30 (30)	0 (0)	–	–
Company 1/Company 2	50/50	50/50	–	–

n – number.

All values are given as numbers (percentages) or mean ± standard deviation.

Table 2

Work Ability Index (WAI) and perceived stress values

Parameter	Cases		Controls	
	mean ± SD	min–max	mean ± SD	min–max
WAI1: current work ability	7.18 ± 1.42	4–10	9.37 ± 0.76	7–10
WAI2: job demands	6.64 ± 1.54	4–9	8.56 ± 0.87	6–10
WAI3: number of diseases	3.26 ± 1.06	1–5	6.63 ± 0.64	5–7
WAI4: impairment due to diseases	2.80 ± 0.75	1–4	5.76 ± 0.50	4–6
WAI5: sick leave	2.22 ± 0.64	1–3	4.02 ± 0.80	2–5
WAI6: prognosis	3.76 ± 1.58	1–7	6.47 ± 0.84	4–7
WAI7: mental resources	2.50 ± 0.50	2–3	3.59 ± 0.55	2–4
WAI total	28.34 ± 3.18	23–37	41.76 ± 2.27	36–48
PSS-10 total	23.18 ± 1.90	20–26	20.01 ± 3.33	12–31

PSS-10 – 10-item Perceived Stress Scale; SD – standard deviation; min – minimum; max – maximum.

supervisor support, greater fear of job loss, and higher levels of exhaustion after work, whereas job satisfaction did not differ significantly between the groups. Within the cases group, fear of job loss and exhaustion after work were the most prominent psychosocial characteristics, whereas supervisor support showed the lowest ratings. In the control group, fear of job loss and exhaustion were reported less often, while overall job satisfaction and autonomy were comparable between the groups (Table 3).

No statistically significant difference in work ability was observed among participants with HTN alone, DM alone, and comorbidity, although employees with HTN tended to have the lowest WAI scores. Among employees with HTN and/or DM, shift work and night work were associated with significantly higher WAI scores (Table 4), whereas no significant associations were observed in the control group (data not shown).

Correlation analysis demonstrated that work ability in employees with HTN and/or DM was positively associated with job autonomy and supervisor support, whereas no significant associations between psychosocial factors and WAI were observed in the control group (Table 5).

Table 3

Comparative analysis of main outcomes and psychosocial factors

Variable	Cases	Controls	*t*-test	*p*-value	Cohen *d*
WAI total	28.34 ± 3.18	41.76 ± 2.27	-34.32	< 0.001	4.85
PSS-10 total	23.18 ± 1.90	20.01 ± 3.33	8.28	< 0.001	1.17
Autonomy (KP4)	3.28 ± 1.05	2.84 ± 1.08	2.92	0.004	0.41
Supervisor support (SP7)	2.50 ± 0.88	2.87 ± 1.01	-2.76	0.006	0.39
Fear of job loss (SZ8)	4.28 ± 0.70	3.03 ± 1.03	10.04	< 0.001	1.42
Exhaustion after work (ST9)	4.24 ± 0.77	2.77 ± 0.99	11.70	< 0.001	1.65
Job satisfaction (ST10)	2.88 ± 0.79	2.89 ± 1.09	-0.07	0.941	-0.01
Sick leave, 12 months	35 (35)	18 (18)	$\chi^2 = 6.57$	0.010	–
Impaired WAI, ≤ 27	44 (44)	0	$\chi^2 = 53.88$	< 0.001	–

WAI – Work Ability Index; PSS-10 – 10-item Perceived Stress Scale; KP4 – influence over work methods question 4 (job autonomy); SP7 – supervisor support question 7; SZ8 – fear of job loss question 8; ST9 – exhaustion after work question 9; ST10 – job satisfaction question 10.
All values are given as mean ± standard deviation or numbers (percentages).

Table 4

Work ability according to shift and night work schedule (cases group)

Group	WAI	*t*-test	*p*-value	Cohen *d*
Shift work (n = 74)	28.78 ± 3.25	2.41	0.018	0.58
Non-shift work (n = 26)	27.08 ± 2.61			
Night work (n = 66)	29.00 ± 3.19	3.01	0.003	0.65
Day work only (n = 34)	27.06 ± 2.77			

WAI – Work Ability Index; n – number.
All values are given as mean ± standard deviation.

Table 5

Pearson correlations of psychosocial factors with WAI

Factor	Cases		Controls	
	r	*p*	*r*	*p*
Autonomy (KP4)	0.549	< 0.001	-0.028	0.781
Supervisor support (SP7)	0.516	< 0.001	0.179	0.074
Job satisfaction (ST10)	0.376	< 0.001	-0.100	0.321
Fear of job loss (SZ8)	-0.317	0.001	0.115	0.254
Colleague support (SP6)	-0.217	0.030	-0.040	0.689
Sufficient time (ZP3)	0.195	0.052	0.021	0.837
Exhaustion (ST9)	-0.167	0.098	0.118	0.241
Strenuous work (ZP2)	-0.166	0.100	0.124	0.219
PSS-10 total	-0.054	0.596	-0.024	0.815
Age	-0.013	0.900	-0.045	0.660

WAI – Work Ability Index; KP4 – influence over work methods question 4 (job autonomy); SP7 – supervisor support question 7; ST10 – job satisfaction question 10; SZ8 – fear of job loss question 8; SP6 – coworker support question 6; ZP3 – sufficient time to complete work question 3; ST9 – exhaustion after work question 9; ZP2 – strenuous work question 2; PSS-10 – 10-item Perceived Stress Scale.
***Note*: Correction for multiple comparisons was performed using the Benjamini-Hochberg procedure [false discovery rate (FDR) < 0.05]. Only correlations surviving FDR correction were considered statistically significant.**

Table 6

Multiple linear regression analysis – predictors of WAI (cases group)

Predictor	B	SE	Beta	*t*-test	*p*-value
Autonomy (KP4)	1.565	0.413	0.515	3.790	< 0.001
Satisfaction (ST10)	1.763	0.497	0.441	3.550	< 0.001
Night work	2.924	1.085	0.438	2.694	0.009
Shift work	-3.029	1.211	-0.420	-2.501	0.014
Exhaustion (ST9)	0.972	0.465	0.235	2.091	0.040
Fear (SZ8)	-1.053	0.491	-0.231	-2.146	0.035
Colleague support (SP6)	1.049	0.441	0.228	2.377	0.020
Supervisor support (SP7)	0.526	0.409	0.146	1.287	0.202
Strenuous work (ZP2)	-0.850	0.585	-0.169	-1.452	0.150
PSS-10	0.148	0.159	0.089	0.934	0.353
Age	-0.075	0.040	-0.204	-1.890	0.062

WAI – Work Ability Index; SE – standard error; KP4 – influence over work methods question 4 (job autonomy); ST10 – job satisfaction question 10; ST9 – exhaustion after work question 9; SZ8 – fear of job loss question 8; SP6 – coworker support question 6; SP7 – supervisor support question 7; ZP2 – strenuous work question 2; PSS-10 – 10-item Perceived Stress Scale.
***Note*: R^2 = 0.565; adjusted R^2 = 0.505; F (11,88) = 9.43; p < 0.001.**

Table 7

Logistic regression – predictors of impaired work ability (WAI ≤ 27) in the pooled sample

Predictor	OR	95% CI	*p*-value
Group, cases vs. controls	156.36*	9.44–2,588.59	< 0.001
Shift work, yes	1.12	0.56–2.22	0.746
Night work, yes	0.98	0.49–1.96	0.953
Fear of job loss, SZ8 ≥ 4	3.62	1.45–9.04	0.006
Low autonomy, KP4 < 3	2.74	1.18–6.36	0.019
Gender, male	1.32	0.67–2.58	0.420
Age *per* year	1.03	0.99–1.08	0.124

WAI – Work Ability Index; OR – odds ratio; CI – confidence interval; SZ8 – fear of job loss question 8; KP4 – influence over work methods question 4 (job autonomy).
***Note*: *Haldane-Anscombe correction (+0.5) applied due to the empty cell (0 controls with WAI ≤ 27). Other OR values were calculated directly from the logistic model.**

Multiple linear regression identified job autonomy, job satisfaction, and night work as significant independent predictors of work ability among employees with HTN and/or DM. The model explained 56.5% of the variance in WAI and was statistically significant (Table 6), whereas the corresponding model in the control group was not statistically significant (data not shown).

Logistic regression performed on the pooled sample identified HTN and/or DM diagnosis as the strongest independent predictor of impaired work ability. Pronounced fear of job loss and low job autonomy were also independently associated with impaired WAI, whereas shift work, night work, sex, and age were not significant predictors (Table 7).

Sick leave during the previous 12 months was more frequent among employees with HTN and/or DM than among controls. Although participants with comorbid HTN and/or DM had the highest frequency of sick leave, differences between diagnostic subgroups were not statistically significant. Disability commission referral was recorded only among employees with HTN and/or DM.

Discussion

The central finding of this study is the important role of organizational and psychosocial work-related factors in shaping work ability among employees with HTN and DM. By including a control group of employees without HTN and DM from the same companies, with comparable proportions of shift and night work and matched sex and age categories, this study enabled a clearer differentiation between the effects of chronic cardiometabolic diseases themselves and the effects of psychosocial and organizational workplace conditions. In the cases group, job autonomy and supervisor support demonstrated the strongest positive associations with WAI, whereas these associations did not reach statistical significance in the control group. These findings are consistent with the Karasek demand-control model, according to which greater job control may have a protective role in employee health and functioning [27, 28]. The psychosocial dimensions assessed in this study were derived from the JCQ [29]. Together, these findings suggest that employees with chronic cardiometabolic diseases may be more sensitive to

psychosocial workplace conditions than their healthy colleagues.

An important methodological consideration relates to the structure of the WAI itself, which incorporates components directly associated with health status, including physician-diagnosed diseases and functional limitations. Consequently, part of the observed difference between cases and controls may reflect instrument characteristics rather than actual differences in functional work ability.

The mean WAI in the cases group (28.34) corresponded to moderate work ability, whereas the control group mean (41.76) fell within the good work ability category. The difference of approximately 13 points was substantial and statistically significant. Previous research has shown that chronic health conditions and both individual and work-related factors are important determinants of work ability [30, 31]. The magnitude of the difference identified in our study may partly reflect differences in occupational context, disease burden, lifestyle habits, and work organization. Nevertheless, the findings support the view that chronic cardiometabolic diseases substantially affect employees' functional work ability.

Multiple regression analysis explained 56.5% of the variance in WAI in the cases group, indicating a relatively strong contribution of psychosocial and organizational factors to work ability among employees with HTN and/or DM. By contrast, the same model applied to the control group explained only a small proportion of variance and was not statistically significant. In the cases group, job autonomy, job satisfaction, and night work emerged as the strongest independent predictors of WAI. Similarly, logistic regression analysis showed that, independent of disease status, pronounced fear of job loss and low autonomy were associated with impaired work ability. Taken together, these findings indicate that psychosocial workplace factors may not affect all employees equally, but rather exert a more pronounced influence among workers with chronic cardiometabolic diseases.

Pronounced fear of job loss, registered in 86% of cases and 32% of controls, represents an important finding of this study. Previous research suggests that subjective job insecurity is an important psychosocial stressor associated with adverse mental and physical health outcomes [32, 33]. The elevated prevalence of fear of job loss even in the control group may reflect broader labor-market conditions. However, its negative association with WAI was observed only among employees with HTN and/or DM, suggesting that workers with chronic disease may be particularly vulnerable to the detrimental effects of occupational insecurity.

An important finding of this study is that employees with HTN and/or DM working night shifts demonstrated significantly higher WAI compared with those working only day shifts, whereas no such difference was observed in the control group. This finding may be explained by the healthy worker effect, according to which workers with better health status and preserved functional capacity are more likely to remain in more demanding jobs and shift schedules [34]. The absence of a similar gradient in the control group further supports the interpretation that this selection effect may be more evident among employees with chronic disease.

No statistically significant association was observed between the total PSS-10 score and work ability in either group, despite substantially higher perceived stress among cases. One possible explanation may relate to the relatively low variability of stress values within the cases group, whereas in the control group the generally high WAI values may have limited the observable association, potentially reflecting a ceiling effect of good work ability [35]. In this context, specific psychosocial workplace characteristics may represent more sensitive indicators of work-related functioning than general measures of perceived stress.

Participants with isolated HTN demonstrated somewhat lower WAI compared with those with DM or comorbidity, although all diagnostic subgroups had considerably lower WAI compared with controls. Given the borderline statistical significance and the lack of detailed data on disease severity and disease control, differences between diagnostic subgroups should be interpreted cautiously. Cardiovascular disease and DM have also been associated with early exit from paid employment, with work-related factors contributing to this relationship [36].

The findings of this study highlight the importance of organizational and psychosocial dimensions of work ability among employees with chronic diseases. By introducing a control group, it was demonstrated that the difference between diseased and healthy employees is not solely a consequence of medical status, but also reflects the interaction between chronic diseases and psychosocial work conditions. Workplace interventions aimed at strengthening job autonomy, improving supervisor support, and reducing psychosocial risks may contribute to preserving work ability among employees with chronic non-communicable diseases. Previous evidence suggests that organizational interventions combined with workplace health-promotion programs may improve employee work ability [37].

Limitations of the study

Several limitations should be acknowledged. First, the cross-sectional design does not allow causal inference regarding the observed associations. Second, frequency matching rather than individual matching resulted in a residual age difference between groups, although age was statistically controlled in regression analyses. Third, all participants were recruited from public sector companies, which may limit the generalizability of findings to other occupational settings. Another limitation is that the study did not include detailed clinical indicators of disease severity or quality of disease control, such as blood pressure values, glycosylated haemoglobin levels, disease duration, treatment adherence, or the presence of chronic complications. Consequently, the observed associations between HTN and/or DM and work ability should be interpreted at the level of disease presence rather than according to the severity or

level of clinical control of these conditions. Another limitation concerns the psychometric properties of some instruments used in this study. Although cultural adaptation procedures and basic psychometric indicators were assessed, full formal validation of all instruments for the Serbian working population has not yet been established. Therefore, the findings should be interpreted in light of these methodological considerations.

The very wide confidence interval for the OR of HTN and/or DM diagnosis in logistic regression reflects statistical imprecision caused by complete separation of the outcome (0 controls with WAI $\leq$ 27). Although the obtained OR was high, this result should not be interpreted as indicating severe clinical disease, since the majority of participants had medically controlled HTN and/or DM and remained occupationally active. The inflated estimate primarily reflects statistical separation rather than disease severity.

Future studies should employ longitudinal designs and include larger and more heterogeneous occupational samples to provide additional insight into the long-term impact of psychosocial and organizational factors on work ability among individuals with chronic diseases.

The inclusion of a control group represents an important methodological strength of this study, allowing a more precise understanding of the interaction between health status and psychosocial workplace factors.

Conclusion

Work ability of employees with arterial hypertension and diabetes mellitus is significantly lower than in employees without these chronic diseases and is determined by organizational and psychosocial factors of the work environment. Work autonomy, supervisor support, and job satisfaction represent key protective factors, while job insecurity has a negative effect—and one that is specifically more pronounced in diseased than in healthy employees. The obtained results indicate the need for improving work organization and reducing psychosocial risks in the work environment, with targeted monitoring of employees with chronic non-communicable diseases. An integrated approach encompassing medical supervision, health promotion, and organizational changes in the workplace can contribute to extending the working life and reducing the risk of losing work ability in the working-age population.

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CASE REPORT
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Catheter ablation of left ventricular outflow tract premature ventricular complexes in a patient with mechanical aortic and mitral valves

Kateterska ablacija komorskih ekstrasistola iz izlaznog trakta leve komore kod bolesnika sa mehaničkim aortnim i mitralnim zaliscima

Mihailo Vukmirović, Blagoje Babić

Clinical Center of Montenegro, Cardiology Department, Podgorica, Montenegro;
University of Montenegro, Medical Faculty, Podgorica, Montenegro

Abstract

Background. Catheter ablation of premature ventricular complexes (PVCs) originating from the left ventricular outflow tract (LVOT) is highly challenging in patients with dual prosthetic mechanical cardiac valves due to restricted ventricular access and the high risk of valvular damage. We report a case of successful catheter ablation of symptomatic, frequent PVCs from LVOT in a patient with mechanical aortic and mitral valves. **Case Report.** A 39-year-old man with previous mechanical aortic and mitral valve replacements presented with symptomatic, frequent PVCs in a bigeminal pattern refractory to medical therapy. Baseline electrocardiography suggested an LVOT origin. Given the double mechanical valves, standard retrograde or transseptal approaches into the left ventricle were precluded. Instead, electroanatomical mapping was safely restricted to the aortic root, superior to the mechanical aortic valve. Intracardiac echocardiography (ICE) was used throughout the procedure for continuous visualization of cardiac structures and precise catheter positioning. Applying radiofrequency energy within the right coronary sinus of Valsalva immediately suppressed the PVCs. Post-procedural ICE ruled out pericardial effusion as well as mechanical valvular interference. At the 12-month clinical follow-up, the patient remained entirely free of PVC recurrence. **Conclusion.** This case demonstrates that catheter ablation of PVCs from LVOT is feasible and safe in patients with mechanical aortic and mitral valves using a supravalvular approach, strictly guided by ICE.

Keywords:
ablation, techniques; arrhythmias, cardiac; echocardiography; heart valve prosthesis; pre-excitation syndromes.

Apstrakt

Uvod. Kateterska ablacija prevremenih komorskih ekstrasistola (*premature ventricular complexes* – PVCs), koje potiču iz izlaznog trakta leve komore (*left ventricular outflow tract* – LVOT), predstavlja veliki izazov kod bolesnika sa dve mehaničke proteze srčanih zalistaka zbog ograničenog pristupa srčanim komorama i visokog rizika od oštećenja zalistaka. Prikazujemo slučaj uspešne kateterske ablacije simptomatskih, učestalih PVCs iz LVOT kod bolesnika sa mehaničkim aortnim i mitralnim zaliscima. **Prikaz bolesnika.** Muškarac star 39 godina, sa prethodno ugrađenim mehaničkim aortnim i mitralnim zaliscima, javio se zbog simptomatskih, učestalih komorskih ekstrasistola po bigeminijskom obrascu, rezistentnih na medikamentnu terapiju. Početni elektrokardiogram ukazivao je na poreklo iz LVOT. S obzirom na dva mehanička zaliska, standardni retrogradni ili transseptalni pristupi levoj komori bili su onemogućeni. Umesto toga, elektroanatomsko mapiranje je bezbedno ograničeno na koren aorte, iznad mehaničkog aortnog zaliska. Intrakardijalna ehokardiografija (*intracardiac echocardiography* – ICE) korišćena je tokom cele procedure radi kontinuirane vizualizacije srčanih struktura i preciznog pozicioniranja katetera. Primena radiofrekventne energije unutar desnog koronarnog sinusa Valsalve dovela je do momentalne supresije PVCs. Postproceduralnom ICE isključen je perikardni izliv kao i poremećaji u radu mehaničkih zalistaka. Tokom perioda praćenja od 12 meseci, bolesnik je bio u potpunosti bez recidiva PVCs. **Zaključak.** Ovaj slučaj pokazuje da je kateterska ablacija PVCs iz LVOT izvodljiva i bezbedna kod bolesnika sa mehaničkim aortnim i mitralnim zaliscima korišćenjem supravalvularnog pristupa, strogo vođenog ICE-om.

Ključne reči:
ablacija, tehnike; srce, poremećaji ritma; ehokardiografija; zalisci srca, veštački; preeksitacijski sindromi.

Correspondence to: Mihailo Vukmirović, Clinical Center of Montenegro, Cardiology Department, Kruševac bb, 81 000 Podgorica, Montenegro. E-mail: mihailovukmirovic@t-com.me

Introduction

Premature ventricular complexes (PVCs) are common clinical arrhythmias that can become highly symptomatic or lead to tachycardia-induced cardiomyopathy when frequent. In these scenarios, catheter ablation serves as a highly effective, Class I recommended therapeutic strategy [1, 2].

Catheter ablation of PVCs arising from the left ventricular outflow tract (LVOT) requires precise electrocardiographic interpretation, identification of the earliest intracardiac activation, and close awareness of adjacent anatomical structures. Successful ablation in this region relies heavily on accurate localization of the arrhythmogenic focus and a meticulous mapping strategy to avoid injuring nearby critical structures, particularly the coronary arteries and the valvular apparatus [3].

The presence of mechanical prosthetic valves exponentially increases the complexity of these procedures. A mechanical mitral valve typically precludes standard transseptal access to the left ventricle, while a mechanical aortic valve prevents traditional retrograde transaortic crossing due to the high risks of catheter entrapment, valvular dysfunction, and thromboembolic events. Consequently, data regarding catheter ablation of LVOT-PVCs in patients with mechanical aortic and mitral valves remain extremely limited, though emerging studies suggest that such procedures are feasible with careful planning and advanced imaging modalities [4–6].

We report a case of successful catheter ablation of symptomatic, frequent LVOT-PVCs in a patient with dual mechanical valves—aortic and mitral. The arrhythmogenic focus was successfully mapped and ablated within the right coronary sinus of Valsalva entirely above the mechanical aortic valve, guided by a combination of fluoroscopy, electroanatomical mapping, and intracardiac echocardiography (ICE).

Case report

A 39-year-old man with a history of mechanical aortic and mitral valve replacements was referred for catheter ablation due to symptomatic ventricular ectopy and worsening heart failure despite optimal medical therapy. Baseline 12-lead electrocardiography (ECG) demonstrated sinus rhythm with a high burden of frequent PVCs presenting in a bigeminal pattern. The PVCs morphology was highly suggestive of an LVOT origin (Figure 1).

The procedure was performed under the guidance of fluoroscopy, three-dimensional electroanatomical mapping, and ICE (Figures 2, 3, and 4). Venous access was obtained *via* bilateral femoral vein punctures. A decapolar catheter was advanced into the coronary sinus for reference. Throughout the procedure, ICE was utilized for the direct, continuous visualization of intracardiac structures, monitoring catheter-valve proximity, and ruling out acute complications. Arterial access was achieved *via* the right femoral artery, and a radiofrequency ablation catheter was advanced through a long sheath.

Catheter manipulation was performed carefully in the aortic root region to avoid any interaction with the mechanical aortic valve (Figure 2).

Three-dimensional electroanatomical mapping accurately delineated the aortic root anatomy and localized the site of successful ablation within the right sinus of Valsalva, superior to the mechanical aortic valve (Figure 3).

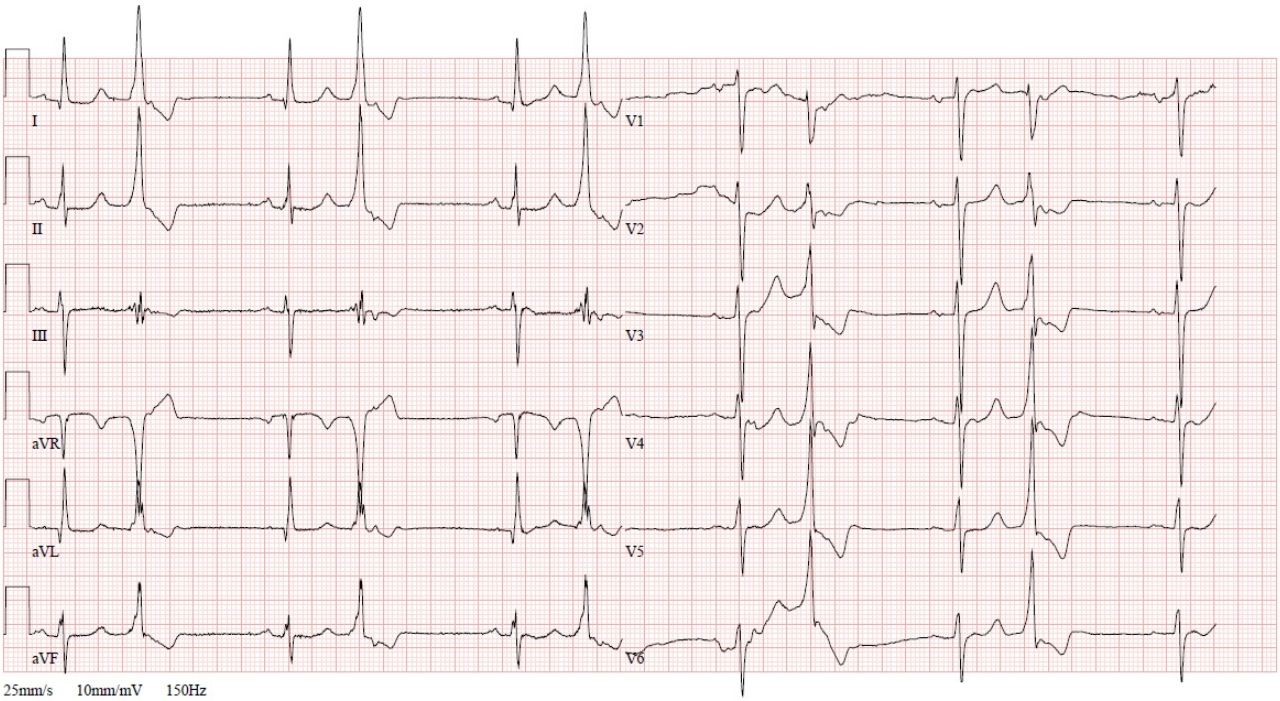

Fig. 1 – Baseline electrocardiogram showing ventricular bigeminy.

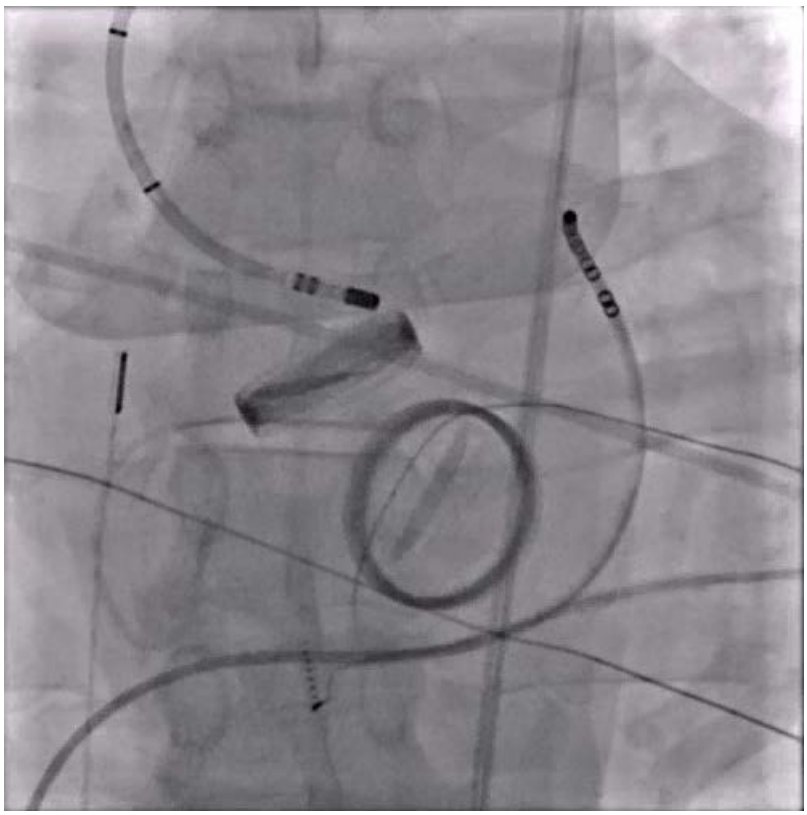

Fig. 2 – Left anterior oblique view of catheter position at the ablation site.

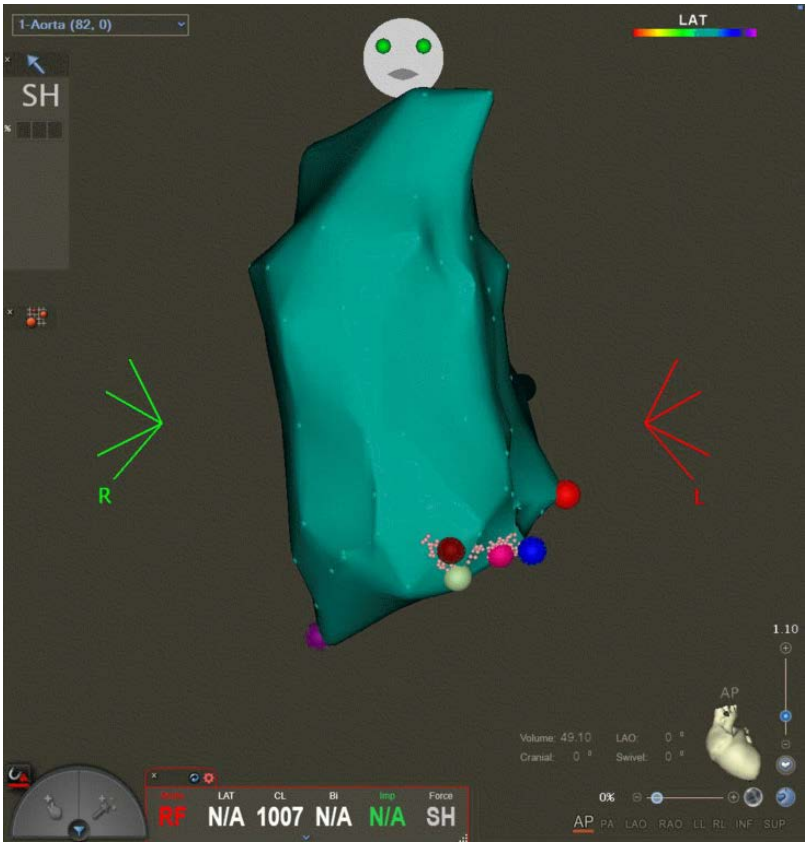

Fig. 3 – Three-dimensional (3D) electroanatomical map of the aortic root. Antero-posterior projection on the 3D mapping system. The dark purple dot represents the right coronary sinus, and the red dot represents the left coronary sinus. The remaining dots indicate mapping sites. Ablation was performed at the location marked by the blue dot.

ICE was utilized throughout the procedure for direct visualization of the mechanical aortic prosthesis and catheter positioning (Figure 4).

Given the dual mechanical valves, electroanatomical and activation mapping of the LVOT region was performed with extreme caution, strictly avoiding any catheter passage across the mechanical aortic valve disc. Mapping was focused within the aortic root. Intracardiac electrograms were recorded at the successful ablation site in the right sinus of Valsalva (Figure 5).

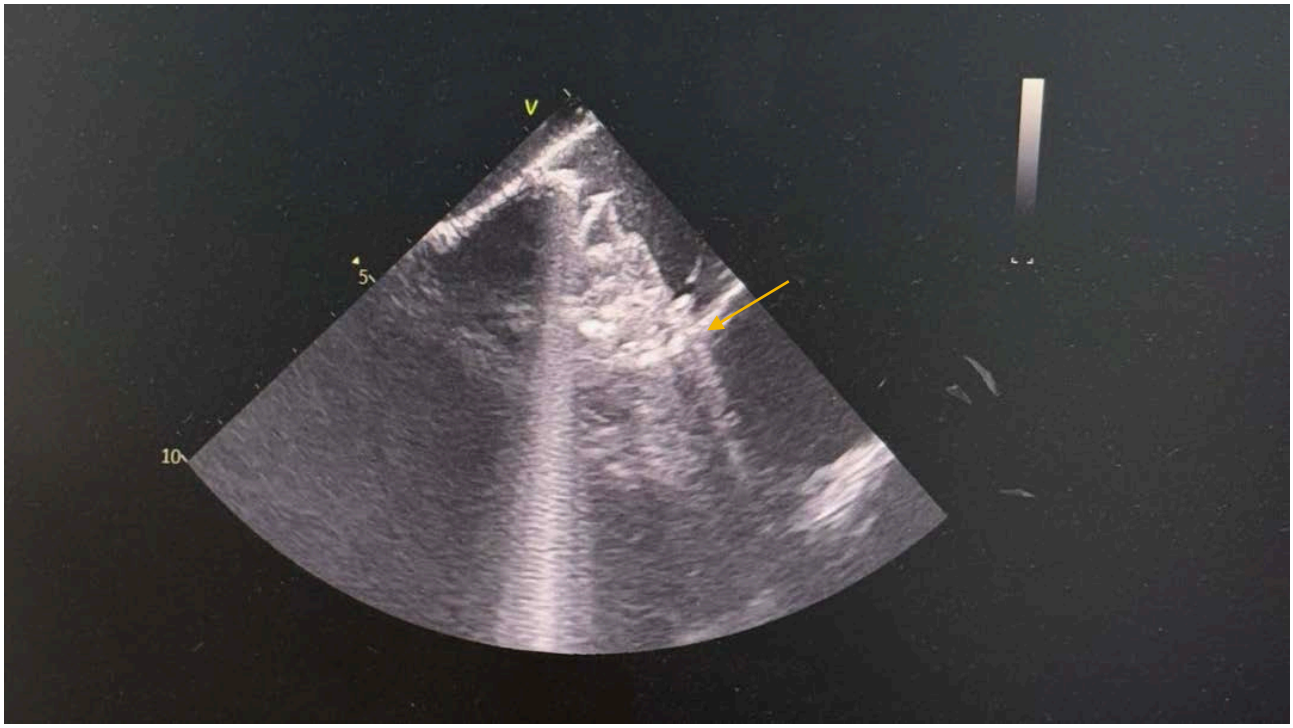

Fig. 4 – Intracardiac echocardiography during the procedure. The yellow arrow indicates the tip of the ablation catheter on intracardiac ultrasound.

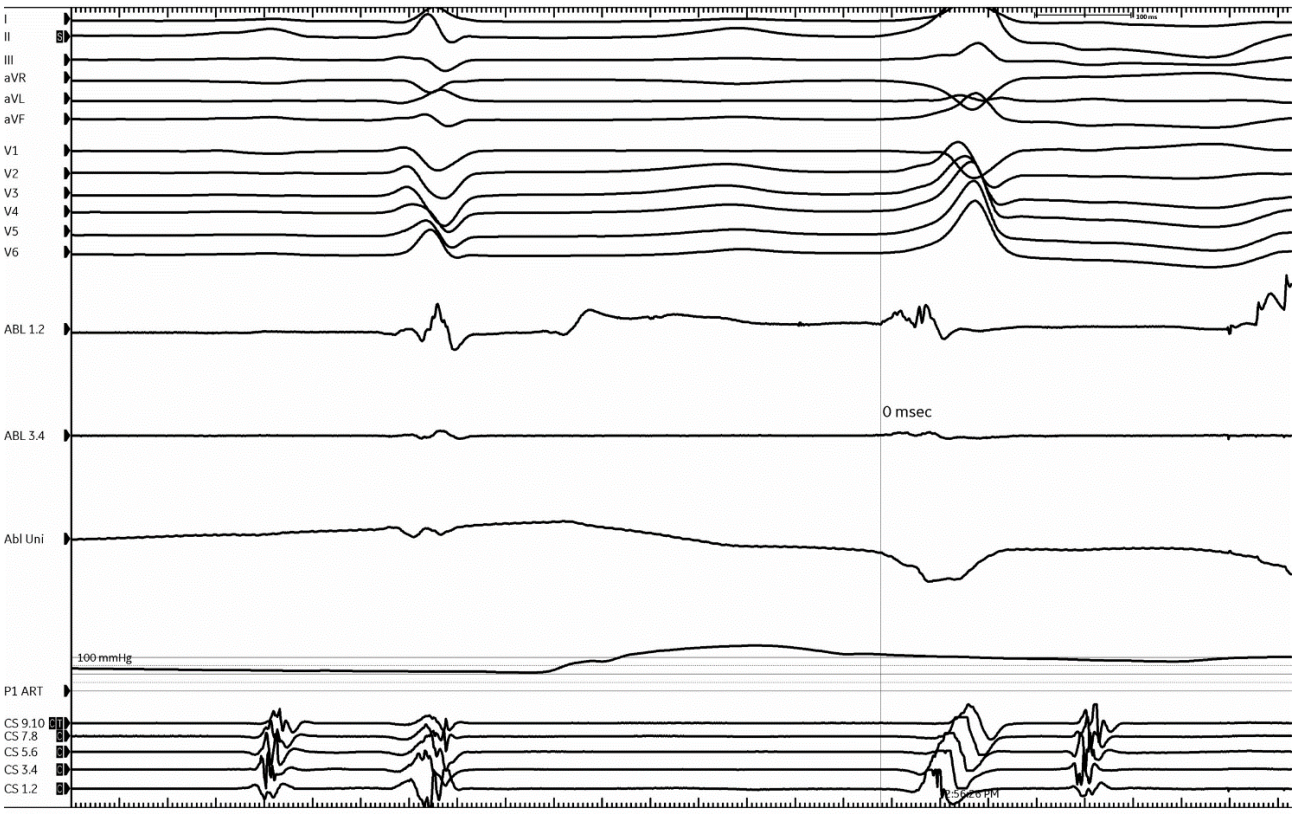

Fig. 5 – Intracardiac electrogram at the successful ablation site.

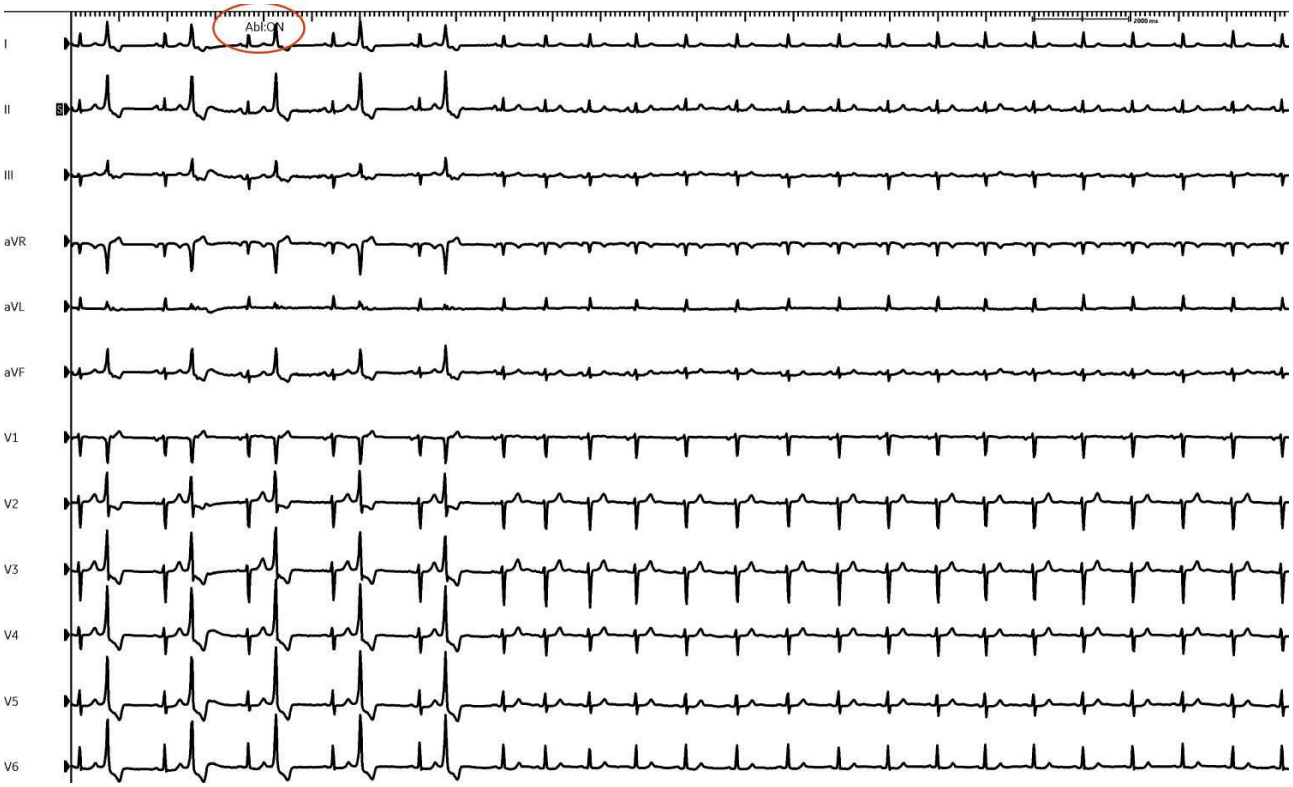

Fig. 6 – Suppression of ventricular ectopy during radiofrequency ablation. The red circle indicates the onset of ablation, i.e., the delivery of radiofrequency energy, immediately followed by the disappearance of premature ventricular complexes.

Although local intracardiac signals showed only modest activation prematurity, radiofrequency energy was delivered at this locus with a maximum power of 30 W. Intraprocedural rhythm tracings demonstrated prompt suppression of PVCs shortly after the initiation of radiofrequency energy delivery (Figure 6). Ventricular ectopy terminated immediately within the first few seconds of application, with a total radiofrequency delivery time of 197 seconds.

Fluoroscopic and electroanatomical imaging confirmed the precise position of the catheter tip within the right coronary sinus, safely clear of the mechanical aortic valve apparatus (Figure 2). Post-procedural ICE confirmed the absence of pericardial effusion or valvular interference. During the 12-month clinical follow-up, the patient remained entirely asymptomatic with no documented recurrence of PVCs.

Discussion

This case highlights the clinical feasibility and safety of catheter ablation for LVOT-PVCs in a patient with mechanical aortic and mitral valve replacements. The primary procedural challenges in this population are the absolute restriction of traditional access routes into the left ventricle and the catastrophic risks associated with catheter-prosthesis interaction.

In patients with standard anatomy, LVOT-PVCs are routinely approached *via* a retrograde transaortic route or a transseptal approach to map the subvalvular LVOT, the aortic cusps, and adjacent structures. Conversely, in patients with a double mechanical valve configuration, the mechanical mitral prosthesis precludes the transseptal approach, and retrograde transaortic crossing is strictly contraindicated because of the high risk of catheter entrapment, valvular disc immobilization, and thromboembolic stroke. Although published data on ventricular arrhythmia ablation with dual mechanical valves remain sparse, our findings align with limited reports showing that these procedures, though technically demanding, are achievable through meticulous strategic planning and advanced imaging modalities [4–6].

Ablation within the aortic root carries inherent risks due to its proximity to the coronary artery ostia and the mechanical valve apparatus. Traditionally, coronary angiography has been mandated to ensure a safe clearance from the coronary ostia during radiofrequency delivery. However, ICE has emerged as a transformative tool, providing high-resolution, real-time visualization of the aortic cusp anatomy, the coronary ostia, and precise catheter-tip stability [7, 8]. Hoffmayer et al. [7] demonstrated that aortic root ablations could be safely performed using a combination of ICE and electroanatomical mapping without routine coronary angiography, provided there is direct visual confirmation of the catheter tip relative to the coronary openings. Similarly, Al Asmar et al. [8] reported that experienced operators could successfully perform mapping and radiofrequency ablation within the sinuses of Valsalva under ICE guidance without supplementary angiography, with no immediate or 30-day adverse events. These data strongly support using ICE as the primary safety modality in anatomically sensitive aortic root procedures.

In our patient, ICE served as the critical safety cornerstone. It provided continuous monitoring of catheter position and stability within the aortic root, ensuring the catheter remained safely clear of the mechanical aortic valve discs. Furthermore, ICE allowed immediate, real-time exclusion of pericardial effusion or mechanical valvular interference after ablation. To further mitigate risks, radiofrequency energy was delivered cautiously using an irrigated-tip catheter at a moderate power of 30 W. The immediate termination of the PVCs during the initial seconds of radiofrequency delivery, the lack of acute recurrence during the intraprocedural waiting period, and the durable elimination of frequent ectopy at the 12-month follow-up collectively validate the safety and efficacy of this supravalvular strategy.

The principal teaching point of this case is that dual mechanical heart valves should not be considered an absolute contraindication for the ablation of LVOT-PVCs. When the arrhythmogenic focus lies supravalvularly within the sinuses of Valsalva, successful ablation can be safely achieved through careful pre-procedural planning, electroanatomical mapping, and rigorous intracardiac echocardiographic guidance.

Conclusion

Catheter ablation of LVOT-PVCs in patients with mechanical aortic and mitral valve replacements is technically demanding but clinically feasible. When the arrhythmogenic focus is situated supravalvularly, electroanatomical mapping within the aortic root enables successful localization and ablation while completely avoiding hazardous catheter passage across the prosthetic valve apparatus. In this complex anatomical setting, integrating intracardiac echocardiography provides critical real-time visualization of the coronary ostia and valve structures, significantly improving procedural safety and efficacy.

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In the originally published version of the article titled **"Analysis of the diagnostic value of fluorescence rapid on-site evaluation compared with bacterial culture in urinary tract infection: a retrospective study"** by Feng Helian, Jianmei Song, Xu Wang, Jiaqi Li, Guoli Li, Xiaoyang Li, published in *Vojnosanitetski Pregled* 2026; 83(8): 487–94. (DOI: https://doi.org/10.2298/VSP260219042H), Guoli Li was not correctly designated as a corresponding author.

This article has been corrected *Online* [1].

With this correction, Guoli Li has been added as a co-corresponding author alongside Xiaoyang Li. The contact details for the new corresponding authors are as follows: Xiaoyang Li, Guoli Li, Capital Medical University, Beijing Chaoyang Hospital, Laboratory Department, 100 041 Beijing, China. E-mail: xiaoyangli4321@gmail.com, liguoli@tmu.edu.cn

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VSP publishes the following categories and types of manuscripts and communications: Editorial, Original Article, Preliminary Report, Short Report, Case Report and Case Series, General (Narrative) Literature Review, Mini-Review, Systematic Literature Review, Meta-Analysis, Systematic Literature Review with Meta-Analysis, Current Topic, In Focus, Article on the History of Medicine/Dentistry/Pharmacy, Letter to the Editor, Research Letter, Clinical Research, Congress and Scientific Meeting Report, Book Review, In Memoriam, and other contributions.

ORIGINAL ARTICLE

An Original Article presents new and significant findings in a specific field, with a detailed description of the research methods used, the results obtained, and the conclusions drawn. The reference list should include the most recent and most relevant references in the field.

PRELIMINARY REPORT

A Preliminary Report presents research that has not yet been completed, with findings that require further investigation and validation before final conclusions can be drawn, but where the obtained information is of interest to the scientific and professional community. It contains all sections of an Original Article, but in a substantially abbreviated form. Authors are encouraged to subsequently publish a full Original Article with complete, validated data and a comprehensive analysis.

SHORT REPORT

A Short Report presents a completed research study that is small in scope, narrowly focused, and has clear conclusions based on the presented results. It includes all sections of an Original Article, but in a substantially abbreviated form. It is considered the final publication of that specific, limited study and cannot be republished as a full-length article (although follow-up research building on it is encouraged).

REVIEW ARTICLES

GENERAL (NARRATIVE) LITERATURE REVIEW

A General (Narrative) Literature Review provides a review, critical analysis, and synthesis of existing scientific knowledge on a selected topic. Authors cover all available relevant literature over a defined time period, present the results of relevant studies, identify gaps, limitations, or controversies, and indicate directions for future research, offering their own perspective on the issue in the form of concluding remarks.

Authors of this category of article should have published at least five papers in peer-reviewed journals (M20) in the field of the review topic.

MINI-REVIEW ARTICLE

A Mini-Review provides a concise overview of the existing literature and the most recent advances within defined aspects of a particular research field, as well as its new and/or current directions of development.

SYSTEMATIC LITERATURE REVIEW

A Systematic Literature Review synthesizes previously published studies on a specific topic using clearly defined and pre-established methodological procedures for study selection and evaluation. The author must use relevant databases, define inclusion and exclusion criteria, and apply a transparent methodology.

META-ANALYSIS

A Meta-Analysis uses statistical methods to combine quantitative data from multiple primary studies in order to identify overall trends and assess the strength of evidence on a specific topic. Authors must use relevant databases, define inclusion and exclusion criteria, and apply a transparent and reproducible methodology. The research question must be clearly defined using the PICOS framework, and selection guidelines and a study flow diagram (PRISMA) must be provided.

SYSTEMATIC LITERATURE REVIEW WITH META-ANALYSIS

A Systematic Literature Review with Meta-Analysis combines qualitative and quantitative synthesis, using statistical techniques to summarize quantitative results and qualitative synthesis for descriptive/narrative findings. Authors must use relevant databases, clearly define inclusion and exclusion criteria, and apply a transparent and reproducible methodology. The research question must be clearly defined according to the PICOS framework, with specification of the reporting guidelines used (e.g., PRISMA) and inclusion of a PRISMA flow diagram showing study selection.

CURRENT TOPIC

A Current Topic addresses a contemporary, unresolved, or controversial issue of theoretical and practical importance, presenting the authors' own research results or the most recent important data from the literature. The structure of the article is flexible, and brief concluding remarks with a clear message are encouraged.

IN FOCUS

An In Focus article provides a thematic, focused analysis or a brief overview of a scientific issue within the journal's scope, addressing a topic of significance for the scientific community and broader professional audience.

CASE REPORTS

CASE REPORT and CASE SERIES (≥4, ≤9)

Case reports or case series present cases with rare or unusual diagnoses, diagnostic processes, treatment strategies, clinical courses, or treatment outcomes that may be useful for clinical practice and medical education. The CARE guidelines should be followed when preparing the manuscript (https://www.care-statement.org/writing-a-case-report). Written informed consent from the patient is mandatory.

EDITORIAL

Editorials are non–peer-reviewed texts written by the Editor-in-Chief and/or members of the Editorial Board, intended to announce a new volume, special issues, or content of significance for the profession and/or institutions served by the journal, as well as invited editorial texts. Editorials should not contain unpublished or original data, and must include a statement of conflict of interest.

LETTER TO THE EDITOR

A non–peer-reviewed comment or critique of a paper published in VSP. It is written in a free format, with optional citation of relevant literature, and must not contain unpublished results. It is published at the discretion of the Editor-in-Chief.

RESEARCH LETTER

A Research Letter is a short report of original research, containing Introduction, Methods, Results, and Discussion in a condensed form (without separate sections or subheadings) and up to 2 supplementary items (tables/figures). It does not include an abstract or keywords, but must meet all general manuscript requirements for consideration, including the peer-review process.

HISTORY OF MEDICINE/STOMATOLOGY/PHARMACY

Manuscripts presenting material relevant to elucidating specific events and/or portraying notable figures in the history of medicine/stomatology/pharmacy, with particular emphasis on military medicine/stomatology/pharmacy.

CLINICAL RESEARCH

Clinical Research includes original randomized controlled trials and observational studies assessing the impact of one or more interventions or measures on human health outcomes, clinical practice, or health policy.

Manuscripts must be prepared in accordance with international guidelines (e.g., CONSORT – https://www.consort-statement.org/ or STROBE – https://www.strobe-statement.org/) and be registered in a recognized public registry (e.g., ClinicalTrials.gov).

BOOK REVIEW

A Book Review includes bibliographic details of the publication (authors, original title, publisher, place, and year of publication), a brief summary, and critical comments on the content, style, and significance of the book in the relevant field. The manuscript must not exceed 2 pages.

SCIENTIFIC MEETING REPORT

A Scientific Meeting Report presents the activities of a scientific or professional meeting, highlighting the most important presentations, conclusions, or recommendations relevant to the wider readership of VSP.

MANUSCRIPT LENGTH

A complete manuscript consists of: title page, abstracts in Serbian and English with keywords, main text, acknowledgements (if applicable), reference list, and supplementary material (tables, figures, charts, diagrams, drawings).

For Original Article, General (Narrative) Literature Review, Systematic Literature Review, Meta-Analysis, and Systematic Literature Review with Meta-Analysis, the manuscript length may not exceed 5,000 words.

For Mini-Review, Preliminary Report, Short Report, Case Report, Case Series, Current Topic, Clinical Research, and History of Medicine/Stomatology/Pharmacy, the manuscript length may not exceed 3,000 words.

Manuscripts in other categories/sections may have a maximum of 1,500 words.

MANUSCRIPT PREPARATION

TITLE PAGE

The first page of the manuscript should include the following:

1. Title of the manuscript without abbreviations;
2. Full names of all authors (without academic titles, but with ORCID numbers included for those who have them) with symbols assigned in the following order: ***, †, ‡, §, ‖, ¶, **, ††**… etc.;
3. Full official names of the institutions where the authors work, including city and country of the institution (the symbols *, †, ‡, §, ‖, ¶, **, †† … etc. correspond to the institutions of each author);
4. At the bottom of the page, provide the name and surname, postal address, email address, and phone number (mobile/Viber or WhatsApp) of the author responsible for correspondence.

ABSTRACT

The abstract and keywords should be provided on the second page of the manuscript. The abstract should be written in short and clear sentences. For the

categories Original Article, Preliminary Report, Short Report, Systematic Literature Review, Systematic Literature Review with Meta-Analysis, Meta-Analysis, and Clinical Research, the abstract must be structured and include the following sections: Introduction/Aim, Methods, Results, Conclusion. Each section should be written as a separate paragraph beginning with a bolded heading. The most important results should be presented, including numerical values and the level of statistical significance. The conclusion must be directly related to the study results. The abstract must not exceed 300 words.

For the categories Case Report and Case Series, the abstract should have the following structure: Introduction (with the aim stated in the last sentence), Case Report, Conclusion. Each section should be written as a separate paragraph beginning with a bolded heading. The abstract must not exceed 250 words.

For all other manuscript categories: General (Narrative) Literature Review, Mini Review, Current Topic, In Focus, and History of Medicine/Stomatology/Pharmacy, the abstract is unstructured and must not exceed 200 words.

Care should be taken in ensuring that the Serbian and English versions of the abstract are accurate and precise translations of each other. No sentence may appear in one version without being translated into the other.

KEYWORDS

Below the abstract, list five to seven relevant keywords or phrases that indicate the content of the manuscript. It is recommended to avoid repeating words from the title of the paper. When selecting keywords, use Medical Subject Headings (MeSH) (https://www.nlm.nih.gov/mesh/meshhome.html).

STRUCTURE OF THE MAIN TEXT

Original Articles, Preliminary Reports, Short Reports, Meta-Analyses, Systematic Literature Review, Systematic Literature Reviews with Meta-Analysis, and Clinical Research papers must include the following sections: Introduction (a brief overview of the research topic, with the study aim stated in the final paragraph); Methods (a precise description of participant selection and applied methods, including statistical methods, and the approval number of the competent Ethics Committee); Results (presented in a logical order, without duplicating the same results in multiple forms); Discussion (without repeating data already presented in the Results section; only the obtained findings should be discussed, placing them in the context of other relevant studies; the discussion and conclusions should be linked to the study aims, and study limitations should be highlighted if necessary); Conclusion (derived directly from the study results); Acknowledgements (if applicable); References.

Manuscripts in the categories General (Narrative) Literature Review, Mini-Review, Current Topic, and In Focus should contain the following sections: Introduction (with appropriate subheadings), Conclusion, and References.

Manuscripts in the categories Case Report and Case Series should include the following sections: Introduction (the aim of the paper should be stated in the final paragraph of the Introduction), Case Report (the patient's identity must remain anonymous), Discussion, and References.

A Case Report must not have more than five authors.

QUESTIONNAIRES

All questionnaires used as measurement instruments for any of the investigated parameters must be translated into the language spoken by the study participants, with evidence provided of their validation and cultural adaptation to the participants' setting.

TABLES AND FIGURES

Tables and figures, the number of which should be appropriate to the length of the text, should be placed at the end of the main manuscript text, after the References. The exact position of each item should be clearly indicated in the text. The final placement of tables and figures will be determined during manuscript preparation for publication.

Tables

The title should be placed above the table, and explanations (the legend) below it. Tables should be numbered with Arabic numerals in the order in which they appear in the text. Tables must be created exclusively in the Microsoft Word program using the menu Table–Insert–Table, with the exact number of rows and columns defined. Use Times New Roman font, 12-point size, single spacing. Tables must be clear and include all elements necessary for the proper interpretation of the data presented. If the displayed values have ranges or reference values, these must be specified.

In the legend below the table, all abbreviations used in the table and all symbols (e.g., superscript letters or bolded values) must be explained. In addition, the applied statistical methods must be clearly specified.

Figures (Illustrations)

Figures include all forms of graphical material (photographs, drawings, diagrams, and graphs). Figures should be embedded in the manuscript at the end of the text, after the References and after the Tables (if any). Figures should be numbered with Arabic numerals in the order in which they appear in the text. Capital letters A, B, C, etc., should be used to designate parts of multipart figures. Letters, numbers, and symbols must be clear, consistent, and of sufficient size to remain legible after reduction. All elements shown in figures must be saved as images (not as editable graphic objects) so that their position cannot be altered, ensuring the accuracy of the data presented. Only digital images with a minimum resolution of 300 dpi and in JPEG, PNG, or PDF format are accepted. Figures that do not meet these requirements will not be accepted for publication. The dimensions of submitted figures should be approximately the same as the dimensions at which they will be published. If authors are unable to provide digital photographs, original images should be scanned at a resolution of 300 dpi and at their original size and submitted in that form. All text in diagrams and graphs should be written in a sans-serif font for better readability (e.g., Arial, Helvetica), with a font size of no less than 10 pt. Measurement units and scales must be clearly indicated. Decimal numbers in graphs must be presented with a decimal point, and thousands should be separated by a comma (e.g., 1,234.56).

Video supplements (illustrations of the manuscript) may last 1–3 minutes and should be submitted in AVI or MP4 (FLV) format. A separate still image representing the video (video thumbnail) must also be provided for use in the electronic edition and publication in the printed edition, along with a link to the platform where the video is already hosted.

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ABBREVIATIONS

Abbreviations should be used only when necessary, primarily for very long names of chemical compounds or for terms that are already widely recognized in abbreviated form (e.g., DNA). For each abbreviation—except standard units of measurement—the full term must be given at its first occurrence in the text (including the abstract). The use of abbreviations should be avoided in the title and abstract; in the title, abbreviations should be used only if absolutely necessary. For terms mentioned more than 3 times in the text, introducing appropriate abbreviations is recommended.

DECIMAL NUMBERS

In manuscripts written in English, decimal numbers should be written with a decimal point (e.g., 22.7), whereas in manuscripts written in Serbian, a comma should be used (e.g., 22,7). Whenever possible, numbers should be rounded to one decimal place and reported consistently throughout the manuscript (e.g., if one value is 32.2, all others should also be rounded to one decimal place, e.g., 32.0).

UNITS OF MEASUREMENT

Length, height, weight, and volume should be expressed in metric units (meter – m, kilogram (gram) – kg (g), liter – L) or their subunits. Temperature should be expressed in degrees Celsius (°C), and blood pressure in millimeters of mercury (mm Hg). Results of clinical and biochemical measurements should be reported in metric units according to the International System of Units (SI).

ACKNOWLEDGEMENTS

The contributions of individuals who should be acknowledged but do not meet the criteria for authorship should be stated. Financial support (sponsorships, grants, equipment, etc.) should be disclosed, as well as the name of the project within which the research was conducted.

STATISTICAL ANALYSIS

In the Methods section, the applied statistical methods should be described in sufficient detail to allow verification of their correct use and reproduction of the analysis. Results must be presented numerically and clearly, with appropriate measures of variability and reliability (e.g., standard deviation, standard error, confidence interval). The type of study should be specified, and the manner in which it was conducted should be described. Inclusion and exclusion criteria must be stated. The software and the version of the computer program used for statistical data analysis should be reported. In the Results section, as well as in the legends of tables and/or figures, the statistical method used to analyze the presented results must be indicated. The p values should always be written with a leading zero (e.g., $p > 0.05$, not $p > .05$).

REFERENCES

References should be numbered with Arabic numerals according to the order of their first appearance in the text (including tables and figure legends). It is recommended that the majority of cited references be published within the last ten years. At least 80% of the cited references should be original research articles, while books, book chapters, and review articles should account for no more than 20% of the total number of references. All references, regardless of the language of the original source, must be cited in English, with the original language indicated in parentheses after the reference.

All data on the references must be accurate, and the cited works should be easily accessible to readers. A DOI number must be provided for each reference. Citation of articles published in journals indexed in Current Contents, Index Medicus (MEDLINE), Excerpta Medica, Scopus, and Web of Science is recommended.

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References should be formatted according to the Vancouver style established by the ICMJE (https://connect.ebsco.com/s/article/Citing-Articles-in-Vancouver-ICMJE-Style?language=en_US).

Citation examples:

Article with 1 to 6 authors

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Article with more than 6 authors

Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med 2017; 13(3): 479–504. DOI: 10.5664/jcsm.6506

Volume with a Supplement

Smith JA, Brown LM. Effects of vitamin D on immune response. J Nutr Sci 2024; 15(Suppl 2): S45–53.

Issue with a Supplement

Zhou Q, Shi R, Kopjar B, Wang H, Chen D, Li H, et al. Adjacent Intervertebral Disc Changes in Patients with Isobar Semirigid Dynamic Stabilization System. Global Spine J 2017; 4(1 Suppl): s-0034-1376699.

Volume with Part (Pt)

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995; 32(Pt 3): 303–6.

Issue with Part (Pt)

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994; 107(986 Pt 1): 377–8.

Issue with no Volume

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995; (320): 110–4.

No Volume or Issue

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993: 325–33.

Pagination with Roman numerals

Fisher GA, Sikic BI. Drug resistance in clinical oncology and hematology. Introduction. Hematol Oncol Clin North Am 1995; 9(2): xi–xii.

Book

Printed Book

Ritter JM, Flower RJ, Henderson G, Loke YK, MacEwan D, Robinson E, et al. Rang & Dale's Pharmacology. 10th ed. London: Elsevier; 2023. p. 3630.

Book in electronic format

Shreeve DF. Reactive attachment disorder: a case-based approach [Internet]. New York: Springer; 2012 [cited 2012 Nov 2]. 85 p. Available from: http://dx.doi.org/10.1007/978-1-4614-1647-0

Chapter

In an edited book

Metcalf CS, Smith MD, Wilcox KS. Pharmacotherapy of the Epilepsies. In*: Brunton LL, Knollmann BC,* editors. Goodman & Gilman's The pharmacological basis of therapeutics. 14th ed. NY: McGrawHill; 2023. p. 385–411.

In an edited electronic (online) book

Halpen-Felsher BL, Morrell HE. Preventing and reducing tobacco use. In: *Berlan ED, Bravender T,* editors. Adolescent medicine today: a guide to caring for the adolescent patient [Internet]. Singapore: World Scientific Publishing Co.; 2012 [cited 2012 Nov 3]. Chapter 18. Available from: http://www.worldscientific.com/doi/pdf/10.1142/9789814324496_0018

Website

Homepage

Diabetes Australia. Diabetes globally [Internet]. Canberra ACT: Diabetes Australia; 2012 [updated 2012 June 15; cited 2012 Nov 2]. 85 p. Available from: http://www.diabetesaustralia.com.au/en/Understanding-Diabetes/Diabetes-Globally/

Part of a website

Australian Medical Association [Internet]. Barton ACT: AMA; c1995-2012. Junior doctors and medical students call for urgent solution to medical training crisis; 2012 Oct 22 [cited 2012 Nov 2]; [about 3 screens]. Available from: https://ama.com.au/media/junior-doctors-and-medical-students-call-urgent-solution-medical-training-crisis

Conference Proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15–19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Article from Conference Proceedings

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: *Lun KC, Degoulet P, Piemme TE, Rienhoff O,* editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6–10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561–5.

Dissertation

Knežević D. The importance of decontamination as an element of complex therapy of poisoning with organophosphorous compounds [Ph.D. Thesis]. Belgrade: School of Veterinary Medicine; 1988. (Serbian)

Other published articles

News article

Vujadinović J. The inconsistency between federal and republican regulation about pharmacies. In between double standards. Borba 2002 February 28; p. 5. (Serbian)

Holy Bible

Serbian Bible. Belgrade: British and Foreign Biblical Society; 1981. Book of Isaiah 2: 19–22. (Serbian)

Dictionaries and similar references

Kostić AĐ. Multilingual Medical Dictionary. 4th Ed. Belgrade: Nolit; 1976. Erythrophobia; p. 173–4.

Other examples of citing publications can be seen at https://www.nlm.nih.gov/bsd/uniform_requirements.html

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Рад који не испуњава услове овог упутства не може бити разматран и биће враћен ауторима да га допуне и исправе**.**

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Сви аутори и рецензенти морају бити регистровани корисници система са јединственом е-маил адресом. Регистрацију је могуће извршити на: http://aseestant.ceon.rs/index.php/vsp/user. Техничко упутство за коришћење система електронске пријаве доступно је на: https://aseestant.ceon.rs/index.php/vsp/about/submissions.

Уколико имате проблем са подношењем рукописа путем платформе *Aseestant* можете се обратити за помоћ Редакцији часописа слањем е-мејла на адресу: vsp@vma.mod.gov.rs.

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Уколико је део резултата поднетог рукописа претходно саопштен на научном/стручном скупу или је део докторске дисертације, у Пропратном писму Уредништву потребно је навести званичан назив скупа, место и време одржавања, и да ли су саопштени резултати публиковани и у којој форми (нпр. исти или другачији наслов или сажетак), а у Напомени на крају рукописа то треба посебно назначити.

Радови се објављују на енглеском језику. Поједине категорије радова (нпр. историја медицине/стоматологије/фармације) се по одлуци Уредништва ВСП могу објавити и на српском језику. Све категорије рукописа осим категорија уводник, писмо уреднику, истраживачко писмо, приказ књиге, извештај са научног или стручног скупа се објављују са апстрактима на српском и енглеском језику (у склопу рукописа). О структури и обиму апстракта видети детаљније у одељку Апстракт овог Упутства.

За писање рукописа користити програм *Word*, фонт *Times New Roman*, величину слова 12, проред 1,5. Величину странице подесити на формат А4, са левом маргином од 4 цм а преостале три 2 цм. Текст куцати без дељења речи (хифенације), а 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 суперскрипту, редоследом којим се појављују у тексту.

Странице нумерисати редом у доњем десном углу, почев од прве стране (изузимајући насловну страну).

При писању текста на енглеском језику придржавати се језичког стандарда *American English*. Обавезно је коришћење међународног система мера (SI). Изузетак чине крвни притисак (mm Hg) i temperatura (°C).

Приликом писања користе се стандардне скраћенице. Избегавати скраћенице у наслову и апстракту осим уколико је неопходно. Пун назив са скраћеницом у загради наводи се у њеном првом помињању, а даље у тексту само скраћенице, како у апстракту тако и у главном тексту. У закључку рада (не апстракта) нема скраћеница.

Не користити комерцијална имена лекова и других препарата, а уколико је то неопходно уз њихове називе обавезно навести и генеричка имена. Уређаји (апарати) се означавају фабричким називима, а податке о произвођачу (назив и место) 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 субскрипту.

Избегавати фонтове *bold* и курзив (*italic*) јер су резервисани за поднаслове. Изузеци су обавезно писање курзивом оних назива који се тако морају писати (нпр. гени или стране речи - латински).

Групе испитаника морају бити јасно дефинисане и доследно именоване кроз цео рад. За исти појам користити један, јединствен термин кроз цео рад. У одељку Резултати избегавати реченице које почињу са: „Табела X показује “ или „Слика X приказује“. Реченица треба да опише резултат, а ознака табеле или слике да стоји у загради на крају описа. Реченице не би требало почињати скраћеницом, бројем или датумом. Избегавати предугачке реченице које умањују јасноћу текста и дати предност краћим јасним реченицама. Закључак формулисати новим реченицама, без преписивања већ изречених. Превод радова на енглески језик посредством *Google Translate* може изазвати неразумевање текста и стога се не препоручује.

У избору кључних речи користити *Medical Subject Headings – MeSH* (https://www.nlm.nih.gov/mesh/meshhome.html). Кључне речи у прихваћеном рукопису не подлежу ауторској коректури, пошто су оне дескриптори из Тезауруса које одређују стручни индексери.

ОБАВЕЗНА ПРАТЕЋА ДОКУМЕНТА

ИЗЈАВА АУТОРА И АУТОРСТВО

За сваки рукопис који се подноси на разматрање за објављивање у ВСП неопходно је да аутор(и) достави(е) Образац за изјаву о ауторству (Изјаву аутора) да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, да су рукопис прочитали и одобрили сви аутори који испуњавају критеријуме ауторства, и контакт податке свих аутора у раду (имејл адресу, број мобилног телефона). У овом обрасцу се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. Сви аутори морају Изјаву аутора потписати својеручно.

За додатне информације о различитим врстама сукоба интереса видети препоруке Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME;* http://www.wame.org*)*.

ВСП поштује препоруке критеријума за ауторство које даје ICMJE (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). Ауторство се заснива на испуњењу сва четири критеријума: значајном доприносу концепцији рада, добијању резултата или анализи/тумачењу резултата; критичкој ревизији рукописа од знатног интелектуалног значаја; одобрењу финалне верзије рукописа која ће бити објављена и преузимању одговорности за све аспекте објављеног садржаја. Сви други учесници који су допринели изради рада, али нису испунили прописане критеријуме требало би да буду наведени у Захвалници уз прецизирање доприноса раду. Потребно је да особе наведене у Захвалници дају писмену сагласност.

ЕТИЧКА САГЛАСНОСТ

Сва истраживања која укључују људе и/или хумани материјал морају бити спроведена у складу са препорукама ICMJE (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/protection-of-research-participants.html) и Хелсиншком декларацијом, ревизија 2024 (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Скенирану страну дозволе Етичке комисије (ЕК) надлежне институције које је одобрила истраживање, на којој се види датум издавања и предмет истраживања, аутори су у обавези да доставе истовремено са рукописом. Дозвола ЕК се доставља на језику на коме је издата и енглеском језику (може и оверена копија).

У одељку Методе мора бити наведено да је студија одобрена од стране надлежног ЕК, уз навођење назива институције и броја одлуке, као и да је спроведена у складу са етичким принципима за истраживања која укључују људе и/или хумани материјал.

Анонимност пацијената мора бити заштићена у складу са ICMJE препорукама. За сва истраживања која укључују податке о пацијентима који омогућавају директну или индиректну идентификацију, аутори су обавезни да прибаве писани пристанак информисаног пацијента, да у рукопису назначе да је пристанак пацијента прибављен, и да га по потреби доставе Уредништву.

У случају истраживања на животињама, аутори су дужни да доставе одобрење надлежног ЕК који води бригу о поштовању међународних стандарда о употреби лабораторијских животиња у истраживачке сврхе.

Уредништво може одбити радове за које процени да нису изведени у складу са међународним етичким стандардима.

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

Уколико се користе претходно објављене илустрације (фотографије, схеме) уз обавезно цитирање извора преузимања потребно је доставити дозволу (писано одобрење часописа у коме су објављене) за њихову објаву у ВСП. Уколико се користе туђе необјављене илустрације (фотографије, схеме) потребно је доставити дозволу аутора илустрација, за њихову објаву у ВСП.

ПЛАГИЈАРИЗАМ

Од 2012. године сви рукописи достављени на разматрање у ВСП подвргавају се провери на потенцијални (ауто)плагијаризам посредством *SCIndeks Assistant – Cross Check* (*iThenticate*). Рукописи код којих се докаже (ауто)плагијаризам биће одбијени. У зависности од степена и врсте утврђеног (ауто)плагијаризама ауторима се може изрећи забрана објављивања у ВСП-у (различите дужине трајања), уз обавештење надлежних тела у институцијама у којима аутори раде и релевантних професионаних удружења.

КОРИШЋЕЊЕ AI

Генеративна вештачка интелигенција (*artificial intelligence*-AI) или технологије које користе помоћ AI (AI-потпомогнуте) могу се користити само уз поштовање начела транспарентности (употреба AI мора бити јасно наведена у рукопису), одговорности (аутори остају у потпуности одговорни за тачност и оригиналност садржаја), проверљивости (сви учесници у публицистичком процесу морају проверити да AI није унела измишљене податке, цитате или тврдње) и поверљивости (ауторима и рецензентима је забрањено учитавање рукописа поднетих у ВСП у јавне AI сервисе).

Употреба AI алата је допуштена само за ограничене језичке и техничке интервенције у тексту рукописа: исправку граматике и правописа, стилско дотеривање ауторског текста, помоћ при форматирању, техничку асистенцију (попут исправљања кода). Аутори могу користити AI алате искључиво за креирање AI-потпомогнутог, али не и AI -генерисаног садржаја.

Аутори који су користили AI-потпомогнут садржај у обавези су да потпуно и тачно наведу употребу AI алата (тачан назив AI алата, датум приступа, коришћене упите и сврху употребе), гарантују оригиналност научног доприноса, избегавају било какву фабрикацију или манипулацију и поштују правила научне етике. Информације о коришћењу AI се наводе у одељку Методе или Захвалница.

Забрањено је користити AI алате за генерисање већег дела садржаја рукописа, креирање научних идеја, података и резултата, анализу и интерпретацију резултата, формирање закључака, измену слика, табела или графикона (укључујући графичке сажетке), измену података или референци.

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

AI ни у ком случају не може бити аутор или коаутор рада, нити може као аутор бити цитиран у одељку Литература.

Ради заштите поверљивости, ниједан део необјављеног истраживања достављеног ВСП не сме бити унет у велики језички модел од стране аутора или рецензента.

Аутори који су користили неки од AI алата су у обавези да приликом подношења рукописа поднесу и Изјаву о коришћењу AI.

ТИПОВИ РУКОПИСА

У ВСП се објављују следеће категорије и типови рукописа и саопштења: уводник, оригинални рад, претходно саопштење, кратко саопштење, приказ случаја и серија случајева, општи (наративни) преглед литературе, мини преглед, систематски преглед литературе, мета-анализа, систематски преглед литературе са мета-анализом, актуелна тема, у фокусу, рад из историје медицине/стоматологије/фармације, писмо уреднику, истраживачко писмо, клиничко истраживање, извештај са конгреса и научног скупа, приказ књиге, *In memoriam* и други прилози.

ОРИГИНАЛНИ ЧЛАНАК

Приказује нова и значајна открића у одређеној области уз детаљан опис коришћених метода истраживања, добијених резултата и изведених закључака. Листа референци треба да укључи најновије и најважније референце из области рада.

ПРЕТХОДНО САОПШТЕЊЕ

Представља приказ истраживања која нису завршена, са налазима који захтевају додатна истраживања и валидацију пре коначних закључака, али су добијене информације од интереса за научну и стручну јавност. Садржи сва поглавља као оригинални научни чланак, али у знатно скраћеном обиму. Аутори се подстичу да касније објаве пуну оригиналну научну студију са комплетним, валидираним подацима и свеобухватном анализом.

КРАТКО САОПШТЕЊЕ

Представља завршено истраживање које је мало по обиму, уско фокусирано са јасним закључцима на основу представљених резултата. Садржи сва поглавља као оригинални научни чланак, али у знатно скраћеном обиму. Сматра се коначном публикацијом тог специфичног, малог истраживања. Не може се поново објавити као чланак пуног обима (иако се подстиче накнадно истраживање које се надовезује на њега).

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

Преглед, критичка анализа и синтеза постојећих научних сазнања о изабраној теми. Аутори обухватају сву доступну припадајућу литературу за одређени временски период, приказују резултате релевантних истраживања, идентификују недостатке, ограничења или контроверзе и указују на правце будућих истраживања, дајући своје виђење проблема у виду закључног става. Аутори чланка ове категорије могу бити они који су објавили минимално пет радова публикованих у часописима с рецензијом (М20) из области прегледног рада.

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

Сажет преглед постојеће литературе и најновијих достигнућа унутар дефинисаних аспеката одређене истраживачке области и њени нови и/или актуелни правци развоја.

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

Синтеза претходно објављених истраживања о одређеној теми коришћењем јасно дефинисаних и унапред одређених методолошких поступака за селекцију и евалуацију. Аутор мора да користи релевантне базе података, постави критеријуме укључивања и искључивања студија и примени транспарентну методологију.

МЕТА-АНАЛИЗА

Користи статистичке методе за комбиновање квантитативних података из више примарних студија како би се идентификовали општи трендови и проценила снага доказа о одређеној теми. Аутор мора да користи релевантне базе података, дефинише критеријуме за укључивање и искључивање и примени транспарентну и репродуцибилну методологију. Неопходно је јасно дефинисање истраживачког питања (PICOS оквир), навођење смерница за одабир и дијаграма тока за селекцију студија (PRISMA).

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

Комбинује квалитативну и квантитативну синтезу, користећи статистичке технике за сумирање квантитативних резултата а квалитативну синтезу за описне/наративне налазе. Аутор мора користити релевантне базе података, јасно дефинисати критеријуме за укључивање и искључивање студија, и применити транспарентну и репродуцибилну методологију. Истраживачко питање мора бити јасно дефинисано према PICOS оквиру, уз навођење коришћених смерница за извештавање (нпр. PRISMA) и укључивање PRISMA дијаграма тока за приказ селекције студија.

АКТУЕЛНА ТЕМА

Разматра савремено, нерешено или контрадикторно питање од теоријског и практичног значаја, уз изношење сопствених резултата истраживања или најновијих важних података из литературе. Конструкција чланка је слободна а пожељне су кратке закључне напомене са јасном поруком.

У ФОКУСУ

Тематска, фокусирана анализа и/или кратак осврт на научни проблем који је у тематској области часописа, а који обрађује питање од значаја за научну заједницу и ширу стручну јавност.

КАЗУИСТИКА

ПРИКАЗ СЛУЧАЈА и СЕРИЈА СЛУЧАЈЕВА (≥4, ≤9)

Приказ случајева са ретком и необичном дијагнозом, дијагностичким процесом, стратегијама лечења, клиничким током, или исходом лечења, који могу бити од користи за клиничку праксу и медицинско образовање. Приликом писања потребно је користити CARE смернице (https://www.care-statement.org/writing-a-case-report). Неопходан је пристанак информисаног пацијента.

УВОДНИК

Уводници су нерецензирани текстови главног и одговорног уредника и/или чланова Уредништва намењени најави новог волумена, тематског броја, садржаја који су од значаја за струку и/или институције чијим члановима је часопис намењен као и уреднички текстови по позиву. Уводници не треба да садрже необјављене или оригиналне податке, а морају укључити изјаву о сукобу интереса.

ПИСМО УРЕДНИКУ

Нерецензирани коментар/критика текста објављеног у ВСП. Пишу се у слободној форми, уз евентуално навођење података из литературе. Не смеју садржати необјављене резултате. Објављују се према одлуци главног и одговорног уредника.

ИСТРАЖИВАЧКО ПИСМО

Кратки приказ оригиналног истраживања, који садржи увод, методе, резултате и дискусију у сажетом облику (без поделе у посебне целине са поднасловима) и максимално до 2 прилога (табеле/слике). Не садржи апстракт и кључне речи али мора да испуни све опште услове за разматрање рукописа (укључујући процес рецензије).

ИСТОРИЈА МЕДИЦИНЕ/СТОМАТОЛОГИЈЕ/ФАРМАЦИЈЕ

Материјал значајан за расветљавање појединих догађаја и/или приказ значајних личности из историје медицине/стоматологије/фармације, а посебно војне медицине/стоматологије/фармације.

КЛИНИЧКО ИСТРАЖИВАЊЕ

Оригинална рандомизована контролисана испитивања и опсервационе студије утицаја једног или више средстава или мера на исход здравља људи, клиничку праксу и здравствену политику. Рукописи морају бити припремљени у складу са међународним смерницама (нпр. CONSORT – https://www.consort-spirit.org/ или STROBE – https://www.strobe-statement.org/) и регистрована у неком од међународно признатих јавних регистара (нпр. ClinicalTrials.gov).

ПРИКАЗ КЊИГЕ

Садржи библиографске податке о публикацији (аутори, изворни наслов, издавач, место и година издања), њен кратак садржај и критичке коментаре садржаја, стила и значаја књиге у датој области. Рукопис не сме бити дужи од 2 странице.

ИЗВЕШТАЈ СА НАУЧНОГ ИЛИ СТРУЧНОГ СКУПА

Приказ активности научног или стручног скупа, уз истицање најважнијих реферата или закључака, односно препорука од значаја за шири круг читалаца ВСП.

ОБИМ РУКОПИСА

Целокупни рукопис рада чине: насловна страна, апстракти на српском и енглеском језику са кључним речима, главни текст рада, захвалност (по потреби), списак литературе, прилози (табеле, слике, графикони, схеме, цртежи).

Обим рукописа за категорије оригинални рад, општи (наративни) преглед литературе, систематски преглед литературе, мета-анализа, систематски преглед литературе са мета-анализом износи до 5 000 речи.

Обим рукописа за категорије мини преглед, претходно саопштење, кратко саопштење, приказ случаја, серија случајева, актуелна тема, клиничко истраживање, историја медицине/стоматологије/фармације износи до 3 000 речи.

Рукописи за остале категорије/рубрике могу имати највише 1 500 речи.

ПРИПРЕМА РАДА

НАСЛОВНА СТРАНА

На првој страници рукописа треба навести следеће:

1. Наслов рада без скраћеница;
2. Пуна имена и презимена аутора (без титула, уз навођење ORCID броја за све ауторе који га имају) са ознакама следећим редом ***, †, ‡, §, ‖, ¶, **, †† …** итд.
3. Пун званичан назив установа у којима аутори раде, место и државу у којој се установе налазе (знаци *, †, ‡, §, ‖, ¶, **, †† … итд.показују редом установе у којима аутори раде);
4. На дну странице навести име и презиме, адресу за контакт, е-маил адресу и број телефона (мобилног/*Viber* или *WhatsApp*) аутора задуженог за кореспонденцију.

АПСТРАКТ

На другој страни рада пишу се апстракт и кључне речи. Апстракт се пише кратким и јасним реченицама. За категорије оригинални рад, претходно саопштење, кратко саопштење, систематски преглед литературе, систематски преглед литературе са метаанализом, мета-анализа, клиничко истраживање, апстракт је структурисан и треба да има следеће делове: Увод/Циљ, Методе, Резултати, Закључак. Сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) и ниво статистичке значајности. Закључак мора бити директно повезан са резултатима рада. Обим апстракта не сме да пређе 300 речи.

За категорије приказ случаја и серија случајева апстракт има следећу структуру: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак. Сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Обим апстракта не сме да пређе 250 речи.

За остале категорије радова, општи (наративни) преглед литературе, мини преглед, актуелна тема, у фокусу, историја медицине/стоматологије/фармације апстракт нема посебну структуру и не сме да пређе 200 речи.

Водити рачуна да српска и енглеска верзија апстракта буду међусобно тачни и прецизни преводи. Ниједна реченица не сме постојати у једној верзији а да није преведена у другој.

КЉУЧНЕ РЕЧИ

Испод апстракта навести пет до седам релевантних кључних речи или израза који указују на садржај рада. Препорука је да се не понављају речи из наслова рада. У избору кључних речи користити *Medical Subject Headings – MeSH* (https://www.nlm.nih.gov/mesh/meshhome.html).

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

Неопходно је да оригинални рад, претходно саопштење, кратко саопштење, мета-анализа, систематски преглед литературе, систематски преглед литературе са мета-анализом, клиничко истраживање садрже поглавља: Увод (кратак приказ предмета истраживања уз навод циља рада у последњем пасусу), Методе (прецизан опис одабира испитаника и примењених метода, укључујући статистичке методе, број дозволе сагласности надлежног ЕК), Резултати (приказани логичким редоследом без дуплирања приказа истих резултата на више начина), Дискусија (без понављања података који су већ наведени у одељку Резултати; дискутовати само добијене налазе довођењем у везу са другим релевантним студијама, повезати дискусију и закључке са циљевима рада, по потреби нагласити лимитације истраживања), Закључак (који проистиче из резултата датог истраживања), Захвалница (по потреби), Литература.

Рукописи из категорија општи (наративни) преглед литературе, мини преглед, актуелна тема, у фокусу садрже следеће целине: Увод (са одговарајућим поднасловима), Закључак, Литература.

Рукописи из категорије приказ случаја, серија случајева садрже следеће целине: Увод (циљ рада навести као последњи пасус Увода), Приказ болесника (идентитет болесника мора остати анониман), Дискусија, Литература.

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

Сви коришћени упитници који су употребљени као мерни инструменти за било који од испитиваних параметара, морају бити преведени на језик говорног подручја испитаника уз навођење доказа о извршеној валидацији и културолошкој адаптацији поднебљу испитаника.

ПРИЛОЗИ

Прилоге чији број треба да буде усклађен са дужином текста поставити на крај главног текста рукописа иза Литературе, а у самом тексту јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

Табеле

Наслов треба написати изнад табеле, а објашњења (легенду) испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле израдити искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће је чинити. Куцати фонтом *Times New Roman*, величином слова 12, с једноструким проредом. Табеле морају бити јасне и имати све елементе неопходне за правилно разумевање шта је у њима приказано. Уколико приказане вредности имају „опсег“ или „референтне вредности“, то се мора додати.

У легенди испод табеле треба објаснити све скраћенице наведене у табели и све ознаке (нпр. слова у суперскрипту или болдоване вредности). Такође, неопходно је прецизирати примењене статистичке методе.

Слике (илустрације)

Под сликама подразумевамо све облике графичких прилога (фотографије, цртежи, схеме и графикони). Слике треба уградити у рукопис на крају текста, после литературе и после табела (ако их има). Слике се означавају арапским бројевима према редоследу навођења у тексту. Велика слова А, Б, Ц итд. треба користити за означавање делова вишеделних слика. Слова, бројеви и симболи треба да су јасни и уједначени, а довољне величине да приликом умањивања буду читљиви. Додаци приказани на сликама морају бити сачувани као фотографије (не као измењиви графички елементи), тако да се њихов положај не може мењати, како би се обезбедила тачност података приказаних на слици. Примају се искључиво дигиталне фотографије са минималном резолуцијом од 300 *dpi* и формата JPEG, PNG или PDF. Слике које не задовољаавају наведене услове неће бити прихваћене за објаву. Димензије достављених слика би требало да буду приближне димензијама у којима ће слика бити објављена. Уколико аутори нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини и као такве их доставити. Сви подаци на схемама и графиконима треба да буду исписани безсерифним фонтом ради лакше читљивости (нпр. *Arial*, *Helvetica*), величина слова не мања од 10 *pt*. Мерне јединице и скале морају бити јасно назначене. Децимални бројеви на графиконима морају бити приказани са тачком, а раздвајање хиљада мора бити означено зарезом (нпр. 1,234.56).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видео-приказа у е-издању и објављена у штампаном издању, као и линк ка платформи где је видео већ постављен.

У легенди испод илустрација треба објаснити све скраћенице, симболе, бројеве или слова који се користе за објашњење појединих делова слике. У случају графикона прецизирати примењене статистичке методе (по потреби), а код фотомикрографије навести детаље о врсти коришћеног бојења и увећању.

Уколико се приказују фотографије особа (болесника), лик мора бити „замућен“ или је потребно обезбедити писану дозволу лица са фотографије за њено коришћење. На прилозима (снимци рендгена, скенера, ултразвука, итд.) потребно је уклонити све што може да идентификује болесника. Уколико је слика већ негде објављена потребно је цитирати извор уз писано одобрење ако се ради о заштићеном материјалу.

СКРАЋЕНИЦЕ

Скраћенице користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (нпр. ДНК). За сваку скраћеницу, осим стандардне јединице мере, навести пун назив при првом навођењу у тексту (укључујући апстракт). У наслову и апстракту избегавати коришћење скраћеница, у наслову их користити само ако су неопходне. За појмове који се у тексту помињу више од три пута препоручује се увођење одговарајућих скраћеница.

ДЕЦИМАЛНИ БРОЈЕВИ

У тексту рада на енглеском језику децималне бројеве писати са тачком (нпр. 22.7), а у тексту на српском језику са зарезом (нпр. 22,7). Кад год је то могуће, број заокружити на једну децималу и писати доследно кроз цео рад (нпр. ако је једна вредност 32.2, све остале морају имати једну децималу, нпр. 32.0).

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), притисак крви у милиметрима живиног стуба (mm Hg). Резултате клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (SI).

ЗАХВАЛНИЦА

Изнети допринос особе којој треба одати признање, али која не испуњава критеријуме за ауторство. Навести финансијску помоћ (спонзорства, стипендије, опрема и друго), као и назив пројекта у оквиру кога је истраживање спроведено.

СТАТИСТИЧКА АНАЛИЗА

У одељку Методе детаљно описати примењене статистичке методе како би била омогућена провера исправности њихове примене и репродукција анализе. Резултати морају бити нумерички јасно приказани уз одговарајуће показатеље варијабилности и поузданости (нпр. стандардна девијација, стандардна грешка, интервал поверења). Прецизирати тип студије и описати начин на који је изведена. Навести критеријуме укључења и искључења. Навести софтвер и верзију компјутерског програма у коме је извршена статистичка обрада података. У одељку Резултати као и у легендама табела и/или прилога навести статистички метод који је коришћен за анализу приказаних резултата. Вредности *p* се увек пишу са почетном нулом (нпр. $p > 0.05$ а не $p > .05$).

ЛИТЕРАТУРА

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту (укључујући табеле и легенде прилога). Препоручује се да већина цитираних радова буде млађа од десет година. Препоручује се да број цитираних оригиналних радова буде најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Сви радови, без обзира на језик извора, цитирају се на енглеском језику, а изворни језик наводи се у загради, иза цитиране референце.

Сви подаци о цитираној литератури морају бити тачни, а цитирани радови лако приступачни читаоцима. Уз сваку референцу навести DOI број. Препоручује се цитирање само радова објављених у часописима које индексирају *Current Contents, Index Medicus* (*Medline*), *Excerpta Medica, Scopus, Web of Science*.

Није дозвољено цитирање апстраката, секундарних публикација, усмених саопштења, необјављених радова, службених и поверљивих докумената, Википедије, препринт објава и *in press* чланака, повучених радова (*retracted article*), радова објављених у предаторским часописима.

Приликом цитирања сајтова, не може се цитирати насловна страна већ се мора цитирати она страна са које је информација преузета. Свака наведена референца мора бити доступна за проверу *online*. Уколико референца не постоји на интернету (нпр. архивски материјал и сл.), аутор мора да достави извор одакле је преузео цитирану литературу односно може сликати или скенирати документ и послати на е-мејл: strliteratura@gmail.com.

Референце се цитирају према Ванкуверском стилу који је успоставио ICMJE (https://connect.ebsco.com/s/article/Citing-Articles-in-Vancouver-ICMJE-Style?language=en_US).

Примери цитирања:

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Остале примере навођења публикација можете видети на https://www.nlm.nih.gov/bsd/uniform_requirements.html

НАКНАДЕ ЗА ОБЈАВЉИВАЊЕ ЧЛАНКА

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Ул. Црнотравска 17
11 000 Београд
Србија
Телефон: (+381 11) 3608-997
Е-пошта: vsp@vma.mod.gov.rs
Е-пошта за информације у вези са претплатом: strliteratura@gmail.com
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