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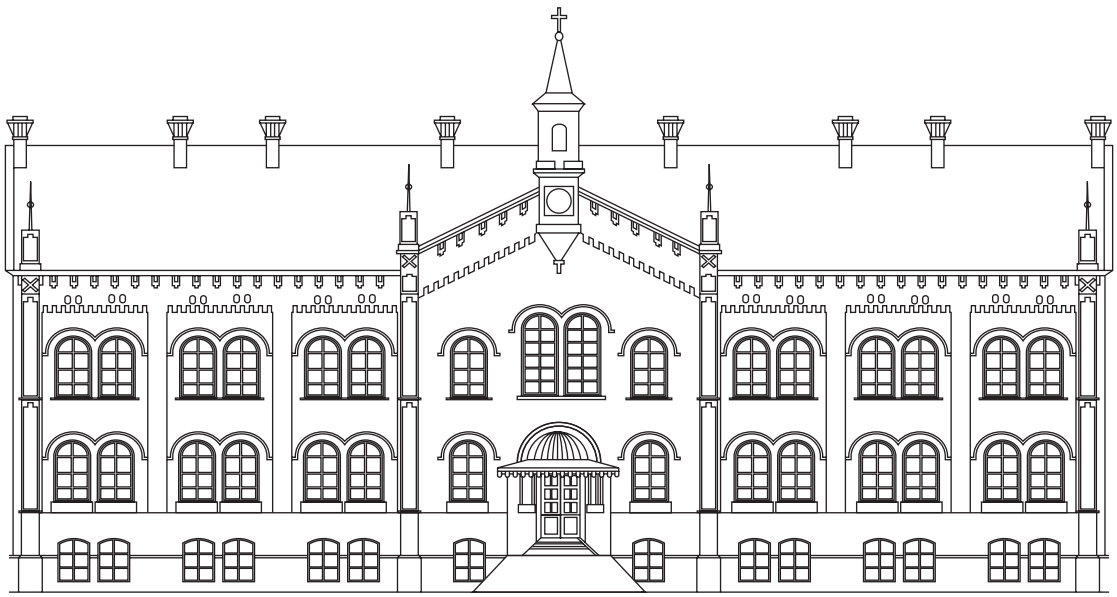

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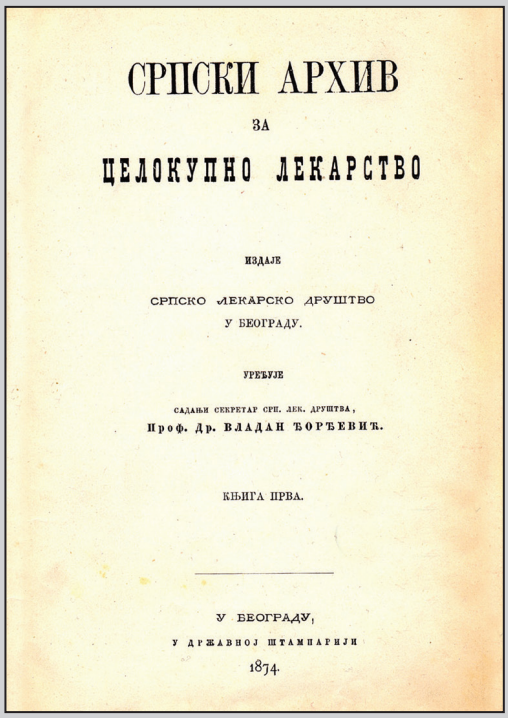

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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У БЕОГРАДУ,

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1874.

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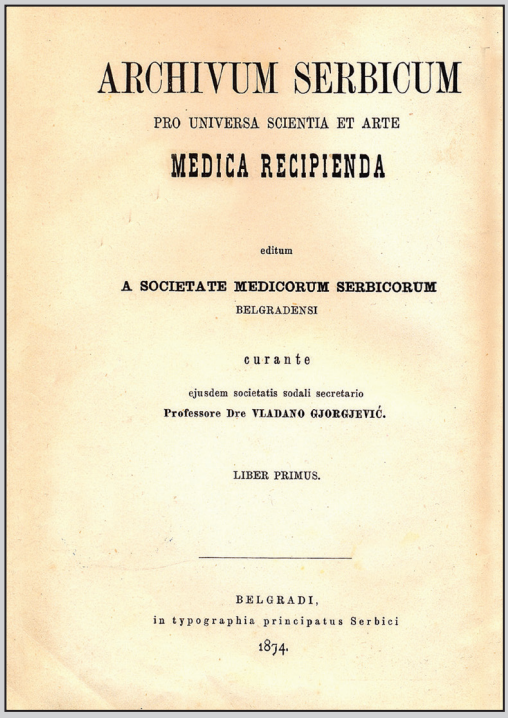

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1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Work ability impairment in patients with temporomandibular dysfunction

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SUMMARY

Introduction/Objective Temporomandibular dysfunction (TMD) is followed by orofacial pain and psychological problems that influence patients' quality of life and work ability. Hypothesis: work ability is affected by psychosocial status changes following temporomandibular dysfunction. The aim of this prospective study was to evaluate the impairment of Work ability in patients with temporomandibular dysfunction.

Methods Forty-four patients with TMD were admitted to the Clinic for Prosthodontics, School of Dental Medicine, University of Belgrade. Patients were treated with medication therapy with ibuprofen, ibuprofen and diazepam, or a stabilizing maxillary occlusal splint. Clinical and functional assessment was based on the diagnostic protocol Research Diagnostic Criteria for TMD. The study protocol was composed of data on clinical signs, a numerical pain scale and VAS, and a work ability-related questionnaire (Symptoms Check List, SCL-90R). The statistical software package SPSS for Windows (18.0) was used for data processing. Univariate regression analysis was used to examine the relationship of each factor individually, while multivariate regression analysis was used to examine the factors of difference.

Results A statistically significant difference was not recorded in work ability, social life, and daily activities in patients regardless of the chosen therapeutic approach. There was a statistically significant difference in work ability between respondents in relation to psychosocial status ($p = 0.002$). Univariate regression analysis showed significant values assessing work ability, social life, and daily activities (0.010*, 0.001*, 0.029*) respectively. In multivariate regression analysis, the assessment of social life was significant ($p = 0.028$).

Conclusion Work ability is influenced by temporomandibular dysfunction proportionally to the level of psychosocial status.

Keywords: temporomandibular dysfunction; orofacial pain; work ability

INTRODUCTION

Temporomandibular dysfunction (TMD) is often followed by orofacial pain, dysfunction of the masticatory muscles and temporomandibular (TM) joints. All these symptoms affect the quality of life of patients. This unpleasant sensory experience, can affect daily activities, sleep, and social activities, but one of the most significant aspects is the reduction in work ability [1, 2, 3]. According to some authors, work ability is described as the ability to perform daily tasks, to focus on solving problems, and to perform work duties [1, 2, 3]. The symptoms of temporomandibular dysfunction, as well as the accompanying sensations related to them, impair the efficient performance of daily activities, which reduces the individual's ability to be productive [4, 5, 6].

Hypothesis: work ability is affected by psychosocial status changes following temporomandibular dysfunction.

The aim of this prospective study was to evaluate the impairment of work ability in patients with temporomandibular dysfunction.

METHODS

At the Clinic for Prosthodontics, School of Dental Medicine in Belgrade, 44 patients aged 25–65 years, who showed painful temporomandibular dysfunction symptoms, were admitted as part of the study group. The clinical examination determined that the following candidates met the inclusion criteria for the study: 1. Subjects with intact teeth, 2. Subjects who were not surgically or orthodontically treated, 3. Subjects who were not receiving medication therapy, 4. Presence of painful symptoms in the region of the face and jaws.

Exclusion criteria were the following: 1. Patients with pain of other origin: odontogenic, neurogenic, vascular, inflammatory, or related to tumor changes in the surrounding structures (ear, throat, eye, nose, and sinuses), 2. Patients who had some other chronic disease that impairs the general health condition and gives a false picture of temporomandibular dysfunction, 3. Patients younger than 25 and older than 65 years, 4. Patients who did not consent to participate in the study.

A detailed clinical examination of the orofacial system was performed in all subjects in

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order to determine the presence of symptoms and signs of temporomandibular dysfunction. Patients with symptoms and signs of temporomandibular dysfunction were included in the study. All subjects were healthy and thoroughly informed about the research protocol. Clinical examination and functional analysis of the orofacial system were based on the diagnostic protocol Research Diagnostic Criteria for TMD (RDC/TMD) (Appendix 1). One or more symptoms of painful muscle and/or joint dysfunction were recorded: 1. pain in the preauricular region, 2. pain or sensitivity when palpating the masticatory muscles, 3. limited and/or painful movements of the lower jaw, 4. deflection of the lower jaw during mouth opening, and 5. presence of sound phenomena when opening the mouth. The study protocol was composed of a combination of data on clinical signs, a numerical pain scale and VAS, and a work ability-related questionnaire (Symptoms Check List, SCL-90R) (Appendix 2).

In the list of Axis II tests related to pain and the psychosocial status of the subjects, data were obtained based on the answers to the questions offered in the RDC/TMD protocol. In response to these questions, the respondent was asked to choose a value on the offered numerical scale from 0 to 10. Pain intensity was expressed by values 0–100, which were calculated by multiplying the mean value obtained from the answers to questions 7, 8, and 9 by 10. The answer to question number 10, which referred to the time interval (number of days) of the respondent's absence from school or work, was also scored. 0–6 days = 0 points, 7–14 days = 1 point, 15–30 days = 2 points, and 31 or more days = 3 points. Changes in social contacts and work ability were expressed in values 0–100 and were the result of answers to questions 11, 12, and 13. The respondent chose a value on the offered numerical scale from 0 to 10.

Mean value (daily activities, social activities, and work activities) × 10, 0–29 = 0 points, 30–49 = 1 point, 50–69 = 2 points, and 70 or more = 3 points. After the evaluation and summing up of the results, the patients were classified into four categories: 0 – temporomandibular dysfunction had no impact on social contacts or work ability in the last six months. I – slightly altered social contacts and work ability. II – moderately altered social contacts and work ability. III – greatly altered social contacts with moderate work incapacity. IV – greatly altered social contacts with significant work incapacity.

During the study, patients were treated in three therapeutic groups, with three therapy modalities in order to reduce the symptoms of painful temporomandibular dysfunction. Treatment modalities included medication therapy with ibuprofen, a combination of ibuprofen and diazepam (Brufen®, Galenika-Abbott, Belgrade, Serbia; Bensedin®, Galenika-Abbott, Belgrade, Serbia), or a stabilizing maxillary occlusal splint. Ibuprofen (400 mg, twice a day) and diazepam (5 mg, one hour before bedtime), or ibuprofen alone (400 mg, twice a day), were administered. A maxillary stabilizing occlusal splint was made according to the following rules: the splint provided simultaneous contacts of the supporting cusps of the lower lateral teeth with the flat surface of the splint in the position of the central relation of the lower jaw. The splint was made of three-layered thermoplastic ERCOLOC-PRO film with a thickness of 3 mm. The therapy was administered over the course of a month, and the follow-up visits were conducted once a week.

The statistical software package SPSS for Windows (18.0) was used for data processing. At the beginning of the research, all variables were described using standard descriptive methods. Attributive features were described by absolute and relative numbers, and numerical measures of central tendency (arithmetic mean and median) and variability measures (standard deviation, minimum and maximum values), as well as the 95% confidence interval. The significance value was set at $p < 0.05$.

Univariate regression analysis was used to examine the relationship of each factor individually in relation to the VAS scale results after therapy. Factors that proved to be statistically significant in the univariate model were processed by multivariate analysis. Multivariate regression analysis was used to examine the factors of difference in order to distinguish outcomes and evaluate the effectiveness of the therapy.

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

RESULTS

Psychosocial parameters according to therapeutic groups are presented in Table 1.

A statistically significant difference was not recorded in work ability, social life, and daily activities in patients regardless of the chosen therapeutic approach (Table 2).

Table 1. Psychosocial parameters according to therapeutic groups

Psychosocial therapy parameters modality		Mean	Med.	SD	Min.	Max.	95% CI
Work ability	ibuprofen	1.75	1	2.113	0	7	0.62–2.88
	splint	0.9	0	2.100	0	9	-0.08–1.88
	ibuprofen + diazepam	2.13	1	2.232	0	6	0.26–3.99
Social life	ibuprofen	2.13	1	1.996	0	6	1.06–3.19
	splint	1.3	0.5	2.029	0	8	0.35–2.25
	ibuprofen + diazepam	2.5	2	2.268	0	6	0.6–4.4
Daily activity	ibuprofen	3.94	3	2.568	0	9	2.57–5.31
	splint	2.45	2	2.139	0	8	1.45–3.45
	ibuprofen + diazepam	3.13	2.5	1.458	2	6	1.91–4.34

Table 2. Psychosocial parameters in relation to the type of therapy

Psychosocial parameters (X ± SD)	Therapy modality			Significancea
	ibuprofen + diazepam	Splint	ibuprofen	
Work ability	2.13 ± 2.232	0.9 ± 2.1	1.75 ± 2.113	p = 0.069
Social life	2.5 ± 2.268	1.3 ± 2.029	2.13 ± 1.996	p = 0.144
Daily activity	3.13 ± 1.458	2.45 ± 2.139	3.94 ± 2.568	p = 0.091

*Statistically significant;
[a]Kruskal–Wallis test

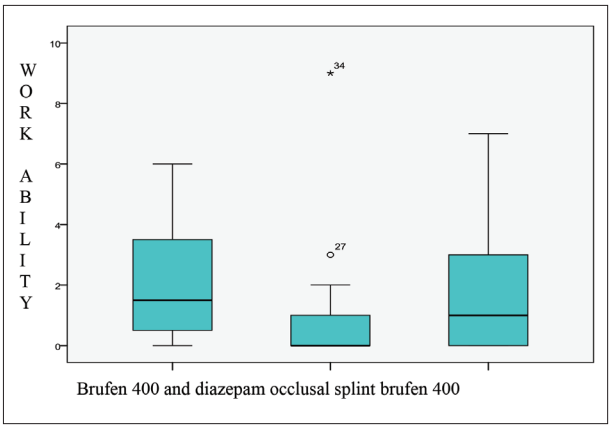

Figure 1. Work ability and therapy modality

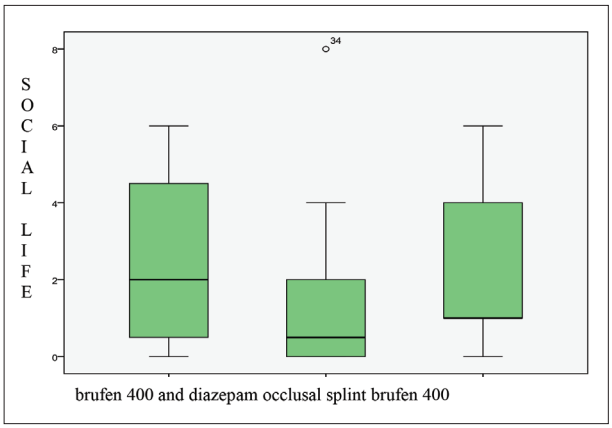

Figure 2. Social life and therapy modality

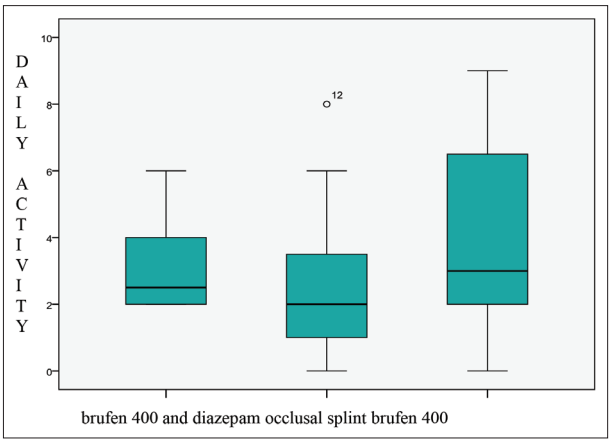

Figure 3. Daily activity and therapy modality

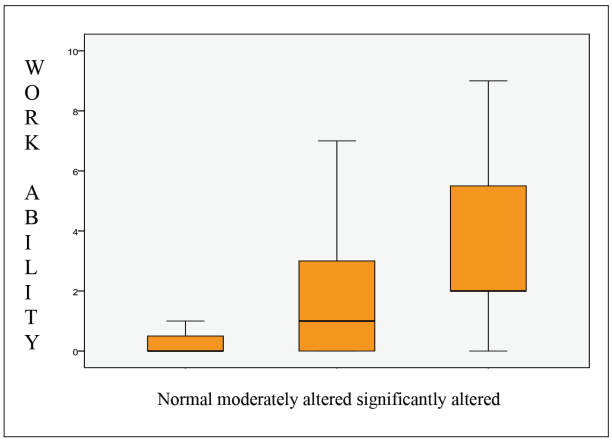

Figure 4. Work ability and psychosocial status

Table 3. Work ability and psychosocial status

Psychosocial status		Mean	Med.	SD	Min.	Max.	95% CI
Work ability	Normal	0.25	0	0.447	0	1	0.01–0.49
	Moderately altered	1.57	1	1.964	0	7	0.68–2.47
	Significantly altered	3.71	2	3.094	0	9	0.85–6.58

Table 4. Work ability and psychosocial status

Observed parameters (X ± SD)	Psychosocial status			Significance[a]
	Normal	Moderately altered	Significantly altered	
Work ability	0.25 ± 0.447	1.57 ± 1.964	3.71 ± 3.094	p = 0.002*

*Statistically significant;
[a]Kruskal–Wallis test

Table 5. Uni- and multivariate regression analysis in relation to VAS

Observed parameters	Univariate		Multivariate $R^2 = 0.528$	
	#B (95%CI)	Significance	B (95%CI)	Significance
Work ability	3.211 (0.818–5.604)	0.010*	-3.024 (-7.327–1.279)	0.162
Social life	4.088 (1.732–6.444)	0.001*	4.517 (0.516–8.517)	0.028*
Daily activity	2.600 (0.287–4.912)	0.029*	0.186 (-2.314–2.687)	0.881

*statistically significant;
#unstandardized coeficient B

Work ability, social life, and daily activities of the patients in relation to the effects of different therapy modalities are presented in Figures 1, 2, and 3.

Work ability and psychosocial status data are presented in Table 3.

There was a statistically significant difference in work ability between respondents in relation to psychosocial status (Table 4).

Work ability in relation to psychosocial status is presented in Figure 4.

Values of work ability were lower in respondents with altered psychosocial status.

In the assessment of pain on the VAS scale, a univariate regression analysis showed significant values for work ability, social life, and daily activities (Table 5). The assessment of social life was significant as a meritoric indicator (predictor) of post-therapy pain intensity in multivariate analysis. In all subjects with TMD, regardless of the post-therapy improvement, the impact of pain on their social life was significant.

DISCUSSION

In temporomandibular dysfunction, orofacial pain is frequently present and is accompanied by changes in patients' psychosocial status. This is reflected in changes in patients' behavior, as well as in reduced work ability [7, 8]. In this study, we wanted to evaluate, whether there is a decrease in work ability in patients with temporoman-

dibular dysfunction, in relation to the applied modality of therapy, as well as in relation to psychosocial factors. When assessing the psychosocial parameters (work ability, social life, and daily activities) based on therapy modality, no statistically significant difference was noted, meaning that the selection of therapy modality did not affect the psychosocial parameters (Tables 1 and 2). When work ability was assessed based on psychosocial status, it significantly differed depending on the level of psychosocial changes experienced by the patient. This suggests that patients with psychosocial challenges due to TMD had lower work ability (Tables 3 and 4). Univariate regression analysis was used to examine each factor individually in relation to the post-therapy effects. The assessment of work ability, social life, and daily activities showed significant values based on the VAS scale (0.010*, 0.001*, 0.029*) respectively. Factors statistically significant in the univariate model were processed by multivariate analysis, which showed that social life was a significant parameter of the post-therapy effect ($p = 0.028^*$).

Similarly, other authors suggested that psychological factors, especially work stress, may influence the development of TMD [2, 3, 5]. Psychological factors were associated with the severity of the TMD symptoms. On the other hand, the severity of TMD may influence the psychological status of patients. This influences not only work ability, but also patients' social life and daily activities, which diminish their quality of life [9–12].

CONCLUSION

Work ability is influenced by temporomandibular dysfunction proportionally to the level of psychosocial status.

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Умањена радна способност болесника са темпоромандибуларном дисфункцијом

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САЖЕТАК

Увод/Циљ Темпоромандибуларну дисфункцију (ТМД) прате орофацијални бол и психолошки проблеми који утичу на квалитет живота и радну способност болесника.
Хипотеза: На радну способност утичу промене психосоцијалног статуса током ТМД-а. Циљ ове проспективне студије био је да се процени смањење радне способности код болесника са ТМД-ом.

Методе Четрдесет четири болесника са ТМД-ом примљена су на Клинику за протетику Стоматолошког факултета Универзитета у Београду. Болесници су лечени медикаментозном терапијом ибупрофеном, или ибупрофеном и дијазепамом, или стабилизујућим максиларним оклузалним сплинтом. Клиничка и функционална процена засноване су на дијагностичком протоколу Истраживачки дијагностички критеријуми за ТМД. Протокол студије састојао се од података о клиничким знацима, нумеричке и скале бола *VAS*, и упитника везаног за радну способност (*Symptoms Check List, SCL-90R*). За обраду података коришћен је статистички софтверски пакет *SPSS for Windows* (18.0). Униваријантна регресиона анализа коришћена је за испитивање односа сваког фактора појединачно, а мултиваријантна регресиона анализа за испитивање разлике између фактора.

Резултати Није забележена статистички значајна разлика када су у питању радна способност, друштвени живот и свакодневне активности код болесника без обзира на изабрани терапијски приступ. Постоји статистички значајна разлика у радној способности између испитаника у односу на психосоцијални статус ($p = 0,002$). Униваријантна регресиона анализа показала је статистичку значајност код процене радне способности, друштвеног живота и свакодневних активности (0,010, 0,001, 0,029) респективно. У мултиваријантној регресионој анализи процена друштвеног живота показала је статистичку значајност ($p = 0,028$).

Закључак ТМД утиче на радну способност пропорционално нивоу измене психосоцијалног статуса.

Кључне речи: темпоромандибуларна дисфункција; орофацијални бол; радна способност

Appendix 1.

DIAGNOSTIC PROTOCOL RDC/TMD, Dworkin & LeResche (1992)

INSTITUTION …… PATIENT NO……
NAME AND SURNAME…… GENDER…… YEAR OF BIRTH……
OCCUPATION……TEL……

Read each question carefully and circle only one answer:

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

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

3. In the last 6 months, have you felt pain in the area of the face, jaw, temple, in front of the ear or in the ear itself? (Axis II)
   No 0
   Yes 1
   (if you have not felt pain in the last 6 months, go to question no. 14)

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

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

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

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

8. In the last 6 months, on a scale of 0-10, what was your worst pain? (Axis II)
(no pain) (excruciating pain)

0 1 2 3 4 5 6 7 8 9 10

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

10. In the last 6 months, how many days did you miss work or school because of pain in the face?
. . . . . days

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

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

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

14
a. Has it ever happened to you that you can't open your mouth all the way, ie. have you had the feeling that your jaw was "locked" in some position?
No 0
Yes 1
b. Was the limitation of mouth opening so pronounced that it prevented you from eating?
No 0
Yes 1

15
a. Do you hear a popping sound when you open or close your mouth or when you yawn?
No 0
Yes 1
b. Do you hear a grinding noise when opening, closing or yawning?
No 0
Yes 1

c. Have you been told or noticed that you grind your teeth or clench your jaw during sleep?
No 0
Yes 1
d. Do you grind your teeth or clench your jaw during the day?
No 0
Yes 1
e. Do you feel pain or have a feeling of stiffness in your jaw in the morning after waking up?
No 0
Yes 1
f. Do you have "ringing" or any noises in your ears?
No 0
Yes 1
g. Have you noticed a change in your bite when you bite down on your back teeth?
No 0
Yes 1

16
a. Have you had any other joint diseases (rheumatoid arthritis, lupus)?
No 0
Yes 1
b. Has anyone in your family had similar joint diseases?
No 0
Yes 1
c. Have you had or do you have swelling and pain in the area of the jaw joints?
No 0
Yes 1
d. Does the pain you feel in the area of the jaw joints last longer than a year?
No 0
Yes 1

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

18. Have you had a headache in the past 6 months?
No 0
Yes 1

19. What type of activity does the existing problem limit or prevent? (Axis II)
No Yes
a. chewing 0 1
b. drinking liquids 0 1
c. taking solid food 0 1
d. taking soft food 0 1
e. laughing 0 1
f. brushing teeth and washing face 0 1
g. yawning 0 1
h. swallowing 0 1

i. speech 0 1
j. facial appearance 0 1

20
a. Do you use any medications?
No 0
Yes 1
b. How long have you been using the medication? .....
c. What kind of medicines do you use?
d. What dose of medicine are you using?
e. Do you take medicine regularly?
No 0
Yes 1

Appendix 2.
Symptoms Check List, SLC-90 (Axis II)

Circle only one of the offered numbers given with the offered questions.
0 ..... not at all
1 ..... very little
2 ..... moderately
3 ..... expressed
4 ..... exceptionally

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

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Direct and indirect antimicrobial activity of the root extract of *Onosma visianii* Clem

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SUMMARY

Introduction/Objective The escalating resistance of numerous pathogens to currently available therapeutic agents has sparked a renewed interest in the search of novel antimicrobial compounds. Plants have become a potentially valuable source of these compounds. This study aimed to assess the direct and indirect antimicrobial effects of *Onosma visianii* Clem root extract on reference and clinical bacterial strains using broth microdilution and a modified ethidium bromide/acridine orange (EB/AO) fluorescence assay.

Methods The ethanolic extract obtained from the dried root of *Onosma visianii* Clem was used to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) by broth microdilution, as well as the half maximal inhibitory concentration (IC_{50}) values for reference and clinical bacterial strains. The EB/AO fluorescence method was employed to assess the effect on efflux pumps in methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE), using 50% of the MIC value of the extract.

Results The MIC, MBC, and IC_{50} values for Gram-positive bacteria were all below 15 μg/mL. The extract demonstrated strong antibacterial activity against VRE and, in particularly, against MRSA isolates (MIC = 7.81 μg/mL and MBC = 7.81 μg/mL). Furthermore, treatment with 50% of the MIC concentration produced a significant inhibitory effect on efflux pumps in Gram-positive bacteria, ranging from 18% to 26% compared with untreated cells.

Conclusion The root extract of *Onosma visianii* Clem exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, with particularly strong effects against VRE and MRSA. In addition, the observed inhibition of bacterial efflux pumps underscores its promise as a candidate for pharmacological evaluation of the antibiotic properties of the *Onosma visianii* Clem root extract.

Keywords: *Onosma visianii* Clem; antimicrobial effect; efflux pumps

INTRODUCTION

For decades, antibiotics have been widely used in human and veterinary medicine, as well as in agriculture, due to their high efficacy and low toxicity, and have proven highly successful in treating bacterial infections. The rising prevalence of antibiotic resistance, driven in part by indiscriminate drug use, poses a major threat that could return us to a pre-antibiotic era [1]. Among the mechanisms of multidrug resistance are bacterial efflux pumps, which expel antibiotics from the cell, thereby reducing their efficacy [2]. Inhibiting these pumps represents a potential strategy to enhance the susceptibility of resistant pathogens to existing antibiotics [3]. The escalating resistance of many pathogens to existing drugs has revitalized interest in plants as a rich yet underexplored source of novel antimicrobials, with only about 1% of traditionally used species having undergone phytochemical investigation [4]. Advances in modern research technologies now enable the detailed characterization of plant-derived compounds, spurring a growing number of microbiological studies on their antimicrobial properties as alternative therapies – an approach of particular relevance in developing regions, where up to 80% of people still rely on traditional medicines [5, 6]. *Onosma visianii* Clem (Boraginaceae) has long been employed in traditional medicine for treating a range of conditions, including wounds and burns, while other species in this family exhibit notable anti-inflammatory effects [7]. Building on our previous findings that naphthoquinone 1-7 (a shikonin derivative) isolated from *O. visianii* roots possesses cytotoxic and antibacterial activity [8], the present study aims to assess the direct and indirect antimicrobial effects of *O. visianii* root extract on reference and clinical bacterial strains using broth microdilution and a modified ethidium bromide/acridine orange (EB/AO) fluorescence assay.

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METHODS

The study was designed as an *in vitro* experimental study and was conducted at the Center for Microbiology, Institute of Public Health, Kragujevac, Serbia. The extract used in the experiments was obtained through ethanolic extraction of the dry root of *O. visianii* Clem at the University of Kragujevac, Faculty of Science, Kragujevac, Serbia.

Reagents and bacteria

The extract of *O.visianii* Clem was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Merck, St. Louis, MO, USA) and diluted with nutrient broth (Oxoid, Thermo Fisher Scientific, Basingstoke, United Kingdom) to achieve a concentration of 2000 μg/mL, ensuring that the concentration of DMSO in the stock did not exceed 3.5%. The initial volume of the stock used for examination was 100 μL [9].

The following reference strains [American Type Culture Collection (ATCC)] were included in the study: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 10145), and *Escherichia coli* (ATCC 25922). Additionally, clinical bacterial strains, including vancomycin-resistant *Enterococcus faecalis* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Pseudomonas aeruginosa*, were also examined.

Antibacterial activity

The McFarland (Hemofarm a.d., Vršac, Serbia) turbidity standard was used to standardize the approximate number of bacteria to 0.5 McFarland. Bacterial strains were subcultured and incubated at 37°C in a thermostat for 24 hours prior to use. A bacterial suspension with a density of 0.5 McFarland was prepared using the direct colony method. From this suspension, 100 μL was mixed with 2000 μL of physiological saline in a test tube, resulting in a bacterial concentration of 5×10^6 CFU/mL. From this bacterial suspension, 10 μL (equivalent to 5×10^4 bacteria) was inoculated into the wells of a microtiter plate, resulting in a final concentration of 5×10^5 CFU/mL [10].

In a microtiter plate with rounded-bottom wells [60 wells in five horizontal rows of a 96-well microtiter plate, (Spektar, Čačak, Serbia)], 100 μL of nutrient broth was added to each well. In the first two rows, 100 μL of the extract stock was added to the initial wells, and then it was serially diluted using the double dilution method into the subsequent wells. The concentrations of the extract ranged from 1000 to 0.488 μg/mL. Subsequently, 10 μL of the prepared bacterial suspension was added to each well. Validity control and blanks for the extract and nutrient broth were included. The inoculated microtiter plates were incubated for 24 hours at 37°C, and the minimum inhibitory concentrations (MICs) were determined [11]. The MIC is defined as the lowest concentration of the test substance at which no visible increase in bacterial growth is observed, indicated by clear wells.

The minimum bactericidal concentration (MBC) was determined by subculturing 10 μL of suspension from each well where no turbidity was observed onto nutrient agar.

After 24 hours of incubation, the plates were examined for visible growth, and the MBCs were determined. MBC is defined as the concentration of the extract that does not result in visible growth on the plate [11].

The direct cytotoxicity of the extract was calculated using equation (1).

$$\text{Cytotoxicity} = ((\text{Acontrol-Atest}) / \text{Acontrol}) \times 100 \quad (1)$$

In this context, A_control refers to the absorbance measurement of the validity control, which is a bacterial suspension without the extract, whereas A_test denotes the absorbance measurement of the test sample (bacterial suspension containing the extract) [9].

Efflux pump activity assay

To determine the activity of efflux pumps in bacteria, the dyes AO/EB (Sigma-Aldrich,) were used. AO is a dye that binds to deoxyribonucleic or ribonucleic acids (DNA or RNA) in organisms and fluoresces in different colors, aiding in the differentiation of cellular organelles. EB, when bound to DNA, exhibits enhanced orange fluorescence when exposed to ultraviolet light. The fluorescence intensity of AO/EB was used to assess the efflux pump activity of certain bacteria [12].

For the efflux pump activity assay, bacterial suspensions of two untreated clinical strains, *Pseudomonas aeruginosa* and MRSA, were prepared. The fluorescence intensity of AO/EB in both untreated bacteria decreased over time as the efflux pumps eliminated the dyes.

Since Gram-positive bacteria eliminated EB/AO more efficiently, the efflux pump activity assay using the *Onosma visianii* Clem root extract was conducted only on Gram-positive bacteria. Two clinical strains, VRE and MRSA, were treated with the *Onosma visianii* Clem root extract. The experimental procedure for each tested isolate was the same as that for determining the MIC values [13]. A bacterial suspension at 50% MIC was used for the efflux pump activity assay [14].

A bacterial suspension with a density of 0.5 McFarland was prepared using the direct colony method, as outlined in European committee on antimicrobial susceptibility testing guidelines [15]. A suspension was prepared on a slide consisting of 10 μL of bacterial suspension and 1 μL of dye solution (100 μg/mL AO and 100 μg/mL EB) dissolved in distilled water [16]. The slides were examined under a fluorescence microscope at 400 × magnification and photographed in real-time at three time points: 0 minutes, 15 minutes, and 30 minutes. The fluorescence intensity was determined using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Comparison was made with untreated strains of VRE and MRSA, using bacterial suspensions with a concentration of 5×10^5 CFU/mL [17]. The accumulation rate of EB/AO, characterized by an increase

Table 1. Antibacterial activity of *Onosma visianii* Clem root extract on reference and clinical bacteria strains

Species	MIC ± SD (μg/ml)	MBC ± SD (μg/ml)	IC_{50} ± SD (μg/ml)
Enterococcus faecalis (ATCC 29212)	**7.81 ± 4.50**	**7.81 ± 0**	9.74 ± 6.80
Staphylococcus aureus (ATCC 25923)	**0.48 ± 0**	**0.48 ± 0**	**< 0.48**
Pseudomonas aeruginosa (ATCC 10145)	500 ± 288.67	> 1000	185.59 ± 1.18
Escherichia coli (ATCC 25922)	250 ± 0	500 ± 0	244.08 ± 76.56
Enterococcus faecalis (VRE)	15.62 ± 0	15.62 ± 0	5.98 ± 0.76
Staphylococcus aureus (MRSA)	7.81± 4.50	7.81 ± 0	2.64 ± 1.03
Pseudomonas aeruginosa	1000 ± 0	> 1000	265.03 ± 72.84
Escherichia coli	1000 ± 0	> 1000	765.5 ± 0

MIC – minimum inhibitory concentration; SD – standard deviation; MBC – minimum bactericidal concentration; IC_{50} – half of the maximum inhibitory concentration; VRE – Vancomycin-resistant *Enterococci*; MRSA – Methicillin-resistant *Staphylococcus aureus*

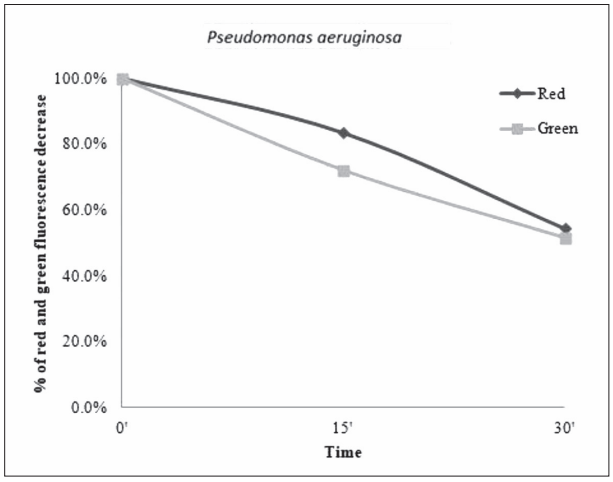

Figure 1. Fluorescence intensity ethidium bromide / acridine orange of untreated *Pseudomonas aeruginosa* at three time points: 0', 15', and 30'

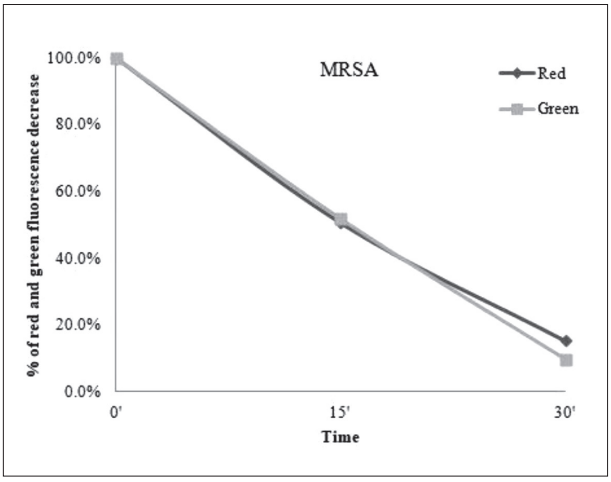

Figure 2. Fluorescence intensity ethidium bromide / acridine orange of untreated methicillin-resistant *Staphylococcus aureus* at three time points: 0', 15', and 30'

in red and green fluorescence intensity, was determined as the fluorescence index relative to the baseline (0 minutes) for both treated and untreated samples at the three time points. Statistical data processing was performed using standard deviation, and the IC_{50} value was calculated by measuring absorbance at 450 nm and using GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA).

RESULTS

Antibacterial activity

O. visianii Clem root extract displayed potent antibacterial activity, with markedly higher efficacy against Gram-positive than Gram-negative bacteria (Table 1). Among reference strains, *Staphylococcus aureus* (ATCC 25923) was the most susceptible (MIC, MBC, and IC_{50} = 0.48 μg/mL), whereas *Enterococcus faecalis* (ATCC 29212) showed reduced sensitivity (MIC/MBC = 7.81/7.81 μg/mL; IC_{50} = 9.74 μg/mL). In contrast, Gram-negative reference strains were far less susceptible, with MIC/MBC values of 250/500 μg/mL for *Escherichia coli* (ATCC 25922) and 500/1000 μg/mL for *Pseudomonas aeruginosa* (ATCC 10145).

A similar pattern was observed for clinical isolates (Table 1). The extract exhibited the greatest activity against MRSA (MIC/MBC = 7.81 μg/mL; IC_{50} = 2.64 μg/mL), followed by VRE (MIC/MBC = 15.62 μg/mL; IC_{50} = 5.98 μg/mL). In contrast, clinical *P. aeruginosa* and *E. coli* isolates were highly resistant, with MIC/MBC values of 1000 μg/mL and IC_{50} values of 265.03 and 765.5 μg/mL, respectively.

Efflux pump activity assay

To investigate the potential of *O. visianii* Clem root extract to interfere with bacterial drug-resistance mechanisms, we assessed efflux pump activity in reference and clinical strains using AO and EB fluorescence assays. Fluorescence intensity measurements revealed that, after 30 minutes, *P. aeruginosa* eliminated approximately 50% of the dyes, whereas MRSA exhibited markedly stronger efflux pump activity, expelling about 90% within the same time frame (Figures 1 and 2).

Because the extract displayed stronger inhibitory effects on Gram-positive bacteria, which also showed higher baseline efflux activity, subsequent assays focused on MRSA and VRE. In MRSA treated with *Onosma visianii* Clem root extract at 50% of its MIC, 72.9% (EB) and 64.8% (AO) of the dyes were retained after 30 min compared with the initial 100%, whereas untreated MRSA retained only 55.6% and 44%, respectively, indicating extract-mediated inhibition of efflux pumps (Figure 3).

Similarly, in VRE treated with *O. visianii* Clem root extract at 50% MIC, 80.9% (EB) and 74.2% (AO) of the

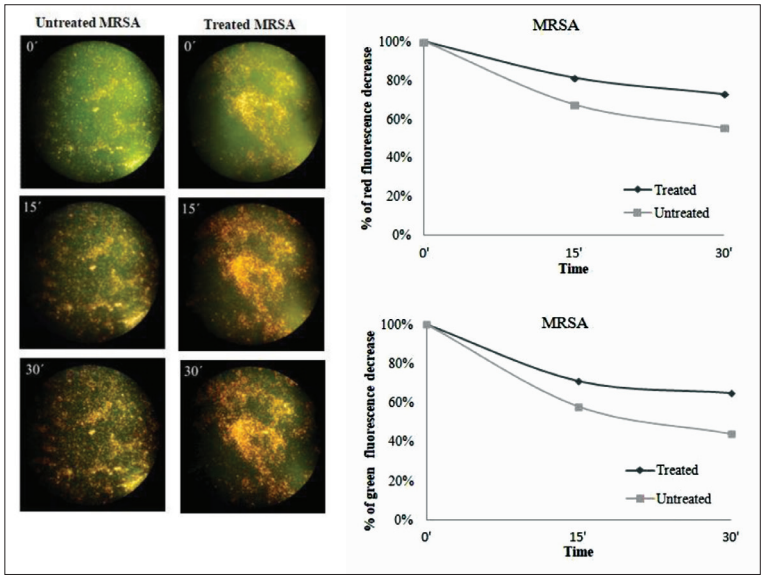

Figure 3. Fluorescence intensity ethidium bromide / acridine orange of treated and untreated methicillin-resistant *Staphylococcus aureus* (MRSA) with *Onosma visianii* Clem root extract at the concentration 50% of the minimum inhibitory concentrations value at three time points: 0', 15', and 30'

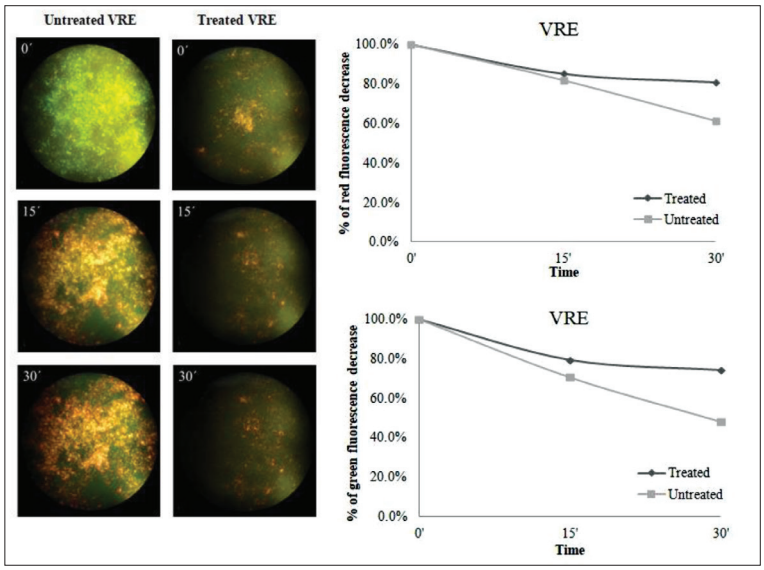

Figure 4. Fluorescence intensity ethidium bromide / acridine orange of treated and untreated vancomycin-resistant *Enterococcus faecalis* (VRE) with *Onosma visianii* Clem root extract at a concentration of 50% minimum inhibitory concentrations value at three time points: 0', 15', and 30'

dyes were retained after 30 min compared with 61.1% and 48% in untreated controls, again demonstrating reduced efflux activity in the presence of the extract (Figure 4).

DISCUSSION

The use of antibiotics in healthcare has been widespread for many years; however, their overuse and unregulated application have led to the emergence of antibiotic resistance, which has become a global public health concern. Consequently, increasing attention has been directed toward investigating the effects and mechanisms of plant extracts, as numerous plant species have been employed for centuries in traditional medicine.

Different mechanisms of action have been identified for the antibacterial activity of medicinal herbs. Some plant extracts inhibit cell wall synthesis, while others accumulate in the bacterial membrane and disrupt its structure and function, leading to cell damage and death [18]. Moreover, certain plant extracts have shown effectiveness against bacterial efflux pumps, which play a key role in mediating multidrug resistance in both Gram-positive and Gram-negative bacteria [19]. Efflux pumps perform an essential function in safeguarding bacteria from toxic materials by actively expelling drugs and toxins. Inhibiting or weakening these efflux pumps can improve the efficacy of antibiotics against resistant bacterial strains [20, 21]. The present study examined the antibacterial properties and the efficacy of the dried root extract of *Onosma visianii* Clem in inhibiting efflux pumps.

Previous results obtained from studies conducted at our university which demonstrated the antibacterial and cytotoxic effects of *Onosma visianii* Clem represent the basis for our research. Vukić et al. [8] conducted a study examining the antibacterial activity of seven distinct naphthoquinones extracted from the dried root of *Onosma visianii* Clem.

Each of the individual naphthoquinones demonstrated efficacy against clinical Gram-positive and Gram negative bacteria with MIC50 and MIC90 values on Gram-positive varying from 6.40 μg/mL to 12.79 μg/mL and 6.82 μg/mL to 13.60 μg/mL, respectively [8]. Conversely, our research assessed the activity of the full ethanolic extract, which encompasses all secondary metabolites to determine whether it is more effective, and compared it against a variety of clinical and reference bacteria. Additionally, our MIC values exhibited enhanced antimicrobial effects against *Staphylococcus aureus* (MIC 0.48 μg/mL) when compared to all isolates evaluated in the prior study. On the other hand, the activity against our Gram-negative bacteria was lower. Our findings also indicated the bactericidal properties of the extract and included IC_{50} values.

Moreover, we advanced our research by evaluating the extract's efficacy against strains of MRSA and VRE. A related study on the extract of *Consolida orientalis* reported MIC values for MRSA between 0.15 and >5 mg/mL, while VRE exhibited MIC values from 0.625 to 2.5 mg/mL. In comparison, our results demonstrate much stronger activity [22]. In another investigation, Amri et al. [23] analyzed the ethanolic extract of *Eupatorium odoratum* against Gram-positive and Gram-negative bacteria, finding no effectiveness against Gram-negative strains. Takongmo Matsuete et al. [24] also noted weaker activity, with a reported MIC value for *Pseudomonas aeruginosa* of > 1000 μg/mL.

The variation in vulnerability can be explained by the distinct structural characteristics of the cell walls found in Gram-positive and Gram-negative bacteria [25]. Gram-negative bacteria typically have a more intricate mechanism for multidrug resistance because of their double membrane structure, which allows for the function of tripartite efflux pump systems [26]. The efflux pump activity assay revealed that the extract of *Onosma visianii* Clem inhibited the efflux pump activity in both MRSA and VRE, as demonstrated by the higher retention of fluorescent dyes in treated bacteria compared to untreated bacteria. Efflux pumps are crucial in contributing to multidrug resistance, and blocking their function can improve the potency of antibiotics [27]. In a comparable study, the bioflavonoid *Scutellaria baicalensis* was shown to affect the efflux pumps of *Staphylococcus aureus*, with EB dye retention at 73% after 30 minutes [28]. Similarly, Anokwah et al. [29] investigated the ethanolic extract of *Loeseneriella Africana*. When *S. aureus* was treated with 50% MIC, it retained 50% EB after 60 minutes, a value significantly lower than that observed in our study. Previous reports have also suggested that polyphenols rich plants can inhibit the efflux pumps Multidrug Resistance 1 and LmrP found in *E. faecalis*, which are critical contributors to multidrug resistance [30]. Our results are consistent with recent findings showing that bioactive compounds in plant extracts may combat multidrug-resistant bacteria by targeting efflux pump mechanisms [31]. The effectiveness of the extract is influenced by its chemical composition, the extraction method, and the timing of plant collection [32]. Despite the long history of this plant in traditional medicine, standardization of the extract is necessary before practical application. In addition, it is essential to evaluate its potential toxicity and the stability of its active components. Although the extract demonstrates promising *in vitro* activity, further standardization, toxicity testing, and stability assessment are required before practical application.

CONCLUSION

The root extract of *Onosma visianii* Clem exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, with particularly strong effects against VRE and MRSA. In addition, the observed inhibition of bacterial efflux pumps underscores its promise as a candidate for pharmacological evaluation of the antibiotic properties of the *Onosma visianii* Clem root extract.

AUTHOR CONTRIBUTIONS

SH played a key role in conceptualizing and designing the study, acquiring and processing data, and drafting the manuscript. SM contributed to the study's conceptual framework, analyzed and interpreted the data, and revised the manuscript. SP participated in the conception and design of the study and was responsible for data analysis and interpretation. AT was involved in study design and contributed to data analysis and interpretation. SR took part in data analysis and interpretation and assisted with manuscript revisions. IP contributed to the study's conceptualization and the acquisition, analysis, and interpretation of data. AH was engaged in data analysis and interpretation. MM was involved in the conception and design of the study, supervised data acquisition, analysis, and interpretation, and revised the manuscript.

Conflict of interest: None declared.

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Директна и индиректна антимикробна активност екстракта корена *Onosma visianii* Clem

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САЖЕТАК

Увод/Циљ Растућа резистенција бројних патогена на тренутно доступне терапијске агенсе изазвала је поновно интересовање за истраживање нових антимикробних једињења. Биљке су постале потенцијално вредан извор ових једињења. Циљ ове студије био је да се процени директна и индиректна антимикробна активност екстракта корена *Onosma visianii* Clem на референтне и клиничке бактеријске сојеве, користећи методу микродилуције у бујону и модификовани етидијум бромид / акридин оранџ (*EB*/*AO*) тест флуоресцентне детекције.

Методе Етанолни екстракт припремљен из сувог корена *Onosma visianii* Clem коришћен је за одређивање минималних инхибиторних концентрација (*MIC*) и минималних бактерицидних концентрација (*MBC*) методом микродилуције у бујону, као и за одређивање вредности половине максималне инхибиторне концентрације (IC_{50}) за референтне и клиничке бактеријске сојеве. Метода *EB*/*AO* флуоресцентног бојења коришћена је за процену утицаја на ефлукс пумпе код сојева *Staphylococcus aureus* резистентног на метицилин (*MRSA*) и *Enterococcus faecalis* резистентног на ванкомицин (*VRE*), користећи концентрацију од 50% *MIC* вредности екстракта.

Резултати Вредности *MIC*, *MBC* и IC_{50} за Грам-позитивне бактерије биле су испод 15 *μg/mL*. Екстракт је показао јако антимикробно дејство према *VRE* и посебно према *MRSA* сојевима (*MIC* = 7,81 *μg/mL* и *MBC* = 7,81 *μg/mL*). Поред тога, третман са 50% *MIC* концентрације изазвао је значајну инхибицију ефлукс пумпи код Грам-позитивних бактерија, у распону од 18% до 26% у односу на нетретиране ћелије.

Закључак Екстракт корена *Onosma visianii* Clem показао је антимикробно дејство према Грам-позитивним и према Грам-негативним бактеријама, са нарочито израженим ефектом према *VRE* и *MRSA*. Осим тога, уочена инхибиција бактеријских ефлукс пумпи истиче обећавајући потенцијал екстракта корена биљке *Onosma visianii* Clem као кандидата за фармаколошка испитивања његових могућих антибиотских својстава.

Кључне речи: *Onosma visianii* Clem; антимикробно дејство; ефлукс пумпе

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The influence of sedentary and shift work on work ability and risk factors for cardiovascular diseases

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SUMMARY
Introduction/Objective In modern society, there is an increasing number of occupations that require sedentary work, and an increasing need for such work to be performed in shifts. This study examined how these two work modalities affect the work ability and health status of workers.
Methods The study included taxi drivers and sanitary workers, further subclassified into two groups depending on their shift work. The Work Ability Index (WAI) questionnaire was used to assess the work ability of participants. Also, anthropometric measurements and the determination of standard biochemical parameters and the levels of soluble adhesion molecules – intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) in blood samples, before and after work, were used to evaluate the impact of sedentary and shift work on the risk factors for cardiovascular diseases (CVDs).
Results Night-shift taxi drivers (TD-NS) had significantly lower total WAI scores compared to day-shift sanitary workers (SW-DS). The WAI3, anthropometric measurements, and lipid profile of study participants indicated that these two groups also differed in the number of obese and hypertensive individuals. Although the study groups did not differ in sICAM-1 levels, sVCAM-1 levels of TD-NS were significantly higher than those of SW-DS both before and after work.
Conclusion Sedentary and shift work have synergistic effects in reducing work ability and promoting CVD development by modifying traditional risk factors.
Keywords: sedentary work; shift work; work ability; obesity; hypertension

INTRODUCTION

The World Health Organization (WHO) has recognized the improvement of working conditions and working environment as one of the strategic objectives of public health, promoting and maintaining the highest level of physical, mental, and social well-being of workers in all occupations [1]. Due to the increased number of sedentary work positions nowadays, and given that a sedentary lifestyle is associated with multiple health problems, including cardiovascular diseases (CVDs), obesity, diabetes mellitus, and early mortality [2, 3], work-related sedentary behavior is becoming an increasingly recognized problem in occupational medicine.

CVDs continue to be a major public health problem and a leading cause of morbidity and mortality globally. A recent meta-analysis showed that sedentary behavior increases the risk of fatal and non-fatal CVDs by 30%, and, interestingly, a significantly higher risk of developing coronary heart disease (47%) compared to stroke (15%) was observed [4]. One plausible explanation is that sedentary behavior modulates traditional CVD risk factors such as increased body mass index (BMI), hypertension, elevated heart rate, and adiposity. However, sedentary behavior also leads to the dysfunction of vascular endothelium, which is a hallmark of the major CVD risk factors and an early event during CVD development [2, 3, 5]. In particular, prolonged sitting has been shown to impair the normal hemodynamics of peripheral arteries, in terms of reduced blood flow and shear stress. Consequently, enhanced endothelial oxidative stress, accompanied by decreased nitric oxide (NO) production, increased production of endothelin-1 (ET-1) and other pro-inflammatory mediators, leads to vascular dysfunction [6].

In addition to occupational sedentary behavior, shift work can also have several adverse effects on health, and most of the harmful effects were attributed to disruption of the circadian rhythm and consequent hypertension, dyslipidemia, insulin resistance, and obesity [3, 5], all known as CVD risk factors.

In the current study, we investigated the effects of occupational sedentary behavior and shift work on subjects' work ability and overall presence of morbidities. To determine how these two work modalities affect risk factors for CVD development, we analyzed subjects'

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Table 1. Socio-demographic characteristics of the study participants

Characteristics		Day-shift taxi drivers	Night-shift taxi drivers	Day-shift sanitary workers	Night-shift sanitary workers	p
Age	Years (mean ± SD)	40.25 ± 7.85	38.3 ± 7.96	37.95 ± 7.86	37 ± 9.32	0.631[1]
Gender	male n (%)	16 (80)	19 (95)	13 (65)	17 (85)	0.104[2]
	female n (%)	4 (20)	1 (5)	7 (25)	3 (5)	
Marital status	married n (%)	9 (45)	7 (35)	10 (50)	7 (35)	0.754[2]
	single n (%)	5 (25)	6 (30)	2 (10)	8 (40)	
	informal relationship n (%)	2 (10)	3 (15)	1 (10)	2 (10)	
	Separated n (%)	1 (5)	2 (10)	3 (15)	2 (10)	
	Divorced n (%)	3 (15)	2 (10)	4 (20)	1 (5)	
Level of education	primary n (%)	2 (10)	3 (15)	6 (30)	8 (40)	0.077[2]
	Secondary n (%)	15 (75)	16 (80)	14 (70)	12 (60)	
	Tertiary n (%)	3 (15)	1 (5)	0 (0)	0 (0)	
Total years of service	Years (mean ± SD)	16.20 ± 8.13	14.90 ± 7.99	14.65 ± 8.81	13.55 ± 9.01	0.774[1]
Years of service at the current workplace	Years (mean ± SD)	6.2 ± 2.63	5.45 ± 2.5	8.5 ± 5.83	8.75 ± 5.42	0.186[1]

[1]Kruskal–Wallis test;
[2]χ^2 test

clinical-anthropometric and laboratory-biochemical parameters, as well as the levels of soluble adhesion molecules, intercellular adhesion molecule-1 (sICAM-1), and vascular cell adhesion molecule-1 (sVCAM-1), as parameters of vascular dysfunction.

METHODS

Study population

The study enrolled 80 subjects, classified into 4 groups: day-shift taxi drivers (TD-DS) (n = 20), night-shift taxi drivers (TD-NS) (n = 20), day-shift sanitary workers (SW-DS) (n = 20), and night-shift sanitary workers (SW-NS) (n = 20). Study participants were recruited through periodic medical examinations at the Institute for Workers' Health Protection, Niš, Serbia, in the period from January to May 2024. All study participants were promptly informed about the relevant details concerning their participation in the study and gave written informed consent; the study was approved by the Ethics Committee. The main socio-demographic characteristics of the study groups are presented in Table 1.

Accordingly, the study groups were homogeneous in terms of age structure (H = 1.727; p = 0.631), gender (χ^2 = 6.154; p = 0.104), marital status (χ^2 = 8.390; p = 0.754), and the level of education (χ^2 = 11.403; p = 0.077). Also, there were no statistically significant differences between the groups regarding the years of total work experience (H = 1.11; p = 0.774) and the years of work experience at the current workplace (H = 4.81; p = 0.186).

Work Ability Index (WAI) Questionnaire

All study participants completed an anonymous self-report questionnaire regarding their work ability. In order to assess the impact of sedentary and shift work on participants' work ability, a standardized questionnaire, Work Ability Index (WAI) [7], was used. In addition to the WAI questionnaire, study participants were also asked to anonymously report their current occupation, age, total years of work experience, and length of service at their current workplace.

Anthropometric and clinical parameters

Anthropometric measurements included body weight, height, waist, and hip circumference. A digital body scale with a stadiometer (SD301, Birotehna, Smederevo, Serbia) with a capacity of 250 kg and an accuracy of 0.1 kg was used to measure participants' body weight and height. BMI was calculated as the ratio of body weight (kg) to the square of body height (m^2). Waist circumference was measured using a non-stretch tape placed parallel to the floor at the midpoint between the iliac crest and the lowest rib. Hip circumference was measured around the widest portion of the buttocks. Based on the obtained values, the waist-to-hip and the waist-to-height ratio was calculated. Clinical parameters included measurement of systolic and diastolic blood pressure and heart rate. Blood pressure and heart rate were measured using the digital device M3 Comfort (Omron Healthcare, Kyoto, Japan), according to the manufacturer's instructions.

Laboratory-biochemical parameters

The subjects' blood was sampled by venipuncture of the medial cubital vein, before the start of the work shift and immediately after. Biochemical analyses included the hemogram, lipidogram, glycemia, and C-reactive protein. All analyses were carried out at the Institute for Workers' Health Protection, Niš, Serbia, according to a standardized protocol.

Serum levels of sICAM-1 and sVCAM-1 were determined by enzyme-linked immunosorbent assay (ELISA). Briefly, after blood sampling, 2 mL of serum was separated, transported on ice to the Scientific Research Center for Biomedicine of the Faculty of Medicine, University of Niš, and stored at -80°C until analysis. Before analysis, samples were slowly thawed, mixed gently, and then sICAM-1 and

sVCAM-1 levels were determined using Human ICAM-1/CD54 Allele-specific Quantikine ELISA-kit and Human VCAM-1/CD106 Quantikine ELISA-kit (R&D Systems, Minneapolis, MN, USA), respectively. The optical density of the samples was read on Spark Multimode Plate Reader (Tecan Trading AG, Männedorf, Switzerland) at a wavelength of 450 nm. sICAM-1 and sVCAM-1 concentrations (ng/mL) were calculated using software TableCurve 2D v5.0 (Grafiti LLC, Palo Alto, CA, USA).

Statistical analysis

Statistical parameters were collected in Microsoft Office Excel 2013 (Microsoft, Redmond, WA, USA), while data analysis was performed using IBM SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics included standard statistical methods for qualitative and quantitative assessment of the obtained parameters. Absolute numbers, relative numbers (%), arithmetic mean ($\bar{x}$), standard deviation (SD), median, minimum, and maximum values were used as measures of central tendency. The normality of the dataset distribution was tested with the Shapiro-Wilk test. The comparison of numerical values between the four groups of study participants, if the distribution was normal, was performed using the ANOVA test and Tukey's honestly significant difference (HSD) test for post hoc analyses. In the case in which the datasets were nonnormally distributed, the Kruskal-Wallis test was used, followed by post hoc Dunn's test using a Bonferroni correction for multiple comparisons. Comparison of numerical values between the two groups, if the data distribution was normal, was performed using a paired Student's t-test. In cases where the distribution was not normal, the Mann-Whitney test was used. The χ^2 test was used to test the statistical significance of absolute frequency differences between samples. Pearson's correlation coefficient was used to determine the association between variables. p values < 0.05 were considered statistically significant.

Ethics: The study was approved by the Ethics Committee of the Faculty of Medicine, University of Niš, Serbia (number: 12-16502/2-8, date: 21.12.2023).

RESULTS

Effects of sedentary and shift work on work ability

The work ability index of the study participants by items and its correlations with age, total years of service, and years of service at the current workplace are shown in Figure 1.

The study groups did not differ significantly in terms of the particular items of the work ability questionnaire (Figure 1A). However, in the item WAI3, which concerns the number of current diseases diagnosed by a physician, it was observed that hypertension is significantly more common among TD-NS compared to SW-DS ($p = 0.002$; $\chi^2 = 9.231$). Also, TD-NS were significantly more obese compared to SW-DS ($p = 0.008$; $\chi^2 = 7.059$). Although there were no significant differences in the scores of WAI items, the overall WAI score differed significantly between the study groups ($p = 0.009$; $H = 11.552$). Post hoc comparisons indicated that TD-NS had significantly lower

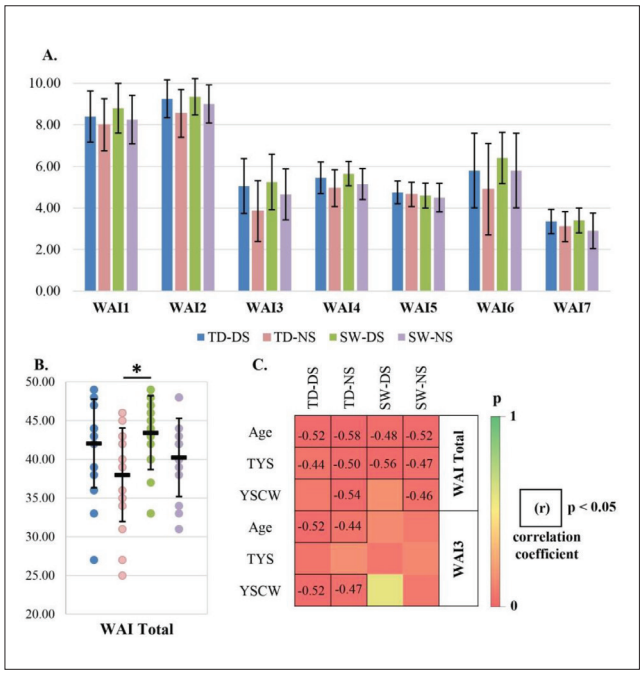

Figure 1. Work ability index (WAI) of study participants: A. individual WAI item scores; B. total WAI score, and C. correlation analyses;
TD-DS – taxi drivers day shift; TD-NS – taxi drivers night shift; SW-DS – sanitary workers day shift; SW-NS – sanitary workers night shift; TYS – total years of service; YSCW – years of service at the current workplace

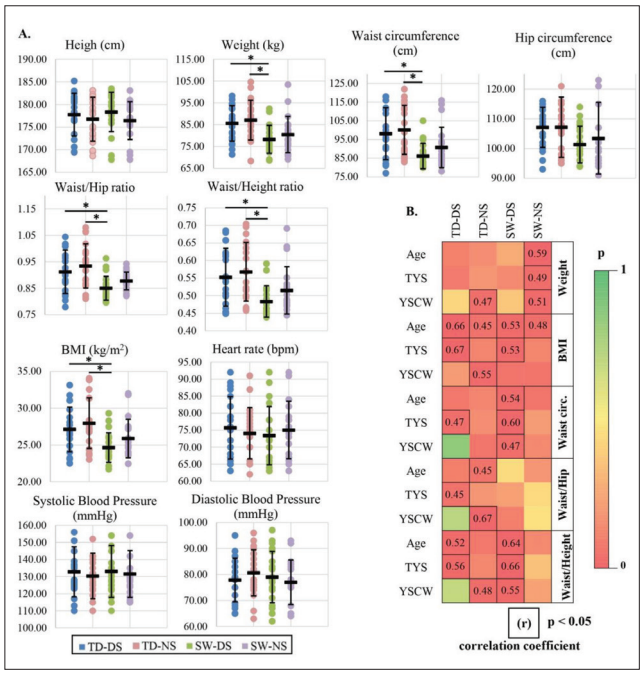

Figure 2. Anthropometric and clinical parameters of study participants: A. statistically significant differences and B. correlation analyses;
TD-DS – taxi drivers day shift; TD-NS – taxi drivers night shift; SW-DS – sanitary workers day shift; SW-NS – sanitary workers night shift; BMI – body mass index; TYS – total years of service; YSCW – years of service at the current workplace

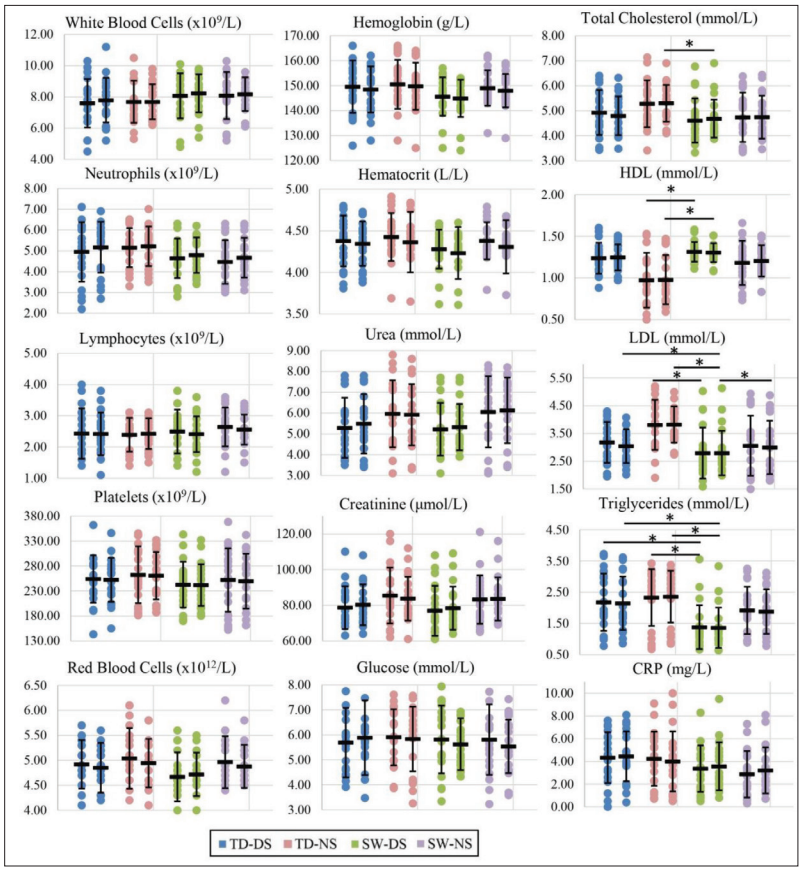

Figure 3. Biochemical parameters of study participants before (first column) and after (second column) work shift;
TD-DS – taxi drivers day shift; TD-NS – taxi drivers night shift; SW-DS – sanitary workers day shift; SW-NS – sanitary workers night shift; HDL – high-density lipoprotein; LDL – low-density lipoprotein; CRP – C-reactive protein

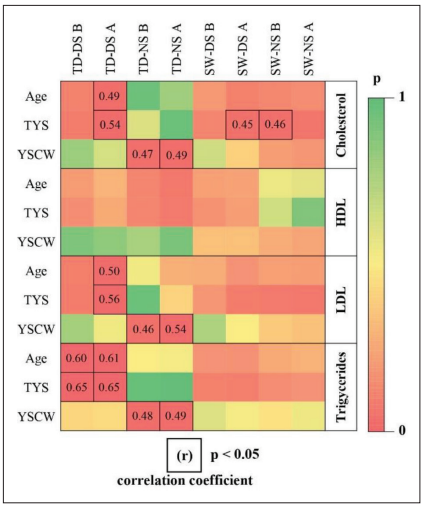

Figure 4. Correlation analysis of the lipid profile of study participants with the characteristics of their work engagement;
TD-DS – taxi drivers day shift before (B) and after (A) the shift; TD-NS – taxi drivers night shift before (B) and after (A) the shift; SW-DS – sanitary workers day shift before (B) and after (A) the shift; SW-NS – sanitary workers night shift before (B) and after (A) the shift; TYS – total years of service; YSCW – years of service at the current workplace; HDL – high-density lipoprotein; LDL – low-density lipoprotein

WAI scores than SW-DS ($p = 0.002$; $z = 3.061$) (Figure 1B). Correlation analyses showed that the total WAI score was negatively correlated with age and total years of work experience in all study groups; however, a negative correlation with years of work at the current workplace was observed only in the groups of TD-NS and SW-NS. Conversely, the WAI3 score was negatively correlated with age and years of work at the current job only in taxi drivers (Figure 1C).

Effects of sedentary and shift work on anthropometric and clinical parameters

As observed in WAI3, anthropometric measurements showed that TD-NS were significantly more obese than SW-DS, according to increased body weight ($p = 0.001$; $z = 3.249$), BMI ($p < 0.001$; $z = 3.436$), waist circumference ($p < 0.001$; $z = 3.722$), waist-to-hip ratio ($p < 0.001$; $z = 3.636$), and waist-to-height ratio ($p < 0.001$; $z = 3.718$). TD-DS also had significantly higher body weight ($p = 0.005$; $z = 2.817$), BMI ($p = 0.008$; $z = 2.654$), waist circumference ($p < 0.01$; $z = 3.065$), waist-to-hip ratio ($p < 0.005$; $z = 2.832$), and waist-to-height ratio ($p < 0.005$; $z = 2.787$) compared to SW-DS. There were no statistically significant intergroup differences in the monitored clinical parameters, including heart rate and systolic and diastolic blood pressure (Figure 2A).

In general, anthropometric parameters of obesity showed a tendency to positively correlate with the age and total work experience of the study subjects, which sporadically reached the level of statistical significance. However, only in the group of TD-NS was there a constant positive correlation between years of work experience at the current workplace and obesity parameters, except for waist circumference (Figure 2B).

Effects of sedentary and shift work on laboratory-biochemical parameters

The values of biochemical parameters in the blood differed significantly between the studied groups (Figure 3).

High-density lipoprotein (HDL) levels of TD-NS were significantly lower than those of SW-DS, both before ($p < 0.001$; $z = 3.665$) and after ($p < 0.001$; $z = 3.689$) the work shift. TD-NS also had significantly higher low-density lipoprotein (LDL) levels compared to SW-DS before the start of the shift ($p = 0.004$; $q = 4.942$). After the shift, LDL levels in this subject group were higher in comparison to TD-DS ($p = 0.004$; $z = 2.869$) as well as SW-DS ($p < 0.001$; $z = 3.647$) and SW-NS ($p < 0.001$; $z = 3.328$). A similar trend was observed for triglycerides. Both TD-NS ($p = 0.001$; $z = 3.198$) and TD-DS ($p = 0.006$; $z = 2.749$) had increased levels of triglycerides compared to SW-DS

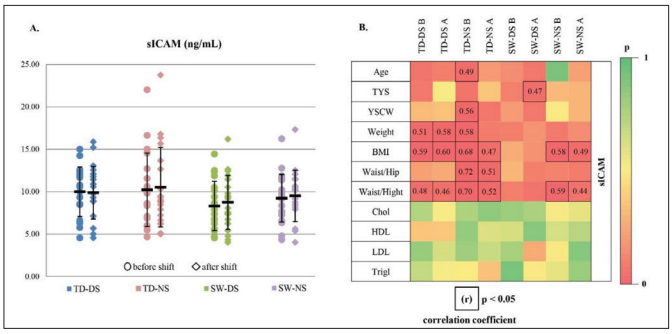

Figure 5. sICAM levels of study participants: A. statistically significant differences and B. correlation analyses;
TD-DS – taxi drivers day shift before (B) and after (A) the shift; TD-NS – taxi drivers night shift before (B) and after (A) the shift; SW-DS – sanitary workers day shift before (B) and after (A) the shift; SW-NS – sanitary workers night shift before (B) and after (A) the shift; TYS – total years of service; YSCW – years of service at the current workplace; BMI – body mass index; Chol – cholesterol; HDL – high-density lipoprotein; LDL – low-density lipoprotein; Trigl – triglycerides

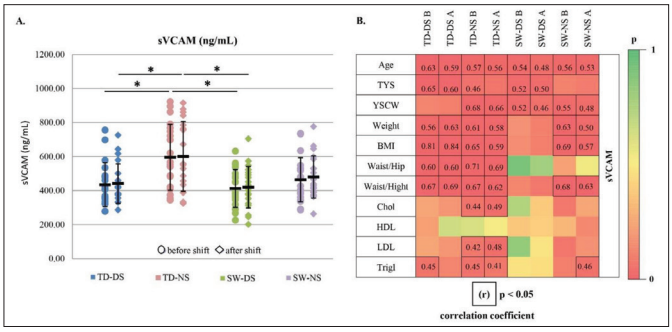

Figure 6. sVCAM levels of study participants: A. statistically significant differences and B. correlation analyses;
TD-DS – taxi drivers day shift before (B) and after (A) the shift; TD-NS – taxi drivers night shift before (B) and after (A) the shift; SW-DS – sanitary workers day shift before (B) and after (A) the shift; SW-NS – sanitary workers night shift before (B) and after (A) the shift; TYS – total years of service; YSCW – years of service at the current workplace; BMI – body mass index; Chol – cholesterol; HDL – high-density lipoprotein; LDL – low-density lipoprotein; Trigl – triglycerides

before starting the shift. The same was observed after the shift had ended ($p < 0.001$; $z = 3.668$ and $p = 0.002$; $z = 3.106$, respectively). No significant changes were recorded in the levels of the tested parameters before and after the shift within the same group of subjects (Figure 3).

Considering that the most significant differences in the biochemical parameters of the study participants were determined in the lipid profile, we correlated them with the characteristics of the participants' work engagement (Figure 4). A significant positive correlation between years of experience at the current workplace and levels of total cholesterol, LDL, and triglycerides was observed only in the group of TD-NS. In the group of TD-DS, a positive correlation was noted between age and total length of service with levels of total cholesterol, LDL, and triglycerides, mainly after the end of the shift.

Effects of sedentary and shift work on vascular dysfunction

The function of the subjects' vascular endothelium was assessed by the levels of soluble adhesion molecules, sICAM-1 and sVCAM-1.

Levels of sICAM-1 did not differ significantly between study groups before ($p = 0.279$; $h = 3.843$) and after the shift ($p = 0.695$; $h = 1.444$), nor were there significant differences between sICAM-1 levels before and after the shift within the same group (Figure 5A). In general, sICAM-1 levels were positively correlated with most anthropometric parameters of obesity in all study groups except for SW-DS (Figure 5B).

In contrast, sVCAM-1 levels of TD-NS were significantly higher than those of TD-DS and SW-DS both before ($p = 0.005$; $z = 2.830$ and $p = 0.002$; $z = 3.103$, respectively) and after the shift ($p = 0.008$; $z = 2.660$ and $p = 0.004$; $z = 2.871$, respectively) (Figure 6A). Similar to sICAM-1 levels, sVCAM-1 levels did not differ significantly before and after the shift within the same study group (Figure 6A).

The levels of sVCAM-1 were positively correlated with age in all study groups. In most groups, sVCAM-1 levels were also positively correlated with years of total work experience and years of service at the current job. Similar to sICAM-1, sVCAM-1 was positively correlated with anthropometric parameters of obesity in all study groups except for SW-DS. In addition, a significant positive correlation of sVCAM-1 with cholesterol, LDL, and triglyceride levels was observed in the group of TD-NS (Figure 6B).

DISCUSSION

During working hours, sanitary workers are constantly physically active, unlike taxi drivers, whose occupation is extremely sedentary. Therefore, we examined how these two work modalities affect the work ability and health status of workers, taking into account shift work.

Our study showed that TD-NS had significantly lower work ability, measured by the total WAI score, compared to SW-DS, indicating that sedentary and shift work have cumulative effects in reducing work ability. Sedentary behavior is associated with loss of skeletal muscle mass, strength, and the development of various metabolic diseases and CVDs [8], which may contribute to the reduction of the total WAI score. In our study, the WAI3 score, which refers to the current health status of study participants, was negatively associated with length of service at the current job only among taxi drivers, indicating that sedentary work primarily affects work ability by compromising the health status of workers. Accordingly, there was a significantly higher number of obese and hypertensive individuals in the group of TD-NS compared to SW-DS. This finding is not surprising considering that obesity is an independent risk factor for the development of hypertension as well as other CVDs [8]. Numerous other studies have also linked sedentary and shift work to an increased risk of CVD development [9, 10]; however, the precise mechanisms underlying this association are still not well understood. A recent study showed that there was a slightly higher rate of CVDs, diabetes, and unhealthy sleep status among shift workers in comparison to non-shift workers, as well as among night-shift workers compared to those who had not been working night shifts [3]. To determine how sedentary

and shift work modify traditional risk factors for CVDs, we examined anthropometric, basic clinical, and laboratory parameters of our subjects, as well as the levels of soluble adhesion molecules (sICAM-1 and sVCAM-1) as markers of endothelial dysfunction.

Anthropometric parameters confirmed the findings of WAI3, showing that both TD-NS and TD-DS were more obese than SW-DS, according to body weight, BMI, waist circumference, waist-to-hip, and waist-to-height ratio. These results indicate that sedentary work has a greater impact on physical fitness than shift work, reducing energy expenditure and regular daily activities, which over time leads to an increase in body weight. These findings are supported by a study that showed the association of sedentary behavior with increased values of obesity-predictive biomarkers [11]. Most obesity parameters were positively correlated with years of work experience at the current workplace only in the group of TD-NS, again suggesting cumulative effects of these two work modalities. Basic clinical parameters, including systolic and diastolic blood pressure and heart rate, did not differ significantly between groups. In WAI3, a higher number of hypertensive patients was documented in the group of night-shift taxi drivers, but blood pressure values did not differ from those of the other groups. This can be explained by the fact that hypertensive patients were medically evaluated and therefore adequately treated.

Among the laboratory parameters, the most pronounced alterations were documented in the participants' lipid profile. Night-shift taxi drivers had increased total cholesterol, LDL, and triglyceride values, and lower HDL levels compared to SW-DS, both before and after the shift. Additionally, TD-DS also had increased values of LDL and triglycerides compared to SW-DS. Together, these results indicate an association between sedentary work and dyslipidemia, which is even more pronounced if the work is performed in shifts. Observed alterations of the lipid profile are typical for obesity and associated with a higher risk for the development of CVDs [12]. Among TD-NS, a positive correlation was recorded between total cholesterol, LDL, and triglyceride levels and years of work at the current job, both before and after the shift. Taking this into account, and the fact that there were no significant differences in lipid parameters within each group before and after the shift, it can be concluded that the effects of sedentary and shift work on lipid status are chronic in nature.

The main cause of most CVDs is atherosclerosis, characterized by inflammation and dysfunction of the vascular endothelium. One of the features of endothelial dysfunction is the upregulation of cell adhesion molecules, such as ICAM-1 and VCAM-1, physiologically involved in leukocyte recruitment [13]. Accordingly, higher plasma levels of ICAM and VCAM have been documented in patients suffering from hypertension, atherosclerosis, coronary heart disease, left atrial and left ventricular systolic dysfunction [14, 15, 16]. Levels of sICAM-1 did not significantly differ among our study participants, but they tended to correlate positively with obesity parameters (BMI and waist-to-height ratio) in all groups except SW-DS, who had the smallest BMI (24.64 ± 2.01), and generally were not obese. The association between obesity and endothelial dysfunction is well established [17]. In contrast to sICAM-1, sVCAM-1 levels were elevated in the group of TD-NS compared to TD-DS and SW-DS, both before and after the shift. The levels of sVCAM-1 were more strongly correlated with almost all obesity parameters except in the SW-DS group. Furthermore, a positive correlation was observed with total cholesterol, LDL, and triglyceride levels in the group of TD-NS, indicating that sVCAM-1 is a more sensitive marker of endothelial dysfunction associated with obesity than sICAM-1. No differences in sICAM-1 and sVCAM-1 levels were observed before and after the shift in all study groups, suggesting that sedentary and shift work also have primarily chronic effects on endothelial dysfunction. Accordingly, the strongest correlation between sVCAM-1 levels and years of service at the current workplace was observed in the group of TD-NS. Other studies have also indicated the detrimental effects of sedentary behavior and shift work on endothelial function; however, in these studies, the level of endothelial dysfunction was evaluated by the flow-mediated vasodilatation technique [5, 18]. This can be considered the main advantage of our study, because we used sICAM-1 and sVCAM-1, which are not only markers of endothelial dysfunction, but also molecules directly involved in the pathogenetic mechanisms linking sedentary and shift work to CVD development [19]. Levels of sVCAM-1 were also positively correlated with age in all groups, indicating a progressive decline in endothelial function during senescence, consistent with other studies and the fact that the incidence of CVD is significantly higher in the older population [20, 21].

CONCLUSION

Our study showed that sedentary and shift work have synergistic effects in reducing work ability, which may be associated with an increased incidence of CVDs. We have shown that these work modalities together promote obesity, dyslipidemia, and endothelial dysfunction, which are traditional risk factors for CVD development. The increased frequency of sedentary and shift work in modern society should motivate managers, in collaboration with occupational medicine specialists, to consider ways to mitigate the harmful effects of such work on their employees. Possible solutions include increasing physical activity during working hours and changing dietary habits as cost-effective strategies.

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Утицај седентарног и рада у сменама на радну способност и фактори ризика за настанак кардиоваскуларних болести

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САЖЕТАК

Увод/Циљ У модерном друштву је све већи број послова који захтевају седентарни начин рада, уз повећану потребу за сменским радом. У овом истраживању испитивали смо како ова два радна модалитета утичу на радну способност и здравље радника.

Методе Истраживање је обухватало таксиста и санитарне раднике који су даље подељени у две групе, у зависности од њиховог рада у сменама. Упитник за израчунавање индекса радне способности (*Work Ability Index*) коришћен је за прикупљање података о радној способности испитаника. Како бисмо испитали утицај седентарног начина рада и рада у ноћним сменама на факторе ризика за настанак кардиоваскуларних болести, извршена су антропометријска мерења, као и одређивање стандардних биохемијских параметара и нивоа солубилних адхезивних молекула – интерћелијског адхезивног молекула-1 (*sICAM*-1) и васкуларног ћелијског адхезивног молекула-1 (*sVCAM*-1) у крви узоркованој пре и након посла.

Резултати Возачи таксија који раде у ноћним сменама имали су значајно нижи индекс радне способности у односу на санитарне раднике који раде у дневним сменама. Подаци о броју дијагностикованих обољења код испитаника, антропометријска мерења и липидни статус указали су на разлике у броју гојазних и хипертоничних особа између ове две групе испитаника. Иако није било разлике у вредностима *sICAM*-1, нивои *sVCAM*-1 код возача у ноћним сменама били су значајно виши него код санитарних радника у дневној смени, и пре и после посла.

Закључак Седентарни и сменски рад имају синергистички ефекат на смањење радне способности и промовишу развој кардиоваскуларних болести модификацијом традиционалних фактора ризика.

Кључне речи: седентарни начин рада; рад у сменама; радна способност; гојазност; хипертензија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The role of *GSTM1* and *GSTT1* deletion variants and alcohol consumption profile in the development of alcoholic liver cirrhosis

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SUMMARY

Introduction/Objective Alcoholic liver cirrhosis (ALC) is the primary cause of alcohol abuse-related mortality, resulting from alcohol-induced oxidative stress. Glutathione S-transferases (GSTM1, GSTT1) are enzymes essential for detoxification, and their deficiency may contribute to the onset of chronic inflammation and disease progression. This study sought to investigate the relationship between *GSTM1* and *GSTT1* deletions and ALC, as well as alcohol consumption patterns in cirrhosis development.

Methods The analysis included 114 ALC patients and 262 controls, with *GSTM1* and *GSTT1* deletions assessed via multiplex PCR.

Results Findings indicated that individuals with the *GSTM1* null genotype had a three-fold increased risk of developing ALC (95% CI, 1.87–4.81; $p < 0.0001$), whereas *GSTT1* null genotypes showed no significant impact. Individuals with both *GSTM1* null and *GSTT1* null genotypes exhibited an 11-fold heightened risk of ALC (OR = 11.21, 95% CI = 3.30–38.14, $p < 0.001$). Furthermore, patients who commenced alcohol consumption at 22.5 years or older developed cirrhosis more rapidly than their younger counterparts ($p < 0.001$).

Conclusion *GSTM1* null and combined *GSTM1/GSTT1* null genotypes constitute significant risk factors for ALC, with patients who started drinking at an older age experiencing accelerated disease progression irrespective of alcohol intake levels.

Keywords: alcoholic liver cirrhosis (ALC); drinking profile; *GSTM1* and *GSTT1*; deletion variants; null genotypes

INTRODUCTION

The consumption of alcoholic beverages causes approximately three million deaths globally (5.3%) annually, mostly due to liver failure. Fatalities linked to alcoholic liver disease (ALD) constitute approximately 21.3%, establishing ALD as one of the primary causes of alcohol-related mortality [1, 2]. ALD encompasses a spectrum of hepatic disorders (alcohol-associated steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma) associated with prolonged alcohol intake [3].

Alcoholic liver cirrhosis (ALC) is a multifactorial disease influenced by environmental, behavioral, metabolic, and genetic factors. It develops following an extended period of chronic liver inflammation, usually caused by long-term alcohol intake. The risk of ALC correlates with drinking patterns, and its progression increases significantly with consumption exceeding three drinks/day for men and two for women [4]. However, chronic liver inflammation does not always progress to cirrhosis, and the effects of alcohol consumption vary among individuals with equivalent intake levels [5, 6].

Phase II metabolizing enzymes, such as glutathione S-transferases (GSTs), protect cells from oxidative stress, particularly from secondary cytotoxic metabolites of reactive oxygen species (ROS) [7]. Homozygous deletions of cytosolic GST θ and μ enzymes, encoded by *GSTT1* and *GSTM1* genes ("null" genotypes), are associated with the absence of these enzymes, increasing susceptibility to ROS, and predisposing hepatocytes to chronic liver inflammation, tissue damage, and ALC development.

Given the inconsistent findings regarding the association between *GSTM1* and *GSTT1* deletion variants and ALC [8–11], we investigated the association between *GSTM1* and *GSTT1* gene deletion variants and alcohol consumption patterns and susceptibility to ALC onset.

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METHODS

Study participants

A total of 114 patients (12 females and 102 males), diagnosed with ALC at the Clinic of Gastroenterology and Hepatology, Zvezdara University Hospital Medical Center, Belgrade, Serbia, between 2015 and 2018, were included. ALC was diagnosed in the presence of clinical or biological signs of liver damage, in individuals consuming more than 20 g/day in women (≈2 drinks) or 30 g/day in men (≈3 drinks) [4].

Liver cirrhosis was diagnosed under standard clinical and laboratory criteria. Comprehensive blood analyses, including complete blood counts, electrolytes, and biochemical markers for the diagnosis of ALC in liver function tests (LFTs), including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, coagulation status (prothrombin time/international normalized ratio), serum albumin, and serum bilirubin concentrations, were conducted. Radiological imaging confirmed signs of cirrhosis. Ultrasonography and computed tomography verified the presence of a nodular liver surface, splenomegaly, collateral vessels, and ascites, while esophagogastroduodenoscopy was conducted to screen for esophageal varices. Neuropsychological testing was performed to detect hepatic encephalopathy (confusion, asterixis, fetor hepaticus). In several patients, the diagnosis was established through liver biopsy.

The severity of liver failure was assessed utilizing LFTs through the Child–Pugh (CP) scoring system. Three CP categories were present: A (asymptomatic or compensated cirrhosis, low mortality risk), B (intermediate disease with moderately impaired hepatic function, decompensated cirrhosis), and C (decompensated cirrhosis, the most severe form with advanced hepatic dysfunction) [6].

The drinking profile, namely the volume of daily alcohol consumption, duration of regular drinking, age at which drinking started, and the type of beverage (beer, wine, or spirits), was quantitatively recorded at the hospital's first visit. Daily alcohol intake (g) was calculated as the number of standard drinks × 10 g. One drink was 100 mL of wine (13%), 30 mL of spirits (40%), or 250 mL of beer (5%). Alcohol exposure duration was estimated from the self-reported drinking onset age to cirrhosis diagnosis.

The control group comprised 262 subjects who came for a preventive health check to Zvezdara University Hospital Medical Center or were blood donors, who self-reported as either abstainers or individuals who consumed < 10 g of alcohol/day with no evidence of liver disease or other pathological conditions.

All participants were unrelated and of Serbian origin. Written informed consent was obtained from all study participants. This study was conducted in accordance with the Declaration of Helsinki, and the Ethics Committee of the Zvezdara University Hospital Medical Center approved the study (Approval Reg. No 8-6-2018, dated 06-01-2018).

Genotyping

Blood samples from study participants were collected in EDTA-coated vacutainers. Genomic DNA was extracted using a DNA extraction kit (Gene JET Whole Blood Genomic DNA Purification Mini Kit; Thermo Scientific, USA) according to the manufacturer's protocol. DNA was stored at -20°C until further analysis.

The genotyping was performed by multiplex polymerase chain reaction (multiplex PCR) for *GSTM1* and *GSTT1*, with the β-globin gene as an internal control. Multiplex PCR reactions contained 2X Multiplex PCR Master Mix (Qiagen®, Hilden, Germany), 0.5 μM of each primer (Metabion, Planegg, Germany) and 0.2 μg of genomic DNA [12]. PCR products were separated on 3% agarose gel, stained with GreenSafe (NZYtech, Lisboa, Portugal), and visualized under UV light. *GSTM1* and *GSTT1* null genotypes displayed no fragments corresponding to 215 bp for *GSTM1* and 480 bp for *GSTT1*, and the 110 bp fragment of the control β-globin gene was observed in every PCR reaction. For validation, 10% of samples were randomly selected and re-genotyped.

Statistical analysis

The $\chi 2$ test was used to evaluate differences between groups for categorical variables. Normally distributed continuous variables were analyzed using the independent t-test and one-way ANOVA, as appropriate, while non-normally distributed variables were analyzed with the Mann–Whitney or Kruskal–Wallis test. Genotype frequencies were directly counted. Univariate binary logistic regression assessed the association between different genotypes and the development of ALC. To determine the relationship between the drinking profile and the duration of alcohol exposure prior to the diagnosis of cirrhosis, Pearson correlation coefficients were calculated. Kaplan–Meier curves analyzed the time to decompensation for patients stratified by median age at the start of drinking alcoholic beverages and compared statistically using the log-rank test. The commencement of the curves was the self-reported time at the initiation of alcohol consumption, and the follow-up time was the duration of alcohol exposure prior to the diagnosis of cirrhosis. Statistical analysis was performed using IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) with statistical significance at $p < 0.05$.

To identify patients at elevated risk of developing ALC, polygenic risk scores (PRSs) based on two deletion variants were developed. It was calculated using R v.4.3.0, as a function

$$f_\beta(x) = \frac{\sum_{i=1}^{n} \beta_i g(x_i)}{\sum_{i=1}^{n} \beta i},$$

where x_i represents points assigned to subjects based on deletion status. Homozygous carriers of *GSTM1* and *GSTT1* deletions were assigned 1 point for each gene; heterozygous carriers, 0.5; and carriers of wild-type alleles, 0. The effect weights of deletions, β, were calculated

Table 1. *GSTM1* and *GSTT1* genotype frequencies in ALC patients and control subjects

Parameter	Patients N (%) (N = 114)	Control subjects N (%) (N = 262)	Adjusted* odds ratio	Lower 95% CI	Upper 95% CI	p
GSTM1						
Non-null ("+/+","+/-")	34 (29.8)	146 (55.7)	Reference			
Null ("-/-")	80 (70.2)	116 (44.3)	3.00	1.87	4.81	**< 0.001**
GSTT1						
Non-null ("+/+","+/-")	99 (86.8)	228 (87.0)	Reference			
Null ("-/-")	15 (13.2)	34 (13.0)	1.02	0.53	1.96	0.95
GSTs genotypes with active genes						
2 active genes	30 (26.3)	116 (44.3)	Reference			
1 active gene	73 (64.0)	142 (54.2)	1.97	1.22	3.23	**0.007**
No active genes	11(9.7)	4 (1.5)	11.21	3.30	38.14	**< 0.001**

Non-null – at least one copy of the gene; null – deletion of both copies of the gene; active gene – at least one copy of the one gene (*GSTM1* or *GSTT1*); GSTM1 – glutathione S-transferase M1; GSTT1 – glutathione S-transferase T1;
*sex- and age-adjusted, logistic regression

from a genome-wide association study [10]. For *GSTM1* (null genotype/deletion), β was 1.308333, and for *GSTT1* (null genotype/deletion), β was 0.69. Differences in PRS distribution between controls and ALC patients were assessed using the nonparametric Wilcoxon rank-sum test for continuous data, with statistical significance at $p < 0.05$.

Ethics: The study was approved by the Ethics Committee of the Zvezdara University Hospital Medical Center (Approval Reg. No 8-6-2018, dated 06-01-2018).

RESULTS

Study participants

Nine times fewer females than males were observed (12 *vs.* 102 in the ALC group and 30 *vs.* 232 in the control group, respectively, p = 0.736). The mean age of participants in the ALC and control groups was 58.23 and 58.56 years, respectively (p = 0.794). No statistically significant differences were observed between patients with ALC and control subjects regarding age or sex distribution.

At the time of hospital admission, patients in CP class A, exhibiting asymptomatic and compensated cirrhosis, were the least represented (14 patients, 12.3%). In contrast, patients with decompensated cirrhosis, both with advanced disease (CP class C, 53 patients, 46.5%), and with CP class B (47 cases, 41.2%) were more prevalent. The median age of onset for alcohol consumption among the patients with ALC was 22.5 years (19–30 years). On average, alcohol consumption commenced 34.6 years prior to cirrhosis diagnosis, with a median daily alcohol intake of 72 g (60–91.5 g). The majority of patients with ALC consumed spirits (76.3%), followed by beer (71%), and 21.1% reported wine consumption. As participants commonly consumed more than one type of beverage, these categories were not mutually exclusive.

Association between *GSTM1* and *GSTT1* deletion variants and onset of ALC

The *GSTM1* and *GSTT1* genotype frequencies in ALC patients and control subjects are shown in Table 1. The majority of ALC patients had deleted both *GSTM1* gene alleles (null genotype), significantly higher than in control subjects (70.2% *vs.* 44.3%, p < 0.001). The *GSTM1* null genotype was significantly associated with the risk of ALC development (p < 0.0001). The risk of developing cirrhosis was three times higher for the *GSTM1* null genotype carriers (OR = 3.00, 95% CI = 1.87–4.81) than in carriers of non-null *GSTM1* genotype. In contrast, the *GSTT1* null genotype was similarly distributed between patients and control subjects (13.2% *vs.* 13.0%, p = 0.96), with no increased risk of disease onset among *GSTT1* null genotype carriers (OR = 1.02, 95% CI = 0.53–1.96) (Table 1).

Furthermore, patients with ALC were divided into three groups: carriers of *GSTM1* non-null/*GSTT1* non-null genotypes (two active genes), carriers of one deleted gene (*GSTM1* or *GSTT1*; one active gene), and carriers of double-null genotypes (*GSTM1* null/*GSTT1* null; no active genes). ALC patients with one active gene had twice the risk of developing ALC compared to those with both active genes (OR = 1.97, 95% CI = 1.22–3.23, p = 0.007). Additionally, the significant risk of ALC disease development was associated with ALC patient carriers of combined *GSTM1* null/*GSTT1* null genotypes (OR = 11.21, 95% CI = 3.30–38.14, p < 0.001), showing an 11-fold higher risk of disease development compared to carriers of *GSTM1* non-null/*GSTT1* non-null genotypes (Table 1).

Clinical characteristics and drinking profile of ALC patients according to GSTs genotypes

Patients with ALC were stratified according to GST null genotypes (*GSTM1*: non-null *vs.* null; *GSTT1*: non-null *vs.* null; *GSTM1*/*GSTT1*: null/null *vs.* one active gene and *vs.* two active genes), and drinking profiles, and clinical characteristics (CP class, biochemical laboratory test results) were compared across the groups (Table 2, Table 3). The median age at the onset of at-risk alcohol consumption was similar in all groups (22–23 years). The duration of alcohol exposure was significantly longer among carriers of the *GSTT1* null genotype compared with *GSTT1* non-null carriers (p = 0.006). Patients with an active *GSTM1* gene (non-null genotype) and carriers of two active genes (*GSTM1*/*GSTT1* non-null/non-null genotype) were associated with significantly increased daily alcohol consumption compared to patients without the *GSTM1* gene (null genotype) and those with one or two genes deleted (one active gene or null/null genotypes) (p = 0.019 and p = 0.024, respectively) (Table 2). Our results demonstrated that, in terms of beverage type, carriers of the *GSTM1* non-null genotype consumed beer more frequently than carriers of the null genotype (p = 0.029) (Table 3).

Table 2. Drinking profile of ALC patients according to *GSTs* genotypes

Drinking profile	*GSTM1*			*GSTT1*			*GSTM1/GSTT1*			
	Non–null N (34)	Null N (80)	p	Non–null N (99)	Null N (15)	p	Null/null N (11)	One active gene N (73)	Two active genes N (30)	p
Initial age of alcohol consumption (years)	22.5 (19–26.5)	22.5 (18–30)	0.78	22 (19–30)	23 (18–26)	0.970	23 (18–30)	22 (18–30)	22.5 (19–26.25)	0.967
Duration of alcohol exposure (years)	35.2 ± 10.5	34.3 ± 9.8	0.63	33.6 ± 9.8	41.1 ± 8.7	**0.006**[a]	40.7 ± 7.8	33.8 ± 10	34.4 ± 10.2	0.093
Daily alcohol consumption (g)	82.5 (60–114)	68.5 (50–83)	**0.019**[b]	72 (60–90)	60 (48–96)	0.664	60 (48–100)	72 (51.5–80)	95 (60–115.5)	**0.024**[c]

The data are expressed as the means ± standard deviations, or medians (25th–75th percentile) unless otherwise noted; active gene – the presence of at least one copy of the one gene (*GSTM1* or *GSTT1*); GSTM1 – glutathione S-transferase M1; GSTT1 – glutathione S-transferase T1;
[a]Student's t-test
[b]Mann–Whitney U test;
[c]Kruskal–Wallis test

Table 3. Clinical characteristics and drinking profile of alcoholic liver cirrhosis patients according to glutathione S-transferases (*GSTs*) genotypes

Profile/Characteristics		*GSTM1*			*GSTT1*			*GSTM1/GSTT1*			
		Non-null N (34)	Null N (80)	p	Non-null N (99)	Null N (15)	P	Null/Null N (11)	One active gene N (73)	Two active genes N (30)	p
Drinking profile											
Type of beverage	beer N (%)	29 (85.3)	52 (65)	**0.029**[d]	72 (72.7)	9 (60)	0.311	5 (45.5)	51 (69.9)	25 (83.3)	0.056
	wine N (%)	7 (20.6)	17 (21.3)	0.937	20 (20.2)	4 (26.7)	0.516	3 (27.3)	15 (20.5)	6 (20.0)	0.866
	spirits N (%)	27 (79.4)	60 (75)	0.612	74 (74.7)	13 (86.7)	0.312	11 (100)	51 (69.9)	25 (83.3)	0.052
Clinical characteristics											
CP class	A, N (%)	3 (8.8)	11 (13.7)	0.549	10 (10.1)	4 (26.7)	0.088	4 (36.4)	7 (9.6)	3 (10)	0.038[d]
	B, N (%)	17 (50)	30 (37.5)	0.215	39 (39.4)	8 (53.3)	0.307	5 (45.4)	28 (38.4)	14 (46.7)	0.706
	C, N (%)	14 (41.2)	39 (48.8)	0.458	50 (50.5)	3 (20)	**0.049**[d]	2 (18.2)	38 (52)	13 (43.3)	0.102

The data are expressed as the means ± standard deviations, or medians (25th–75th percentile) unless otherwise noted; active gene refers to the presence of at least one copy of the one gene (*GSTM1* or *GSTT1*);
GSTM1 – glutathione S-transferase M1; GSTT1 – glutathione S-transferase T1; CP – Child–Pugh;
[a]Student's t-test:
[b]Mann–Whitney U test;
[c]Kruskal–Wallis test;
[d]χ^2 test

The majority of our ALC patients were categorized as CP score class C (45.5%). In this class, patients with a *GSTT1* non-null genotype were more frequent than those with a null genotype (p = 0.049) (Table 3). No significant differences in biochemical laboratory test results were observed among patients with respect to *GSTM1* and *GSTT1* genotypes in the ALC group.

No significant correlation was observed between daily alcohol consumption and the duration of alcohol exposure (Pearson correlation, r = 0.036, p = 0.705). However, a significant negative correlation was found between the age at initiation of alcohol consumption and the duration of exposure (r = -0.475, p < 0.001).

Patients who were 22.5 years of age or older when they initiated drinking developed cirrhosis more rapidly than younger patients (Log-rank p < 0.001) (Figure 1).

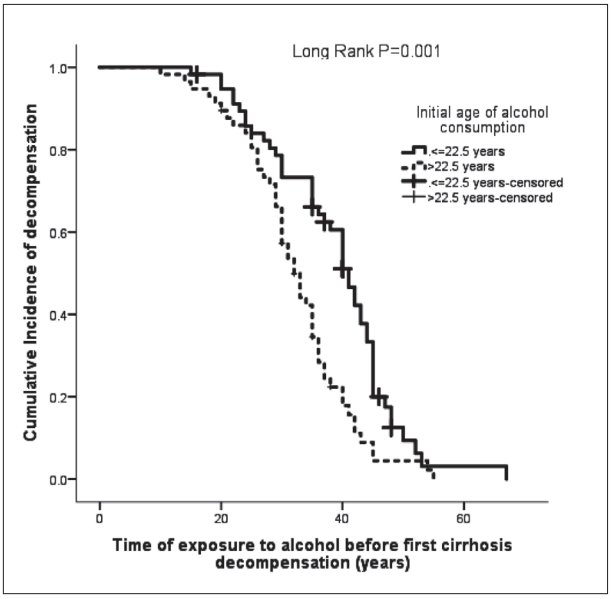

Figure 1. Kaplan–Meier analysis shows that patients who began drinking at ≤ 22.5 years (solid line) developed first cirrhosis decompensation significantly later than those who started after 22.5 years (dashed line) (log-rank p = 0.001); censored cases included nine and five patients, respective-ly, without decompensation

Two-variant PRS and risk for ALC development

The risk of developing ALC was assessed using PRS, which accounts for deleterious *GSTM1* and *GSTT1* variants. The distribution of PRSs differed significantly between patients and the control group. The ALC group showed a rightward shift toward higher PRS values compared with control subjects, indicating an increased cumulative genetic risk associated with these deletions. The disparity between the groups was highly significant with p = 2e-05 (Figure 2).

The *GSTM1* null genotype demonstrated a significant association with ALC, conferring a three-fold increased

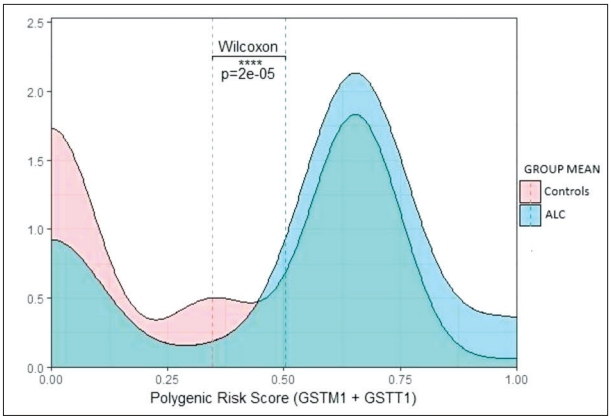

Figure 2. Distribution of *GSTM1* and *GSTT1* deletion variant polygenic risk score (PRS) between controls and alcoholic liver cirrhosis (ALC) patients; ALC patients (blue) had significantly higher PRS values than controls (red) (Wilcoxon test, p = 2e-05); the X-axis shows PRS (0–1), the Y-axis shows density, and dashed lines mark group means

risk, whereas the *GSTT1* deletion alone had no effect. The absence of both genes substantially increased the disease risk. Although no genotype-related biochemical differences were detected, the *GSTT1* null genotype was linked to a longer duration of alcohol exposure, and a later onset of drinking correlated with a more rapid progression to cirrhosis. Furthermore, the PRS, incorporating *GSTM1* and *GSTT1* deletion variants, effectively distinguished ALC patients from controls, indicating the contribution of combined genetic and behavioral factors to disease risk.

DISCUSSION

Excessive alcohol consumption causes individual mental, behavioral, medical, and social problems and imposes a substantial burden on public health and the global economy [2, 3, 13].

Our results demonstrated that patients who initiated alcohol consumption later in life progressed to cirrhosis faster than those who started at a younger age. This could reflect a selection bias, as the prevalence of liver disease increases with age [14]. This is likely due to diminished tissue regeneration and impaired metabolism in older individuals [15], making the liver more vulnerable to alcohol-induced injury and fibrosis, ultimately leading to cirrhosis [16]. Some studies have corroborated that a later onset of alcohol consumption is associated with accelerated disease development, which aligns with our findings [17, 18].

Chronic liver inflammation followed by diffuse hepatic fibrosis causes cirrhosis and eventually leads to liver failure [19, 20]. Alcohol metabolism in hepatocytes generates significant ROS, causing cellular injury and lipid peroxidation, increasing oxidative stress and chronic inflammation, which are crucial for the development of ALC [21]. Glutathione S-transferases (GSTs) detoxify harmful electrophilic compounds and ROS by conjugation to reduced glutathione (GSH) [22]. Limited GSH concentrations during stress leave hepatocytes vulnerable to toxic ethanol metabolites [23, 24]. Individuals lacking one or two GST genes exhibit lower antioxidant capacity against elevated ROS levels than those with both active GST genes [25]. In our study, carriers of the *GSTM1* null genotype exhibited a three-fold increased risk of ALC development, whereas carriers of the double-null genotype (*GSTM1* and *GSTT1*) demonstrated an 11-fold higher risk. This cumulative effect of low GSH concentrations in hepatocytes and the absence of GST enzymes likely contributes to chronic liver inflammation, tissue damage, and cirrhosis. Our results are consistent with the literature data from diverse populations [10, 26] and with conditions associated with elevated oxidative stress investigated within the Serbian population [12, 27].

In our study, *GSTT1* null genotype carriers consumed alcohol long before developing ALC (approximately 41 years) compared to carriers of at least one *GSTT1* allele (approximately 34 years). These findings suggest that the *GSTT1* null genotype did not affect ALC development. A minority of carriers of double-deleted alleles were detected in the group with severe cirrhosis, suggesting that a deficiency in these enzymes may have fatal consequences for patients classified as CP class C. In our patients, carriers of the *GSTM1* null genotype had significantly lower daily alcohol consumption. Furthermore, patients with combined null/null genotypes had significantly lower daily alcohol consumption than patients with one or two active genes. This finding indicates an association between the *GSTM1* null genotype, alone and in combination, and ALC development, irrespective of the quantity of alcohol consumed. Patients with the null/null combination consumed beer at an almost significantly lower rate and spirits at an almost significantly higher rate than patients with one or two active genes. These findings may suggest a predisposition to developing cirrhosis irrespective of the type of beverage and daily alcohol intake in individuals possessing double-deleted GST genes.

Multiple risk factors and comorbidities can accelerate cirrhosis progression. The combination of null alleles in the *GSTM1* and *GSTT1* genes as a risk factor for developing ALC was further corroborated using PRS calculation. The mean PRS was significantly higher in patients with ALC compared to control subjects. Our findings suggest that PRS may be an effective tool for predicting the risk of developing ALC, as we recently showed in another study [28].

One limitation of this study was the control group selection, comprising individuals without liver or other pathological conditions who self-reported as abstainers or who consumed < 10 g of alcohol/day. This limitation can be mitigated by matching alcohol use disorder individuals with patients with similar drinking habits and demographics. Most ALC patients were diagnosed at an advanced stage of liver disease with decompensation; thus, the diagnosis time matched the decompensation time. Assessing daily alcohol intake is challenging because it relies on self-reported data. Patients may be reluctant to report the actual quantities consumed. ALC develops through interactions between genes involved in alcohol metabolism and oxidative stress and environmental factors. Including factors such as body mass index (BMI), diabetes, drinking

habits, and additional genetic markers would help estimate the independence of GSTs as cirrhosis risk factors. In addition, no other comorbidities were investigated. The results from this study should be interpreted as those of a single-center study.

CONCLUSION

Our results demonstrated that *GSTM1* null and combined *GSTM1*/*GSTT1* null genotypes are significant risk factors for the development of alcoholic liver cirrhosis. In addition, patients who started alcohol consumption at age > 22.5 years develop cirrhosis significantly faster, regardless of the amount of alcohol consumed. Further research on additional genetic variants involved in alcohol metabolism, as well as the examination of other risk factors contributing to the onset and progression of ALC in our patients, should be conducted.

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Data availability: The data underlying this article cannot be shared publicly due to a nondisclosure agreement with the institution. The data will be shared upon request to the corresponding author.

Conflict of interest: None declared.

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Улога делеционих варијанти гена *GSTM1* и *GSTT1* и профила конзумирања алкохола у развоју алкохолне цирозе јетре

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САЖЕТАК

Увод/Циљ Алкохолна цироза јетре (АЦЈ) примарни је узрок смртности узроковане злоупотребом алкохола, и настаје као последица оксидативног стреса изазваног алкохолом. Глутатион *S*-трансферазе (*GSTM1*, *GSTT1*) јесу ензими неопходни за детоксификацију и њихов недостатак може утицати на појаву хроничне инфламације и прогресију болести. Циљ ове студије био је да истражи повезаност између делеционих варијанти *GSTM1* и *GSTT1* и АЦЈ, као и утицај профила пијења алкохола на развој цирозе.

Методе Анализа је обухватила 114 болесника са АЦЈ и 262 испитаника у контролној групи, а мултиплекс ПЦР-ом су одређене делеционе варијанте *GSTM1* и *GSTT1*.

Резултати Резултати су показали да особе са *GSTM1* нултим генотипом имају три пута повећан ризик од развоја АЦЈ (95% *CI*, 1,87–4,81; $p < 0,0001$), при чему *GSTT1* нулти генотипови нису показали значајан утицај на развој болести. Особе са оба нулта генотипа (*GSTM1/GSTT1*) показале су 11 пута повећан ризик од АЦЈ (*OR* = 11,21, 95% *CI* = 3,30–38,14; $p < 0,001$). Болесници који су почели да конзумирају алкохол са 22,5 или више година брже су развили цирозу од оних који су почели да пију у млађем узрасту ($p < 0,001$).

Закључак *GSTM1* нулти и комбиновани *GSTM1/GSTT1* нулти генотипови представљају значајне факторе ризика за развој АЦЈ код старијих болесника код којих је убрзана прогресија болести без обзира на ниво уноса алкохола.

Кључне речи: алкохолна цироза јетре; профил пијења алкохола; *GSTM1*; *GSTT1*; делеционе варијанте; нулти генотипови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association of ABO/Rh blood groups with clinical features in Behçet's disease – a retrospective study

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SUMMARY

Introduction/Objective Behçet's disease is a chronic multisystem inflammatory disorder with heterogeneous manifestations. Identifying associated factors may improve understanding of pathogenesis and support individualized management. This study aimed to evaluate the association between ABO blood groups, Rh factor, and clinical and laboratory features of Behçet's disease.

Methods This retrospective study included 160 patients with Behçet's disease followed at the Rheumatology Department of Fırat University Hospital between January 2010 and May 2025. Demographic data and laboratory parameters, including hematological, biochemical, and inflammatory markers, were retrieved from electronic medical records. Patients were grouped as blood group O or non-O and as Rh-positive or Rh-negative, and comparative analyses were performed to evaluate differences in clinical and laboratory findings.

Results Of the 160 patients, 140 were Rh-positive and 20 Rh-negative. Age and most laboratory parameters were similar between Rh groups, except for higher erythrocyte sedimentation rate values in Rh-positive patients ($p = 0.043$). Compared with blood group O patients ($n = 50$), non-O patients ($n = 110$) had higher white blood cell counts ($p = 0.008$), neutrophil counts ($p = 0.010$), and alanine aminotransferase levels ($p = 0.009$), while hemoglobin levels were lower in group O patients ($p = 0.048$). Clinical manifestations were largely comparable; however, articular involvement was more frequent in Rh-negative than in Rh-positive patients (50% *vs*. 27.1%, $p = 0.037$).

Conclusion ABO blood groups and Rh factor were not associated with most clinical or laboratory features of Behçet's disease. Increased articular involvement in Rh-negative patients suggests a potential association warranting further investigation.

Keywords: Behçet disease; blood group; Rh factor; arthritis

INTRODUCTION

Behçet's disease is a chronic, relapsing, multisystem inflammatory disorder characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, arthritis, gastrointestinal involvement, and neurological manifestations. It predominantly affects young adults between the ages of 20 and 30 and is classified as a variable vessel vasculitis due to its capacity to involve blood vessels of all sizes and types. The exact etiology remains unclear, but genetic predisposition, particularly the *HLA-B51* allele, and environmental triggers are believed to play significant roles in disease pathogenesis. Clinical presentation is highly heterogeneous, and disease severity can vary considerably between patients. Early recognition and comprehensive management are crucial to prevent irreversible organ damage and improve long-term outcomes [1, 2, 3].

In Behçet's disease, various factors such as age, sex, geographical region, and blood type can influence both the course of the disease and the pattern of symptoms experienced. These factors may affect the frequency, severity, and distribution of clinical manifestations, as well as the likelihood of specific organ involvement. For instance, certain populations may present with predominantly mucocutaneous features, while others are more prone to ocular or neurological complications. Understanding these demographic and geographical variations is essential for anticipating disease behavior, tailoring management strategies, and improving long-term patient outcomes [4, 5, 6].

Blood group types and Rh factor status may influence the symptom patterns observed in Behçet's disease. Some research suggests that individuals with non-O blood groups (A, B, or AB) may be more susceptible to vascular complications, such as thrombosis. Nonetheless, several studies have reported no significant association between ABO or Rh blood groups and disease prevalence or manifestations, highlighting the need for larger and more comprehensive investigations [7, 8].

In this study, we aimed to explore the potential relationship between blood group types, Rh factor status, and the clinical characteristics of Behçet's disease. Previous research has suggested that immunohematological factors, including ABO blood groups and Rh positivity, may influence the manifestation and progression of various autoimmune and inflammatory

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disorders [9]. Given the heterogeneous nature of Behçet's disease, which presents with diverse patterns of mucocutaneous, ocular, vascular, and neurological involvement, identifying potential associations with blood group profiles could provide valuable insights into disease pathogenesis, prognosis, and individualized management strategies.

METHODS

A total of 160 patients diagnosed with Behçet's disease and followed up at the Rheumatology Department were included in this study. This was a single-center, retrospective observational study conducted in the rheumatology department of our institution, and patients who were under follow-up between January 2010 and May 2025 were enrolled. The study was conducted in accordance with the principles of the Declaration of Helsinki.

In our study, demographic data of the patients – including age and gender – were recorded. Additionally, laboratory parameters such as white blood cell (WBC) count, neutrophil (Neu) count, lymphocyte (Lymph) count, hemoglobin (Hb) level, platelet (Plt) count, urea, creatinine, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were collected using the hospital's electronic medical record system.

The diagnosis of Behçet's disease in patients was made based on the 2013 International Criteria for Behçet's Disease. According to these criteria, points were assigned as follows: two points for the presence of oral aphthous ulcers, two points for genital ulcers, two points for ocular lesions, one point for skin manifestations, one point for central nervous system involvement, one point for vascular lesions, and one point for a positive pathergy test. Patients with a total score of four or more were classified as having Behçet's disease and were included in the study [10].

Mucocutaneous findings included oral ulcers, genital ulcers, acneiform lesions, and overall mucocutaneous involvement. Ocular involvement was defined as the presence of uveitis confirmed by ophthalmologic examination. Pathergy test results were recorded as positive or negative according to standard clinical assessment. Neurologic involvement was defined based on documented neurological findings and/or imaging consistent with central nervous system involvement. Articular involvement was defined as the presence of clinically documented inflammatory arthritis or arthralgia.

Patients were classified into two groups based on their blood type: Group O and non-O blood types. In addition, they were further divided into two subgroups according to their Rh factor status (Rh-positive and Rh-negative). Comparative analyses were then conducted between these groups to evaluate potential differences in symptomatology and laboratory parameters, aiming to identify whether specific blood group profiles or Rh status were associated with distinct clinical patterns or laboratory findings in Behçet's disease.

Statistical analysis

The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Variables that followed a normal distribution were presented as mean ± standard deviation, whereas non-normally distributed variables were reported as median and interquartile range. For continuous variables, comparisons between groups were made using the Student's t-test if the data were normally distributed, and the Mann–Whitney U test if not. Categorical variables were compared using the χ^2 test; when the expected frequencies were low, Fisher's exact test was used instead. A p-value of less than 0.05 was considered statistically significant.

Ethics: This study involving human participants was reviewed and approved by the local Committee on Ethics (decision date: 04.09.2025; decision number: 2025/12-16). Because this was a retrospective chart-review using de-identified records, the procedures were not explained to participants and no written informed consent was obtained (consent requirement was waived for this retrospective analysis).

RESULTS

A total of 160 patients were included in the analysis, comprising 50 individuals in the Group O and 110 in the non-O Group (Table 1). The comparison of demographic and laboratory parameters showed that age was similar between the two groups (41.67 ± 10.68 *vs.* 42.77 ± 11.99 years, p = NS). WBC count (8.71 *vs.* 7.32 × 10^9/L; p = 0.008) and Neu count (5.68 *vs.* 4.66 × 10^9/L; p = 0.010) were significantly higher in the non-O Group compared with the Group O. Hb levels were lower in Group O patients compared with the non-O Group [13.35 (9.9–16.1) *vs.* 14.15 (4.6–16.4) g/dL; p = 0.048]. No significant differences were observed in Lymph count, Plt count, urea, creatinine, uric acid, GGT, ESR, or CRP values between the groups (all p > 0.05). However, ALT levels were significantly higher in the non-O Group than in the Group O [19 (6–170) *vs.* 14 (7–81) U/L; p = 0.009] (Table 2).

Table 1. Distribution of ABO blood groups in the study population (n = 160)

Blood group category	n	% (of total)
O	50	31.3%
Non-O (Total)	110	68.8%
A	71	44.4%
B	32	20%
AB	7	4.4%

Values are expressed as n (%); percentages were calculated based on the total study population (n = 160); non-O includes blood groups A, B, and AB

Of 160 patients, 140 were Rh-positive, and 20 were Rh-negative. The mean age was similar between Rh-positive (42.80 ± 11.77 years) and Rh-negative patients (39.85 ± 10.01 years), with no statistically significant difference (p = 0.554). WBC counts were slightly higher in

Table 2. Demographic and laboratory parameters between blood groups

Parameter	O group (median, range) n : 50	Non-O group (median, range) n: 110	p
Age (years)	42 (19–68)	43 (18–71)	
WBC × 10^9/L	7.32 (0.86–25.10)	8.71 (0.97–27.26)	**0.008***
Neu × 10^9/L	4.66 (1.22–22.40)	5.68 (1.24–17.50)	**0.010***
Lymph × 10^9/L	1.96 (1.27–4.79)	2.13 (1.10–4.61)	0.530*
Hb g/dL	13.35 (9.9–16.1)	14.15 (4.6–16.4)	**0.048***
Plt × 10^9/L	267 (129–530)	277 (23–602)	0.821*
Urea mg/dL	26 (11–58)	27 (10–87)	0.296*
Creatinine mg/dL	0.8 (0.41–1.16)	0.8 (0.4–1.4)	0.326*
Uric acid mg/dL	4.05 (2.6–8)	4.5 (2–10.4)	0.232*
ALT U/L	14 (7–81)	19 (6–170)	**0.009***
GGT U/L	13 (5–102)	23.50 (10–177)	0.197*
ESR mm/hour	21.5 (1–82)	19.5 (2–143)	0.857*
CRP mg/L	6.1 (0.03–157)	6.13 (0.04–131)	0.994*

WBC – white blood cell count; Neu – neutrophils; Hb – hemoglobin; Plt – platelets; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein;
*Mann–Whitney U test

Table 3. Demographic and laboratory parameters between Rh groups

Parameter	Rh+ group (n: 140)	Rh– group (n: 20)	p
Age (years)	42.80 ± 11.77	39.85 ± 10.01	0.554
WBC × 10^9/L	8.44 (0.96–27.26)	7.19 (0.86–15.92)	0.254*
Neu × 10^9/L	6.34 (1.22–42)	5.63 (2.50–11.58)	0.456*
Lymph × 10^9/L	2.15 ± 0.87	1.99 ± 0.86	0.461
Hb g/dL	13.8 (4.6–16)	14.1 (10.4–16.4)	0.407*
Plt × 10^9/L	281 (129–750)	82.7 (2.3–407)	0.159*
Urea mg/dL	27 (10–87)	83.2 ± 14.5	0.942*
Creatinine mg/dL	0.80 (0.40–1.40)	0.76 (0.41–1.10)	0.954*
Uric acid mg/dL	4.48 ± 1.35	4.24 ± 1.02	0.563
ALT U/L	18 (6–170)	16 (12–45)	0.404*
GGT U/L**	21.50 (1–177) (n = 92)	55.5 (5–102) (n = 13)	0.333*
ESR mm/hour	22.5 (1–143)	14 (3–41)	0.043*
CRP mg/L	6.16 (0.03–157)	4.65 (3–131)	0.484*

WBC – white blood cell count; Neu – neutrophils; Hb – hemoglobin; Plt – platelets; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein;
*Mann–Whitney U test
**GGT values were analyzed in patients with available measurements only (n varies due to missing data); data are presented as mean ± standard deviation or median (range), as appropriate

Rh-positive patients [median: 8.44 (0.96–27.26) × 10^9/L] compared with Rh-negative patients [7.19 (0.86–15.92) × 10^9/L], but the difference was not significant (p = 0.254). Similarly, Neu counts showed no significant variation between Rh-positive [6.34 (1.22–42) × 10^9/L] and Rh-negative [5.63 (2.50–11.58) × 10^9/L] groups (p = 0.456). Lymph counts were also comparable between groups (2.15 ± 0.87 *vs.* 1.99 ± 0.86 × 10^9/L; p = 0.461). Hb and Plt levels did not differ significantly (p = 0.407 and p = 0.159, respectively). Likewise, urea and creatinine levels showed no meaningful differences (p = 0.942 and p = 0.954, respectively), and uric acid, ALT, GGT, and CRP values were comparable across both groups (all p > 0.05). The only parameter showing a statistically significant difference was the ESR, which was higher in Rh-positive patients [median: 22.5 (1–143) mm/h] compared with Rh-negative patients [14 (3–41) mm/h] (p = 0.043) (Table 3).

The comparison of clinical findings between patients with Group O (n = 49) and non-O blood groups (n = 111) revealed no statistically significant differences in mucocutaneous, ocular, or systemic manifestations. Oral ulcers were highly prevalent in both groups (98% in Group O *vs.* 97.3% in the non-O group, p = 0.641), while genital ulcers were more frequent in the non-O group (78.4%) compared with the Group O group (67.3%), although this difference was not statistically significant (p = 0.137). Mucocutaneous involvement was nearly universal in both groups (98% *vs.* 100%, p = 0.306). Acneiform lesions were observed in 49% of patients in the Group O and in 63.6% of those in the non-O group (p = 0.166). Uveitis was present in 44.9% of Group O patients compared with 36% of non-O patients (p = 0.158). Pathergy test positivity was relatively low and comparable between groups (50% *vs.* 46.4%, p = 0.836). Similarly, neurologic involvement rates were comparable (12.2% *vs.* 14.4%, p = 0.713), and articular involvement did not differ significantly between the two groups (32.7% *vs.* 28.8%, p = 0.793). Overall, these findings indicate no significant association between ABO blood group and the clinical spectrum of Behçet's disease (Table 4).

In the comparison of clinical manifestations between Rh-positive and Rh-negative patients, oral ulcers were highly prevalent in both groups, observed in 97.9% of Rh-positive and 95% of Rh-negative patients, with no statistically significant difference (p = 0.417). Genital ulcers were more frequent among Rh-positive patients (77.1%) compared with Rh-negative patients (60%); however, this difference did not reach statistical significance (p = 0.098).

Mucocutaneous involvement was present in all Rh-positive patients (100%) and in 95% of Rh-negative patients (p = 0.125). Acneiform lesions were reported in 57.9% of Rh-positive and 65% of Rh-negative individuals (p = 0.567), while uveitis occurred in 39.3% and 35% of patients, respectively (p = 0.713). Pathergy test positivity showed similar rates between the two groups (11.4% *vs.* 15%, p = 0.457), and neurologic involvement was also comparable (14.3% *vs.* 10%, p = 0.457). Importantly, articular involvement was significantly more frequent in Rh-negative patients (50%) than in Rh-positive patients (27.1%) (p = 0.037), suggesting a potential association between Rh factor and joint involvement in Behçet's disease (Table 5).

DISCUSSION

In our study, patients were first categorized according to blood groups and Rh factor into Rh-positive (n = 140) and Rh-negative (n = 20) groups. Comparative analyses were conducted between these groups using demographic variables, such as age, and a range of laboratory parameters, including WBC count, Neu count, Lymph count, hematocrit, Hb, Plt count, urea, creatinine, uric acid, ALT, aspartate aminotransferase (AST), ESR, and CRP. No statistically significant differences were found between Rh-positive

Table 4. Comparison of symptoms by blood group category

Lesion type / category	Condition	O (n = 49)	Non-O (n = 111)	Total (n = 160)	p
Oral ulcer	Absent	1 (2%)	3 (2.7%)	4 (2.5%)	0.641
	Present	48 (98%)	108 (97.3%)	156 (97.5%)	
Genital ulcer	Absent	16 (32.7%)	24 (21.6%)	40 (25%)	0.137
	Present	33 (67.3%)	87 (78.4%)	120 (75%)	
Mucocutaneous	Absent	1 (2%)	0 (0%)	1 (0.6%)	0.306
	Present	48 (98%)	111 (100%)	159 (99.4%)	
Acneiform lesion	Absent	25 (51%)	40 (36.4%)	65 (40.9%)	0.166
	Present	24 (49%)	70 (63.6%)	94 (59.1%)	
Uveitis	Absent	27 (55.1%)	71 (64%)	98 (61.3%)	0.158
	Present	22 (44.9%)	40 (36%)	62 (38.8%)	
Pathergy test	Negative	6 (50%)	15	21 (13.1%)	0.836
	Positive	6 (50%)	13	19 (11.9%)	
Neurologic involvement	Absent	43 (87.8%)	95 (85.6%)	138 (86.3%)	0.713
	Present	6 (12.2%)	16 (14.4%)	22 (13.8%)	
Articular involvement	Absent	33 (67.3%)	79 (71.2%)	112 (70%)	0.793
	Present	16 (32.7%)	32 (28.8%)	48 (30%)	

Values are expressed as n (%); O – blood group O; Non-O – non-blood group O (A, B, or AB); categorical variables were compared using the χ^2 or Fisher's exact test; $p < 0.05$ was considered significant

Table 5. Comparison of clinical manifestations according to Rh factor

Lesion type / category	Condition	Rh (+) (n: 140)	Rh (–) (n: 20)	Total	p
Oral ulcer	Absent	3 (2.1%)	1 (5%)	4 (2.5%)	0.417
	Present	137 (97.9%)	19 (95%)	156 (97.5%)	
Genital ulcer	Absent	32 (22.9%)	8 (40%)	40 (25%)	0.098
	Present	108 (77.1%)	12 (60%)	120 (75%)	
Mucocutaneous	Absent	0 (0%)	1 (5%)	1 (0.6%)	0.125
	Present	140 (100%)	19 (95%)	159 (99.4%)	
Acneiform lesion	Absent	58 (41.4%)	7 (35%)	65 (40.6%)	0.567
	Present	81 (57.9%)	13 (65%)	94 (58.8%)	
Uveitis	Absent	85 (60.7%)	13 (65%)	98 (61.3%)	0.713
	Present	55 (39.3%)	7 (35%)	62 (38.8%)	
Pathergy test	Negative	15 (10.7%)	6 (30%)	21 (13.1%)	0.457
	Positive	16 (11.4%)	3 (15%)	19 (11.9%)	
Neurologic involvement	Absent	120 (85.7%)	18 (90%)	138 (86.2%)	0.457
	Present	20 (14.3%)	2 (10%)	22 (13.8%)	
Articular involvement	Absent	102 (72.9%)	10 (50%)	112 (70%)	**0.037**
	Present	38 (27.1%)	10 (50%)	48 (30%)	

Values are expressed as n (%); O – blood group O; Non-O – non-blood group O (A, B, or AB); categorical variables were compared using the χ^2 or Fisher's exact test; $p < 0.05$ was considered significant

and Rh-negative groups in most demographic and laboratory parameters, except for ESR, which was higher in Rh-positive patients. Sincan et al. [11], in their study involving 3000 blood donors, reported no significant differences in complete blood count parameters, except for red cell distribution width. Similarly, in our study, no statistically significant differences were observed between the two groups in either complete blood count or biochemical parameters [11].

Numerous studies have explored whether blood group antigens influence laboratory markers in healthy individuals. For example, Al-Mawali et al. [12] found that in a large healthy population, there were no significant differences in RBC, WBC, or Plt indices across ABO blood groups, underscoring a minimal hematological impact of these blood types in the general population. In our study, when blood groups were categorized into group O and non-O, statistical analyses were performed to compare age, WBC, Neu, Lymph, hematocrit, Hb, Plt, urea, creatinine, uric acid, ALT, AST, ESR, and CRP values. Significant differences were found in WBC ($p = 0.008$), Neu count ($p = 0.010$), Hb ($p = 0.048$), and ALT levels ($p = 0.009$), while other parameters showed no statistically significant differences between the groups. These statistical variations may be attributable to the non-normal distribution of the variables and the relatively small sample size, which could limit the robustness of the findings.

A previous study has explored the association between blood groups and the clinical manifestations of rheumatologic diseases, including Behçet's disease. Findings from these investigations suggest that certain ABO and Rh blood group types may influence disease susceptibility, severity, and symptom patterns [13, 14]. For instance, some research has reported variations in mucocutaneous or ocular manifestations depending on blood group type [13]. Although the underlying mechanisms remain unclear, it has been proposed that immunohematological factors, such as antigenic determinants on red blood cells, could modulate inflammatory and immune responses, thereby affecting disease expression [14]. In our study, comparison between blood group O and non-O patients revealed no statistically significant differences in the frequency of oral ulcers, genital ulcers, uveitis, mucocutaneous lesions, acneiform eruptions, neurological involvement, articular involvement, or pathergy test positivity. This indicates that, in our cohort, ABO blood group did not appear to influence the distribution of Behçet's disease manifestations.

However, when comparing Rh-positive and Rh-negative patients, a statistically significant difference was observed in articular involvement, which was more frequent in Rh-negative individuals ($p = 0.037$). The reason for this association is not fully understood, but it is possible that immunohematological variations linked to the Rh antigen could influence immune complex deposition or inflammatory cascades within synovial tissues. Rh antigens are known to modulate immune cell recognition and cytokine profiles, potentially contributing to differential inflammatory responses [15, 16]. Previous studies investigating the association between Rh factor and autoimmune disease susceptibility are limited and have produced inconsistent results, with no clearly established mechanistic link [17]. In our cohort, Rh factor status appeared to be associated with differences in clinical expression of Behçet's disease, particularly with respect to articular involvement ($p = 0.037$). These findings suggest that Rh status may represent a potential modifier of disease phenotype and warrant further evaluation in larger, prospective studies.

This study has several important limitations that should be acknowledged. First, the relatively small sample size

may reduce the statistical power and limit the robustness of the conclusions. Second, its retrospective design introduces potential biases inherent to such studies. Furthermore, being a single-center investigation restricts the generalizability of the findings to wider populations. Another key limitation is the lack of adjustment for potential confounders such as age, sex, and disease duration, which may have influenced the observed associations. These limitations highlight the need for future multicenter, prospective studies with larger cohorts to validate and extend the present results.

CONCLUSION

Our study demonstrates that ABO blood groups do not significantly influence the distribution of clinical manifestations in Behçet's disease. However, Rh factor status, particularly Rh negativity, appears to be associated with increased frequency of articular involvement. These findings suggest that immunohematological factors linked to the Rh system may play a role in modulating disease expression. Further large-scale, prospective studies are warranted to clarify the mechanisms underlying this association and to evaluate the potential of Rh factor as a modifier of disease phenotype in Behçet's disease.

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Data availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Повезаност *ABO/Rh* крвних група са клиничким карактеристикама Бехчетове болести – ретроспективна студија

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САЖЕТАК

Увод/Циљ Бехчетова болест је хронични мултисистемски инфламаторни поремећај са хетерогеним манифестацијама. Идентификација повезаних фактора може побољшати разумевање патогенезе и подржати индивидуализовани приступ лечењу. Ова студија имала је за циљ да процени везу између *ABO* група крви, *Rh*-фактора и клиничких и лабораторијских карактеристика болести.

Методе У ову ретроспективну студију укључено је 160 болесника са Бехчетовом болешћу лечених на Одељењу за реуматологију Универзитетске болнице „Фират" од јануара 2010. до маја 2025. године. Демографски подаци и лабораторијски параметри, укључујући хематолошке, биохемијске и инфламаторне маркере, преузети су из електронских медицинских картона. Болесници су подељени у групе према крвној групи (0 или не-0) и *Rh*-статусу (позитивном или негативном), а упоредне анализе су спроведене ради процене клиничких и лабораторијских разлика.

Резултати Од 160 болесника, 140 је било *Rh*-позитивно, а 20 *Rh*-негативно. Старост и већина лабораторијских параметара били су слични између *Rh* група, осим виших вредности брзине седиментације еритроцита код *Rh*-позитивних болесника ($p = 0,043$). У поређењу са болесницима нулте крвне групе ($n = 50$), болесници који нису нулта крвна група ($n = 110$) имали су већи број леукоцита ($p = 0,008$), неутрофила ($p = 0,010$) и виши ниво аланин аминотрансферазе ($p = 0,009$), док су нивои хемоглобина били нижи код болесника са нултом крвном групом ($p = 0,048$). Клиничке манифестације биле су упоредиве; међутим, захваћеност зглобова била је чешћа код *Rh*-негативних него код *Rh*-позитивних болесника (50% у односу на 27,1%, $p = 0,037$).

Закључак *ABO* крвне групе и *Rh*-фактор нису били повезани са већином клиничких и лабораторијских карактеристика Бехчетове болести. Повећана захваћеност зглобова код *Rh*-негативних болесника указује на потенцијалну повезаност која захтева даље истраживање.

Кључне речи: Бехчетова болест; крвна група; *Rh*-фактор; артритис

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparative evaluation of inflammatory biomarkers and total bilirubin for the early detection of complicated appendicitis in adults

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SUMMARY

Introduction/Objective Acute appendicitis (AA) is the most frequent cause of emergency surgical interventions, and numerous biomarkers can be used diagnostically to differentiate patients with AA from those with pain of other etiologies, as well as to predict disease progression.
The aim of this paper is to determine the accuracy of neutrophil-lymphocyte ratio (NLR), procalcitonin (PCT) and total bilirubin (TBil) in the diagnosis of complicated AA (CoAA) and their comparison with the Alvarado score (AS) as well as the histopathological (HP) findings.

Methods AA was diagnosed preoperatively in 67 patients using AS. Examined parameters and AS were determined before surgery and compared postoperatively with HP findings. Depending on the HP findings, the respondents were classified into three groups: gangrenous and gangrenous-perforated appendicitis, which are classified into CoAA, phlegmonous (PhAA) and catarrhal AA (CAA).

Results The results of the univariate analysis show that a one-unit increase in NLR increased the probability of CoAA by 20% (1.02 to 1.51, $p < 0.05$). PCT ≥ 0.5 ng/ml increases the probability of CoAA by 26.84 times (3.30 to 218.55; $p < 0.001$), while TBil > 21μmol/l increases the probability of CoAA by 4.80 times (1.41 to 16.37, $p < 0.05$). ROC curve showed that PCT was the best predictor of CoAA compared to CAA/PhAA, with a cut-off of 0.56, as well as CAA in relation to PhAA/CoAA with a cut-off of 0.37.

Conclusion PCT, TBil, and NLR can be used in daily clinical practice as powerful, easily available, inexpensive parameters in the diagnosis of CoAA in adults.

Keywords: inflammatory biomarkers; total bilirubin; complicated acute appendicitis

INTRODUCTION

The lifetime prevalence of acute appendicitis (AA), as the most frequent surgical condition, is around one in seven cases, with an incidence of 1.5–1.9 per 1000. The male: female ratio is 1.4. According to literature, there is a correlation between the number of hospitalizations for appendicitis and atmospheric pressure and temperature [1, 2]. Despite the high frequency of AA, correct diagnosis before surgery is a challenge that can tempt even highly experienced surgeons [3]. The literature reports a negative appendectomy rate of 15–25%. In women of reproductive age, this rate has almost doubled due to the prevalence of gynecological diseases, reaching as high as 30–50%. In young male patients, the rate of negative appendectomy is relatively low (5–22%). In children, the diagnosis may be incorrect in 30–46% of cases. Untimely diagnosis and delayed surgical treatment led to perforation and subsequent complications. Therefore, an adequate and easily accessible test that can confirm or rule out complicated forms of appendicitis and can be useful in making decisions about emergency surgical treatment [4].

The modern diagnostic principle aims primarily of confirmation or elimination of the diagnosis of AA and to differentiate complicated from uncomplicated forms of the disease, which also determines the therapeutic modalities [5]. Due to this reason, there is still research orientation towards finding biomarkers of sufficient specificity and sensitivity that would more clearly suggest the degree of appendicular inflammation and which, are easily available, minimally invasive, cheap, and can be repeated if necessary [6, 7]. To reduce the rate of negative appendectomies, i.e., cases of missed AA, the objective of this work was to determine the accuracy of inflammatory biomarkers [neutrophil-lymphocyte ratio (NLR), procalcitonin (PCT), and total bilirubin (TBil)] and their comparison with Alvarado score (AS) and histopathological (HP) findings in adult patients operated on for AA.

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METHODS

A prospective study was conducted and included 67 patients older than 18 years in whom appendectomy was performed due to AA during a six-month period at the Department of Surgery of the Clinical Hospital Center Kosovska Mitrovica. AA was diagnosed using AS (Table 1) [8]. AS is a clinically validated scoring system. Its validity has been demonstrated in large prospective and retrospective studies, with reported sensitivity ranging from 72% to 88% and area under the ROC curve values exceeding 0.75 in different populations. A cut-off value of ≥ 6 was used as clinically appropriate for identifying patients with suspected AA and for guiding further diagnostic evaluation, and has demonstrated an optimal balance between sensitivity and specificity. After preoperative blood sampling, serum NLR, PCT, and TBil levels were determined. The patients were treated with an open appendectomy. Definitive diagnosis of the removed appendices was established by HP analysis of samples. The results of the parameters and AS were compared with the HP findings. According to the HP assessment, the severity of AA was categorized into three groups: gangrenous and gangrenous perforating appendicitis were classified as complicated AA (CoAA) in contrast to catarrhal AA (CAA) and phlegmonous AA (PhAA).

Table 1. Alvarado score

Symptoms	Alvarado score
Pain migration	1
Anorexia	1
Nausea/vomiting	1
Signs	
Tenderness in right iliac fossa	2
Bloomberg's sign	1
Elevated temperature (> 37.2°C)	1
Laboratory	
Leukocytosis (> 10×10^9)	2
Neutrophils > 75%	1
Total	10

Statistical analyses

Continuous variables are presented as means, standard deviations, medians, minimum and maximum values. Student's t-test and Mann–Whitney test were used to compare continuous variables. For comparisons involving three independent groups, ANOVA analysis was used together with appropriate post hoc procedures (Tukey's method and Tamhane's T2 test), as well as Kruskal–Wallis test and Mann–Whitney test for group comparisons. The association between categorical variables was assessed using Pearson's χ^2 test.

Univariate logistic regression was performed to assess the impact of individual independent parameters on changes in the odds ratio for a positive or negative outcome. The diagnostic performance of the parameters in differentiating AA types was examined using ROC curve analysis and appropriate cut-off values were determined. Statistical significance was set at $p < 0.05$. All statistical procedures were performed using SPSS for Windows, Version 16.0. (SPSS Inc., IL, Chicago,USA).

Ethics: The study was conducted in accordance with the standards of the institutional ethics committee (number 3482 dated 27.05.2025.).

RESULTS

The average age of the patients was 38.72 ± 16.46 years, ranging from 18 to 80 years, with 35 (52.24%) male patients and 32 (47.76%) female patients (the male:female ratio is 1.09). CoAA occurred more frequently in older patients, compared to CAA and PhAA ($p < 0.05$). Into the first pathohistological group, CAA were classified 16 patients – 23.88% (eight men and eight women), the second group, PhAA consisted of 33 patients – 49.25% (17 men and 16 women), while in the third group, gangrenous and gangrenous perforating (CoAA) included 18 patients – 26.87% (10 men and eight women) (Table 2).

The minimum and maximum, as well as the average values of the examined parameters are given in Table 3.

Table 2. Histopathological forms of acute appendicitis in relation to gender and average age of patients

Histopathology	Number (%)	Sex		Age (years)
		Male	Female	
CAA	16 (23.88%)	8	8	35 ± 17.91 (29)
PhAA	33 (49.25%)	17	16	36.94 ± 15.94 (35)
CoAA	18 (26.87%)	10	8	45.28 ± 15.12[ab*] (46)
Σ	67 (100%)	35	32	38.72 ± 16.46 (36)

CAA – catarrhal acute appendicitis; PhAA – phlegmonouse acute appendicitis; CoAA – complicated acute appendicitis; parameters are given as nembers, mean value, standard deviation, median;
*$p < 0.05$;
a–*vs* CAA;
b–*vs* PhAA

Table 3. Average values of the tested parameters

Parameter	X ± SD	Med	Min	Max
AS	7.94 ± 1.82	8	2	10
NLR	6.24 ± 3.4	5.34	1.53	16.67
PCT (ng/ml)	0.50 ± 0.19	0.51	0.19	0.97
TBil (μmol/L)	17.05 ± 12.16	14.50	2.40	94.30

AS – Alvarado score; NLR – neutrophil-lymphocyte ratio; PCT – procalcitonin; TBil – total bilirubin; parameters are given as mean value (X), standard deviation (SD), median (Me), minimum (Min) and maximum (Max) value

The most authoritative criteria for the definitive diagnosis of AA were positive AS value (AS ≥ 6) and the HP finding. In Table 4 are given the average values of the tested parameter compared to the AS. All three parameters, NLR and PCT ($p < 0.001$) and TBil ($p < 0.01$), had significantly higher values in positive AS. The average values of the investigated parameters compared to the HP finding of AA are given in Table 5. AS was statistically significant in

Table 4. Average values of the tested parameters compared to Alvarado score (AS)

Parameter	AS negative (≤ 5)	AS positive (≥ 6)	p
NLR	4.85 ± 2.70 (4.52)	**8.17 ± 3.47 (7.16)**	**0.0000*** **
PCT (ng/ml)	0.40 ± 0.14 (0.42)	**0.63 ± 0.17 (0.61)**	**0.0000*** **
TBil (μmol/L)	15.16 ± 14.14 (12.51)	**19.69 ± 8.22 (19.20)**	**0.0019** **

NLR – neutrophil-lymphocyte ratio; PCT – procalcitonin; TBil – total bilirubin; parameters are given as mean value (X) ± standard deviation deviation (SD), median (Me);
**p < 0.01;
***p < 0.001 (Student t-test or Mann–Whitney U-test)

Table 5. Average values of the tested parameters compared to histopathological findings

Parameter	CAA (n = 16)	PhAA (n = 33)	CoAA (n = 18)	p
AS	6.94 ± 1.18 (7)	**7.70 ± 2.05a** (7)**	**9.28 ± 0.83a***b** (9.00)**	**0.0000*** **
NLR	4.71 ± 2.65 (4.62)	6.09 ± 3.65 (5.19)	**7.88 ± 3.11a**b* (7.01)**	**0.0083** **
PCT (ng/ml)	0.25 ± 0.05 (0.25)	**0.50 ± 0.06a*** (0.51)**	**0.72 ± 0.15ab*** (0.69)**	**0.0000*** **
TBil (μmol/L)	17.64 ± 20.94 (12.8)	14.95 ± 6.48 (14.20)	20.37 ± 9.32ab* (19.50)	0.0600

CAA – catarrhal acute appendicitis; PhAA – phlegmonouse acute appendicitis; CoAA – complicated acute appendicitis; AS – Alvarado score; NLR – neutrophil-lymphocyte ratio; PCT – procalcitonin; TBil – total bilirubin; parameters are given as mean value (X) ± standard deviation (SD) and median (Me)a–vs CAA, b–vsPhAA, c–vsCoAA;
*p < 0.05;
**p < 0.01;
***p < 0.001 (ANOVA, Kruskal–Wallis test, Student's t-test, Mann–Whitney U-test)

Table 6. Frequency of elevated values of the tested parameters compared to Alvarado score (AS)

Parameter	Value	AS		p
		≤ 5	≥ 6	
PCT (ng/ml)	< 0.5 ≥ 0.5	58.97% 41.03%	10.71% **89.29%**	**0.0001*** **
TBil (μmol/L)	≤ 21 > 21	92.31% 7.69%	57.14% **42.86%**	**0.0007*** **
Histopathology	CAA and PhAA CoAA	94.87% 5.13%	42.86% **57.14%**	**0.0000*** **

PCT – procalcitonin; TBil – total bilirubin; CAA – catarrhal acute appendicitis; PhAA – phlegmonouse acute appendicitis; CoAA – complicated acute appendicitis;
***p < 0.001 (χ2 test)

Table 7. Frequency of elevated values of the tested parameters compared to histopathological findings

Parameter	Value	Histopathology			p
		CAA	PhAA	CoAA	
AS	**≤ 5** **≥ 6**	93.75% 6.25%	66.67% 33.33%	11.11% **88.89%ab*****	0.0000***
PCT (ng/ml)	< 0.5 ≥ 0.5	100 % 0%	42.42% **57.58%a*****	5.56% **94.44%a***b***	0,0000***
TBil (μmol/L)	≤ 21 > 21	93.75% 6.25%	81.82% 18.18%	55.56% **44.44%a***	0.0205*

AS – Alvarado score; CAA – catarrhal acute appendicitis; PhAA – phlegmonous acute appendicitis; CoAA – complicated acute appendicitis;
avs. CAA;
bvs. PhAA;
cvs. CoAA;
*p < 0.05;
**p < 0.01;
***p < 0.001 (χ2 test)

Table 8. Univariate logistic regression analysis estimating the probability of predicting Alvarado score

Parameter	OR	Limits 95% CI		p
		Lower	Upper	
NLR	1.44	1.17	1.79	0.0007***
PCT ≥ 0.5ng/ml	**9.20**	2.84	29.77	0.0002***
TBil > 21μmol/l	**9.00**	2.23	36.33	0,0020**

OR – odd ratio (probability ratio positive Alvarado score and negative Alvarado score); CI – confidence interval;
**p < 0.01;
***p < 0.001

CoAA compared to CAA (p < 0.001) and PhAA (p < 0.01), as well as in PhAA compared to CAA (p < 0.05). NLR values were statistically higher in CoAA compared to CAA (p < 0.01) and PhAA (p < 0.05). PCT values were higher in CoAA in relation to PhAA and CAA and in PhAA in relation to CAA (p < 0.001). TBil was elevated in CoAA compared to PhAA and CAA at a statistical significance level of p < 0.05.

Table 6 shows the frequency of elevated values of the tested parameters compared to AS. In patients with AS ≥ 6, PCT and TBil values were higher, as well as more frequent HP findings of CoAA (p < 0.001).

Compared to HP, the AS finding in CoAA was statistically more significant than in PhAA and CAA (p < 0.001). PCT was elevated in CoAA compared to CAA (p < 0.001), as well as in PhAA (p < 0.05). This parameter was elevated in PhAA compared to CAA (p < 0.001). TBil had elevated values in CoAA compared to CAA (p < 0.05) as shown in Table 7.

In order to determine the importance of each of the examined parameters in predicting the degree of appendicitis definitively established by HP finding, univariate logistic regression analysis was conducted. NLR, PCT, and TBil were correlated with AS values of ≥ 6: a one-unit increase in NLR values led to an increase in the probability AS ≥ 6 by 44%, while the value of PCT ≥ 0.5ng/ml increases 9.20 and TBil 9 times the probability of AS ≥ 6 (Table 8). The examined parameters as factors of interest for the HP finding of CoAA are shown in Table 9. A positive AS value increases the probability of occurrence of CoAA by 24.67 times. The probability of CoAA occurrence increases by 20% with a unit increase in NLR (1.02 to 1.51, p < 0.05).

TBil > 21 μmol/l increases the probability of CoAA 4.80 times (1.41–16.37, p < 0.05), while PCT ≥ 0.5 nng/ml by 26.84 times (3.30–218.55; p < 0.01).

For NLR, PCT, TBil, and AS, which were shown by univariate logistic regression analysis to be factors of interest for the HP findings, as the gold standard for establishing the type of AA, their diagnostic potential (sensitivity and specificity) was determined by analyzing ROC curves. Two cut-off values were calculated for each parameter, the first separating CAA from PhAA or CoAA and the second separating CoAA from CAA or PhAA. Based on the parameter values, it is evident that PCT showed the best diagnostic characteristics for differentiating CAA from PhAA/CoAA and for CoAA compared to CAA/PhAA. In the first case, the AUC is 1.000 with a statistical significance of p < 0.001. The cut-off is 0.37, confidence interval 0.946–1.000, it has the highest sensitivity and specificity and

Table 9. Univariate logistic regression analysis estimating the probability of predicting acute appendicitis histopathology

Parameter	OR	Limits 95% CI		p
		Lower	Upper	
Alvarado score ≥ 6	**24.67**	4.94	123.12	**0.0001*****
NLR	**1.20**	1.02	1.51	**0.0249***
PCT ≥ 0.5ng/ml	**26.84**	3.30	218.55	**0.0021****
TBil > 21 µmol/l	**4.80**	1.41	16.37	**0.0122***

NLR – neutrophil-lymphocyte ratio; PCT – procalcitonin; TBil – total bilirubin; OR – odds ratio (between catarrhal acute appendicitis and phlegmonous acute appendicitis on one side and complicated acute appendicitis on the other), CI–confidence interval;
*p < 0.05;
**p < 0.01;
***p < 0.001

Table 10. Diagnostic characteristics of Alvarado score (AS), neutrophil-lymphocyte ratio (NLR), neutrophil and procalcitonin (PCT) for distinguishing catarrhal acute appendicitis from phlegmonous acute appendicitis / complicated acute appendicitis

Parameter	Area below ROC curve (95% CI)	SE	p	Cut-off	Se (%)	Sp (%)	PPV (%)	NPV (%)	OA (%)
AS	0.775 (0.662–0.889)	0.053	0.0001***	8.5	52.94	93.75	96.43	37.50	62.69
NLR	0.676 (0.520–0.832)	0.080	0.0342*	3.29	86.27	43.75	83.02	30.43	30.43
PCT	**1.000 (0.946–1.000)**	**0.000**	**0.000*****	**0.37**	**100**	**100**	**100**	100	**100**
TBil	0.611 (0.456–0.759)	0.079	0.161	16.5	81.3	45.1	88.46	31.71	53.73

*p < 0.05;
***p < 0.001;
TBil – total bilirubin; CI – confidence interval; SE – standard error; Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value; OA – overall accuracy

Table 11. Diagnostic characteristics of Alvarado score (AS), neutrophil-lymphocyte ratio (NLR), and procalcitonin (PCT) for distinguishing complicated acute appendicitis from catarrhal acute appendicitis/phlegmonous acute appendicitis

Parameter	Area below ROC curve (95% CI)	SE	p	Cut-off	Se (%)	Sp (%)	PPV (%)	NPV (%)	OA (%)
AS	0.823 (0.719–0.927)	0.053	0.0001***	> 8	88.89	75.51	57.14	72.55	79.10
NLR	0.728 (0.608–0.848)	0.061	0.0045**	> 4.52	100	44.90	40	44.90	59.70
PCT	**0.963 (0.885–0.994)**	**0.029**	**0.000*****	**> 0.56**	**94.44**	**91.84**	**80.95**	97.83	**92.54**
TBil	0.70 (0.58–0.81)	0.079	0.011*	> 23.2	38.9	95.9	70	81	79

*p < 0.05;
**p < 0.01;
***p < 0.001;
TBil – total bilirubin; CI – confidence interval; SE – standard error; Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value; OA – overall accuracy

highest overall accuracy (Figure 1, Table 10). In the second case, PCT was also shown to have the best predictive characteristics for CoAA compared to CAA/PhAA. The AUC is 0.963, and the statistical significance of p < 0.001. The cut-off value is 0.56 with a confidence interval of 0.885–0.994, with sensitivity and specificity of 94.44% and 91.84%, as well as the highest values of positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. AS has been shown to have good predictive characteristics for CoAA compared to CAA/PhAA. The AUC is 0.823, with a cut-off value > 8, sensitivity 88.89% and specificity 75.51%, high overall accuracy, PPV and NPV (Figure 2, Table 11).

DISCUSSION

Despite the increased use of laboratory tests, radiological diagnostic methods and clinical scoring systems for timely diagnosis, AA remains the most common dilemma of the surgical team. In recent years, laparoscopic appendectomy has been the gold standard for surgical treatment of appendicitis. Numerous studies have shown the advantages of laparoscopic appendectomy over open appendectomy: reduced pain intensity, lower complication rates, shorter hospitalization, and better quality of life postoperatively [9]. In order to avoid unnecessary operative explorations, as well as overlooked AAs in everyday surgical practice, there is a constant effort to find more reliable and precise diagnostic tools. Therefore, the search for an ideal biomarker that would be used exclusively or combined with other parameters or as part of the stratification results has been ongoing for a long time [6, 7].

Neutrophils represent the first line of defense against infection agents, primarily bacteria. Lymphocytes are immunocompetent cells that coordinate the immune response and assist neutrophil activity. Neutrophilia and lymphocytopenia are components of the cellular response in systemic inflammation [10, 11]. In a study by Omari et al. [12] investigating risk factors for appendicitis in the elderly, 94% of the perforated group had a left shift compared with 61% of the nonperforated group.

The NLR is derived directly from the differential blood count. The fact is that from previous studies, NLR is a more sensitive parameter than leukocyte count, and the NLR value can be used to identify those patients who have a higher risk of complications and a more careful observation must be considered. Numerous studies have shown that NLR are increased in AA and are correlated with the severity of inflammation. NLR has been suggested as a valuable predictor of gangrenous appendicitis in patients undergoing surgery for AA [13, 14]. In 2014, Kahramanca et al. [15] published a study comparing normal and inflamed appendices and found an NLR cut-off value of 4.68. In complicated appendicitis, the NLR cut-off value was estimated to be 5.74, with 70.8% sensitivity and 48.5% specificity. In a limited number of published studies, the diagnostic value of NLR was higher than that of conventional laboratory assessments [leucocyte count, C-reactive protein (CRP)] [15]. Godinez-Vidal et al. [16] compared NLR with other biomarkers to assess disease severity. It was shown that NLR > 12 may be associated with generalized peritonitis

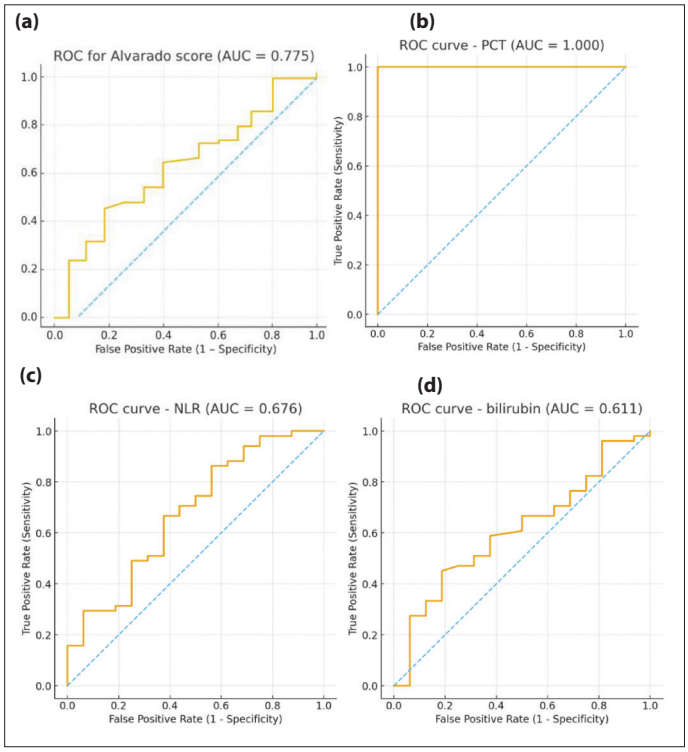

Figure 1. ROC curve and predictive characteristics of a) Alvarado score (AS); b) procalcitonin (PCT); c) neutrophil-lymphocyte ratio (NLR); d) total bilirubin (TBil) for distinguishing catarrhal acute appendicitis from phlegmonous acute appendicitis / complicated acute appendicitis

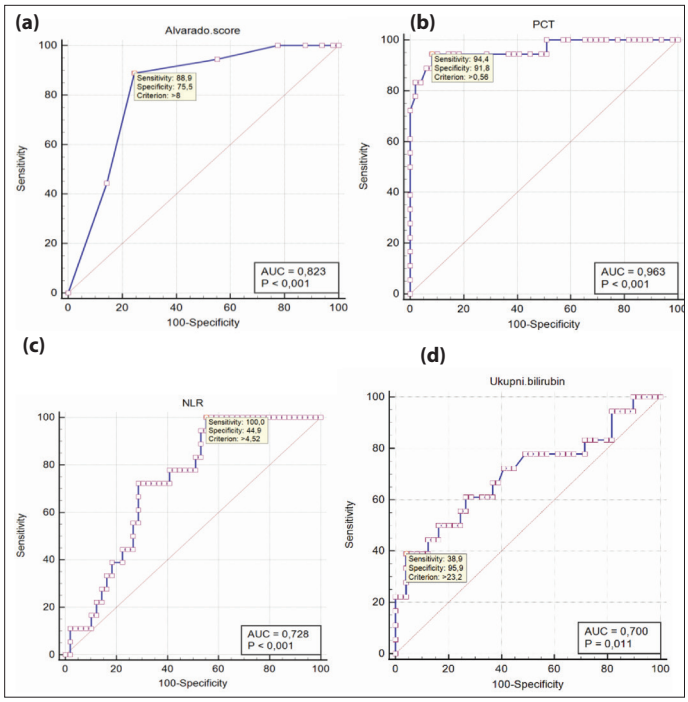

Figure 2. ROC curve and predictive characteristics of a) Alvarado score (AS); b) procalci-tonin (PCT); c) neutrophil-lymphocyte ratio (NLR); d) total bilirubin (TBil) for distinguish-ing complicated acute appendicitis from catarrhal acute appendicitis/phlegmonous acute appendicitis

and perforated appendicitis [16]. The results of our study show good performance of NLR, which can be accepted as an easily applicable, inexpensive and available additional parameter contributing to the diagnosis of appendicitis. The odds ratio for AS ≥ 6 and AS ≤ 5 increased by 44% with a unit increase in NLR (1.17–1.79, $p < 0.001$), and the probability of HP findings of CoAA and PhAA/CAA increases by 20% with a unit increase in NLR (1.02–1.51, $p < 0.05$) (Tables 8 and 9). The cut-off value between CAA and PhAA/CoAA was 3.29, with statistical significance of $p < 0.05$ (Table 10). The cut-off value between CoAA and CAA/PhAA was > 4.52, the AUC is 0.728 with a statistical significance of $p < 0.01$ (Table 11). These findings are in concordance with available data from the literature and indicate that NLR can be accepted as an additional parameter that contributes to the diagnosis of appendicitis.

Hyperbilirubinemia results from an imbalance between bilirubin production and excretion. Some studies have shown that bacterial endotoxins, such as toxins produced by *Escherichia coli*, reduce liver cell secretion, contributing to intrahepatic cholestasis and sinusoidal damage [17, 18]. Emmanuel et al. [19] found in their study that the specificities of white cell count and CRP were less than hyperbilirubinemia for simple appendicitis (60% and 72%) and perforated or gangrenous appendicitis (19% and 36%). The results of Nevler et al. [20] suggest that bilirubin levels may be an important diagnostic factor, similar to leucocyte, CRP, and AS. The sensitivity of TBil in predicting complicated appendicitis was found 91.43% (76.94% to 98.20%), whereas the specificity of this test was 88% (78.44% to 94.36%), PPV and NPV were 78.03% and 95.65%, respectively, in the study of Bakshi and Mandal [21]. In our study, TBil > 21 μmol/l increases the probability of CoAA by 4.80 times (1.41–16.37, $p < 0.05$) (Table 9). The result of ROC analysis for bilirubin showed AUC = 0.70 (95%CI:0.58–0.81, $p < 0.05$), with specificity and sensitivity of 95.9% and 38.9%, PPV – 70% and NPV – 81%, indicating acceptable/good discrimination of complicated from uncomplicated appendicitis (Table 11).

PCT is a prohormone of calcitonin, and the main site of synthesis is the liver, but also the neuroendocrine cells of the lungs and small intestine. Under physiological conditions, PCT is not released into the circulation. PCT is initially detected in plasma six to 12 hours after intake, increases and reaches a peak after 12–24 hours, and remains in the form of a plateau in the following 2–3 days. Numerous studies have addressed the role of PCT in the diagnosis of CoAA, all with the aim of implementing proper treatment and preventing unnecessary appendectomy [22]. Reviewing the literature, there is no firm consensus regarding the role of PCT in the diagnosis of appendicitis. Wu et al. [23] found that PCT was statistically more significant in patients with CoAA, with an AUC of 0.69 compared to 0.61 for CRP. Prediction of disease severity can be made depending on the quantitative values of PCT [23]. The aim of the study conducted by Sand et al. [24] was to examine the diagnostic significance of PCT in AA. They concluded that

PCT is elevated, especially after gangrene and perforation of the appendix. Extremely small sensitivity does not recommend its routine use for AA [24]. Research by Haghi et al. [25] suggests that PCT and Interleukin 6 together may provide useful evidence for decision-making. Negative results for either of these biomarkers may help to exclude AA and reduce the number of negative appendectomies [25]. In our research compared to AS the level of PCT ≥ 0.5 ng/ml was shown to increase the odds ratio for positive and negative AS value by 9.20 times (2.84–29.77; $p < 0.001$) and increases the probability of occurrence of CoAA by 26.84 times (3.30–218.55; $p < 0.01$) (Tables 8 and 9). These data demonstrate that PCT values can influence the accuracy of AA diagnosis, predict the severity of inflammation, and may serve as independent markers for CoAA.

AS is a scoring system used to determine the risk of AA in patients with abdominal complaints. Data from the literature indicate that AS is used to diagnose AA [8]. We selected a cut-off value of ≥ 6 for the AS based on previously published validation studies showing that this cut-off provides an optimal balance between sensitivity and specificity for the diagnosis of AA. Lower cut-offs increase sensitivity at the expense of specificity, whereas higher cut-offs (≥ 7) may lead to missed diagnoses. Therefore, a cut-off value of ≥ 6 was considered clinically appropriate for identifying patients with suspected AA and for further diagnostic evaluation [26, 27]. In our work, the ROC curve showed that AS with a cut-off value of > 8 was a good predictor of CoAA compared to CAA/PhAA, with AUC = 0.823(0.719–0.927), which is lower only compared to PCT (Figure 2, Table 11). All this indicates a high predictive ability of AS, especially for estimating the probability of CoAA. This is in line with the findings of other studies that report AS as a superior diagnostic aid [28].

CONCLUSION

This study demonstrated very good diagnostic properties of NLR, AS, and especially PCT and their ability to predict CoAA. Elevated TBil may be one of the markers of a complicated course of the disease, especially when combined with other parameters. These parameters are inexpensive for patients, easily accessible, minimally invasive and can be repeated if necessary and do not require any special apparatus or training. Results are acquired quickly, unnecessary additional diagnostics can be reduced, the rate of negative appendectomies is reduced, as is the rate of AA complications. NLR, TBil, PCT together with the AS should be used in daily practice, preferably in combination, as powerful diagnostic parameters and predictors of CoAA. It is certainly necessary to conduct further research in this area in the near future. Further multicenter prospective studies with larger samples and longer study periods are needed to confirm the significance of NLR, TBil, and PCT in the diagnosis of AA in adults, especially its complicated form.

Conflict of interest: None declared.

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Упоредна евалуација инфламаторних биомаркера и укупног билирубина за рано откривање компликованог апендицитиса код одраслих

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САЖЕТАК

Увод/Циљ Акутни апендицитис (АА) најчешћи је узрок хитног хируршког лечења. Бројни биомаркери се могу користити за предвиђање прогресије болести, као и за разликовање болесника са АА од оних са болом друге етиологије.

Циљ овог рада био је да се утврди тачност односа неутрофила и лимфоцита, прокалцитонина и укупног билирубина у дијагнози компликованог АА (КоАА), као и да се они упореде са Алварадо скором и хистопатолошким налазима.

Методе АА је дијагностикован преоперативно код 67 болесника коришћењем Алварадо скора. Испитивани параметри и вредности скора одређивани су преоперативно, и постоперативно упоређивани са хистопатолошким налазима. У зависности од налаза, испитаници су класификовани у три групе: гангренозни и гангренозно-перфоративни апендицитис (сврстани у КоАА), флегмонозни и катарални апендицитис.

Резултати Резултати униваријантне анализе показују да повећање односа неутрофила и лимфоцита за једну јединицу повећава вероватноћу КоАА за 20% (1,02 на 1,51, $p < 0,05$). Вредност прокалцитонина ≥ 0,5 *ng/ml* повећава вероватноћу КоАА за 26,84 пута (3,30 на 218,55; $p < 0,001$), док укупни билирубин > *21 µmol/l* повећава вероватноћу појаве КоАА за 4,80 пута (1,41 на 16,37, $p < 0,05$). *ROC* крива је показала да је прокалцитонин најбољи предиктор КоАА у односу на катарални и флегмонозни (са *cut-off* 0,56), као и за катарални у односу на остале групе (са *cut-off* 0,37).

Закључак Прокалцитонин, укупни билирубин и однос неутрофила и лимфоцита могу се користити у свакодневној клиничкој пракси као поуздани, лако доступни, економски исплативи параметри у дијагностици КоАА код одраслих.

Кључне речи: инфламаторни биомаркери; укупни билирубин; компликовани акутни апендицитис

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

MRI-based clinical anatomical evaluation of vertebral morphometric changes associated with disc herniation stages at the L4–L5 level

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SUMMARY

Introduction/Objective This study aimed to evaluate vertebral body and intervertebral disc morphometry at the L4–L5 level in individuals with lumbar disc herniation, with a particular focus on identifying morphometric parameters relevant to radiological interpretation from a clinical anatomical perspective.

Methods Lumbar MRI scans of 98 individuals aged 40–60 with single-level L4–L5 disc herniation and 184 healthy individuals (defined as subjects with normal discs or disc bulging without protrusion or extrusion) were retrospectively reviewed. Anterior, middle, and posterior morphometric measurements of the L4–L5 intervertebral disc and the L4–L5 vertebral bodies were obtained on standardized sagittal planes and compared between groups as well as between protrusion and extrusion subgroups.

Results No significant differences were observed in L4 and L5 vertebral body heights between healthy and pathological groups ($p > 0.05$). However, anterior, middle, and posterior disc heights at the L4–L5 level were all significantly reduced in the pathological group compared with healthy controls ($p < 0.05$). Within the pathological group, posterior disc height and the percentage of posterior disc height loss were significantly lower in extruded cases than in protruded cases ($p < 0.05$), whereas anterior and middle disc heights showed no significant differences ($p > 0.05$). In addition, the disc height asymmetry ratio was significantly lower in extruded cases, reflecting a posterior-dominant disc collapse pattern ($p < 0.05$).

Conclusion While a general reduction in disc height was observed in the pathological group, the decrease in posterior disc height was more pronounced, particularly in extruded cases. The preservation of vertebral body morphometry highlights morphometric changes specific to disc pathology. From a clinical anatomical perspective, posterior disc morphometry may be considered a supportive parameter in the radiological evaluation of lumbar disc herniation.

Keywords: lumbar disc herniation; L4–L5 level; disc height; vertebral morphometry; magnetic resonance imaging (MRI)

INTRODUCTION

Low back pain is a common musculoskeletal condition affecting a large proportion of the population at least once during their lifetime, with prevalence increasing with age [1]. Disc herniation is the displacement of the nucleus pulposus into the vertebral canal through a tear in the annulus fibrosus caused by intervertebral disc degeneration. Lumbar disc herniation (LDH) is considered one of the most common causes of low back pain and is seen in 50%–70% of the population, increasing with age [2, 3]. In the lumbar (L) region, the L4–L5 and L5–S1 discs are the most common levels of herniation, as they bear the greatest mechanical load due to the biomechanics of the spine [4, 5, 6].

Advances in magnetic resonance imaging (MRI) technology have enabled detailed examination of disc degeneration and geometric changes in vertebral structures owing to high-resolution soft tissue contrast, thereby making morphometric analysis of the lumbar spine more reliable [7–10]. In addition, there is increasing evidence that disc herniation may affect not only the disc itself but also the morphometry of the lower and upper vertebrae, with recent MRI-based studies showing that disc herniation is associated with lumbar morphometric changes and adjacent vertebral endplate alterations [11, 12]. It has been reported that disc height, vertebral body height, and oblique angles may reflect biomechanical load distribution within the intervertebral space and may vary across herniation stages, with recent MRI-based evidence also linking lower lumbar endplate morphology to disc degeneration in lumbar disc herniation [13, 14]. However, studies specifically comparing the effects of different herniation stages at the L4–L5 level on the morphometry of adjacent vertebrae are limited in the literature [15, 16]. Current studies have generally evaluated the complete lumbar segment and have not focused specifically on particular segments [17, 18, 19]. However, the L4–L5 level has particular clinical significance as it is the segment that experiences the greatest biomechanical load and where herniation is

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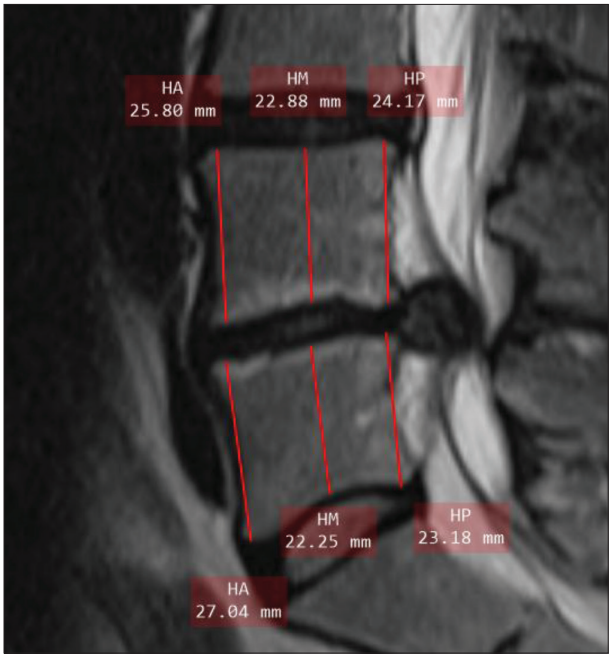

Figure 1. Sagittal T2-weighted lumbar MRI image of a 55-year-old female patient with an extruded disc herniation at the L4–L5 level

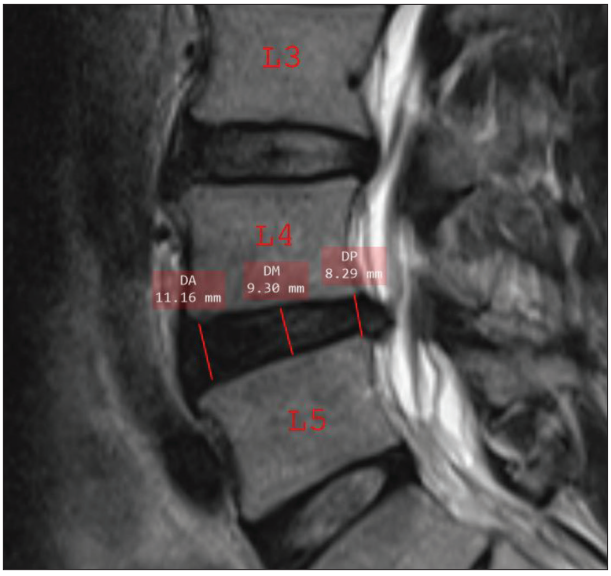

Figure 2. Sagittal T2-weighted lumbar MRI image of a 49-year-old male patient with a protruded disc herniation at the L4–L5 level

most frequently observed. From both a diagnostic assessment and surgical planning perspective, detailed examination of morphometric features at this level will significantly contribute to understanding the pathological processes. Thus, this study aimed to evaluate the effects of disc herniation stage at the L4–L5 level on the morphometry of the disc and adjacent vertebral bodies, with a segment-specific focus and a separate analysis of protrusion and extrusion stages, and by introducing novel morphometric indices (DHAR, PDI, and MDI).

METHODS

In this retrospective study, lumbar MRI scans obtained at Artvin State Hospital between 2020 and 2025 were reviewed in 282 eligible individuals. Participants were classified into a healthy group (n = 184) and a pathological group (n = 98) with single-level L4–L5 disc herniation (protrusion/extrusion).

Inclusion criteria were as follows: age between 40 and 60 years, having undergone a lumbar MRI scan, and the image quality being of an evaluable standard. Exclusion criteria were the following: history of lumbar surgery, detection of vertebral fracture, presence of scoliosis of 20° or greater, insufficient image quality, disc herniation at three or more levels, presence of sequestration (free fragment).

Radiological evaluation and measurement protocol

Morphometric measurements were obtained from sagittal T2-weighted MRI sections by measuring the anterior (HA), middle (HM), and posterior (HP) heights of the L4 and L5 vertebral bodies, as well as the anterior (DA), middle (DM), and posterior (DP) heights of the L4–L5 intervertebral disc. All measurements were performed on images acquired using an MRI scanner (Siemens Healthineers, Erlangen, Germany) and evaluated through the hospital's Picture Archiving and Communication System (PACS) using the built-in, calibrated measurement tools of the system under standardized window settings (contrast and brightness). Each measurement was performed twice, and mean values were used for statistical analysis.

In addition to absolute morphometric disc height measurements, four relative indices were defined to better capture the geometric architecture of disc height: disc height asymmetry ratio (DHAR), posterior disc index (PDI), middle disc index (MDI), and posterior–middle gap (P–M gap). The loss rate (%) was calculated using the following formula: [(Healthy Group Average Value – Pathological Group Average Value) / (Healthy Group Average Value) × 100].

Participants were divided into two groups: the healthy group and the pathological group. The healthy group included individuals with normal discs or disc bulging at the L4–L5 level, whereas the pathological group consisted of cases with disc herniation at the protrusion or extrusion stage. Disc bulging cases were included in the healthy group as they do not represent true herniation and were therefore considered separately from the pathological group. Example sagittal T2-weighted MRI images of extruded and protruded cases are presented in Figure 1 and Figure 2, respectively. Herniation staging was based on radiology reports archived in the PACS system and was cross-checked to minimize potential classification errors.

Statistical analyses

Comparisons between protruded and extruded groups were carried out using the Mann–Whitney U test. To highlight not only statistical significance but also the practical relevance of the findings, effect sizes were calculated using

Table 1. Participants' demographic characteristics

Groups	n	Female (n)	Male (n)	Age (Mean ± SD)
Healthy	184	109	75	49.2 ± 5.1
Pathological (disc protrusion/ extrusion)	98	59	39	52.5 ± 5.3
Total	282	168	114	50.3 ± 5.6

Table 2. Comparison of L4 and L5 vertebral body heights between the healthy group and the pathological groups

Vertebra	Measurement area	Healthy group Mean ± SD (mm)	Pathological group Mean ± SD (mm)	p*
L4	HA	25.9 ± 0.8	25.7 ± 0.9	0.462
	HM	23.4 ± 0.7	23.3 ± 0.8	0.317
	HP	24.1 ± 0.9	24 ± 1	0.411
L5	HA	27.6 ± 0.9	27.4 ± 1	0.274
	HM	23.8 ± 0.8	23.7 ± 0.9	0.233
	HP	24.1 ± 0.7	24 ± 0.8	0.361

HA – anterior vertebral height; HM – middle vertebral height; HP – posterior vertebral height;
*Mann–Whitney U test

Table 3. Comparison of intervertebral disc heights at the L4–L5 level between healthy group and pathological groups

Level	Measurement Area	Healthy group (Mean ± SD, mm)	Pathological group (Mean ± SD, mm)	p*
L4–L5	DA	11.8 ± 0.7	10.9 ± 0.8	0.004
	DM	10.4 ± 0.6	9.6 ± 0.7	< 0.001
	DP	9.7 ± 0.8	8.9 ± 0.9	< 0.001

DA – anterior disc height; DM – middle disc height; DP – posterior disc height;
*Mann–Whitney U test

Table 4. Comparison of L4–L5 disc heights in protruded (n = 59) and extruded (n = 39) cases

Measurement area	Protruded (Mean ± SD, mm)	Extruded (Mean ± SD, mm)	p*
DA	11 ± 0.9	10.8 ± 0.95	0.284
DM	9.8 ± 0.72	9.5 ± 0.82	0.091
DP	9.1 ± 0.85	8.8 ± 0.81	0.041*

DA – anterior disc height; DM – middle disc height; DP – posterior disc height;
*Mann–Whitney U test

Table 5. Comparison of disc height loss rates in protruded and extruded cases

Measurement area	Protruded average loss (%) ± SD	Extruded average loss (%) ± SD	p*
Anterior	5.1 ± 2.3	7.6 ± 2.5	0.29
Middle	7.7 ± 2.4	11.5 ± 2.8	0.078
Posterior	10.3 ± 2.7	16.5 ± 3	0.037*

*Mann–Whitney U test

the rank-biserial correlation coefficient (r) and interpreted as small (≈ 0.1), moderate (≈ 0.3), or large (≥ 0.5). A p value of < 0.05 was set as the threshold for statistical significance. All analyses were performed using IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA).

Ethics: This study was conducted retrospectively following approval by the Artvin Çoruh University Scientific Research and Publication Ethics Committee (Decision No: E-18457941-050.99-168439, Date: February 13, 2025).

RESULTS

A total of 282 individuals were included in the study. Of the participants, 168 were female and 114 were male. The mean age in the pathological group was 52.5 ± 5.3 years, while the mean age in the healthy group was 49.2 ± 5.1 years (Table 1).

When comparing the anterior, middle, and posterior corpus heights of the L4 and L5 vertebrae between the healthy group and the pathological group, no statistically significant difference was observed (p > 0.05) (Table 2).

In the healthy group, anterior, middle and posterior disc height values were found to be significantly higher than those in the pathological group (p < 0.05) (Table 3).

When comparing disc heights between protruded and extruded cases within the pathological group, posterior disc height was found to be significantly lower in extruded cases (p = 0.041). No statistically significant difference was observed between these groups regarding anterior and middle disc heights (p > 0.05) (Table 4).

No statistically significant difference was observed in anterior and middle disc heights in protruded and extruded cases; however, a significantly greater percentage of posterior disc height loss was observed in extruded cases compared with protruded cases (p = 0.037) (Table 5).

The disc height asymmetry ratio (DHAR) was significantly lower in extruded cases compared with protruded cases (p = 0.048), indicating a disproportionate reduction in posterior disc height relative to the anterior segment. No statistically significant differences were observed between groups for the posterior disc index (PDI), middle disc index (MDI), or the posterior–middle height gap (P–M gap) (p > 0.05) (Table 6).

When the morphometric profiles of extruded and protruded cases were compared, posterior disc height, the percentage of posterior disc height loss, and the disc height asymmetry ratio showed significant differences between the groups. No statistically significant differences were observed for the remaining parameters (Table 7).

DISCUSSION

The most important finding of the present study is that disc height reduction at the L4–L5 level is particularly pronounced in the posterior segment in patients with disc herniation, and that this reduction is more evident in extruded cases compared with protruded cases. When the healthy and pathological groups were compared, no significant differences were observed in vertebral body heights; however, intervertebral disc heights were reduced across all segments in the pathological group, with the posterior disc segment showing the greatest decrease. Within the pathological cohort, posterior disc height was significantly lower in extruded cases than in protruded cases, whereas the percentage of posterior disc height loss was significantly greater in extruded cases than in protruded cases. In addition, relative indices reflecting the geometric distribution of disc height were evaluated, and among

Table 6. Novel posterior-dominant disc collapse indices in protruded and extruded cases

Index (Novel)	Formula	Protruded (Mean ± SD)	Extruded (Mean ± SD)	p*
DHAR	DP/DA	0.83 ± 0.09	0.81 ± 0.08	0.048*
PDI (%)	(DA – DP) / DA × 100	17.3 ± 4.1	18.5 ± 4.4	0.094
MDI (%)	(DA – DM) / DA × 100	10.9 ± 3.6	12 ± 3.9	0.081
P–M Gap (mm)	DM – DP	0.7 ± 0.28	0.7 ± 0.31	0.992

DHAR – disc height asymmetry ratio (DP/DA); PDI – posterior dominance index, percentage of posterior disc height loss relative to anterior disc height; MDI – middle drop index, percentage of middle disc height loss relative to anterior disc height; P–M Gap – absolute difference between middle and posterior disc heights; DA – anterior disc height; DM – middle disc height; DP – posterior disc height;
*Mann–Whitney U test

Table 7. Morphometric profile distinguishing protrusion and extrusion at the L4–L5 Level

Parameter	Protruded (Mean ± SD)	Extruded (Mean ± SD)	p*	Effect size (r)
DA (mm)	11 ± 0.9	10.8 ± 0.95	0.284	0.02
DM (mm)	9.8 ± 0.72	9.5 ± 0.82	0.091	0.03
DP (mm)	9.1 ± 0.85	8.8 ± 0.81	0.041*	0.13
Loss DA (%)	5.1 ± 2.3	7.6 ± 2.5	0.290	0.03
Loss DM (%)	7.7 ± 2.4	11.5 ± 2.8	0.078	0.03
Loss DP (%)	10.3 ± 2.7	16.5 ± 3	0.037*	0.12
DHAR	0.83 ± 0.09	0.81 ± 0.08	0.048*	0.10
PDI (%)	17.3 ± 4.1	18.5 ± 4.4	0.094	0.05
MDI (%)	10.9 ± 3.6	12 ± 3.9	0.081	0.02
P–M Gap (mm)	0.7 ± 0.28	0.7 ± 0.31	0.992	~0.00

DA – anterior disc height; DM – middle disc height; DP – posterior disc height; Loss DA (%) – percentage loss of anterior disc height relative to the healthy group; Loss DM (%) – percentage loss of middle disc height relative to the healthy group; Loss DP (%) – percentage loss of posterior disc height relative to the healthy group; DHAR – disc height asymmetry ratio (DP/DA); PDI (%) – posterior dominance index, percentage of posterior disc height reduction relative to anterior disc height; MDI (%) – middle drop index, percentage of middle disc height loss relative to anterior disc height; P–M Gap – absolute difference between middle and posterior disc heights;
*Mann–Whitney U Test

these indices, only the disc height asymmetry ratio demonstrated a significant difference between protruded and extruded cases. Although the associated effect sizes were small, posterior disc–related parameters showed consistent differences between the two herniation stages, whereas no significant differences were observed in anterior and middle disc measurements or in the remaining relative indices.

Our findings indicate a reduction in disc height in lumbar disc degeneration and related pathologies, which is consistent with studies in the current literature. In some imaging-based studies, a correlation was found between disc degeneration and a decrease in disc height, and this condition was found to be particularly pronounced at the L4–L5 and L5–S1 disc levels [20, 21]. In a study conducted by Zheng et al. [21], the relationship between lumbar disc degeneration and intervertebral disc height was examined in 85 patients with back pain. It was found that as the severity of degeneration and herniation increased, a significant decrease in disc height occurred. The Pfirrmann classification was used in the study, and an average decrease of 1.25–1.76 mm in disc height at each degree of degeneration was found. They reported that this decrease occurred independently of age, gender, and smoking status. In addition, a positive correlation was observed between the increase in VAS (Visual Analog Scale) score and the decrease in disc height. This finding demonstrates that the increase in the degree of disc degeneration has an effect not only on disc morphometry but also on the severity of clinical symptoms. In another study, lumbosacral MRI images of 72 individuals were evaluated according to the Pfirrmann grading system [20]. A significant decrease in disc height was observed with increasing degeneration severity, independent of age, gender, body mass index, and smoking, with an average reduction of 0.98–1.60 mm per Pfirrmann grade. Similarly, in our study, the fact that anterior, middle, and posterior disc height values were significantly reduced in the pathological group compared with the healthy group supports the clinical implications of this quantitative reduction in disc height that occurs with increasing degeneration. In a study by Singh et al. [22], the lumbar spine parameters of individuals with chronic low back pain were compared with those of a healthy control group using MRI. Significant reductions in disc angle and disc cross-sectional area were observed in the group with chronic low back pain, particularly at the L3–L4 and L4–L5 levels, in relation to the healthy control group [22]. Furthermore, degenerative changes in the intervertebral discs were observed in 54% of patients, whereas these findings were not observed in the healthy group. It was found that reductions in disc angles and disc cross-sectional area may lead to early degeneration due to abnormal load transfer and stress distribution [22]. These results are in correlation with the significant reduction observed in the posterior segment of the disc, particularly in pathological cases in our study. Since changes in the spinal angle directly affect the load distribution in the posterior segment of the disc, the disruption of geometric balance in this region may weaken spinal stability, leading to mechanical imbalance. Thus, morphometric losses occurring in the posterior segment may be associated with the early appearance of clinical symptoms. In another study by Näther et al. [23], the number of degenerated discs in the spine was evaluated according to age, sex, and disc levels. The study showed that disc degeneration increases with age, with the L4–L5 and L5–S1 levels being the most commonly affected. Degenerative disc disease was present in 91.6% of individuals aged 50–61. Although no significant gender differences were identified, a reduction in disc height appears to be associated with a higher likelihood of both disc herniation and bony degenerative changes such as spondylosis. Bakar et al. [24] examined mature single-level lumbar disc herniations in a study evaluating the vertebral disc morphometrics and spinal balance parameters of 30 operated patients and 15 healthy individuals. In the surgical group, L1–L2 and L5–S1 disc heights were found to be significantly reduced compared to the healthy group. In addition, significant decreases were observed in parameters representing sagittal balance, such as the T12 angle of inclination and the L4–S1 Cobb angle.

ROC and regression analyses revealed that a decrease in the average height of the L5–S1 disc below 8.15 mm is a significant morphometric indicator of the presence of lumbar disc herniation. In another study, the morphometric and stereological analysis of the vertebrae of patients with lumbar intervertebral disc herniation and healthy individuals was performed [25]. In the study, although no significant difference was observed in disc heights in the early period between the healthy and patient groups, a marked difference was detected in disc volumes. This finding indicates that disc height can be preserved in patients with herniation in the early stages, but volume loss is an early indicator of degeneration. The study observed that although posterior disc height in particular showed a tendency to differ significantly, it did not confirm statistical significance in the early stages. In light of all these findings, the significant reduction observed in posterior disc height in our study suggests that it may represent an important morphometric marker of advanced lumbar disc herniation.

A study examining the relationship between lumbar disc herniation and vertebral morphometry investigated 249 middle-aged Finns [26]. It was found that for every 1 cm^2 increase in the cross-sectional area of the L4 vertebral axial diameter, the likelihood of disc displacement increased by 10%. Hornung et al. [27] found that one of the strongest morphometric indicators predicting early resorption in patients with lumbar disc herniation was the height of the L4 posterior vertebral body. The study reported that a higher posterior vertebral height would accelerate the resorption process by expanding the space between the disc material and the epidural space. This finding indicates that vertebral morphometrics may play a role not only in disc pathologies but also in the natural healing of the disc. In another study on vertebral body morphometry, it was reported that a geometric difference of more than 10% in the anteroposterior dimension between adjacent vertebral bodies, particularly at the L5–S1 level, increases the risk of disc herniation by sixfold [28]. These findings suggest that disproportionality in vertebral morphometry may lead to an imbalance in axial load transmission, resulting in increased stress accumulation in the posterior fibers of the disc [28]. In our study, no significant change was observed in the supero-inferior heights of the vertebral body in disc herniation. This finding may be explained by the fact that in the early stages of degenerative loading, the pathology primarily manifests at the intervertebral disc level, and the vertebral body morphometry remains stable in the short term. Furthermore, with the reduction in disc height, part of the axial load may be partially compensated by the posterior elements and facet joints, thereby reducing the impact on the corpus and preserving vertebral height. Furthermore, factors such as the MR slice geometry, lordosis angle, and sample size used in the evaluation may have prevented small differences in the corpus from becoming statistically significant. Therefore, our findings may suggest that structural changes develop primarily at the disc level at the onset of the degenerative process, while effects on vertebral corpus morphometry may require longer-term remodeling to become detectable. Very recent MRI-based studies published in 2026 have further strengthened the clinical and biomechanical relevance of disc morphometry in lumbar disc herniation. Šprláková-Puková et al. [29] demonstrated that specific morphometric parameters, particularly middle disc height, may serve as significant predictors of clinical symptom resolution, and that patients with favorable morphometric profiles may have a substantially higher likelihood of spontaneous recovery without surgical intervention. Furthermore, a long-term longitudinal MRI study by Lund et al. [30] revealed that intervertebral disc degeneration progresses in a level-specific and age-dependent manner and is associated with future low back pain outcomes. Taken together, these findings support the concept that morphometric evaluation of the intervertebral disc, especially segment-specific and posterior disc measurements, may provide valuable insight into both the progression and clinical implications of lumbar disc herniation.

From a clinical perspective, a marked reduction in posterior disc height on MRI can offer a simple and objective way to better understand the severity of lumbar disc herniation. Beyond traditional disc degeneration grading systems, measuring posterior disc height provides a clear, segment-specific, and quantitative description of disc morphology, particularly in cases of extrusion. While this finding should not be interpreted as a definitive staging criterion or a factor determining treatment choice, it may help clinicians achieve a more detailed anatomical and radiological appreciation of herniation patterns. Posterior disc morphometry can serve as a supportive anatomical reference during routine clinical assessment and follow-up of patients with lumbar disc herniation.

The limitations of this study include the fact that only L4–L5 level disc herniation was addressed, with other lumbar segments excluded from the assessment. The participants were aged 40–60 years; therefore, the results are limited to a single age group. Measurements were performed manually; automatic or three-dimensional segmentation techniques were not used. Furthermore, information that could affect morphometric measurements, such as participants' physical activity level, occupational stress, history of chronic disease, and regular medication use, could not be obtained due to the retrospective nature of the analysis. For these reasons, the findings need to be supported by further prospective studies with larger samples and multivariate analyses.

CONCLUSION

The findings of this study indicate that intervertebral disc height is markedly reduced in the presence of lumbar disc herniation, with this reduction becoming more pronounced in the posterior disc segment as the disease progresses. Posterior disc segment is more susceptible to collapse in advanced stages of herniation. Degenerative alterations initially occur at the intervertebral disc level, while vertebral corpus morphometry remains relatively preserved. Among the relative indices reflecting the

geometric distribution of disc height, only the disc height asymmetry ratio is expected to be different between protruded and extruded cases. Taken together, these findings suggest that posterior disc morphometry may represent a supportive morphometric parameter in the radiological assessment of lumbar disc herniation severity.

Conflict of interest: None declared.

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Клиничко-анатомска евалуација морфометријских промена пршљенова повезаних са стадијумима дискус хернije на нивоу *L4–L5* заснована на магнетној резонанци

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САЖЕТАК

Увод/Циљ Циљ ове студије био је да се процени морфометрија тела пршљенова и интервертебралног диска на нивоу *L4–L5* код особа са лумбалном дискус хернијом, са посебним фокусом на идентификацију морфометријских параметара релевантних за радиолошку интерпретацију из перспективе клиничке анатомије.

Методе Ретроспективно су анализирани лумбални магнетнорезонантни снимци 98 особа старости 40–60 година са једнонивојском дискус хернијом на нивоу *L4–L5* и 184 здрава испитаника (са нормалним дисковима или дискус бублгингом на нивоу *L4–L5*). Предња, средња и задња морфометријска мерења интервертебралног диска *L4–L5* и тела пршљенова *L4* и *L5* добијена су на стандардизованим сагиталним равнима и упоређена између група, као и између подгрупа са протрузијом и екструзијом.

Резултати Нису уочене значајне разлике у висини тела пршљенова *L4* и *L5* између здравих и патолошких група ($p > 0,05$). Међутим, предња, средња и задња висина диска на нивоу *L4–L5* биле су значајно смањене у патолошкој групи у поређењу са здравом контролном групом ($p < 0,05$). Унутар патолошке групе, задња висина диска и проценат губитка задње висине диска били су значајно нижи у случајевима екструзије у односу на протрузију ($p < 0,05$), док предња и средња висина диска нису показале значајне разлике ($p > 0,05$). Поред тога, однос асиметрије висине диска био је значајно нижи у случајевима екструзије, што одражава образац колапса диска који је доминантан у задњем делу ($p < 0,05$).

Закључак Иако је у патолошкој групи уочен општи пад висине диска, смањење његове задње висине било је израженије, нарочито у случајевима екструзије. Очување морфометрије тела пршљенова указује на морфометријске промене специфичне за патологију диска. Из перспективе клиничке анатомије, постериорна морфометрија диска може се сматрати додатним параметром у радиолошкој евалуацији лумбалне дискус хернije.

Кључне речи: лумбална дискус херниja; ниво *L4–L5*; висина диска; морфометрија пршљенова; магнетна резонанца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effects of dexmedetomidine on stress response during tracheal intubation under general an-esthesia in patients with poorly controlled hypertension

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SUMMARY

Introduction/Objective The aim was to investigate the effects of dexmedetomidine (DEX) on hemodynamics and stress responses during tracheal intubation under general anesthesia in patients with poorly controlled hypertension.

Methods This study is a prospective, randomized, controlled clinical study, it included 43 patients divided into an experimental group (n = 28) and a control group (n = 15). The experimental group received an intravenous infusion of 0.5 μg/kg DEX before anesthesia induction, while the control group was given an equal volume of normal saline. Heart rate (HR) and arterial blood pressure were recorded at multiple time points after induction and intubation, and the rate–pressure product (RPP) was calculated. Statistical analysis was performed using independent sample t-test, Mann–Whitney U test or χ^2 test.

Results At first and third minute after intubation, significant increases in HR, systolic blood pressure, diastolic blood pressure and mean arterial pressure were observed in the control group ($p < 0.01$), whereas the experimental group showed no significant changes. All post-intubation hemodynamic parameters were significantly lower in the experimental group ($p < 0.001$), with no difference in RPP. No adverse events or serious complications within seven days post-surgery occurred in either group.

Conclusion DEX effectively inhibits the sympathetic activation response induced by tracheal intubation, maintains hemodynamic stability, and demonstrates a favorable safety profile.

Keywords: dexmedetomidine; hypertension; intratracheal intubations; hemodynamics

INTRODUCTION

During the induction of general anesthesia, tracheal intubation – including laryngoscopy and endotracheal tube placement – induces intense mechanical and nociceptive stimulation, triggering a stress response in the central nervous system and leading to pronounced sympathetic activation [1, 2]. In patients with poorly controlled hypertension, this stress response manifests not only as tachycardia and abrupt elevation in blood pressure, but also as increased catecholamine release, which may precipitate myocardial ischemia, arrhythmias, or even heart failure, significantly compromising intraoperative safety and postoperative recovery [3].

Commonly used general anesthetics such as propofol and remifentanil offer advantages including rapid onset, stable anesthesia, quick recovery, and effective analgesia. However, their use in hypertensive patients has notable limitations [4, 5, 6]. High doses or rapid infusion of propofol can cause respiratory and circulatory depression [7]. Although remifentanil provides strong analgesia and rapid recovery, it is associated with postoperative hyperalgesia and agitation [8]. Additionally, other antisympathetic agents such as β-blockers have restricted indications and may not fully meet the individualized perioperative needs of all hypertensive patients.

Dexmedetomidine (DEX) is a highly selective α2-adrenergic receptor agonist with notable sedative, analgesic, and sympatholytic properties [9, 10]. By reducing central sympathetic outflow and catecholamine release, it effectively attenuates hemodynamic fluctuations during intense stimuli such as tracheal intubation [11]. The efficacy of DEX in reducing stress responses during anesthesia induction and emergence; however, systematic evidence regarding its efficacy and safety throughout the intubation process in patients with poorly controlled hypertension remains scarce.

Based on the α2-adrenergic agonist properties of DEX, we hypothesize that its administration in patients with poorly controlled essential hypertension can effectively attenuate sympathetic activation during tracheal intubation under general anesthesia, thereby improving intraoperative stability of blood pressure and heart rate (HR). This study aims to provide a new pharmacological strategy for perioperative management in hypertensive patients, potentially reducing the risk of cardiovascular and cerebrovascular complications.

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METHODS

Study Design

This was a prospective, randomized, controlled clinical study designed to evaluate the effects of DEX on hemodynamic and stress responses during tracheal intubation in patients with poorly controlled hypertension.

Subjects

Patients [American Society of Anesthesiologists (ASA class II)] who underwent general anesthesia with tracheal intubation at Xingwen County Traditional Chinese Medicine Hospital, Yibin, Sichuan Province, were enrolled in this study between March 2022 and March 2025. The inclusion criteria were as follows:

(1) diagnosis of essential hypertension according to the Chinese Medical Association Hypertension Prevention and Treatment Guidelines [12], with a history of standard antihypertensive therapy but poor blood pressure control (systolic blood pressure [SBP] 140–180 mmHg or diastolic blood pressure [DBP] 90–110 mmHg);
(2) age ≥ 18 years, body weight 50–80 kg;
(3) ASA physical status II (mild to moderate systemic diseases, but these conditions have not resulted in significant functional limitations);
(4) scheduled for elective surgery under general anesthesia with tracheal intubation;
(5) no acute conditions such as myocardial infarction, stroke, or severe infection based on preoperative examinations (complete blood count, biochemistry, electrocardiogram (ECG), chest X-ray, etc.);
(7) provision of written informed consent and ability to cooperate with research-related assessments.

Exclusion criteria included:

(1) secondary hypertension;
(2) severe cardiac, hepatic, or renal dysfunction (New York heart association class III–IV heart failure; Child–Pugh class B–C liver function; glomerular filtration rate [GFR] < 30 mL/min);
(3) significant arrhythmia (e.g., sinus bradycardia with HR < 50 bpm, second- or third-degree atrioventricular block), severe tachycardia, or evidence of myocardial ischemia;
(4) history of allergy to DEX, propofol, remifentanil, or related anesthetics;
(5) pregnancy or lactation;
(6) neuropsychiatric disorders, severe cognitive impairment, or inability to accurately report adverse reactions;
(7) active endocrine or metabolic diseases (e.g., hyperthyroidism, diabetic ketoacidosis);
(8) other conditions deemed inappropriate for inclusion by the investigators.

Randomization and blinding

Eligible patients were randomly assigned in a 1:1 ratio to either the DEX group or the control (saline) group using a computer-generated randomization sequence. The allocation was concealed in sealed, opaque envelopes opened by an independent research nurse just prior to anesthesia induction.

To maintain blinding, the study drugs (0.5 μg/kg DEX or an equal volume of normal saline, both diluted to 20 mL) were prepared by the hospital pharmacy in identical syringes labeled only with the patient study number. The anesthesiologist performing the intubation and data collection was blinded to group assignment.

Briefly, a total of 60 patients were initially randomized. After randomization, 17 patients were excluded from the final analysis.

Although this study included all eligible patients during the study period (n = 43), a post-hoc power analysis confirmed that this sample size provided adequate power (> 80%) to detect clinically significant differences in hemodynamic parameters.

Surgical procedure

Hypertensive patients took their usual morning antihypertensive medications on the day of surgery (excluding angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers). All patients followed a preoperative fasting protocol of 12 hours for solids and eight hours for clear fluids, which was the institutional standard at the time of the study. To mitigate potential intravascular volume depletion, upon arrival in the operating room and prior to any study drug administration or anesthesia induction, all patients received a standardized preload infusion of 5 ml/kg of lactated Ringer's solution via an upper limb venous access. Subsequently, all patients received pure oxygen via face mask (flow rate 6 L/min). Under local anesthesia, radial artery catheterization was performed and connected to a monitor for continuous ECG, pulse oxygen saturation (SpO_2), and invasive blood pressure (IBP) monitoring. Patients in the experimental group received an intravenous infusion of 0.5 μg/kg DEX (Jiangsu Hengrui Pharmaceuticals Co., Ltd., Lianyungang, Jiangsu Province, China) diluted to 20 mL, administered via an upper limb vein pump over 10 minutes before induction of general anesthesia. The control group received an equal volume of normal saline over the same duration. Immediately after infusion, anesthesia induction was initiated with the following protocol: intravenous injection of sufentanil (Humanwell Healthcare Group Co., Ltd., Wuhan, Hubei, China) at 0.3 μg/kg over 10 seconds, followed by target-controlled infusion of propofol (Jiangsu Nhwa Pharmaceutical Co., Ltd., Xuzhou, Jiangsu Province, China) using the Marsh pharmacokinetic model, with a plasma target concentration set at 3.5 mcg/mL. After loss of consciousness, vecuronium bromide (Hubei Keyi Pharmaceutical Co., Ltd., Wuhan, Hubei, China) at 0.1 mg/kg was administered intravenously, and face-mask-assisted

ventilation was provided. Tracheal intubation was performed when train-of-four stimulation showed disappearance of T1–T4. If HR decreased to < 50 bpm, 0.4 mg atropine was administered intravenously; if SBP fell to ≤ 10.6 kPa (≈80 mmHg), 1 mg dopamine was administered.

Standardized operating procedure for endotracheal intubation

To minimize the interference of procedural factors with the study results, a standardized operating procedure was implemented for the endotracheal intubation of all enrolled patients:

Assessment of intubation difficulty and operator qualifications: All endotracheal intubation procedures were performed by two anesthesiologists with more than 15 years of clinical experience in endotracheal intubation under general anesthesia. Prior to intubation, airway assessment was conducted using the Mallampati classification system. All patients were classified as grade II, indicating favorable airway conditions and a predicted low intubation difficulty.

Intubation equipment: The same model of video laryngoscope was used for laryngoscopic exposure and endotracheal intubation in all patients, ensuring a clear visual field, gentle manipulation, and technical consistency throughout the procedure.

Procedure duration: The target time for the entire intubation process – from laryngoscope insertion to confirmation of correct endotracheal tube placement – was set at within one minute. Actual recorded data demonstrated that intubation was successfully completed within this timeframe for all patients, with no cases of significantly difficult intubation or multiple intubation attempts required. Statistical comparison of the specific intubation duration between the two groups was not conducted in this study, as all procedures were performed in accordance with the same principles of rapidity and gentleness and completed within a narrow time window.

Procedural consistency: Prior to the study, the two principal operators engaged in thorough discussions to standardize intubation techniques, manipulation force, and the emphasis on minimizing hemodynamic fluctuations. This step was designed to reduce variability arising from individual operator habits.

Through the implementation of the aforementioned standardized measures, we aimed to minimize the impact of technical variables – other than the study drug intervention (DEX / normal saline) – on the stress response to endotracheal intubation, thereby enabling a clearer delineation of the effects of the drug intervention itself.

Data collection

Patient demographics, including age, sex, body mass index (BMI), disease type, duration of hypertension, and surgical type were collected using a standardized form. Hemodynamic parameters were recorded, including HR, SBP, DBP, mean arterial pressure (MAP), and the rate–pressure product (RPP; HR × SBP) before intubation and within 10 minutes after general anesthesia induction. Adverse events were monitored intraoperatively, including hypotension (SBP < 90 mmHg), hypertensive crisis (SBP > 180 mmHg), arrhythmia, and hypoxia (SpO_2 < 90%).

The specific time points for hemodynamic recording were defined as follows: T0 (0 minutes): immediately after confirmation of correct endotracheal tube placement; T1, T3, T5, and T10: one, three, five, and 10 minutes after T0, respectively. Measurements labeled "pre-intubation" were taken after anesthesia induction but before the commencement of laryngoscopy.

Selection of hemodynamic baseline: To isolate the cardiovascular stress response specifically attributable to tracheal intubation from the effects of anesthetic induction agents, the hemodynamic values measured immediately after anesthesia induction but before laryngoscopy were designated as the baseline (time 0 min) for all subsequent comparisons. Although preoperative baseline values (before anesthesia induction) were also recorded, the induction agents (propofol and sufentanil) uniformly caused significant hemodynamic alterations in all patients. Therefore, using the post-induction, pre-intubation state as the reference point allows for a clearer assessment of the additive stress induced by the intubation procedure itself. This analytical approach was applied identically to both the experimental and control groups.

Postoperative follow-up at 24 hours, 48 hours, and seven days included monitoring for infection, bleeding, respiratory/cardiovascular complications, and mortality. Data were entered via an electronic case report form. Hemodynamic parameters were automatically recorded by a multi-parameter monitor to ensure accuracy and traceability.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0. (IBM Corp., Armonk, NY, USA). Continuous variables (e.g., age, BMI, hemodynamic parameters) were described as mean ± standard deviation or median (interquartile range) based on normality. Categorical variables were expressed as frequency (percentage). Intergroup comparisons were conducted using independent samples t-test, Mann–Whitney U test, or χ^2 test, as appropriate. A two-sided α level of 0.05 was defined for statistical significance.

Ethics: This study was approved by the Medical Ethics Committee of Xingwen County Traditional Chinese Medicine Hospital, Yibin, Sichuan Province, and strictly adhered to the ethical principles of the Declaration of Helsinki.

RESULTS

Baseline characteristics

Between March 2022 and March 2025, 60 patients were enrolled and randomized. Following the exclusion of 17 patients for protocol violations or data incompleteness, 15

Table 1. General characteristics of patients

Parameters	Experimental group	Control group	Statistical measure (χ^2 or t value)	p
Gender			0.720	0.396
Male	15	6		
Female	13	9		
Age (years)	57.75 ± 7.80	59.07 ± 8.38	0.514	0.610
Body mass index (kg/m^2)	24.95 ± 1.36	25.03 ± 1.65	0.171	0.865
History of hypertension (years)	4.53 ± 2.62	2.93 ± 2.43	1.958	0.057

Table 2. Type of surgery performed on the patient

Types of surgery	Number (n)	Percentage (%)
Laparoscopic cholecystectomy	28	65.12%
Ureterolithotripsy	4	9.30%
Knee replacement surgery	3	6.98%
Laparoscopic inguinal hernia repair	2	4.65%
Right femoral fracture open reduction and internal fixation	2	4.65%
Knee arthroscopy	2	4.65%
Rotator cuff repair	1	2.33%
Right humeral fracture open reduction and internal fixation	1	2.33%

from the control group (six due to protocol deviation in anesthetic management, seven due to incomplete hemodynamic data recording, two due to cancellation of surgery after induction), and two from the DEX group (due to incomplete data recording). Consequently, the final per-protocol analysis set comprised 28 patients (15 males, 13 females) in the DEX group and 15 patients (six males, nine females) in the control group.

As shown in Table 1, no significant difference was observed in gender distribution between the two groups ($\chi^2 = 0.720$, $p = 0.396$). The mean age was 57.75 ± 7.80 years in the experimental group and 59.07 ± 8.38 years in the control group, with no statistically significant intergroup difference ($t = 0.514$, $p = 0.610$). BMI values were comparable between the two groups (experimental group: 24.95 ± 1.36 *vs*. control group: 25.03 ± 1.65, $t = 0.171$, $p = 0.865$). Additionally, there was no significant difference in the duration of hypertension history between the experimental group (4.53 ± 2.62 years) and the control group (2.93 ± 2.43 years) ($t = 1.958$, $p = 0.057$). These results indicate that the study population was comparable in terms of demographic and basic metabolic characteristics.

The types of surgery performed on the included patients are summarized in Table 2. The procedures consisted of laparoscopic cholecystectomy (28 cases, 65.12%), ureteroscopic lithotripsy (four cases, 9.30%), knee replacement (three cases, 6.98%), laparoscopic inguinal hernia repair and open reduction and internal fixation of right femoral fracture (two cases each, 4.65%), knee arthroscopy (two cases, 4.65%), as well as rotator cuff repair and open reduction and internal fixation of right humeral fracture (one case each, 2.33%).

DEX improves intraoperative stability of blood pressure and HR

This study systematically evaluated changes in cardiovascular parameters at different time points after induction in anesthesia in the experimental and control groups (Figure 1). In the control group, significant increases in HR, SBP, DBP, and MAP were observed at the first and third minute after intubation ($p < 0.01$). Subsequently, at the fifth and tenth minute after intubation, these parameters returned to levels not significantly different from those at 0 minute ($p > 0.05$). In contrast, when compared to their own pre-intubation baseline (0 minute), the experimental group showed no statistically significant changes in HR, SBP, DBP, or MAP at any of the post-intubation time points ($p > 0.05$). Throughout intubation, HR, SBP, DBP, and MAP in the experimental group were significantly lower than those in the control group ($p < 0.001$). No significant differences in the RPP were detected between the two groups at any time point, suggesting that myocardial load was not significantly affected under the experimental conditions. These results indicate that the intervention in the experimental group may influencing the early cardiovascular response following intubation.

Safety of DEX

A systematic safety assessment was conducted for both the experimental and control groups. Intraoperative real-time monitoring showed that no acute adverse events – such as hypotension (SBP < 90 mmHg), hypertensive crisis (SBP > 180 mmHg), arrhythmia, or hypoxia ($SpO_2 < 90\%$) – occurred in either group. During postoperative follow-up (24 hours, 48 hours, and seven days), no cases of infection, bleeding, respiratory complications (e.g., hypoxemia, atelectasis), cardiovascular events (e.g., myocardial ischemia, heart failure), or mortality were observed in either group.

DISCUSSION

The findings of this study demonstrate that preoperative administration of DEX in patients with poorly controlled hypertension undergoing elective surgery significantly reduces HR and blood pressure levels during tracheal intubation compared with the non-intervention control group, indicating a clear clinical benefit in maintaining hemodynamic stability. Notably, during the first- to third-minute post-intubation window – when the stress response is most intense – patients in the experimental group maintained relatively stable HR, SBP, DBP, and MAP, whereas the control group exhibited marked elevations. These results support the efficacy of DEX in attenuating the stress response associated with tracheal intubation.

DEX is a highly selective central α_2-adrenergic receptor agonist, and its primary mechanism of action is closely associated with the inhibition of the central sympathetic

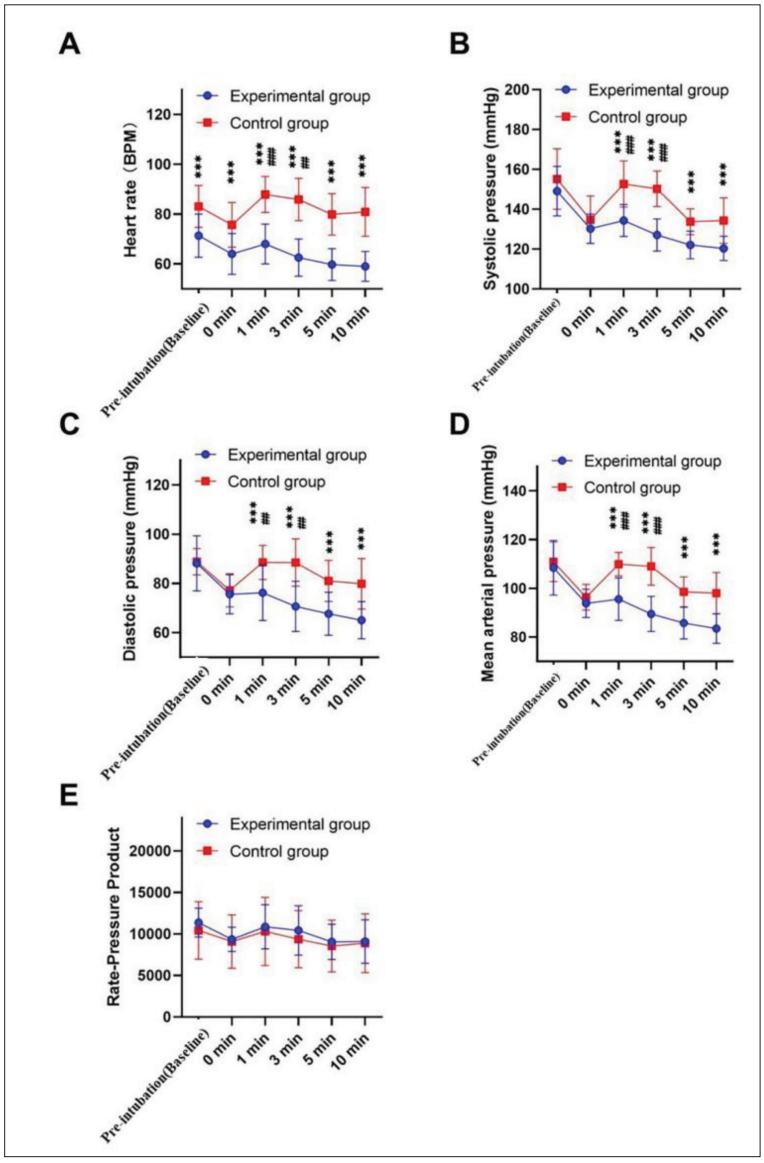

Figure 1. Hemodynamic and other stress responses in surgical patients after anesthesia and intuba-tion; A – heart rate; B – systolic blood pressure; C – diastolic blood pressure; D – mean arterial pressure; E – heart rate-blood pressure product; data points labeled "Pre-intubation (Baseline)" were recorded after anesthesia induction but prior to laryngoscopy; T0 (0 min) denotes the time point immediately after intubation; *** indicates a statistical p-value less than 0.001 between the experimental group and the control group; ## indicates a statistical p-value less than 0.01 between the corresponding time point in the control group and the control group at 0 minutes; ### indicates a statistical p-value less than 0.001 between the corresponding time point in the control group and the control group at 0 minutes

nervous system [13]. By selectively activating α2 receptors in regions such as the locus coeruleus of the brainstem, DEX significantly reduces sympathetic tone, thereby decreasing the release of norepinephrine and other catecholamines [13, 14]. This mechanism plays a key role in maintaining hemodynamic stability during surgery by effectively suppressing sympathetic-mediated increases in HR and blood pressure, particularly during high-stress events such as tracheal intubation [15, 16]. Moreover, DEX exhibits a multifaceted pharmacological profile. It not only provides effective sedation that closely resembles non-rapid eye movement sleep but also has minimal impact on respiratory drive while offering mild analgesic and anxiolytic effects [17, 18]. Its early administration during anesthesia induction allows for the modulation of preoperative psychological stress, thereby creating favorable conditions for smooth intubation and subsequent anesthetic maintenance. With its rapid onset and controllable duration of action, DEX is suitable for both bolus and continuous infusion, facilitating individualized hemodynamic management during surgery. It is particularly noteworthy that DEX offers distinct advantages in patients with reduced autonomic regulatory function or states of sympathetic dominance, such as those with hypertension, cardiac insufficiency, or advanced age [17]. Through central sympathetic inhibition, it reduces peripheral vascular resistance and cardiac afterload, helping to stabilize HR and prevent abrupt blood pressure fluctuations, thereby alleviating cardiovascular stress during surgery. This multi-mechanistic and synergistic profile makes DEX an ideal agent for managing perioperative circulatory instability and reducing the risk of intraoperative complications, especially in patient populations characterized by sympathetic overactivation.

Patients with poorly controlled hypertension face significant cardiovascular risks during tracheal intubation, primarily due to intense sympathetic activation triggered by the procedure [19]. As a potent noxious stimulus, tracheal intubation rapidly elicits central and peripheral sympathetic excitation, prompting the adrenal medulla to release large amounts of catecholamines (e.g., epinephrine and norepinephrine). This leads to marked peripheral vasoconstriction, tachycardia, and increased myocardial contractility. Consequently, blood pressure rises sharply within a short period, often reaching or exceeding critical thresholds. Although these hemodynamic changes are generally transient and reversible in normotensive individuals, they can result in severe or even fatal outcomes in patients with poorly controlled hypertension, whose vascular structure and function are chronically impaired [20, 21, 22]. Under chronic hypertension, pathological alterations such as arterial stiffness, reduced vascular compliance, and endothelial dysfunction are common, significantly diminishing the capacity to regulate and tolerate acute hemodynamic fluctuations. In this context, the transient hypertensive peaks induced by intubation – superimposed on already elevated baseline blood pressure – can readily exceed the autoregulatory thresholds of vital organs (e.g., heart, brain, and kidneys), thereby precipitating acute myocardial ischemia, arrhythmias, left ventricular failure, or even cerebral hemorrhage and aortic

dissection [23, 24]. This risk is particularly pronounced in patients with underlying coronary or cerebrovascular diseases, where intubation-related sympathetic surge may directly trigger life-threatening events. Furthermore, the pronounced increase in HR during intubation augments myocardial oxygen consumption and shortens coronary perfusion time, elevating the risk of myocardial ischemia [25]. In hypertensive patients with left ventricular hypertrophy or diastolic dysfunction, abrupt elevation in blood pressure acutely increases left ventricular afterload, potentially inducing pulmonary congestion or acute heart failure [26]. Therefore, effective suppression of sympathetic excitation and achievement of hemodynamic stability throughout all phases of tracheal intubation have become central challenges in the perioperative management of patients with uncontrolled hypertension. The findings of this study underscore the importance of meticulous hemodynamic control in these patients, particularly during anesthesia induction and intubation. In clinical practice, preoperative evaluation of blood pressure control should be thoroughly conducted, and individualized anesthetic strategies – such as selecting antisympathetic agents like DEX, optimizing induction protocols, minimizing intubation duration, and considering prophylactic pharmacological intervention when necessary – should be implemented based on patient-specific risks [27]. These measures are essential for reducing intubation-related complications and enhancing perioperative safety. Personalized and precise hemodynamic management in high-risk patients represents a cornerstone of modern anesthetic practice and a fundamental safeguard for intraoperative patient safety.

In addition to DEX, multiple pharmacological and non-pharmacological interventions are currently available to maintain perioperative circulatory stability in hypertensive patients. For instance, short-acting β-blockers such as esmolol can rapidly control intraoperative tachycardia, while vasodilators including nitroprusside and nitroglycerin may be used for acute blood pressure management. Adjunctive agents such as midazolam and fentanyl analogues also contribute to attenuating the stress response during intubation [28, 29, 30]. Furthermore, refining the anesthesia induction process, prolonging pre-intubation oxygenation, and avoiding repeated laryngoscopic attempts can help reduce the intensity of intubation-induced stress. However, compared with these conventional approaches, DEX offers a unique combination of sedative, analgesic, and antisympathetic properties. Its multi-mechanistic action aligns more comprehensively with the perioperative requirements of hypertensive patients, particularly under conditions of autonomic hyperactivity.

Despite the promising findings, this study has several limitations. First, as a single-center prospective observational study, it may be subject to selection bias, which limits the generalizability of the results. Second, the relatively small sample size resulted in insufficient statistical power for certain subgroup analyses (e.g., patients of different age groups or varying durations of hypertension). Furthermore, the study primarily focused on immediate intraoperative hemodynamic changes and did not include outcome measures such as postoperative complication rates, patient satisfaction, or long-term prognosis. Future multi-center, large-sample, randomized controlled trials are warranted to further validate the safety and efficacy of DEX in high-risk populations. Additionally, the lack of plasma concentration monitoring of the drug and the absence of more sensitive indicators such as HR variability preclude a deeper understanding of its specific effects on autonomic nervous function. Further investigation is needed to elucidate these mechanisms.

CONCLUSION

As a multi-mechanistic and low-risk perioperative adjunct, DEX represents a valuable intervention for maintaining hemodynamic stability in patients with poorly controlled hypertension during high-stress procedures such as tracheal intubation, and its broader clinical application is warranted.

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Author contributions: All authors contributed to the study conception and design. Chao Li participated in the collection of experimental data. Yuanxin Sun wrote the manuscript. Yuanxin Sun and Chao Li reviewed and edited the manuscript. All authors read and approved the final manuscript.

Conflict of interest: None declared.

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Ефекти дексмедетомидина на стресни одговор током трахеалне интубације у општој анестезији код болесника са лоше регулисаном хипертензијом

Јуансин Сун, Чао Ли

Окружна болница традиционалне кинеске медицине „Сингвен", Одељење за анестезиологију, Јибин, Сечуан, Кина

САЖЕТАК

Увод/Циљ Циљ рада био је испитивање ефеката дексмедетомидина на хемодинамику и стресни одговор током трахеалне интубације под општом анестезијом код болесника са лоше контролисаном хипертензијом.

Методе Ово је проспективна, рандомизована, контролисана клиничка студија која је обухватила 43 болесника подељена у експерименталну групу ($n = 15$) и контролну групу ($n = 28$). Експериментална група је примала интравенску инфузију од 0,5 *μg/kg* дексмедетомидина пре индукције анестезије, док је контролна група примала исту запремину физиолошког раствора. Срчана фреквенција и артеријски крвни притисак забележени су у више временских тачака након индукције и интубације, и израчунат је производ фреквенције и притиска. Статистичка анализа је урађена коришћењем *t*-теста за независне узорке, Ман–Витнијевог *U* теста или χ^2 теста.

Резултати Показано је да је срчана фреквенција након индукције била значајно нижа у експерименталној групи у односу на контролну ($p < 0,001$), без међугрупне разлике у крвном притиску. Након првог и трећег минута од интубације, уочено је значајно повећање срчане фреквенције, систолног, дијастолног и средњег артеријског притиска у контролној групи ($p < 0,01$), док експериментална група није показала значајне промене. Сви хемодинамски параметри након интубације били су значајно нижи у експерименталној групи ($p < 0,001$), без разлике у вредностима производа фреквенције и притиска. Није било нежељених догађаја или озбиљних компликација унутар седам дана након операције ни у једној групи.

Закључак Дексмедетомидин ефикасно инхибира одговор симпатичке активације изазван трахеалном интубацијом, одржава хемодинамску стабилност и показује повољан безбедносни профил.

Кључне речи: дексмедетомидин; хипертензија; трахеална интубација; хемодинамика

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Recreational skiing- and snowboarding-related injuries – a four-year trauma center cohort from Kopaonik mountain ski center

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SUMMARY

Introduction/Objective Skiing and snowboarding are popular winter sports with distinct participant demographics, equipment, and movement patterns. Comparative epidemiological data from Southeast Europe are limited. The aim of this study was to compare injury epidemiology and injury patterns between skiers and snowboarders at the Kopaonik Ski Resort, Serbia.

Methods This retrospective descriptive epidemiological study analyzed ski patrol reports and medical records from the University Clinical Center of Serbia over four winter seasons (2021–2024). Injured participants aged 4–81 years who received on-site ski patrol assistance and were evaluated at the resort-based trauma center were included. Participants were categorized according to sport (skiing or snowboarding). Group differences in demographic, temporal, and environmental characteristics were assessed using χ^2 tests. Injury types and anatomical distribution were compared using χ^2 or Fisher's exact tests, and odds ratios with 95% confidence intervals were calculated.

Results A total of 3368 injured participants were included, of whom 3011 (89.4%) were skiers and 357 (10.6%) snowboarders. Snowboarders were younger and more often male ($p < 0.001$). They were more frequently injured on novice slopes, whereas skiers predominated on advanced slopes ($p = 0.001$). Helmet use was higher among snowboarders ($p = 0.004$). Skiers had higher odds of knee and lower leg injuries, while snowboarders more commonly sustained forearm and hand/wrist injuries. Fractures and dislocations were more frequent among snowboarders, whereas sprains predominated in skiers.

Conclusion Significant sport-specific differences in injury patterns were identified. These findings support the development of targeted injury prevention strategies and optimization of healthcare services for winter sports injuries.

Keywords: snowboarding; skiing; injury

INTRODUCTION

Skiing and snowboarding are popular winter sports, and while they share similar environments, they differ in demographics, movement patterns, equipment, and skill level of participants. Previous studies have shown that snowboarders tend to be younger and more often males compared to skiers [1–5]. Historically, helmet use was higher among snowboarders, but recent reports indicate a similar acceptance level between the two groups [2, 6]. Furthermore, the temporal patterns of injuries vary, with skiers being more frequently injured during weekdays and snowboarders more often on weekends [4, 7]. Available evidence indicates that snowboarders sustain injuries on easier slopes, whereas skiers' injuries are more evenly distributed across intermediate and advanced slopes [2, 8]. Weather conditions have also been identified as an external risk factor for injuries, but data comparing skiers and snowboarders are limited [9, 10, 11].

Additionally, previously published papers showed different injury patterns in those two groups. Upper extremity injuries were predominant in snowboarders, while lower extremity injuries were more frequent in skiers [1, 2, 4, 12, 13, 14].

Kopaonik Ski Center, which was established in 1964, is the largest ski resort in Southeast Europe, encompassing 48 alpine ski slopes, 30 ski trails, and two beginner training grounds, which can accommodate 12,000–14,000 simultaneous skiers. Delibašić et al. [15] have shown that the capacity of the ski lift transportation system at the Kopaonik Ski Center increased by 58%, and the injury rate nearly doubled from 2005. to 2010. Furthermore, snowboarding experienced a significant boom in the 1990s, when most major ski areas developed separate slopes for snowboarders. Snowboarding developed with delay at Mt Kopaonik, where the first snowboard park opened in 2012. The rapid development of the largest ski resort in Southeastern Europe on one side, and the significantly delayed initial adoption may lead to local differences in epidemiology of those injuries, underscoring the need for specific epidemiological data and comparative analyses. Although numerous international

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studies have investigated demographic, temporal, and environmental factors associated with snow-sport injuries, to the best of our knowledge, no previous research has examined those aspects in the Southeastern European context. This retrospective observational study aimed to address two primary objectives:

to analyze and compare differences between injuries in skiers and snowboarders regarding demographic, environmental, and temporal factors;

to compare injury patterns between the two sports in terms of anatomical location and injury type.

Understanding those factors in the local context is essential for developing targeted injury prevention strategies, guiding slope management policies, and improving skier and snowboarder safety. This study presents an opportunity to contribute valuable insights into the field of winter sports safety, particularly in the Southeastern European context.

METHODS

Participants and inclusion criteria

This retrospective descriptive epidemiological study was conducted at Mt Kopaonik Ski Center and included individuals injured while skiing or snowboarding during the period spanning from 2021 to 2024. Included participants were those who received on-site assistance from the Mountaineer Rescue Service of Serbia (ski patrol) and were subsequently medically evaluated at the Trauma Center of the University Clinical Center Serbia, located at the base of the resort. Participants treated only by the ski patrol or those who refused medical evaluation were excluded from the study. Furthermore, only patients with musculoskeletal injuries were included.

Data collection

The study participants were categorized into two distinct cohorts based on their injuries sustained during skiing or snowboarding at the time of injury. Data regarding demographic characteristics and types of injuries were obtained from the hospital database system following a standardized data extraction protocol to ensure consistency. Information regarding helmet usage, environmental, and temporal factors was obtained from ski patrol reports. All participants had a unique ID, which allowed researchers to link them to databases.

Statistical analysis

All analyses were performed in IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA) with significance set at $p < 0.05$. Means and standard deviations were calculated for continuous variables and percentages for categorical variables. Age was analyzed as a continuous variable and categorized into predefined groups (< 15, 15–24, 25–44, 45–64, and ≥ 65 years). Comparisons between injured skiers and snowboarders were performed with the Pearson χ^2 test. Effect sizes (Φ coefficient and Cramér's V) were calculated to determine the strength of associations. To identify cells contributing most to significant χ^2 results the adjusted residuals (AR) were calculated. AR indicated the degree to which the observed frequency differs from the expected frequency. Positive AR values indicated overrepresentation, while negative AR values indicated underrepresentation. An absolute AR value greater than 1.96 was considered statistically significant [16]. AR analyses were considered exploratory, and focused on identifying the largest deviations from expectation. No formal correction for multiple comparisons was applied.

Injury distribution across anatomical regions was compared between sports using the χ^2/Fisher test as above. For each region, we calculated an odds ratio (OR) with a 95 % confidence interval (95% CI) with OR > 1 meaning the injury was more common in skiers, and OR < 1 meaning it was more common in snowboarders.

Ethics: The study was approved by the Ethics Committee of the University Clinical Center, Number 524/4.

RESULTS

The differences between skiers and snowboarders in terms of demographic characteristics, temporal, environmental factors and helmet usage are summarized in Table 1.

The final sample consisted of 3368 injured participants, including 3011 skiers (89.4%) and 357 snowboarders (10.6%). Among the patients included in the study, 1713 were female (50.9% and 1655 were male (49.1%). The mean age of the patients was 31± 14.68 years.

Differences between skiers and snowboarders regarding demographic factors

Compared to skiers, a greater proportion of males was found among snowboarders (58.8% *vs*. 49.9%, $p = 0.001$). Skiers were older than snowboarders (32.3 ± 15 *vs*. 26.3 ± 9.7 years, $p < 0.001$). When age was categorized, a significantly higher than expected number of skiers were injured among the 45–64-year group (AR = 8.2) while snowboarders were overrepresented in the 25–44 years group (AR = 4.7) (Table 1).

Differences between skiers and snowboarders regarding environmental factors

Both skiers and snowboarders were most frequently injured on easy slopes. However, there was a significant difference between the two groups with respect to slope difficulty ($p = 0.001$). A significantly higher than expected number of skiers was injured on advanced/expert slopes (AR = 3.4), while this was true for snowboarders on novice slopes (AR = 2.6). Most of the injured skiers (77.2%) and snowboarders used helmets (83.5%), although this proportion was significantly higher in snowboarders ($p = 0.007$) (Table 1)

Table 1. Details of injured skiers and snowboarders

Parameters		Skiers (3011)	AR[a]	Snowboarders (357)	AR[a]	p	Effect size
Demographic factors							
Gender	Male	1503 (49.9)	-3.2	210 (58.8)	3.2	**0.001**	$\varphi = 0.055$
	Female	1508 (50.1)	3.2	147 (41.2)	-3.2		
Age	Mean age	32.33 ± 15.04		26.33 ± 9.68		0.000[b]	
	< 15	498 (16.5)	-0.7	64 (17.9)	0.7	**0.000**	V = 0.150
	15–24	570 (18.9)	-2.5	87 (24.4)	2.5		
	25–44	1230 (40.9)	-4.7	192 (53.8)	4.7		
	45–64	660 (21.9)	8.2	13 (3.9)	-8.2		
	65+	53 (1.8)	2.1	1 (0.3)	-2.1		
Environmental factors							
Slope difficulty	Novice	392 (13)	-2.6	64 (17.9)	2.6	**0.001**	V = 0.069
	Easy	1380 (45.8)	-0.7	171 (47.9)	0.7		
	Intermediate	784 (26)	0.1	92 (25.8)	-0.1		
	Advanced/expert	455 (15.1)	3.4	30 (8.4)	-3.4		
Helmet	Yes	2325 (77.2)	-2.7	298 (83.5)	2.7	**0.007**	$\varphi = 0.050$
	No	686 (22.8)	2.7	59 (16.5)	-2.7		
Temporal factors							
Day of week	Weekday	1925 (63.9)	2.2	207 (58)	-2.2	**0.027**	$\varphi = 0.038$
	Weekend	1086 (36.1)	-2.2	150 (42)	2.2		
Time of injury	Morning (8–10)	130 (4.3)	0.4	14 (3.9)	-0.4	0.143	V = 0.045
	Noon (10–12)	842 (28)	0.0	100 (28)	0.0		
	Afternoon (12–14)	990 (32.9)	1.3	105 (29.4)	-1.3		
	Late afternoon (14–16)	925 (30.8)	-0.5	114 (31.9)	0.5		
	Night (18–22)	121 (4)	-2.4	24 (6.7)	2.4		
Season of injury	2021	1059 (35.2)	1.6	110 (34.7)	-1.6	0.133	V = 0.041
	2022	831 (27.6)	0.1	98 (27.5)	-0.1		
	2023	617 (20.5)	-2.2	91 (25.5)	2.2		
	2024	504 (16.7)	0.2	58 (16.2)	-0.2		
Month of injury	January	1097 (36.4)	1.7	114 (31.9)	-1.7	0.513	V = 0.031
	February	1054 (35)	-0.7	132 (37.2)	0.7		
	March	632 (21)	-0.5	79 (22.1)	0.5		
	April	56 (1.9)	-0.1	7 (2)	0.1		
	December	172 (5.7)	-1.0	25 (7)	1.0		
Off slope	Yes	65 (2.2)	-0.8	10 (2.8)	0.8	0.437	$\varphi = 0.013$
	No	2946 (97.8)	0.8	347 (97.2)	-0.8		
Weather conditions	Optimal	1473 (48.9)	-2.3	198 (55.5)	2.3	**0.017***	V = 0.049
	Moderate	1292 (42.9)	2.9	125 (35)	-2.9		
	Adverse	246 (8.2)	-0.9	34 (9.5)	0.9		

AR – adjusted residuals;
astatistically significant differences between groups ($p < 0.05$) are denoted by boldface p; AR values >1.96 or <-1.96 indicate statistically significant differences at $p < 0.05$; b t-test; φ to determine the strength of the association between two variables for 2 × 2 comparations; V (Cramer) for multicategory comparations to determine the strength of the association between two variables

Difference between skiers and snowboarders regarding temporal factors

Skiers had a significantly higher proportion of weekday injuries compared to snowboarders (63.9% *vs*. 58%), while snowboarders were more likely to be injured on weekends (42% *vs*. 36.1%, p = 0.027) (Table 1).

A significant difference regarding injury risk with respect to weather conditions was observed (p = 0.017). Although injuries in both groups most often occurred under optimal weather conditions, a higher-than-expected number of ski injuries were sustained under moderate weather conditions (AR = 2.9) (Table 1).

Time of injury, season of injury, and month of injury did not significantly differ between skiers and snowboarders (p > 0.05).

Differences between skiers and snowboarders regarding injuries

The anatomical distribution of injuries differed significantly between skiers and snowboarders (Table 2). Lower-limb injuries were markedly more frequent among skiers, particularly the knee (39.7% *vs*. 10.1%; OR = 5.87, 95% CI 4.13–8.34, p < 0.001) and lower leg (10.2% *vs*. 3.6%, OR = 3,

95% CI 1.70–5.29, p < 0.001). Thigh and ankle injuries did not differ significantly between sports (both p > 0.05).

Upper-limb injuries were more frequent among snowboarders (62.7% *vs*. 26.7%, p < 0.001) with the largest differences in the forearm (OR = 0.14, 95% CI 0.11–0.188, p < 0.001) and hand/wrist (OR = 0.49, 95% CI 0.342–0.713, p < 0.001). Trunk injuries were less common in skiers than snowboarders (OR = 0.64, 95% CI 0.43–0.95, p = 0.027). This difference was mainly due to lower back-pelvis injuries (OR 0.42, 95% CI 0.25–0.70, p = 0.001)

Types of injury

Injury type differed between sports (Table 3). Compared with snowboarders, skiers had higher odds of sprain (27.5% *vs.* 9.8 %; OR = 3.48, 95% CI 2.41–4.93, p < 0.001), whereas fracture (20.7% *vs*. 36.0%; OR = 0.47, 95% CI 0.37–0.59, p < 0.001) and dislocation (5.2% *vs*. 10.6%; OR = 0.47, 95% CI 0.32–0.68, p < 0.001) were more frequent among snowboarders. Differences for contusion, laceration, and concussion were not statistically significant (all p > 0.05).

DISCUSSION

Almost 90% percent of injuries in our cohort were sustained by skiers. Recent studies reported the incidence of ski injuries being between 62–69.7%, indicating that skiing still maintains greater popularity compared to snowboarding [2, 4]. Results from our study demonstrated demographic differences and confirmed globally observed patterns between skiers and snowboarders in the local setting. A higher proportion of younger population and males was found among snowboarders compared to skiers. This is in accordance with data from previous studies suggesting that snowboarding tends to be more popular among younger demographics, particularly males [1–5, 8].

Most injuries in both groups were sustained on easy slopes. Our results align with limited results reported in literature showing that most injuries from both sports occur on easy to intermediate slopes, where the most participants spend the majority of their time [2, 17]. However, our results showed a higher proportion of snowboard related injuries on novice slopes, and a higher proportion of ski related injuries on advanced/expert slopes in skiers. This finding suggests that injured snowboarders are beginners compared to skiers who were more experienced. Lower ability is a clearly recognized risk factor for injury [18, 19, 20]. Promotion of responsible skiing and snowboarding within one's limits and abilities with a special focus on novice snowboarders should be considered an important educational task.

A significantly higher helmet usage among snowboarders compared to skiers found in our study is in line with results from the 2000s [21, 22, 23] but different from recent years results, suggesting similar helmet use between the two groups, and a dramatical increase in helmet adoption for both skiing and snowboarding [2, 6]. The pattern observed in our cohort suggests the need to create more focused helmet adoption safety initiatives for both groups, with a special focus on skiers.

Skiers had a higher proportion of injuries during the weekdays compared to snowboarders (63.9%

Table 2. The anatomical distribution of injuries in skiers and snowboarders common in skiers

Injured body region	n (%)	Skiers n (%)	Snowboarders n (%)	p[b]	OR[a]	CI
Head and neck	332(9.9%)	304 (10.1%)	28 (7.8%)	0.177	1.32	0.88–1.97
Head	228 (6.6%)	204 (6.8%)	24 (6.7%)	0.970	1.00	0.65–1.56
Face	59 (1.8%)	59 (2%)	0 (0%)	0.002[c]	14.4 d	NC
Neck	45 (1.3%)	41(1.4%)	4 (1.1%)	1.00 [c]	1.21	0.43–3.42
Trunk	211 (6.3%)	179 (5.9%)	32 (9%)	0.026*	0.64	0.43–0.95
Chest	103(2.9%)	93 (3.1%)	10 (2.8%)	0.765	1.10	0.57–2.14
Back	4 (0.1%)	3 (0.1%)	1 (0.3%)	0.361 [c]	0.35	0.04–3.42
Abdomen	11 (0.3%)	10 (0.3%)	1 (0.3%)	1.000[c]	1.18	0.15–9.29
Lower back and pelvis	93 (2.8%)	73 (2.4%)	20 (5.6%)	**0.001**	0.41	0.25–0.69
Upper limb	1027 (30.5%)	803 (26.7%)	224 (62.7%)	**0.000**	0.21	0.17–0.27
Shoulder	429 (12.7%)	362 (12%)	67 (18.8%)	**0.000**	0.59	0.44–0.78
Upper arm	79 (2.3%)	73 (2.4%)	6 (1.7%)	0.380	1.45	0.63–3.36
Elbow	41 (1.2%)	32 (1.1%)	9 (2.5%)	0.035* [c]	0.41	0.19–0.87
Forearm	267 (7.9%)	164 (5.4%)	103 (28.9%)	**0.000**	0.14	0.11–0.18
Hand/wrist	211 (6.3%)	172(5.7%)	39 (10.9%)	**0.000**	0.49	0.34–0.71
Lower limb	1766 (52.4%)	1700 (56.5%)	66(18.5%)	**0.000**	5.71	4.34–7.54
Thigh	106 (3.1%)	99 (3.3%)	7 (2%)	0.174	1.75	0.81–3.81
Knee	1231 (36.5%)	1195 (39.7%)	36 (10.1%)	**0.000**	5.86	4.13–8.34
Lower leg	320 (9.5%)	307 (10.2%)	13 (3.6%)	**0.000**	3.00	1.71–5.29
Ankle	96 (2.9%)	89 (3%)	7 (2%)	0.285	1.52	0.70–3.31
Foot	13 (0.4%)	10 (0.3%)	3 (0.8%)	0.152 [c]	0.39	0.11–1.43
Other	32 (1%)	25 (0.8 %)	7 (2%)	0.073 [c]	0.41	0.18–0.97

[a]OR – odds ratio for injury in skiers relative to snowboarders; OR > 1 indicate injuries more; OR < 1 indicate injuries more common in snowboarders;
[b]ps refer to χ^2 or Fishers exact test as appropriate; statistically significant differences between groups (p < 0.05) are denoted by boldface p;
[c]Fishers exact test;
[d]OR calculated using a continuity correction of 0.5 due to zero cases among snowboarders

Table 3. Comparison of injury types between skiers and snowboarders

Type of injury	n (%)	Skiing	Snowboarding	p	OR[a]	CI
Dislocation	193 (5.8%)	156 (5.2%)	37 (10.6%)	**0.000**	0.46	0.32–0.68
Contusion	1249 (37.1%)	1128 (37.8%)	121 (34.6%)	0.241	1.14	0.91–1.45
Fracture	745 (22.1%)	619 (20.7%)	126 (36%)	**0.000**	0.46	0.36–0.58
Laceration	156 (4.7%)	140 (4.7%)	16 (4.6%)	0.922	1.02	0.61–1.74
Sprain	864 (25.7%)	829 (27.5%)	35 (9.8%)	**0.000**	3.49	2.44–4.99
Concussion	129 (3.8%)	114 (3.8%)	15 (4.2%)	0.662	0.89	0.52–1.55
Other	32 (1%)	25 (0.8%)	7 (2%)	**0.037**	0.41	0.19–0.97

[a]OR = odds ratio for injury in skiers relative to snowboarders;
*Statistically significant differences between groups (p < 0.05) are denoted by boldface p

vs. 58%), while snowboarders were more likely to be injured on weekends (42% *vs*. 36.1%, p = 0.027). This is in contrast with previously published works by Chen et al. [7] and Subaşı et al. [4], who reported only minor or non-significant differences between sports. Our results show a clear temporal difference, suggesting that injury timing may be sport-specific and that targeted preventive strategies during peak weekend periods may be particularly relevant for snowboarders. The higher injury counts on weekends in snowboarders can possibly be explained by the fact that snowboarders are younger and less experienced than skiers, and primarily snowboard only on weekends, which is consistent with results observed in this study.

While most injuries in both groups occurred under optimal weather conditions, skiers sustained injuries more often under moderate weather conditions compared to snowboarders. To the best of our knowledge, prior studies have not compared weather-related injury risk by sport but showed that injuries concentrate in favorable or warmer conditions, and that temperature/visibility influence risk in both groups [9, 11]. Our results may reflect specific differences in risk-taking behavior, with snowboarders being more active only during favorable conditions, potentially increasing their exposure to injury only under these conditions. It could also be assumed that skiers are more experienced, thus skiing also when weather conditions are not optimal.

Different anatomical localization in both disciplines of injuries in our cohort also underline their diversity. The most frequently affected area in snowboarders was the upper extremity, with fractures being the most frequent type of injury. On the contrary, skiers sustained significantly more lower extremity injuries, particularly involving the knee, where sprains occurred with notably higher odds. The results of our study confirmed the previously well-established differences in anatomical localization and types of injuries in both disciplines, which can be explained by different mechanisms of injuries [1–4, 13, 14, 24, 25]. Snowboarders whose feet are fixed attempt to fall with reflex arms outstretching, leading to force of injury transition to the upper limb. On the other hand, skiers are more prone to torsional forces on their stationary lower limbs during falls [14]. Evidence-based equipment related injury prevention measures of knee and wrist injuries should be promoted. Wearing wrist guards, especially during the learning phase when fall risk is higher for snowboarders, and lower binding settings for easier release of skis have been scientifically demonstrated to be effective [14, 26, 27, 28].

Strengths and limitations

The major strength of our study is the large sample of injuries collected over four years at Mt Kopaonik Ski Center, the largest ski resort in Southeast Europe. In addition, the analysis of both ski patrol and Trauma Center databases allowed comprehensive analysis and deeper understanding of our cohort.

The first limitation of our study is its retrospective nature. Second, the present study assessed data obtained from a single center. Furthermore, available medical records did not encompass all significant variables, such as mechanism of injury, pre-existing conditions, self-reported skill levels, etc. The most important missing variables are specific injury diagnosis, which was due to imprecise medical documentation. Ski patrol reports may not have captured the full spectrum of injuries, especially those that did not require medical treatment. Hence, it was not possible to provide incidence and prevalence data. Also, the sample size for snowboarders was significantly smaller than for skiers, which may have limited statistical power to detect differences between the two groups.

Future research should incorporate prospective data collection, encompass more precise diagnosis and a broader spectrum of variables.

CONCLUSIONS

Despite the increasing popularity of snowboarding in recent years, skiing remains significantly more popular. Snowboarders are mostly less experienced younger male, who commonly sustain injuries on novice slopes on weekends. Targeted prevention strategies should be focused on promotion of evidence-based equipment related injury prevention, responsible skiing and snowboarding within one's capabilities. Special attention should be focused on more precise diagnostics of injuries and defining a standardized approach for uniform data collection in all ski centers.

Conflict of interest: None declared.

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Повреде у рекреативном скијању и сноубордингу – четворогодишња кохортна студија траума центра у ски-центру Копаоник

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САЖЕТАК

Увод/Циљ Скијање и сноубординг су популарни зимски спортови који се разликују по демографским карактеристикама учесника, опреми и обрасцима кретања. Компаративни епидемиолошки подаци о повредама у овим спортовима у југоисточној Европи су ограничени. Циљ ове студије био је да се упореде епидемиологија и обрасци повређивања код скијаша и сноубордера на Скијалишту Копаоник у Србији.

Методе Спроведена је ретроспективна дескриптивна епидемиолошка студија заснована на анализи извештаја ски-патроле и медицинске документације Универзитетског клиничког центра Србије током четири зимске сезоне (2021–2024). Укључени су повређени учесници узраста од 4 до 81 године који су збринути на терену и прегледани у траума центру на скијалишту. Испитаници су подељени према врсти спорта. Разлике у демографским, временским и амбијенталним карактеристикама анализиране су χ^2 тестом. Тип и анатомска локализација повреда упоређивани су применом χ^2 или Фишеровог егзактног теста, уз израчунавање односа шанси (*OR*) са 95% интервалом поузданости.

Резултати Анализирано је 3368 повређених учесника, од којих су 3011 (89,4%) били скијаши, а 357 (10,6%) сноубордери. Сноубордери су били млађи и чешће мушког пола ($p < 0,001$). Повреде код сноубордера чешће су се јављале на лакшим стазама, док су скијаши доминирали на тежим стазама ($p = 0,001$). Скијаши су имали веће изгледе за повреде колена и потколенице, док су сноубордери чешће задобијали повреде подлактице и шаке/зглоба. Преломи и луксације били су чешћи код сноубордера, а уганућа код скијаша.

Закључак Утврђене су значајне разлике, специфичне за спортове, у обрасцима повређивања, што указује на потребу за циљаном превенцијом повреда и унапређењем здравствене заштите у зимским спортовима.

Кључне речи: сноубординг; скијање; повреде

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Screening of urinary tract infections in children using fluorescent flow cytometry – a single-center study

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SUMMARY

Introduction/Objective Urinary tract infections (UTIs) in children represent a significant public health problem due to their high prevalence and the need for timely diagnosis and adequate treatment. Automated methods using fluorescent flow cytometry are increasingly being used in laboratories for the screening of UTIs. The aim of this study was to determine the cutoff values for leukocyturia and bacteriuria using fluorescent flow cytometry for the screening of urinary tract infections in the pediatric population.

Methods A total of 821 urine samples were cultured, of which 366 samples met the criteria for fluorescent flow cytometry on the automated Sysmex UF- 4000 analyzer. The counts of leukocytes and bacteria were compared with the culture results.

Results Of the total urine cultures tested, 209 (25.5%) were positive, and 599 (73%) were negative. There was a statistically significant difference in the prevalence of uropathogens according to the age of the children ($p < 0.001$). The area under the curve (AUC) for leukocyte count was 0.77 (95% CI: 0.71–0.84), while the AUC for bacterial count was 0.85 (95% CI: 0.81–0.89). A low negative likelihood ratio (0) was observed at the bacterial cutoff of 40.1, and the negative predictive value was high (between 91% and 99%).

Conclusion Determination of leukocyte and bacterial counts in urine in children using fluorescent flow cytometry can serve as an initial test when deciding on urine culture in microbiological laboratories. These results may indicate the necessity of reducing unnecessary urine cultures while providing faster confirmation of negative test results.

Keywords: fluorescent flow cytometry; leukocytes; bacteria; cutoff value; urine culture

INTRODUCTION

Urinary tract infections (UTIs) represent one of the most common bacterial infections in the pediatric population, affecting all age groups of children. They constitute a significant public health problem globally, given their high incidence and the need for timely diagnosis and therapy. Despite the growing problem of antimicrobial resistance, the use of antibiotics remains an indispensable part of the therapeutic approach in the treatment of UTIs in children [1, 2]. The incidence of UTIs in children varies and depends on age, sex, and race. The incidence rate is significantly high in both sexes during the first year of life. Boys have a higher incidence than girls in the first year of life, after which the rate declines. Girls are predominantly affected by UTIs, at a rate of 2–4 times higher than in boys [3, 4]. Functional urination disorders are common in recurrent urinary tract infections. They can cause damage to the upper urinary tract and kidneys, as well as negatively affect the quality of life of children, which is why their early detection and treatment are important [5]. Inadequate selection of antibiotics may lead to therapeutic failure and contribute to the development of antimicrobial resistance. Recurrent UTIs, particularly in children, represent a significant clinical problem due to consequent irreversible damage, including scarring of the renal parenchyma, progressive impairment of renal function, arterial hypertension, and the development of chronic kidney disease. Evidence from research indicates a high prevalence of multidrug resistance among Gram-negative bacteria, which represent the most common etiological agents of UTIs [6, 7]. Microbiological analysis of urine, that is, urine culture, is still considered the most reliable method for the detection and identification of UTI pathogens. The diagnostic process may be complicated by the presence of fecal flora, due to colonization of the perineal region and distal urethra [8, 9]. To minimize sample contamination, suprapubic aspiration and catheterization are considered reference methods for urine collection for culture in young children. Given the invasive nature of certain diagnostic procedures, inflammation in the urinary tract may be indirectly assessed through quantification of white blood cells (WBCs) and bacteria in urine [10, 11].

Modern fully automated urine analysis systems, including combined chemical and sediment analyzers, as well as devices based on digital microscopy and flow cytometry, have become an integral part of routine laboratory practice. These technologies enable standardized, efficient, and reproducible sample processing, thereby supporting clinicians in the early identification and management of

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suspected UTI samples [12, 13]. Fluorescent flow cytometry allows rapid and reliable analysis of urine samples, enabling UTI screening in less than one minute. An additional advantage of this technology is its ability to differentiate between Gram-positive and Gram-negative bacteria [14, 15, 16].

The aim of this study was to examine the most common causative agents of UTIs in children and the role of fluorescent flow cytometry as an auxiliary diagnostic method during microbiological urine analysis.

METHODS

From January to July 2025, we analyzed urine samples from the Clinic of Pediatric Diseases, University Clinical Center of the Republic of Srpska (UCC RS), which were routinely submitted to the microbiology laboratory for culture. The study was conducted over this six-month period to ensure consistent laboratory procedures, stable analytical conditions, and an adequate sample size across all age groups, while minimizing variability related to procedural or organizational changes over longer study periods. Participants were stratified into the following age groups to reflect distinct developmental and physiological stages: 0–6 months, seven months to four years, 5–9 years, 10–14 years, and 15–18 years.

Urine cultures were performed exclusively in hospitalized pediatric patients admitted to the pediatric ward. Both urinalysis and urine culture were requested only when clear clinical indications were present, based on the treating physician's assessment (e.g., signs or symptoms suggestive of urinary tract infection). Exclusion criteria included: urine samples obtained as part of routine screening examinations; outpatient urine samples; samples with incomplete clinical or laboratory data; contaminated urine samples; and repeated urine samples from the same patient, with only the first eligible sample included in the analysis. A total of 906 urine samples obtained through voiding and urinary catheterization were analyzed, of which 821 samples were included in our study. Of these, 366 urine specimens met the criteria, that is, a minimum of 2 mL of urine for fluorescent flow cytometry using the automated analyzer Sysmex UF-4000 (Siemens Corporation).

Fluorescent flow cytometry is an analytical method that enables rapid measurement of light scatter and fluorescence emission produced by adequately illuminated cells or particles. During analysis, cells or particles are suspended in a liquid medium and generate optical signals as they individually pass through a laser light beam. Because measurements are performed separately for each particle or cell, the results represent cumulative individual cytometric characteristics. Urine particle analysis is based on sheath flow principles, whereby each particle within the sample stream is individually illuminated by laser light, generating forward-scattered light, side-scattered light, side fluorescence, and depolarized side-scattered light, which are subsequently converted into electrical signals. In this study, urine samples were analyzed using an automated urine flow cytometer (Sysmex UF-4000, Sysmex Corporation, Kobe, Japan). The system utilizes a blue laser to enhance bacterial detection and depolarized side-scattered light to improve differentiation between erythrocytes and crystals. Fluorescent staining is performed using proprietary reagents with radio-frequency identification (RFID) technology to ensure accurate measurement and differentiation of bacteria. The analyzer enables differentiation of epithelial cells and urinary casts and allows rapid exclusion of negative urinary tract infection samples. The UF-4000 provides quantitative analysis of 17 diagnostic parameters in urine mode and nine diagnostic parameters in body fluid mode, with results reported as particles per microliter using manufacturer-provided algorithms. The method allows for the analysis of physiological and cellular properties of urine and other body fluids, such as cerebrospinal fluid, pleural fluid, synovial fluid, and others. The analytical parameters that the Sysmex UF-4000 can detect and quantify include: erythrocytes, non-lysed erythrocytes, white blood cells (WBCs), WBC clusters, epithelial cells (squamous, non-squamous, transitional, and renal epithelial cells), casts (hyaline and pathological casts), bacteria, fungi, crystals, spermatozoa, and mucus. The detection range for WBCs on the Sysmex UF-4000 is 1–10,000/μL, and for bacteria 5–10,000/μL.

Urine cultures were performed using a 1-μL loop on chromogenic agar plates (BioMérieux, Marcy-l'Étoile, France) to enable identification and quantification of microorganisms, incubated at 37°C for 18–24 hours. After the incubation period, only those samples with significant growth (cutoff ≥ 10^5 CFU/mL) of a single uropathogen were considered to confirm UTIs. For colony counts between 10^4 and 10^5 CFU/mL, results were evaluated in accordance with the sampling method and the child's clinical presentation. Negative samples were sterile or mixed/contaminated with insignificant growth (cutoff ≤ 10^3 CFU/mL) and were not further tested. Mixed cultures, defined as those containing two or more types of uropathogens, required repeat urine sampling.

Identification of microorganisms was performed according to the manufacturer's recommendations for chromogenic agar plates. For cultures where the microorganism could not be reliably determined, identification was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Zhuhai DL Biotech CO) and an automated identification and antibiotic susceptibility testing system, Vitek 2 (BioMérieux, Marcy-l'Étoile, France).

Statistical analysis

For statistical data processing, IBM SPSS Statistics Version 23 (IBM Corp., Armonk, NY, USA) and Microsoft Excel Office 2019 were used. Descriptive statistics (frequencies and percentages) were applied for the distribution of categorical data, and the chi-square (χ^2) test was used to test associations. Receiver operating characteristic (ROC) curve analysis was applied to compare the performance of different parameters, while the values of the area under the curve (AUC) were used to assess diagnostic accuracy in

Table 1. Distribution of patient characteristics with urine culture test by age and sex

Variables	Total	Urine culture test results, N (%)			p
	N = 821	Positive N = 209 (25.5)	Negative N = 599 (73)	Contamination N = 13 (1.5)	
Age					
0–6 months	294	95 (45.5)	197 (32.9)	2 (15.4)	< 0.001*
7 months – 4 years	256	76 (36.4)	174 (29)	6 (46.2)	
5–9 years	100	15 (7.2)	83 (13.9)	2 (15.4)	
10–14 years	74	7 (3.3)	65 (10.9)	2 (15.4)	
15–18 years	97	16 (7.7)	80 (13.4)	1 (7.7)	
Sex					
Male	389	92 (44)	291 (48.6)	6 (46.2)	0.522
Female	432	117 (56)	308 (51.4)	7 (53.8)	

*$\chi^2 = 32.487$, df = 8;
$\chi^2 = 1.301$, df = 2

detecting urinary tract infections (UTIs). A p-value < 0.05 was considered significant.

Ethics: Approval for conducting the study was obtained from the Ethics Committee of UCC RS (01-19-162-2/25) in order to protect the rights of participants in accordance with the current Declaration of Helsinki.

RESULTS

The results showed that out of the total urine cultures tested, 209 (25.5%) were positive, 599 (73%) were negative, and contamination with normal physiological flora was identified in 13 (1.5%) samples. A statistically significant difference was observed in urine culture results in relation to the age of children (p < 0.001). The highest percentage of positive results was recorded in the youngest children (0–6 months) at 45.5%, and the percentage of positive results decreased with age. Although the prevalence of urinary tract infections was higher in girls (56%), the difference compared to boys was not statistically significant (p = 0.522) (Table 1).

There was a statistically significant difference in the distribution of uropathogens in relation to the age of children (p < 0.001). *Escherichia coli* (46.6%) was most frequently isolated in the age group of seven months to four years, with its frequency decreasing with increasing age. *Klebsiella spp*. (68.9%), Enterococcus faecalis (55.6%), and *Acinetobacter spp*. (80%) were significantly represented in urine cultures of the youngest age group of children (0–6 months). The lowest frequencies of urinary tract infection causative agents were observed in children aged 10–14 years, namely *E. coli* (5.2%) and *Klebsiella spp*. (2.2%). Based on the analysis of urinary tract infection causative agents in relation to sex, different patterns of their distribution were observed. E. coli was more frequently isolated in girls (56%), as was E. faecalis (66.7%), while in boys *Klebsiella spp*. (53.3%) and *Proteus mirabilis* (70%) were more frequently isolated. However, the observed statistical value (p > 0.05) indicated that there was no statistically significant difference in the frequency of isolated microorganisms from urine between boys and girls (Table 2).

Results derived from automated flow cytometry

Table 3 presents the mean values and medians with interquartile ranges for the number of WBC and bacteria in two groups of urine samples (negative and positive). In negative samples, the mean WBC count was significantly lower (88.5), while in positive samples it was significantly increased (1,166.9). The WBC median count also showed a large difference: 7.7/μL (IQR 2.7–34.05) in negative samples and 125.6/μL (IQR 19.1–751.6) in positive samples. The mean number of bacteria in negative samples was 781.94, whereas in positive samples it was markedly increased to 12,453.19 (Table 3).

Table 3. Medians and ranges of white blood cell counts and bacterial numbers by the UF-4000 device

UF-4.000	Negative	Positive
WBC ($\bar{x}$)	88.5	1166.9
Median/μL [IQR]	7.7 (2.7–34.05)	125.6 (19.1–751.6)
Bacteria ($\bar{x}$)	781.94	12,453.19
Median/μL [IQR]	32 (11.4–114.6)	689.3 (150.75–7659.95)

WBC – white blood cells

ROC curves for WBC count and bacterial count in urine are presented in Figure 1. The area under the curve (AUC) for WBC count was 0.77 (95% CI: 0.71–0.84), while the AUC for bacterial count was 0.85 (95% CI: 0.81–0.89).

Table 2. Distribution of types of pathogens causing urinary tract infections in children by age and sex

Variables	*Escherichia coli*	*Klebsiella spp.*	*Enterococcus faecalis*	*Proteus mirabilis*	*Acinetobacter spp.*	*Other*	*p*
Age							
0–6 months	41 (35.3)	31 (68.9)	15 (55.6)	1 (10)	4 (80)	3 (50)	0.001*
7 months – 4 years	54 (46.6)	7 (15.6)	8 (29.6)	6 (60)	0 (0)	1(16.7)	
5–9 years	8 (6.9)	1 (2.2)	3 (11.1)	3 (30)	0 (0)	0 (0)	
10–14 years	6 (5.2)	1 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)	
15–18 years	7 (6)	5 (11.1)	1 (3.7)	0 (0)	1 (20)	2 (33.3)	
Sex							
Male	51 (44)	24 (53.3)	9 (33.3)	7 (70)	2 (40)	2 (40)	0.372
Female	65 (56)	21 (46.7)	18 (66.7)	3 (30)	3 (60)	3 (46.7)	

Other – *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Serratia marcescens*, *Candida spp.;*
*$\chi^2 = 47.447$, df = 20;
$\chi^2 = 5.372a$, df = 5;

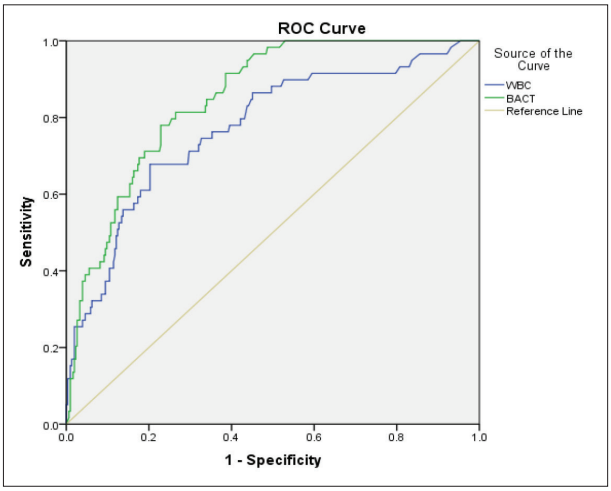

Figure 1. The receiver operating characteristic (ROC) curves for urinalysis test in children;
WBC – white blood cells; BACT – bacterial

Table 4. Diagnostic accuracy performance of UF-4000 with different cut-off for WBC/μL and bacteria/μL

Variables*	Cut-off WBC count/μL				Cut-off BACT count/μL			
	13.4	25.8	39.8	59.4	40.1	54.4	61.9	95
TP (n)	46	38	40	36	57	54	51	48
FP (n)	97	83	70	23	2	5	8	11
TN (n)	202	216	229	244	215	190	198	231
FN (n)	13	21	19	23	84	109	101	86
SE (%)	78	64.4	67.8	61	96.6	91.5	86.4	81.4
SP (%)	67.6	72.2	76.6	81.6	71.9	63.5	66.6	72.9
LR+	2.4	2.3	2.9	3.3	3.4	2.5	2.6	3
LR-	0.3	0.5	0.4	0.5	0	0.1	0.2	0.3
PPV (%)	32.2	31.4	36.4	39.6	40.4	33.1	33.8	35.8
NPV (%)	94	91.1	92.3	91.4	99.1	97.4	96.1	95.5

WBC – white blood cells; BACT – bacterial; TP – true positives; FP – false positives; TN – true negatives; FN – false negatives; SE – sensitivity; SP – specificity; LR(-) – negative likelihood ratio; LR(+) – positive likelihood ratio; PPV – positive predictive value; NPV – negative predictive value

The diagnostic efficiency of bacterial count in urine was significantly higher compared to WBC count ($p < 0.001$) (Figure 1).

In Table 4, three different cutoff values for WBC and bacterial counts were evaluated to assess the performance of urine flow cytometry in predicting urinary tract infection. These cutoff values were selected based on a combination of previously published literature in pediatric populations and our preliminary ROC curve analysis. Lower cutoffs were included to maximize sensitivity and reduce false-negative results, while higher cutoffs were chosen to increase specificity and reduce false-positive results. This approach allows comparison of test performance across a range of clinically relevant thresholds, highlighting the trade-off between sensitivity and specificity for different clinical decision-making scenarios. Sensitivity decreased with increasing cutoff values: WBC count/μL (from 78% at 13.4 to 61% at 59.4). Sensitivity was also considerably higher for bacterial cutoff values, particularly at lower thresholds (96.6% at 40.1 vs. 81.4% at 95). Specificity increased with higher cutoff values and was higher for WBC than for bacteria at the observed thresholds (67.6–81.6% *vs.* 63.5–72.9%). The low negative likelihood ratio value (0) for a bacterial cutoff of 40.1 indicated that it was nearly perfect for ruling out disease. The positive predictive value (PPV) was low (between 31% and 40%) due to a higher number of false-positive test results. The negative predictive value (NPV) was high (between 91% and 99%), indicating that the test was very effective in ruling out disease when negative (Table 4).

DISCUSSION

Our study showed that 25.5% of urine cultures were positive, while 73% were negative. Negative urine cultures represent a significant portion of the overall clinical and microbiological load. The use of highly sensitive diagnostic tests to rule out UTIs in pediatric patients could contribute to reducing the number of unnecessary urine cultures, as well as the inappropriate use of antibiotics in suspected cases of infection [17].

UTIs occur more often in girls than in boys and are among the most commonly observed bacterial infections in the pediatric population [11]. Similar to other literature reports, the results of our study also showed that UTIs were more frequent in girls (56%) compared to boys (44%) [18, 19]. These infections were more frequently recorded in infants and young children than in school-aged children. This is consistent with the literature noting that the peak of UTIs occurs during the first year of life and then between two and four years of age, which corresponds to the period of toilet training [20, 21]. Similar to other studies on the causative agents of UTIs in the pediatric population, in our study *E. coli* was the most frequently isolated bacterium from urine [22, 23, 24]. *Klebsiella* spp., *E. faecalis*, and *Acinetobacter* spp. were the most common isolates from infants' urine, which may imply hospital-acquired infections. In the study by Moghnia et al. [25], of a total of 3996 urine samples processed, 282 showed significant bacteriuria, mostly in boys (185). The most common isolates also produced Extended-spectrum beta-lactamases, particularly *K. pneumoniae* (56%) and *E. coli* (38.3%) [25].

UTIs are among the most common and most serious bacterial infections encountered by pediatricians and general practitioners. Although the diagnosis and treatment of these infections may seem uncomplicated, they still represent some of the most controversial issues in pediatrics. A partial source of controversy regarding UTIs arises from nonspecific clinical presentation, suboptimal methods of urine sample collection, revised guidelines for radiological evaluation of the urinary tract, as well as heterogeneity in therapeutic and preventive approaches [26].

Urine culture is the gold standard in the diagnosis of UTIs in children and is one of the most common laboratory tests. Obtaining a quality urine specimen in children is quite difficult by voiding, especially in newborns, infants, and children who are not toilet trained. In the era of laboratory automation, in order to facilitate the work of laboratory staff and obtain results more quickly, alternative

methods are being sought to accelerate the process of obtaining urine culture results. In patients with negative results on urine dipstick, microscopic, or automated urinalysis, urine culture is not necessary if other causes of fever are present [2].

In our study, we assessed the optimal counts of WBC and bacteria in urine in children using the Sysmex UF-4000 automated analyzer immediately before urine culture. The results showed that WBC values were significantly higher in positive samples [125.6 (19.1–751.6)] compared to negative samples [7.7 (2.7–34.05)]. The median number of bacteria per μL of urine in negative samples was 32 (11.4–114.6), and in positive samples it was 689.3 (150.75–7659.95). Similar results were obtained by Savitri et al. [27], who evaluated the number of WBCs in infants' urine; the values in positive samples for WBC and bacteria were 88.5 (27.9–182.5); 400.4 (39.5–44,914.4), and those in negative samples were 19.7 (0.5–181.8); 51.2 (0–6,283.7). Increased WBCs in urine in children, diagnosed by a leukocyte esterase test or microscopic analysis, indicate inflammation, most often UTI. However, certain noninfectious and systemic conditions can lead to false-positive WBC findings in urine, including infections caused by **g**roup A *Streptococcus*, Kawasaki disease, and physiological changes induced by intensive physical activity [9].

In this study, we evaluated different thresholds of WBC and bacteria in urine associated with positive urine culture results in the pediatric population using sensitivity, specificity, PPV, NPV, and positive and negative LR values. ROC curve analysis assessed the diagnostic value of WBC and bacteria counts in distinguishing participants with positive from those with negative urine culture findings. The obtained area under the curve (WBC AUC = 0.77; bacterial count AUC = 0.85) indicates satisfactory discriminative ability, suggesting that WBC and bacteria counts can, to some extent, differentiate negative from positive urine culture results. These results are consistent with other studies that evaluated the significance and role of automated WBC and bacterial counts using flow cytometry as a screening test for UTIs in children [27, 28].

The optimal cutoff value for leukocyturia and bacteriuria was 40 cells/μL, with sensitivity of 67% and 96.6%, specificity of 76.6% and 71.6%, PPV of 36.4% and 40.4%, and NPV of 92.3% and 99.1%, respectively. The high NPV of leukocyturia and bacteriuria demonstrated the ability of these two parameters to accurately predict negative urine culture results in order to rule out urinary tract infections in children. Liu et al. [29] identified a similar optimal cutoff value of 40.8 WBC/μL, which showed the largest area under the ROC curve and the highest Youden index. The following diagnostic characteristics were achieved: sensitivity 80.7% (95% CI: 0.770–0.840), specificity 77.8% (95% CI: 0.761–0.792), PPV 96.5% and NPV 35.2%. Determining the number of WBCs in urine by flow cytometry provides optimal performance as an initial diagnostic test for urinary infections in febrile children. A study conducted in Belgium showed that if a cutoff value of > 35 WBC/μL of urine was used, which also provided high sensitivity (99.5% [95% CI, 99 to 100%]) and acceptable specificity (80.6% [95% CI, 78 to 83%]), the number of urine samples sent to the laboratory for culture would be reduced by 67% [17].

Determining an appropriate cutoff value within an exclusion strategy represents a significant challenge, since improving the sensitivity of the test often comes at the expense of its specificity, thereby increasing the risk of false-positive results. The presence of WBCs in urine indicates an inflammatory response, which may be caused by various factors, not necessarily infection, thus increasing the risk of false-positive findings. Additionally, sample contamination may result in a clinically significant bacterial count even in the absence of true infection. Since urine culture is the diagnostic gold standard, such discrepancies often cannot be clearly identified without additional analyses [30].

CONCLUSION

The screening method for excluding negative urine cultures in children using fluorescent flow cytometry can assist in clinical decision-making. In children without symptoms and signs of UTIs, WBC and bacterial counts below 40/μL can be used for screening negative cultures. To avoid missing clinically significant cases of UTIs in children, and for a more efficient evaluation of fluorescent flow cytometry as a screening method, further studies in different pediatric populations using complementary diagnostic methods are recommended.

Conflict of interest: None declared.

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Скрининг инфекција уринарног тракта код деце помоћу флуоресцентне проточне цитометрије – студија једног центра

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САЖЕТАК

Увод/Циљ Инфекције уринарног тракта код деце представљају значајан јавноздравствени проблем због високе учесталости и потребе за благовременом дијагностиком и адекватним лечењем. Аутоматизована метода помоћу флуоресцентне проточне цитометрије све више се данас користи у лабораторијама за скрининг уринарних инфекција. Циљ овог истраживања био је одредити граничне вредности леукоцитурије и бактериурије коришћењем методе флуоресцентне проточне цитометрије за скрининг инфекција уринарног тракта у педијатријској популацији.

Методе Укупно је култивисан 821 узорак урина, а критеријум за флуоресцентну проточну цитометрију на аутоматском анализатору *Sysmex UF*-4.000 задовољило је 366 узорака. За препознавање и квантификацију микроорганизама коришћене су хромогене подлоге. Број леукоцита и бактерија поређен је са резултатима култивације.

Резултати Од укупног броја тестираних уринокултура, 209 (25,5%) било је позитивно, а 599 (73%) негативно. Постојала је статистички значајна разлика у заступљености уропатогена у односу на узраст деце ($p < 0,001$). Површина испод *ROC* криве за број леукоцита износила је 0,77 (95% *CI*: 0,71–0,84), а за број бактерија 0,85 (95% *CI*: 0,81–0,89). Ниска вредност негативног односа вероватноће (0,0) забележена је за граничну вредност од 40,1 за бактерије, а негативна предиктивна вредност била је висока (између 91% и 99%).

Закључак Одређивање вредности леукоцита и бактерија у урину код деце помоћу флуоресцентне проточне цитометрије може да буде почетни тест приликом одлучивања за култивацију урина у микробиолошким лабораторијама. Ови резултати би могли указати на неопходност смањења непотребне култивације урина, а истовремено и на брже добијање негативних резултата теста.

Кључне речи: флуоресцентна проточна цитометрија; леукоцити; бактерије; граничне вредности; уринокултура

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Peripapillary capillary vessel density in normal tension glaucoma and primary open-angle glaucoma

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SUMMARY

Introduction/Objective Primary open angle glaucoma (POAG) is a chronic, progressive, optic neuropathy with possible blindness and irreversible changes in the optic nerve head (ONH). Optical coherence tomography (OCT) is non-invasive method that provides structural glaucoma damage evaluation. OCT angiography (OCTA) provides qualitative and quantitative assessment of peripapillary microvasculature. The objective of this study was to determine whether there is a difference in peripapillary capillary vessel density (VCD) in normal tension glaucoma (NTG), POAG and healthy subjects.

Methods This prospective study included 120 eyes: 40 healthy eyes, 30 eyes with NTG and 50 eyes with POAG. Ophthalmological examination, central corneal thickness, OCT, OCTA also visual field were performed.

Results Vessel capillary density total (VCD total) is significantly higher in healthy compared to NTG and POAG (39.25 ± 0.94 *vs*. 36.79 ± 2.50 *vs*. 37.58 ± 1.53; $p < 0.001$). Peripapillary VCD (VCD perip.) is lower significantly in NTG and POAG in comparison with healthy subjects (39.21 ± 2.62 *vs*. 40.18 ± 1.35 *vs*. 41.29 ± 0.81; $p < 0.001$). VCD total is significantly lower in NTG compared to POAG (36.79 ± 2.50 *vs*. 37.58 ± 1.55; $p < 0.001$). We found significantly positive correlation between OCT and OCTA parameters of ONH with mean deviation (MD) index in NTG between retinal nerve fiber layer average with MD ($r = 0.370$, $p < 0.05$) also between VCD perip. inf. with MD ($r = 0.395$, $p < 0.05$). In POAG, we obtained a significantly positive correlation between VCD inf. with MD ($r = 0.277$, $p < 0.05$) also VCD inside disc with MD ($r = 0.395$, $p < 0.01$).

Conclusion Glaucoma patients have a significantly lower vessel density ONH and VCD perip. compared to healthy subjects. VCD total of ONH is significantly lower in NTG compared to POAG. We obtained a positive correlation between OCT and OCTA parameters with MD in NTG and POAG.

Keywords: optical coherence tomography angiography; optic nerve head; intraocular pressure

INTRODUCTION

Primary open angle glaucoma (POAG) is a chronic, progressive, optic neuropathy with possible blindness and irreversible changes in the optic nerve. It causes a reduction of the neuroretinal rim and retinal nerve fiber layer (RNFL) with relative visual field (VF) defects. Changes in the VF are prevented with early diagnosis and treatment of glaucoma [1].

There are morphologically optic nerve head (ONH) changes in glaucoma as well as RNFL thickness decrease [2, 3, 4].

Usually used non-invasive method that provides structural glaucoma damage evaluation is optical coherence tomography (OCT) [5].

OCT angiography (OCTA) is non-invasive and high-resolution method which provides peripapillary vessel density (angioflow vessel density) as well as superficial perifoveal vessel density quantification. Perfused area expressed as vessel density is a percentage of full examined of its limited sectors inside retinal layer [6, 7]. Peripapillary vessel capillary density (VCD perip.) is the most consistent OCTA parameter in the diagnosis and monitoring of glaucoma progression [6], and a useful predictor of VF progression in intermediate and advanced glaucoma patients [8]. Jia et al. [9] were the first to describe vessel density reduced measurement by OCTA in glaucoma.

The aim of this study was to determine whether there is a difference in VCD perip. in normal tension glaucoma (NTG) and primary open-angle glaucoma (POAG) patients as well as healthy subjects.

METHODS

This prospective research included 120 eyes: 40 healthy eyes, 30 eyes with NTG and 50 eyes with POAG. The study was performed in Family Čivčić Practice of Ophthalmology, Belgrade, from April 2022 to March 2023.

Inclusion criteria were: open angle under gonioscopy, early [mean deviation (MD) ≤ 6 dB] and middle (MD ≤ 12 dB) stage of glaucoma patients according to Hodapp´s classification [1], age ≥ 18 years. Different inclusion criteria were specified in:

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– healthy-control group: eyes without glaucoma with intraocular pressure (IOP) 10–21 mmHg, best corrected visual acuity (BCVA) ≥ 0.9, normal cup/disc ratio, RNFL thickness and VCD perip.;

– NTG group: eyes with early and middle stage (Hodapp's classification) NTG, with characteristic change of the ONH and RNFL and MD ≤ 12 dB without elevated IOP; BCVA ≥ 0.5;

– POAG group: eyes with early and middle stage (Hodapp´s classification) POAG, with typical damage of RNFL and ONH and MD ≤ 12 dB with elevated IOP; BCVA ≥ 0.5;

Exclusion criteria: advanced POAG glaucoma stage, secondary glaucoma, myopia ≥ -6 D, ONH drusen and other ONH anomalies, trauma, other ocular diseases, previous laser treatment as well as ocular surgery (glaucoma or cataract), MD > 12 dB, unreliable VF patients (false-positive errors > 15%, false-negative errors > 15%, and fixation losses > 20%).

All patients went through the following examination procedure: slit-lamp biomicroscopy, BCVA, Goldmann applanation tonometry, evaluation of dilated fundus, gonioscopy, central corneal thickness measurement using ultrasonic pachymeter SP-100 Tomey (Tomey, Nagoya, Japan) and 24-2 threshold test with standard automated perimetry AP-1000 Tomey (Tomey). Spectral domain OCT (SOCT REVO 60, software version 11.05, Optopol Technology, Zawiercie, Poland,) was performed in RNFL measurements and ONH analysis. OCTA examination was performed with SOCT REVO 60 with angio mode, software 11.05 (2021) axial resolution 5μm and 60 000 scan per second. The quality of the obtained recordings was QI ≥ 8.

VCD is defined as the percentage of area occupied by capillaries with flow in the scanned region. Measurements were made and analyzed in the radial peripapillary capillaries (RPC) plexus from the inner limiting membrane to the posterior boundary of the RNFL.

In research, certain statistical descriptive methods are considered: arithmetic mean, standard deviation, median, minimum, maximum and percentage. The difference between the mean values of variables with normal distribution was analyzed by one-way ANOVA parametric test. For variables without a normal distribution, the non-parametric Kruskal–Wallis test was used. Additional analyzes were performed with the parametric t- test. Correlation between the investigated parameters was done with the Pearson's correlation test and Spearman-R Order correlation test. Statistical analysis was done in IBM SPSS Statistics for Windows, Version 26.0. (IBM Corp., Armonk, NY, USA). Statistical significance is considered at level value of $p < 0.05$.

Ethics: The study has approval of the Local Ethics Committee and the Medical Faculty University of Novi Sad, Novi Sad, Serbia (Number 01-39/237/1).

Table 1. Demographic and clinical characteristics of patients

Parameters	Health (n = 40) $\bar{x} \pm SD$	NTG (n = 30) $\bar{x} \pm SD$	POAG (n = 50) $\bar{x} \pm SD$	p
Age (years)	47 (18–74)	60.60 ± 14.16	62.36 ± 11.64	< 0.001
Gender (M/F), n	16/24	8/22	15/35	0.442
BCVA	0.98 ± 0.10	0.89 ± 0.18	0.94 ± 0.11	NS*
IOP (mmHg)	16.18 ± 2.73	15.47 ± 2.83	17.36 ± 3.74	< 0.05*
CCT (μm)	561.75 ± 36.45	498.73 ± 19.09	565.28 ± 28.98	< 0.0001*
MD (dB)	0.52 (-3.06–3.45)	-1.57 (-11-1.41)	-1.27 (-4.57–3.61)	< 0.001**

M/F – male/female; BCVA – best corrected visual acuity; IOP – intraocular pressure; CCT – central corneal thickness; MD – mean deviation; NTG – normal tension glaucoma; POAG – primary open angle glaucoma;
*One Way ANOVA;
**Kruskal–Wallis

Table 2. OCT parameters of the optic nerve head

OCT parameters	Health (n = 40) $\bar{x} \pm SD$	NTG (n = 30) $\bar{x} \pm SD$	POAG (n = 50) $\bar{x} \pm SD$	p
RNFL aver. (μm)	123.45 ± 9.31	113.33 ± 18.42	114.26 ± 17.24	< 0.01*
RNFL sup. (μm)	139.38 ± 13	130.21 ± 23.06	127.36 ± 20.53	< 0.05*
RNFL inf. (μm)	144.58 ± 13.55	127 ± 26.54	129.42 ± 24.55	< 0.001*
C/D area ratio	0.29 (0.13–0.47)	0.425 (0.2–0.96)	0.49 ± 0.13	< 0.001**

OCT – optical coherence tomography; NTG – normal tension glaucoma; POAG – primary open angle glaucoma; RNFL – retinal nerve fiber layer; C/D – cup/disc area ratio;
*One Way ANOVA;
**Kruskal–Wallis

Table 3. OCTA parameters of the optic nerve head

OCTA parameters	Health (n = 40) $\bar{x} \pm SD$	NTG (n = 30) $\bar{x} \pm SD$	POAG (n = 50) $\bar{x} \pm SD$	p
VCD total	39.25 ± 0.94	36.79 ± 2.5	37.58 ± 1.55	< 0.001*
VCD sup.	39 ± 1.92	36.64 ± 2.66	37.43 ± 1.91	< 0.001*
VCD inf.	39.19 ± 1.09	36.68 ± 3.03	37.45 ± 2.18	< 0.001*
VCD ins.d	31.53 ± 2.61	27.96 ± 4.17	27.33 ± 4.55	< 0.001*
VCD perip.	41.29 ± 0.81	39.21 ± 2.62	40.18 ± 1.35	< 0.001*
VCD perip.sup.	41.47 ± 1.1	39.62 ± 2.62	40.47 ± 1.31	< 0.001*
VCD perip.inf.	41.13 ± 0.85	38.74 ± 3.01	39.82 ± 2.07	< 0.001*

OCTA – optical coherence tomography angiography; VCD – vessel capillary density; VCD ins.d – vessel capillary density inside disc; VCD perip. – vessel capillary density peripapillary; NTG – normal tension glaucoma; POAG – primary open angle glaucoma;
*One Way ANOVA

RESULTS

This prospective research included 120 eyes: 40 healthy eyes, 30 eyes with NTG and 50 eyes with POAG. There were 39 (32.5%) male patients and 81 (67.5%) female patients. Demographic and clinical characteristics of patients are presented in Table 1. IOP values are significantly higher in POAG compared to NTG and healthy subjects ($p < 0.05$). Central corneal thickness is significantly smaller in NTG compared to POAG and healthy eyes ($p < 0.001$). MD index of VF is significantly higher in NTG and POAG patients compared to healthy eyes ($p < 0.01$).

Table 2 shows the OCT parameters of ONH and the comparison between the groups. Cup/disc area ratio is significantly higher in NTG and POAG compared to healthy subjects ($p < 0.001$). RNFL average thickness is significantly lower in NTG and POAG patients compared to healthy eyes ($p < 0.01$).

Table 4. OCTA parameters of the optic nerve head in NTG and POAG

OCTA parameters	NTG (n = 30) $\bar{x}$ ± SD	POAG (n = 50) $\bar{x}$ ± SD	p
VCD total	36.79 ± 2.5	37.58 ± 1.55	< 0.001˚
VCD sup.	36.64 ± 2.66	37.43 ± 1.91	< 0.05˚
VCD inf.	36.68 ± 3.03	37.45 ± 2.18	< 0.01˚
VCD ins.d	27.96 ± 4.17	27.33 ± 4.55	NS
VCD perip.	39.21 ± 2.62	40.18 ± 1.35	< 0.001˚
VCD perip.sup.	39.62 ± 2.62	40.47 ± 1.31	< 0.001˚
VCD perip.inf.	38.74 ± 3.01	39.82 ± 2.07	< 0.001˚

OCTA – optical coherence tomography angiography; VCD – vessel capillary density; VCD ins.d – vessel capillary density inside disc; VCD perip. – vessel capillary density peripapillary; NTG – normal tension glaucoma; POAG – primary open angle glaucoma;
˚t – Test

Table 5. Pearson's correlation coefficient between RNFL thickness and OCTA parameters of the optic nerve head

OCT parameters	OCTA parameters	Health (n = 40)	NTG (n = 30)	POAG (n = 50)
RNFL average	VCD total	0.2960	0.1227	0.0056
	VCD sup.	0.3915*	0.1315	-0.0637
	VCD inf.	0.1149	0.2314	0.0077
	VCD ins.d	0.2189	0.0200	-0.3468*
	VCD perip.	0.0260	0.0742	0.1973
RNFL superior	VCD total	0.2493	0.0130	-0.0123
	VCD sup.	0.2384	0.1820	-0.0397
	VCD inf.	0.0913	0.0651	-0.0332
	VCD ins.d	0.1208	-0.1586	-0.2669
	VCD perip.	0.1177	-0.0353	0.1723
RNFL inferior	VCD total	0.1029	0.2055	0.0321
	VCD sup.	0.3727*	0.0102	-0.1129
	VCD inf.	0.0312	0.2655	0.0727
	VCD ins.d	0.0450	0.0403	-0.3883**
	VCD perip.	0.1879	0.1255	0.2436
RNFL temporalis	VCD total	0.0552	0.1199	-0.1955
	VCD sup.	0.0629	0.2676	-0.2325
	VCD inf.	0.0768	0.3723*	-0.1442
	VCD ins.d	0.1420	0.2727	-0.2228
	VCD perip.	0.0955	0.1252	-0.1248
RNFL nasalis	VCD total	0.3378*	0.0975	-0.0192
	VCD sup.	0.2301	0.2077	0.1023
	VCD inf.	0.2836	0.2855	-0.1036
	VCD ins.d	0.3158*	0.0848	-0.2049
	VCD perip.	0.2343	0.0934	0.1021

OCT – optical coherence tomography; OCTA – optical coherence tomography angiography; RNFL – retinal nerve fiber layer; VCD – vessel capillary density; VCD ins.d – vessel capillary density inside disc; VCD perip. – vessel capillary density peripapillary; NTG – normal tension glaucoma; POAG – primary open angle glaucoma;
* p < 0.05;
** p < 0.01

OCTA parameters of ONH and comparison between groups are presented in Table 3. VCD total is significantly higher in healthy compared to NTG and POAG patients (39.25 ± 0.94 *vs*. 36.79 ± 2.50 *vs*. 37.58 ± 1.53; p < 0.001). VCD perip. is significantly lower in NTG and POAG compared to healthy subjects (39.21 ± 2.62 *vs*. 40.18 ± 1.35 *vs*. 41.29 ± 0.81; p < 0.001).

OCTA parameters of ONH and comparison between NTG and POAG groups are shown in Table 4. VCD total is significantly lower in NTG compared to POAG (36.79 ± 2.50 *vs*. 37.58 ± 1.55; p < 0.001). VCD perip. is highly statistically significantly lower in NTG compared to POAG (39.21 ± 2.62 *vs*. 40.18 ± 1.35; p < 0.001).

Table 6. Spearman-R correlation OCT and OCTA parameters of the optic nerve head with mean deviation index visual field

OCT and OCTA parameters	Health (n = 40)	NTG (n = 30)	POAG (n = 50)
RNFL average	-0.132	0.370*	-0.124
RNFL superior	-0.089	0.320	-0.155
RNFL inferior	-0.215	0.301	-0.110
VCD total	-0.024	0.357	0.235
VCD superior	-0.040	0.155	0.122
VCD inferior	0.049	0.316	0.277*
VCD ins.d	-0.125	-0.013	0.395**
VCD perip.	0.016	0.295	-0.015
VCD perip.sup.	-0.036	0.103	-0.092
VCD perip.inf.	0.033	0.395*	0.044

OCT – optical coherence tomography; OCTA – optical coherence tomography angiography; RNFL – retinal nerve fiber layer; VCD – vessel capillary density; VCD ins.d – vessel capillary density inside disc; VCD perip. – vessel capillary density peripapillary; NTG – normal tension glaucoma; POAG – primary open angle glaucoma;
* p < 0.05;
** p < 0.01

Pearson's correlation coefficient between RNFL thickness and OCTA parameters of ONH are presented in Table 5. We obtained a positive correlation between RNFL average and RNFL inf. with VCD sup. (p < 0.05) and RNFL nasalis with VCD total and VCD ins.d (p < 0.05) in healthy subjects. We found significantly positive correlation in NTG between RNFL temp. and VCD inf. (r = 0.372, p < 0.05). In POAG, we obtained a significant negative correlation between RNFL average and VCD ins.d (r = - 0.347, p < 0.05) and RNFL inf. with VCD ins.d (r = - 0.388, p < 0.01).

Spearman R correlation OCT and OCTA parameters ONH with MD index VF are shown in Table 6. We found significantly positive correlation between OCT and OCTA parameters of ONH with MD index in NTG among RNFL average and MD (r = 0.370, p < 0.05) and among VCD perip.inf. and MD (r = 0.375, p < 0.05). In POAG we obtained a significantly positive correlation between VCD inf. and MD (r = 0.277, p < 0.05) as well as VCD ins.d and MD (r = 0.395, p < 0.01).

DISCUSSION

OCTA provide qualitative and quantitative analysis of VCD of ONH and peripapillary region in glaucoma patients [6].

During aging, in normal eyes there is a decrease in RNFL thickness, Ganglion Cell Comple thickness, VCD perip., and superficial macular vessel density. Changes that occur with aging should be considered during diagnosis and follow-up of glaucoma patients [10].

Bojikian et al. [11] analyzed 26 healthy, 30 POAG, and 31 NTG subjects. Healthy eyes showed significantly higher RNFL thickness average compared to POAG and NTG (p < 0.0001). Eyes with NTG also had significantly lower flux and vessel area density compared to normal eyes (p < 0.0001).

In our study, we found that VCD total is significantly higher in healthy compared to NTG and POAG patients (39.25 ± 0.94 *vs.* 36.79 ± 2.50 *vs.* 37.58 ± 1.53; p < 0.001). VCD perip. is significantly lower in NTG and POAG compared to healthy subjects (39.21 ± 2.62 *vs.* 40.18 ± 1.35 *vs.* 41.29 ± 0.81; p < 0.001). Lee et al. [12] shows the results of VCD perip. between patients with NTG and POAG compared to glaucoma suspect and control groups. Full VCD is significantly lower in NTG and POAG compared to control group (p < 0.001). They did not find statistically significant VCD between NTG and POAG.

Onishi et al. [13] compared VCD perip. in healthy eyes (49.12 ± 2.80) to POAG (37.63 ± 7.19), p < 0.001; but not statistically significant in NTG eyes (45.33 ± 7.66; p = 0.692). In this study, there was a significantly difference among VCD perip.in POAG and NTG eyes (p = 0.030). Scripsema et al. [14] reported global perfused capillary density in POAG, NTG, and healthy eyes (33.13 ± 6.23; 36.49 ± 3.18; 41.32 ± 1.96). A statistically significant difference between the groups was obtained (p < 0.01).

Chen et al. [15] compared VCD perip. in glaucoma to healthy eyes. In the present study, VCD perip. total was significantly lower in glaucoma eyes than in healthy eyes (43.8% ± 5.7% *vs.* 53.3% ± 3%; p < 0.001). Pearson's correlation coefficient showed that VCD perip. total (r = 0.74) and RNFL thickness (r = 0.65) had strong positive correlation with MD (p < 0.001).

In our study, there is a significantly positive correlation in NTG among RNFL average and MD (r = 0.370, p < 0.05) and among VCD perip. inf. and MD (r = 0.375, p < 0.05). In POAG patients there are a significantly positive correlation between VCD inf. and MD (r = 0.277, p < 0.05) as well as VCD ins.d and MD (r = 0.395, p < 0.01). Kim et al. [16] analyzed peripapillary perfusion in NTG (71 eyes) and control group (71 eyes). Average RNFL thickness were significantly lower in NTG (78.45 ± 12.66μm, p < 0.001) compared to control group (92.39 ± 9.40μm). MD index VF was significantly lower in NTG compared to control group (-4.84 ± 5.34dB *vs.* -0.51 ± 1.40dB; p < 0.001). VCD perip. average decreased significantly in NTG compared to control group (42.77 ± 2.67 *vs.* 45.15 ± 1.74; p < 0.001). The RNFL thickness average correlated positively with average perfusion density (r = 0.610; p < 0.001).

Petrović et al. [17] compared results of VCD and VCD perip. in healthy eyes and different stages of POAG. They discovered that all OCTA parameters are significantly higher in healthy eyes.

Ozturk et al. [18] compared VCD perip. in a control group with different stages of POAG (early, moderate, and severe stages). VCD perip. parameters had the highest diagnostic performance in all POAG patients. VCD perip. helps identify early-stage glaucoma.

Lin et al. [19] analyzed VCD perip. in NTG (74 eyes) and healthy eyes (24 eyes). They found that VCD total is statistically significantly lower in NTG (43.9 ± 6.14) than in healthy eyes (48.41 ± 3.03), p < 0.001. VCD perip. were significantly lower in NTG (45.46 ± 7.87) than in healthy eyes (50.73 ± 3.31), p < 0.002. RNFL thickness significant lower in NTG compared to healthy eyes (85.34 ± 15.22 *vs.* 99.75 ± 6.78; p < 0.001).

Belbase et al. [20] reported on OCTA vessel density in healthy, glaucoma-suspected and POAG eyes. They found significantly lower peripapillary VD in glaucoma compared to glaucoma suspected and healthy eyes (47.42 ± 7.73 *vs.* 52.62 ± 2.4 *vs.* 56.07 ± 2.71, p < 0.001). They obtained a significant positive correlation between peripapillary VD and MD of VF (R^2 = 0.32, p < 0.001).

Elsalhy et al. [21] analyzed glaucoma suspected patients and discovered significantly lower value of the mean RPC plexus compared to healthy individuals (46.6 ± 2 *vs.* 48.8 ± 1.7, p < 0.001).

The finding of VCD perip. reduction clearly separates healthy eyes from eyes with NTG and POAG, which is important for clinical practice [12, 18].

CONCLUSION

Glaucoma patients have a significantly lower vessel density ONH and VCD perip. compared to healthy subjects. VCD total of ONH is significantly lower in NTG compared to POAG. We obtained a positive correlation between OCT and OCTA parameters with MD in NTG and POAG. OCTA is an important imaging method in the diagnosis and monitoring of glaucoma progression.

Note: The paper is part of a doctoral dissertation.

Conflicts of interest: None declared.

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Перипапиларни капиларни проток код нормотензивног и примарног глаукома отвореног угла

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САЖЕТАК

Увод/Циљ Примарни глауком отвореног угла (ПГОУ) је хронична, прогресивна, оптичка неуропатија са могућим слепилом и иреверзибилним променама главе оптичког нерва (ГОН). Оптичка кохерентна томографија (ОКТ) је неинвазивна метода која пружа процену структурног оштећења код глаукома. ОКТ ангиографија (ОКТА) пружа квалитативну и квантитативну процену перипапиларне микроваскулатуре. Циљ ове студије је био да утврди да ли постоји разлика у капиларном протоку перипапиларне регије код нормотензивног глаукома (НТГ), ПГОУ и здравих особа.

Методе Овом проспективном студијом обухваћено је 120 очију: 40 здравих очију, 30 очију са НТГ и 50 очију са ПГОУ. Спроведени су офталмолошки преглед, мерење централне дебљине рожњаче, ОКТ, ОКТА и тестирање компјутеризованог видног поља.

Резултати Укупна густина капиларних крвних судова (ГККС) била је значајно већа код здравих у поређењу са НТГ и ПГОУ (39,25 ± 0,94; 36,79 ± 2,50; 37,58 ± 1,53; $p < 0,001$). Густина укупног перипапиларног капиларног плексуса (ПКП) значајно је мања код НТГ и ПГОУ у односу на здраве (39,21 ± 2,62; 40,18 ± 1,35; 41,29 ± 0,81; $p < 0,001$). Укупна ГККС ГОН је значајно мања код НТГ у односу на ПГОУ (36,79 ± 2,50; 37,58 ± 1,55; $p < 0,001$). Укупни ПКП је значајно мањи код НТГ у поређењу са ПГОУ (39,21 ± 2,62; 40,18 ± 1,35; $p < 0,001$). Значајну позитивну корелацију између ОКТ и ОКТА параметара ГОН са средњом девијацијом индекса видног поља (СДИВП) добили смо код НТГ између слоја нервних влакана ретине и СДИВП ($r = 0,370$, $p < 0,05$) и код перипапиларног капиларног плексуса у доњем сектору и СДИВП ($r = 0,395$, $p < 0,05$). Код ПГОУ значајну позитивну корелацију добили смо између ГККС у доњем сектору и СДИВП ($r = 0,277$, $p < 0,05$) и ГККС унутар диска са СДИВП ($r = 0,395$, $p < 0,01$).

Закључак Болесници са глаукомом имају значајно мању ГККС ГОН и ПКП у односу на здраве особе. Укупна ГККС ГОН значајно је мања код НТГ у односу на ПГОУ. Позитивну корелацију добили смо између ОКТ и ОКТА параметара са средњом девијацијом индекса видног поља код НТГ и ПГОУ.

Кључне речи: оптичка кохерентна томографија – ангиографија; глава оптичког нерва; интраокуларни притисак

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Optical coherence tomography angiography microvascular changes in the macular region in open-angle glaucoma

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SUMMARY

Introduction/Objective Glaucoma is the leading cause of irreversible blindness worldwide. It is hypothesized that glaucomatous microvascular changes in the superficial capillary plexus (SCP) may lead to early structural damage and correlate with visual field defects. The aim of this study was to quantitatively analyze microvascular characteristics of the SCP in the macular region of healthy eyes and in eyes with varying severity of open-angle glaucoma (OAG) using optical coherence tomography angiography (OCT-A).

Methods A total of 144 eyes were included in this cross-sectional study, including 109 eyes with confirmed OAG and 35 healthy eyes. Based on the Hodapp–Anderson–Parrish classification, patients were categorized into early, moderate, and severe stage glaucoma. All subjects underwent visual field, optical coherence tomography, and OCT-A examinations.

Results Significant reductions were observed in macular vessel density across glaucoma stages. Foveal vessel density decreased from 23 ± 1.9% in normal eyes to 13.9 ± 1.8% in severe glaucoma eyes. Foveal and parafoveal vessel densities were significantly reduced even in early-stage glaucoma. OCT parameters progressively decreased with glaucoma severity. Total ganglion cell complex thickness decreased from 108.5 ± 5.6 μm in healthy eyes to 61.2 ± 6.9 μm in severe glaucoma eyes. Total peripapillary retinal nerve fiber layer thickness decreased from 106.2 ± 7.9 μm in healthy eyes to 48.7 ± 7.5 μm in severe glaucoma.

Conclusion Our findings support the hypothesis that macular vessel density decreases progressively with glaucoma severity. These results reinforce the potential clinical utility of OCT-A in detecting early glaucoma and monitoring disease progression.

Keywords: glaucoma; vascular density; intraocular pressure; optical coherence tomography

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness worldwide. It is a multifactorial optic neuropathy characterized by progressive loss of retinal ganglion cells (RGCs) and their axons in both the macular and peripapillary regions, with characteristic structural changes in the optic nerve head (ONH) and corresponding functional visual field defects [1]. Among the many risk factors for glaucoma, intraocular pressure (IOP) is the only modifiable one. It is estimated that in 2020, approximately 76 million people were affected by glaucoma, with projections indicating a 74% increase by 2040 [2].

The primary goal of glaucoma treatment is to preserve patients' visual function and quality of life at an acceptable cost [1]. Lowering IOP is currently the only proven treatment to slow disease progression [3]. However, some patients continue to experience deterioration despite achieving target IOP, suggesting that optic nerve ischemia and reduced ocular blood flow contribute to glaucoma pathogenesis and progression [4, 5].

Optical coherence tomography angiography (OCT-A) is a novel, non-invasive imaging modality that provides detailed visualization of the retinochoroidal microvasculature, including blood flow around the ONH and macula, without the need for contrast dyes [6]. Because RGCs are predominantly supplied by the superficial macular vascular complex, it is hypothesized that glaucomatous microvascular changes in this layer may lead to early structural damage and correlate with visual field defects [7, 8]. Therefore, the macular region is considered a strategic location for detecting and monitoring glaucoma progression.

The aim of this study was to quantitatively analyze microvascular characteristics of the superficial capillary plexus (SCP) in the macular region of healthy eyes and in eyes with varying severity of open-angle glaucoma (OAG). Additionally, we sought to investigate correlations between macular vessel density and traditional structural and functional parameters of glaucoma.

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METHODS

Study design

Subjects

A total of 109 eyes from patients aged ≥ 40 years with confirmed OAG in at least one eye were included. The control group comprised 35 healthy eyes from individuals over 40 years of age.

All participants underwent a comprehensive ophthalmic examination, including: best-corrected visual acuity (BCVA) using the Snellen chart, slit-lamp biomicroscopy, IOP measurement with Goldmann applanation tonometry, central corneal thickness (CCT) measurement (Topcon Aladdin Optical Biometer, Oakland, CA, USA), gonioscopy using a Goldmann contact lens, dilated fundus examination, standard automated perimetry using Octopus 600 (Haag-Streit, Mason, OH, USA), OCT imaging of the ONH and macula, including retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) thickness measurements, and macular vessel density assessment via OCT-A (Topcon Maestro 2, IMAGEnet6 software, Oakland, CA, USA).

Inclusion criteria

The inclusion criteria were age ≥ 40 years, a confirmed diagnosis of OAG for at least one year, and an open anterior chamber angle on gonioscopy.

The control group included eyes with IOP ≤ 21 mmHg, no history of elevated IOP, normal optic nerve appearance with intact neuroretinal rims and RNFL, and a normal visual field with no defects.

Exclusion criteria

Exclusion criteria for both healthy and glaucoma groups included: BCVA < 0.2, spherical refraction ≥ 5 D or myopia > 4 D, pregnancy or breastfeeding, other types of glaucoma (angle-closure), age-related macular degeneration or other inherited/acquired macular diseases, uveitis, diabetic or hypertensive retinopathy/maculopathy, corneal diseases, history of ocular trauma or previous ocular surgeries (except cataract and glaucoma surgery), non-glaucomatous optic neuropathy, use of medications affecting retinal function (tamoxifen, antimalarials, phenothiazines, canthaxanthin, methoxyflurane), neurological disorders (Alzheimer's, Parkinson's, dementia, stroke).

Glaucoma classification

Based on the Hodapp–Anderson–Parrish classification, patients were categorized as follows:

- Early-stage glaucoma: Mean deviation (MD) > -6 dB;
- Moderate-stage glaucoma: MD between -6 and -12 dB;
- Severe-stage glaucoma: MD < -12 dB;
- Control group: Healthy eyes.

OCT angiography and structural measurements

OCT-A Measurements

OCT-A scans were performed using the Topcon Maestro 2 (IMAGEnet6 software, Oakland, CA, USA). This device uses an 840 nm light source with an A-scan rate of 50,000 scans per second, providing high-resolution 3D visualization of retinal vasculature.

Superficial vessel density (SVD) was automatically calculated as the percentage of the scanned area occupied by blood vessels. Measurements focused on the SCP, spanning from the internal limiting membrane (ILM) to the inner plexiform layer (IPL).

A 3×3 mm^2 field centered on the fovea was analyzed, with density assessed in the following:

- Foveal zone (1 mm diameter);
- Parafoveal zones (1–3 mm): superior, inferior, nasal, and temporal sectors.

OCT and OCT-A image quality review was completed according to the Imaging Data Evaluation and Analysis Reading Center protocol on all scans using standard Topcon Maestro 2 software (IMAGEnet6 software). Poor-quality scans were excluded from the study and analysis if one of the following criteria was met: signal strength index < 45, poor clarity, residual motion artifacts, image cropping or local weak signal due to vitreous floaters, segmentation errors, or images with off-center fovea.

OCT structural measurements

All participants underwent ONH and macular imaging, with measurements including:

- ONH: Disc area, rim area, cup-to-disc (C/D) ratios, and RNFL thickness
- GCC: Thickness from ILM to inner nuclear layer, including nerve fiber, ganglion cell, and IPL layers

Statistical analysis

Results are presented as mean ± standard deviation, median (25th–75th) and number (%). Graphical and mathematical methods were used to examine data distribution. The groups were compared using the χ^2 test. Data were analyzed using one-way ANOVA or the Kruskal–Wallis test when three groups were compared. For significant one-way ANOVA results, Tukey post hoc analysis was used. Afterward, for the Kruskal–Wallis test, the Mann–Whitney test was used as a post hoc test. All $p < 0.05$ values were considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics, Version 26 (IBM Corp., Armonk, NY, USA).

Ethics: This cross-sectional study was approved by the Research Ethics Committee of the Belgrade Ophthalmology Center Special Eye Hospital on November 9, 2022 (approval number: 3/30/2022). The study was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

Table 1. Demographic and clinical characteristics of normal, early, moderate, and severe open-angle glaucoma eyes

Parameter	Normal n = 35	Early n = 44	Moderate n = 34	Severe n = 31	p
Age (y)	55.8 ± 8.8	61 ± 10.4	64.4 ± 12.1	67.1 ± 10.7	< 0.001
Sex (female/male)	20 (57.1%) / 15 (42.9%)	27 (61.4%) / 17 (38.6%)	19 (55.9%) / 15 (44.1%)	7 (22.6%) / 24 (77.4%)	0.005
BCVA (Snellen)	0.9 ± 0.1	0.9 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.003
CCT (μm)	562.9 ± 17.7	552.7 ± 31.1	546.6 ± 24.4	541.9 ± 26	0.007
Linear C/D ratio	0.4 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0	< 0.001
Vertical C/D ratio	0.4 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1 ± 0	< 0.001
IOP (mmHg)	16.4 ± 2.2	17.1 ± 2.8	16.5 ± 2.7	16.5 ± 3.2	0.68
MD visual field (dB)	0.5 ± 0.6	-2.5 ± 1.8	-8.8 ± 1.9	-18.4 ± 3.6	< 0.001

BCVA – best corrected visual acuity; CCT – central corneal thickness; IOP – intraocular pressure; MD – mean deviation

Table 2. Optical coherence tomography structural characteristics of normal, early, moderate, and severe open-angle glaucoma eyes

Parameter	Normal n = 35	Early n = 44	Moderate n = 34	Severe n = 31	p
Total GCC thickness (μm)	108.5 ± 5.6	89.6 ± 13.6	77.6 ± 6.6	61.2 ± 6.9	< 0.001
Superior GCC thickness (μm)	107.1 ± 4.9	90.9 ± 12.8	79.2 ± 11.4	62.5 ± 7.5	< 0.001
Inferior GCC thickness (μm)	110 ± 6.9	88 ± 16.3	76.1 ± 9.9	60.6 ± 6.5	< 0.001
Total pRNFL thickness (μm)	106.2 ± 7.9	84 ± 16.4	63.1 ± 10.9	48.7 ± 7.5	< 0.001
Superior pRNFL thickness (μm)	126.1 ± 13.2	98.1 ± 22.2	71.1 ± 19.8	55.4 ± 12.7	< 0.001
Inferior pRNFL thickness (μm)	139 ± 13.6	100.8 ± 26.1	65.9 ± 13.1	50.9 ± 6.6	< 0.001
Average Macular thickness (μm)	279.7 ± 11.1	266.1 ± 15.4	255.3 ± 14.1	237.3 ± 14.7	< 0.001

GCC – ganglion cell complex; pRNFL – peripapillary retinal nerve fiber layer

RESULTS

A total of 35 healthy eyes, 44 early-stage glaucoma eyes, 34 moderate-stage glaucoma eyes, and 31 severe-stage glaucoma eyes met the inclusion criteria.

Demographic and clinical characteristics of normal, early, moderate, and severe OAG eyes are presented in Table 1. The mean age increased progressively from normal eyes (55.8 years) to severe glaucoma eyes (67.1 years). The proportion of males increased significantly with disease severity ($p = 0.005$), with the severe glaucoma group being predominantly male (77.4%). BCVA declined with increasing severity of glaucoma ($p = 0.003$). CCT decreased with increasing glaucoma severity ($p = 0.007$). The linear C/D ratio increased with disease severity ($p < 0.001$). Post hoc tests showed significant differences between all severity levels ($p < 0.001$). Similar to the linear C/D ratio, the vertical C/D ratio significantly increased with severity ($p < 0.001$), with all pairwise comparisons being statistically significant ($p < 0.001$). No statistically significant difference in IOP was observed between the groups ($p = 0.68$). MD worsened with increasing glaucoma severity ($p < 0.001$). Post hoc tests showed significant differences between all groups ($p < 0.001$), confirming progressive visual field loss across stages.

The data on structural characteristics of normal, early, moderate, and severe OAG eyes are presented in Table 2. Total GCC thickness progressively decreased with increasing glaucoma severity, from 108.5 ± 5.6 μm in healthy eyes to 61.2 ± 6.9 μm in severe glaucoma eyes ($p < 0.001$). Tukey HSD tests revealed significant differences between normal and moderate glaucoma (mean difference: –30.9 μm, $p < 0.001$) and between normal and severe glaucoma (mean difference: –47.3 μm, $p < 0.001$). Both superior and inferior GCC thicknesses decreased significantly across all stages of glaucoma ($p < 0.001$). Pairwise comparisons showed significant differences between normal and early glaucoma, as well as between early and moderate glaucoma (all $p < 0.001$).

Regarding peripapillary RNFL (pRNFL) thickness, a progressive reduction was observed with increasing disease severity ($p < 0.001$). Total pRNFL thickness decreased from 106.2 ± 7.9 μm in healthy eyes to 48.7 ± 7.5 μm in severe glaucoma eyes. Pairwise comparisons showed significant differences between normal and early ($p < 0.001$), early and moderate ($p < 0.001$), and moderate and severe glaucoma groups ($p < 0.001$). Similar trends were observed for superior and inferior pRNFL thickness ($p < 0.001$). The average macular thickness decreased significantly with increasing glaucoma severity ($p < 0.001$), from 279.7 ± 11.1 μm in healthy eyes to 237.3 ± 14.7 μm in severe glaucoma.

The data on superficial macular vessel density in different retinal regions are presented in Table 3 and Figure 1. Foveal vessel density progressively decreased from 23 ± 1.9% in healthy eyes to 13.9 ± 1.8% in severe glaucoma ($p < 0.001$). Post hoc analysis showed significant reductions between normal and early glaucoma (mean difference: 4.89%, $p < 0.001$), normal and moderate glaucoma (6.81%, $p < 0.001$), and normal and severe glaucoma (9.13%, $p < 0.001$). Differences between early and moderate glaucoma (1.92%, $p < 0.001$) and early and severe glaucoma (4.24%, $p < 0.001$) were also statistically significant.

Parafoveal vessel density in the superior sector declined significantly from 44.3 ± 2.5% in healthy eyes to 37.2 ± 3.5% in severe glaucoma ($p < 0.001$). Parafoveal vessel density in the inferior sector exhibited a similar pattern, decreasing from 45.4 ± 3.2% in healthy eyes to 36.7 ± 2.5% in severe glaucoma ($p < 0.001$). Parafoveal

Table 3. Superficial macular vessel density measurements in normal, early, moderate, and severe open-angle glaucoma eyes

Parameter	Normal	Early	Moderate	Severe	p
Foveal VD (%)	23 ± 1.9	18.1 ± 1.6	16.2 ± 2.2	13.9 ± 1.8	< 0.001
Parafoveal VD Superior (%)	44.3 ± 2.5	40.7 ± 4.2	39.6 ± 3.7	37.2 ± 3.5	< 0.001
Parafoveal VD Inferior (%)	45.4 ± 3.2	40.3 ± 5.3	37.8 ± 4.3	36.7 ± 2.5	< 0.001
Parafoveal VD Temporal (%)	44.8 ± 2.8	44 ± 4.6	42.4 ± 4.5	39 ± 2.9	< 0.001
Parafoveal VD Nasal (%)	45.1 ± 2.8	43.2 ± 3.9	42 ± 4.5	41.3 ± 3.1	< 0.001

VD – vessel density

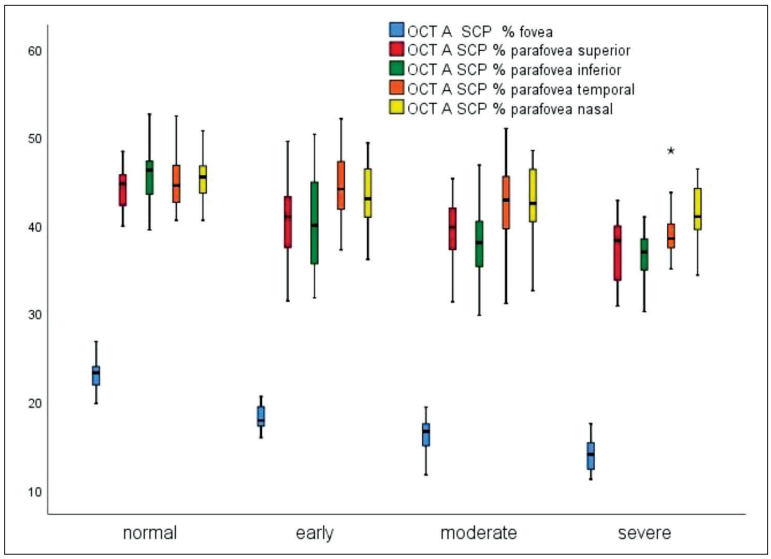

Figure 1. Superficial macular vessel density measurements in normal, early, moderate, and severe open-angle glaucoma eyes;
SCP – superficial capillary plexus

vessel density in the temporal sector was significantly lower in glaucoma eyes, decreasing from 44.8 ± 2.8% in normal eyes to 39 ± 2.9% in severe glaucoma (p < 0.001). Post hoc comparisons revealed a significant difference between normal and severe glaucoma (5.75%, p < 0.001), while differences between early and moderate glaucoma were not statistically significant. Parafoveal vessel density in the nasal sector also declined with disease progression, from 45.1 ± 2.8% in healthy eyes to 41.3 ± 3.1% in severe glaucoma (p < 0.001). Post hoc analysis between normal and early glaucoma showed significant differences for all parameters except for temporal and nasal parafoveal vessel density.

The correlations between OCT-A vessel density measurements and structural parameters in the glaucoma group are presented in Table 4. A strong positive correlation was observed between total GCC thickness and macular vessel density measurements in all parafoveal sectors, with the highest correlation found in the foveal region (r = 0.602, p < 0.001) followed by the temporal sector (r = 0.557, p < 0.001). Significant correlations were also noted in the superior, inferior, and nasal parafoveal regions (p < 0.001; p < 0.001; p = 0.025). Conversely, linear and vertical C/D ratios exhibited strong negative correlations with vessel density parameters. The strongest negative correlation for the linear C/D ratio was in the foveal region (r = -0.607, p < 0.001), and for the vertical C/D ratio, the most pronounced correlation was also in the foveal region (r = -0.579, p < 0.001). Total pRNFL thickness was positively correlated with vessel density, with the strongest correlation observed in the foveal region (r = 0.536, p < 0.001).

Table 4. Correlation between optical coherence tomography angiography (OCT-A) vessel density measurements and structural parameters in glaucoma group

N = 109		OCT-A SCP fovea	OCT-A SCP parafovea superior	OCT-A SCP parafovea inferior	OCT-A SCP parafovea temporal	OCT-A SCP parafovea nasal
GCC thickness total µm	R	0.602**	0.457**	0.435**	0.557**	0.214*
	P	< 0.001	< 0.001	< 0.001	< 0.001	0.025
linear c/d	R	-0.607**	-0.415**	-0.351**	-0.471**	-0.243*
	P	< 0.001	< 0.001	< 0.001	< 0.001	0.011
vertical c/d	R	-0.579**	-0.347**	-0.330**	-0.424**	-0.224*
	P	< 0.001	< 0.001	< 0.001	< 0.001	0.019
pRNFL thickness total µm	R	0.536**	0.326**	0.329**	0.448**	0.157
	P	< 0.001	0.001	< 0.001	< 0.001	0.102

SCP – superficial capillary plexus; GCC – ganglion cell complex;
pRNFL – peripapillary retinal nerve fiber layer;
*p < 0.05;
**p < 0.001

DISCUSSION

Both the mechanical and vascular theories are considered to play a crucial role in the pathogenesis and progression of glaucoma. While mechanical factors, such as increased IOP and ONH deformation, have been extensively studied, the vascular hypothesis remains an area of growing interest. The vascular influence, particularly in the macular region, warrants further investigation, as this area is frequently affected in glaucoma [9]. The irreversible loss of RGCs is believed to be caused, at least in part, by insufficient blood supply. Given their high metabolic demand, RGCs are heavily dependent on the SCP for perfusion. Their unequal distribution within the retina, with the highest concentration in the macular region, suggests that macular vessel density changes may play a role in glaucomatous disease progression [10].

To further explore these vascular changes, we measured and compared macular SVD across different stages of OAG using the Topcon Maestro 2 OCT and OCT-A device. We also assessed the correlations between capillary vessel density and structural parameters.

Our study confirmed that total GCC thickness progressively decreased with increasing glaucoma severity, consistent with findings from the study by Srivastava et al. [11]. Both superior and inferior GCC thickness decreased significantly across glaucoma stages, which aligns with findings from Soares et al.

[12]. Moreover, pairwise comparisons confirmed significant differences between normal and early glaucoma, supporting results from Hassanen et al. [13].

These findings reinforce previous reports suggesting that glaucomatous damage primarily affects the inner retinal layers, leading to the thinning of these structures.

Regarding the pRNFL thickness, we found a significant reduction in all glaucoma stages. Similar trends were observed for superior and inferior RNFL thickness, aligning with findings from previous studies [14]. However, in contrast to our results, Bhat et al. [15] reported that inferior pRNFL thinning was the most sensitive marker for glaucoma detection across all disease stages.

Our results, in line with previous research, showed that macular SVD was significantly reduced in glaucoma eyes compared with healthy controls [16]. Specifically, foveal vessel density progressively decreased with glaucoma severity, consistent with the study conducted by Hwang et al. [17].

Parafoveal vessel density declined significantly across all disease stages, with notable reductions in superior, inferior, temporal, and nasal sectors. The nasal parafoveal vessel density also declined with disease progression, although post hoc analysis showed that differences between normal and early glaucoma were not significant for temporal and nasal parafoveal vessel density. This could be explained by the fact that these regions have higher vessel density in healthy eyes [18].

Kurysheva et al. [19] found that SVD was more sensitive than GCC thickness in distinguishing early glaucoma from healthy eyes, supporting the idea that vascular changes may precede structural thinning in some cases.

To further explore the relationship between vascular and structural changes, we analyzed correlations between macular vessel density and retinal structural parameters. Our results showed a strong positive correlation between total GCC thickness and macular vessel density across all parafoveal sectors, with the strongest correlation in the foveal region, followed by the temporal sector. However, it remains unclear whether RGC loss is primarily driven by vascular insufficiency or whether vessel density reductions are a secondary consequence of structural damage.

Conversely, linear and vertical C/D ratios exhibited strong negative correlations with vessel density parameters, indicating that larger C/D ratios are associated with reduced vessel density. Total pRNFL thickness was positively correlated with vessel density, with the strongest correlation in the foveal region.

This study has several limitations that should be acknowledged. First, all patients were using different antiglaucoma medications, which could potentially influence ocular blood flow and retinochoroidal vessel measurements. Second, we used a 3×3 mm^2 scan instead of a 6×6 mm^2 scan, which might have led to different conclusions. Considering that RGCs are more concentrated in the perifoveal rather than the foveal and parafoveal regions, a larger scan size may yield different results. Finally, this study does not provide longitudinal data on structural and functional changes over time. A longitudinal study would offer a more robust evaluation of the relationship between macular microvascular perfusion and glaucomatous optic neuropathy progression.

CONCLUSION

Our findings support the hypothesis that macular vessel density decreases progressively with glaucoma severity and that vascular and structural parameters are closely correlated. These results reinforce the potential clinical utility of OCT-A in detecting early glaucoma and monitoring disease progression. Future studies should aim to clarify the causal relationship between vascular insufficiency and RGC loss and further explore the longitudinal changes in macular microvascular perfusion in glaucoma.

Conflict of interest: None declared.

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Микроваскуларне промене у жутој мрљи мерене оптичком кохерентном томографијом са ангиографијом код болесника са глаукомом отвореног угла

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САЖЕТАК

Увод/Циљ Глауком је водећи узрок ireверзибилног слепила у свету. Микроваскуларне глаукоматозне промене на нивоу површног капиларног плексуса у жутој мрљи могу да доведу до структуралних промена које корелирају са променама у видном пољу. Циљ ове студије је да се квантитативно анализира површни васкуларни плексус жуте мрље код здравих очију и очију са различитим стадијумима глаукома отвореног угла, употребом оптичке кохерентне томографије са ангиографијом.

Методе У студију су укључена 144 ока – 109 са дијагнозом глаукома отвореног угла и 35 здравих очију. Према Ходап–Андерсон–Паришовој класификацији учињена је даља подела на рани, средњи и узнапредовали стадијум глаукома. Свим болесницима је урађено испитивање видног поља, оптичка кохерентна томографија и оптичка кохерентна томографија са ангиографијом.

Резултати Уочена је статистички значајна редукција густине крвних судова жуте мрље у свим стадијумима глаукома. Фовеална густина крвних судова је редукована са 23 ± 1,9% код здравих очију на 13,9 ± 1,8% код узнапредовалог стадијума глаукома. Фовеална и парафовеална густина крвних судова значајно је редукована и у раном стадијуму болести. Параметри оптичке кохерентне томографије прогресивно су се смањивали са тежином обољења. Укупна вредност дебљине ганглијског ћелијског комплекса редукована је са 108,5 ± 5,6 *μm* код здравих очију на 61,2 ± 6,9 *μm* у узнапредовалом глаукому. Укупна дебљина перипапиларних нервних влакана ретине је смањена са 106,2 ± 7,9 *μm* код здравих на 48,7 ± 7,5 *μm* у узнапредовалом стадијуму глаукома.

Закључак Густина крвних судова жуте мрље прогресивно се смањује са напредовањем болести. Ови резултати подстичу потенцијалну употребу оптичке кохерентне томографије са ангиографијом у клиничкој пракси за детектовање раног стадијума глаукома, као и за праћење његове прогресије.

Кључне речи: глауком; жута мрља; интраокуларни притисак; густина крвних судова

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Analysis of the most common reasons for voluntary blood donor deferral in Southeast Serbia

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SUMMARY
Introduction/Objective Criteria for the selection of blood donors (BDs) and criteria for permanent or temporary postponement of blood donation have a vital role in the safety of blood transfusion. It is a well-known fact that the BDs deferral has a negative effect on the return of both first and regular donors. The aim of the study was to analyze the frequency of deferral and the reasons for deferral of voluntary BDs with an emphasis on temporary deferrals due to low hemoglobin (Hb) levels, in order to identify temporarily deferred donors and advise them properly in order to increase the number of voluntary donors.
Methods The study included voluntary BDs from January 1 to December 31, 2023. A total of 2322 rejected voluntary BDs were included in the study. Donors of both sexes, aged between 18 and 65 years, were included.
Results The overall prevalence of deferred donors was 4.85%. The most common reason for temporary deferral was low Hb level, identified in 1002 BDs (43.15%). Other reasons included low blood pressure (265 donors, 11.41%), medication use (172 donors, 7.41%), recent blood donation (133 donors, 5.73%), high blood pressure (99 donors, 4.26%), thyroid dysfunction (96 donors, 4.13%), and recent surgical intervention (81 donors, 3.49%).
Conclusion The results of this study suggest that implementing parameters that more accurately reflect iron status would help ensure donor safety. To protect the health of BDs and maintain an adequate blood supply, appropriate procedures should be applied, which may include temporary iron supplementation.
Keywords: blood donors; selection criteria; deferral causes; hemoglobin

INTRODUCTION

Transfusion of blood and blood components, as one of the forms of tissue transplantation, is an intervention that saves lives [1].

According to the Law on Transfusion Medicine (Official Gazette of RS No./40/2017), the preparation of blood and blood components includes the activity of promotion, planning, collection and testing, processing and distribution of blood and blood components and is performed in authorized transfusion institutions. Blood donation is a set of activities aimed at motivating, informing, educating, inviting, and gathering blood donors (BDs), with the aim of providing adequate amounts of safe blood. Donating blood is a humane gesture based on voluntariness, gratuity, and anonymity [2]. This definition is in accordance with the recommendations of the World Health Organization related to donation [3].

The Council of Europe supports this approach by promoting and recommending the principle of self-sufficiency based on voluntary, non-remunerated donations [4]. Voluntary blood donation is primarily a privilege, as only healthy individuals can serve as the source of blood – an irreplaceable therapeutic resource that saves lives and improves the quality of life for many patients [5].

The main goal of the transfusion service is to maintain the quality of work, in all segments related to work with donors, to retain regular BDs, to recruit new ones and to persuade those who have stopped donating blood to come again.

Selection of BDs is carried out according to established, exclusively medical criteria for temporary postponement of blood donation or permanent refusal due to ineligibility for blood donation, whether blood donation could endanger the potential donor or recipient. Donor selection is conducted without regard to sex, religion, sexual orientation, political affiliation, profession, social status, or any other comparable factor [5].

Prior to each blood donation, BD goes through the same procedure: filling out the questionnaire for BDs, determining the hemoglobin (Hb) concentration, determining the blood group on the plate (at the first blood donation) and a medical examination. The medical examination consists of an analysis of the answers given by BD in the questionnaire for BDs and, accordingly, a well-taken medical history and physical examination. Following the physical examination, the physician determines the eligibility of the BD in accordance with the established criteria for donor selection and the criteria for permanent and temporary deferral from blood donation. General recommendations include: body weight > 50 kilograms, body temperature up to 37°C, blood pressure not lower than 100/60 mmHg (24/13.3 kPa),

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pulse between 50 and 100 beats per minute, Hb values must be above 135 g/L for men and above 125 g/L for women, the auscultatory findings over the heart and lungs must be normal, the pharynx calm, the lymph glands of the neck and armpits, liver and spleen must not be palpated. In our country, men can donate blood every three months (12 weeks), and women every four months (16 weeks) [4].

About 118,54 million blood donations are collected worldwide [6]. Generally 250,000 blood donations are collected each year in Serbia (Blood Transfusion Institute of Serbia, unpublished data, 2018). To maintain a stable and sufficient blood supply, Serbia's blood transfusion system requires approximately 40 BDs per 1000 inhabitants, representing about 4% of the total population [7]. Approximately 3% is the actual percentage of BDs in the entire population [7] and to guarantee a constant supply donated blood in Serbia arise from voluntary BDs.

The demand for blood and blood products evolves due to different factors. As a consequence of improvements in medicine, rise in the number of transplantations, and aging of the population the demand for blood and blood products is increasing [8].

The aim of the study was to analyze the deferral incidence and reasons for BD deferral with emphasis on temporary deferrals due to low Hb levels, in order to identify temporary deferred donors and properly advise them with the intention of increasing the number of voluntary BDs without compromising the quality of blood and the safety of donors and recipients.

Our further intention was to develop strategies based on the study results to reduce the rate of deferral of BDs and improve the rate of return of rejected donors.

METHODS

The study included BDs from January 1, 2023 to December 31, 2023. A total of 2322 deferred voluntary BDs, were included in the study. BDs of both sexes, aged between 18 and 65 years, were included.

All voluntary BDs included in the study signed the approval. After completing the BD questionnaire form all BDs were screened. This form included the basic profiles of the donors (name, age, sex, address, etc.), medication intake, medical history, tests or treatment, jaundice, high-risk behavior, and any other clinically relevant illness.

Pre-donation screening involved a medical history questionnaire, followed by a physical examination and Hb measurement.

During the research period, a validated quantitative method was used to determine Hb concentration from capillary blood on the Hemo Control analyzer (EKF Diagnostics, Cardiff, United Kingdom), as described in the literature [9]. The devices were calibrated and regularly maintained according to the manufacturer's instructions.

Data were collected from the register of deferred BDs with reference to age and sex. All data are presented in the form of table.

Statistical analysis

Categorical variables were summarized as absolute numbers and percentages. The overall deferral rate was calculated as the proportion of deferred donors among all registered donors. Associations between donor characteristics (sex, age group, and donation status) and deferral status were evaluated using the χ^2 test of independence. For comparisons between two groups (e.g., male *vs*. female donors; first-time *vs*. repeat donors), 2×2 contingency tables were constructed. For comparisons across multiple age groups, a 2×5 contingency table was used. Relative risks (RR) with 95% confidence intervals (CI) were calculated to estimate the strength of associations. All statistical tests were two-sided, and a $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, NY, USA).

Ethics: This retrospective cross-sectional study was conducted at the Blood Transfusion Institute of Niš, Serbia, after obtaining approval from the Ethics Committee of the Blood Transfusion Institute of Niš, Niš, Serbia (No. 1005, from March 3, 2024).

RESULTS

A total of 47,862 potential BDs were screened during the study period, 39,264 males (82.04%) and 8598 females (17.96%). In this period, 45,540 voluntary BDs donated blood, 38,181 male (83.84%) and 7359 females (16.16%), and 2322 BDs donors were deferred (deferral rate – 4.85%). A total of 1239 females (53.36%) and 1083 males (46.64%) were deferred during this period (Table 1).

Table 1. Donor characteristics and deferral status

Characteristic	Total donors (n = 47,862)	Deferred n (%)	Deferral rate (%)
Sex			
Male	39,264	1083 (46.64%)*	2.76
Female	8598	1239 (53.36)*	14.41
Total	47,862	2322 (100)	4.85

*Percentage represents proportion of total deferrals.
Statistical test: $\chi^2(1) = 2022.4$, $p < 0.001$
Relative risk (female *vs*. male): RR = 5.22 (95% CI 4.87–5.60)

The deferral rate was significantly higher among female donors compared with male donors (14.41% *vs*. 2.76%). A χ^2 test demonstrated a significant association between sex and deferral status ($\chi^2(1) = 2022.4$, $p < 0.001$). Female donors had a 5.22-times higher risk of deferral compared with male donors (RR = 5.22, 95% CI 4.87–5.60) (Table 1).

Low Hb was the most frequent cause of deferral, accounting for 1002 cases (43.15% of all deferrals). It was more common among female donors (737 cases; 59.48% of female deferrals) compared with male donors (265 cases; 24.47% of male deferrals). Other causes of deferral included low blood pressure (11.41%), medication use (7.41%), recent donation (5.73%), permanent deferral (4.05%), and other causes (Table 2).

Table 2. Causes of donor deferrals and their proportions

Causes	No. deferred (%)	No. deffered males (%)	No. deffered females (%)
Low hemoglobin	1002 (43.15%)	265 (24.47%)	737 (59.48%)
High hemoglobin	4 (0.17%)	4 (0.37%)	0 (0%)
Low blood pressure	265(11.41%)	98 (9.05%)	167 (13.48%)
High blood pressure	99 (4.26%)	89 (8.22%)	10 (0.81%)
Underweight	13 (0.56%)	2 (0.18%)	11 (0.89%)
Medication	172 (7.41%)	126 (11.63%)	46 (3.71%)
Thyroid disorders	96 (4.13%)	20 (1.85%)	76 (6.13%)
Asthma	33 (1.42%)	25 (2.31%)	8 (0.65%)
Epilepsy	17 (0.73%)	6 (0.56%)	11 (0.89%)
Common cold	65 (2.80%)	48 (4.43%)	17 (1.37%)
Menstruation	46 (1.99%)	0 (0%)	46 (3.71%)
Recent surgery	81 (3.49%)	66 (6.09%)	15 (1.21%)
Recent donation	133 (5.73%)	108 (9.97%)	25 (2.02%)
Recent alcohol intake	5 (0.22%)	5 (0.46%)	0 (0%)
Recent tattoo/piercing	34 (1.46%)	25 (2.31%)	9 (0.73%)
High risk behavior	14 (0.60%)	14 (1.29%)	0 (0%)
Tick bite	45 (1.94%)	40 (3.69%)	5 (0.40%)
Vaccines	6 (0.26%)	4 (0.37%)	2 (0.16%)
Trauma	4 (0.17%)	4 (0.37%)	0 (0%)
Skin lesions	28 (1.21%)	19 (1.75%)	9 (0.73%)
Sick leave	41 (1.77%)	31 (2.86%)	10 (0.81%)
Giving up before donation	11 (0.47%)	6 (0.56%)	5 (0.40%)
Permanent deferral	94 (4.05%)	68 (6.28%)	26 (2.10%)
Others	14 (0.60%)	10 (0.93%)	4 (0.32%)
Total	2322	1083	1239

Overall, 1002 donors (2.09% of all screened donors) were deferred due to low Hb. The incidence of low Hb deferral was higher among first-time donors compared with repeat donors (2.55% *vs*. 1.95%). This difference was statistically significant ($\chi^2(1) = 14.2$, $p < 0.001$). First-time donors had a 31% higher risk of low Hb deferral compared with repeat donors (RR = 1.31, 95% CI 1.15–1.48) (Table 3).

Table 3. Low hemoglobin deferral by donation status

Donation status	Total donors	Low hemoglobin (n)	Incidence (%)
First-time	11,634	297	2.55
Repeat	36,228	705	1.95
Total	47,862	1002	2.09

Statistical test: $\chi^2(1) = 14.2$, $p < 0.001$
Relative risk (first-time vs. repeat): RR = 1.31 (95% CI 1.15–1.48)

Table 4. Deferral rate by age group

Age group (years)	Total screened	Deferred	Deferral rate (%)
18–25	7519	664	8.83
26–35	11,474	446	3.89
36–45	12,377	464	3.75
46–55	10,581	398	3.76
56–65	5911	350	5.92
Total	47,862	2322	4.85

Statistical test: $\chi^2(4) = 496.2$, $p < 0.001$

Deferral rates differed significantly across age groups ($\chi^2(4) = 496.2$, $p < 0.001$). The highest deferral rate was observed among donors aged 18–25 years (8.83%), followed by donors aged 56–65 years (5.92%). Donors aged 26–55 years demonstrated lower and comparable deferral rates ranging from 3.75% to 3.89% (Table 4).

DISCUSSION

The assessment of the suitability of potential BDs depends on the results of Hb measurement, medical history, and physical examination, all of which are mandatory before each blood donation [4].

BD selection and deferral criteria are essential components of transfusion safety and are intended to safeguard the health of both donors and recipients. It is widely known than BD deferral has adverse effect on the return of both, first and repeat donors [10]. Therefore, it is necessary to understand the reasons for BD deferral and to spread productive strategies to maintain those already motivated but temporarily deferred voluntary BDs.

The rate and reasons for deferral differ from region to region and from one center to another. In our study, 2322 out of total 47,862 registered prospective BDs were found unfit to donate due to various reasons. Most of the deferred BDs were females 1239 (53.36%), with men constituting 46.64% (1083) of the donors. The prevalence of deferral in our institute was 4.85%. An international comparison reveals that the deferral rate in this study (4.85%) is relatively lower than the rates reported in Germany (6.2%), France (10.8%), and the United States of America (USA) (12.8%) [11, 12, 13].

Various researches have reported a similar deferral rate, (5.2%) by Nhachigule et al. [14], and (7.2%) by Minj et al. [15]. Some studies have even had a higher deferral rate of (24.2%) like study by Oyedeji et al. [16]. As deferral rates vary, the main reasons for refusing to donate blood also vary. This variation in delay can be caused by many reasons such as geographical variation in health problems, socioeconomic status, different donor selection criteria, sex variation, etc.

The two types of donor deferrals include temporary, in which the donor is deferred for a specific period, defined according to the reason; and permanent, in which the donor is indefinitely deferred from donating blood [17].

We found that temporary deferrals (95.95%) were more common reasons compared to permanent deferrals (4.05%). This data is consistent with studies conducted in France, and the USA [12, 13]. The main permanent deferral conditions were donors positive for transfusion transmissible infections, certain chronic systemic diseases and malignancies.

In our research, low Hb level was the most frequent cause for BD deferral and since this is a temporary cause, it gives us space to educate and counsel BDs about the deferral cause and treatment strategy to overcome it, which will enable them to donate blood in the future. Furthermore, education and motivation of deferred BDs is of primary importance, because it helps them to continue with blood donation later.

Of all blood donation postponements in our study, 1002 BDs (43.15%) were deferred because of low Hb concentration, which was the most common reason for temporary deferral of blood donation. The results of the study from Croatia show similar values (36.3%) [18], as well as the results from Slovenia (30%) [19], Romania (30.6%) [19], and India (51%) [20]. A slightly lower rate of rejection of BDs due to low Hb concentration was recorded in Turkey 20.7% [21], while in developed European countries these rates were significantly lower [19]. Percentage of temporarily rejected BDs due to low Hb concentration was recorded in 59.48% of women and 24.47% of men, which is quite similar to some other studies where this percentage was 44.82% of female donors versus 34.55% of male donors [18].

Each potential BD, regardless of the number of blood donations, has the Hb value determined every time. According to EU Directives and The European Directorate for the Quality of Medicines & HealthCare guidelines, Hb concentration for donors should be > 135 g/L in men and > 125 g/L in women, whereas in other countries, these values differ by 5–10 g/L [4].

One donation of blood (450 ml) includes 210–240 mg of iron, as 1 mL of red cells contains 1.12 mg of iron [22]. Globally, more than 30% of the world's population are estimated to be anemic and many due to iron deficiency. Iron deficiency is a common consequence of repeated blood donations and a strong predictor of low Hb [23]. Insufficient iron stores may hamper the recovery from subsequent donations, thus risking the development of low Hb and/or iron-deficient anemia.

The highest number of deferred donors belonged to the 18–25 age group, which is consistent with the findings reported by Gaikwad et al. [24] and Patil and Jayaprakash [25].

The prevalence of low Hb in adolescent BDs, particularly among females, has been noted in previous studies as well as in the present study [17]. This suggests that low micronutrient levels in young people contribute to these findings, and that improving micronutrient intake could significantly reduce deferrals due to temporary conditions such as anemia [24].

Younger age and female sex were identified as the primary factors associated with absent iron stores, iron deficiency anemia, and deferral from blood donation due to low Hb levels [26].

Our analysis suggested that there is high prevalence of anemia even in first time BDs. Of the total of 1002 rejected donors due to low Hb level, 297 (29.64%) were first time donors. Anemia in females can be associated to physiological conditions such as menstruation and pregnancies. Although anemia in first-time male BDs may be associated with unrecognized medical conditions, occult gastrointestinal bleeding, vitamin b12 deficiency, and hyperthyroidism [27].

It is considered that BDs are healthy persons and that they do not need extensive laboratory tests. However, we have clear evidence that multiple blood donations can put the donor at risk of developing anemia [22].

According to existing recommendations, transfusion centers rely on the method of testing Hb before donation, which is not the best method for testing iron status in any case. And with this problem European transfusion centers deal in different ways. In Denmark, the Czech Republic, and Italy, ferritin has been introduced as a method to control the iron status of BDs [5]. In Italy, ferritin is measured once a year in regular donors. In the Czech Republic, ferritin is measured at the first donation and in selected cases of repeated donations. In Denmark, ferritin testing is performed in all first donations and every tenth donation. The Netherlands has defined the values of ferritin at which it reacts and ferritin controls are performed at the first and fifth donation [5]. Therefore, with values < 15 μg/L, they advise a 12-month suspension of blood donation, and with values < 30 μg/L – six months [20].

The most important predictors for the occurrence of iron deficiency in BDs as the results of the Danish study are sex, number of previous donations, time since the last donation, and menopausal status [28]. All things considered, this large study shows that the most important predictor for low Hb in regular BDs is low ferritin (< 15 μg/L). They suggest that these findings provide compelling evidence for the importance of ferritin monitoring in BDs as a tool for evaluating donor risk [29].

In the USA, Canada, and countries of the European Union, these indicators were a clear signal for conducting studies that introduced iron supplementation after donating blood. In Denmark, the practice of sending 100 iron tablets to the home address of a donor with a determined ferritin level < 15 μg/L has been introduced [5]. If ferritin is at the level of 15–40 μg/L, the donor is sent 60 tablets for supplementation [20]. In Serbia there is still no recommendation to introduce iron supplementation in BDs.

Other causes of deferral among males in present study beside low Hb level were medication (126), recent donation (108), low blood pressure (98) and high blood pressure (89), while in females were low Hb level, low blood pressure (167), thyroid disorders (76) and medication (46). Recent alcohol intake was seen only in male donors (five), as well as trauma (four), high Hb level (four) and high-risk behavior (14).

Blood pressure is declared high or low when it is outside the range of 100–180 mmHg for systolic blood pressure and 60–100 mmHg for diastolic blood pressure [4].

Our study showed that 11.41% of BDs were rejected due to low blood pressure. Low blood pressure represents a relatively common temporary cause of donor deferral in blood donation services. Hypotension may occur due to several physiological factors, including dehydration, prolonged fasting, fatigue, or anxiety associated with the donation process. These factors may trigger a vasovagal response, which can lead to a transient decrease in blood pressure and increase the risk of adverse donor reactions such as dizziness or fainting. Deferring donors with hypotension is therefore an important precaution to ensure donor safety during blood collection. Also, a study by AlNouri et al. [30] announced that the most common medical examination cause of deferral was low blood pressure (11.60%).

In our study, high blood pressure had a deferral rate 4.26% and it is detected much more often in males (8.22%)

than in females (0.81%). This finding may be explained by the generally higher prevalence of hypertension in men, particularly in middle-aged and older populations. Hormonal factors, such as the protective effect of estrogen in premenopausal women, may contribute to lower blood pressure levels in female donors. In addition, lifestyle factors including higher rates of alcohol consumption, smoking, and obesity among men may increase the likelihood of elevated blood pressure during pre-donation screening. The leading cause for permanent deferral in study conducted by Gaikwad et al. [24] was hypertension. This correlates with the results of the study conducted by Patil et al. [25], who announced hypertension as the most common cause of permanent BDs deferral.

One of the most common causes for pre-donation BD deferral is medication, as revealed in our findings (7.41%). All of these deferrals can be avoided if a more detailed list of drugs that should not be taken is made available to BDs. Still, a large number of people were unsure about the exact names or specifications of the medications they were taking.

A study by Oyedeji et al. [16] reported that under medication was 2.9% and Patil et al. [25], reported even higher rate of 8.18%.

CONCLUSION

Our study found that 4.85% of BDs were deferred, with low Hb levels being the most common reason, followed by inadequate blood pressure and medication use. Low Hb levels were particularly prevalent among female donors and younger age groups.

Our study showed that females represent a potential resource for increasing the number of BDs. The results suggest that implementing parameters that more accurately reflect iron status would help ensure donor safety. To protect the health of BDs and maintain an adequate blood supply, appropriate procedures should be applied, which may include temporary iron supplementation for those donors who require it.

It should be emphasized that the demand for blood, both globally and in our country, is increasing, while the donor population is declining due to the significant trend of population aging, particularly pronounced in Western Europe. Unfortunately, this trend is also evident in Serbia, where the average age exceeds 40 years. Therefore, donor care should be a key priority within our healthcare system.

Conflict of interest: None declared.

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Анализа најчешћих разлога за одбијање добровољних давалаца крви на територији југоисточне Србије

Милица Ранђеловић, Зоран Станојковић

Завод за трансфузију крви Ниш, Ниш, Србија

САЖЕТАК

Увод/Циљ Критеријуми за селекцију давалаца и критеријуми за трајно или привремено одлагање давања крви имају виталну улогу у безбедности трансфузије крви и осмишљени су да осигурају здравље даваоца и примаоца. Добро је позната чињеница да одбијање давалаца крви негативно утиче на повратак како нових, тако и редовних давалаца. Циљ студије био је да се анализирају учесталост и разлози за одбијање добровољних давалаца крви са нагласком на привремена одбијања због ниског нивоа хемоглобина (*Hb*), како би се даваоци идентификовали и правилно саветовали у циљу повећања њиховог броја.

Методе Студијом су обухваћени добровољни даваоци крви од 1. јануара до 31. децембра 2023. године. У студију су укључена укупно 2322 одбијена добровољна даваоца крви оба пола, старости између 18 и 65 година.

Резултати Укупна преваленца одбијених давалаца била је 4,85%. Најчешћи разлог за привремено одлагање давања крви био је низак ниво *Hb*, који је утврђен код 1002 даваоца крви (43,15%). Остали разлози су укључивали низак крвни притисак (265 давалаца, 11,41%), медикаментозну терапију (172 даваоца, 7,41%), недавно давање крви (133 даваоца, 5,73%), висок крвни притисак (99 давалаца, 4,26%), поремећај функције штитне жлезде (96 давалаца, 4,13%), недавну хируршку интервенцију (81 давалац, 3,49%).

Закључак Резултати ове студије сугеришу да би се коришћењем параметара који прецизније одражавају статус гвожђа успоставило безбедније давање крви за даваоце. У циљу заштите здравља давалаца крви и обезбеђивања адекватних залиха крви, потребно је применити одговарајуће процедуре, које могу укључивати привремену суплементацију гвожђем.

Кључне речи: даваоци крви; критеријуми селекције; узроци одбијања; хемоглобин

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Modified mylohyoid nerve anesthesia with 4% articaine with epinephrine and dexamethasone for mandibular dentoalveolar surgery

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SUMMARY
Introduction The use of the modified mylohyoid nerve anesthesia (MMA) technique for dentoalveolar surgery is uncommon.
Case outline We present a 51-year-old Caucasian female patient experiencing chronic inflammation and pain in the area of the missing lower left second premolar and molars. Cone-beam CT imaging of the mandible revealed impaction of the mandibular permanent second premolar; 3.5 mL of 4% articaine and 1:100,000 epinephrine were administered with 0.5 mL of dexamethasone using the MMA technique behind the site of the third molar. The pain was measured through the visual analog scale and recorded a value of 8 mm, with the duration of anesthesia being 270 minutes.
Conclusion Modified mylohyoid nerve anesthesia can be utilized independently as a primary anesthesia or as a supplementary option for insufficiently effective or failed Halsted anesthesia.
Keywords: mylohyoid nerve; anesthesia; articaine; dexamethasone; dentoalveolar surgery

INTRODUCTION

Dentoalveolar mandibular surgery frequently involves the treatment of pathologies, such as impacted teeth, retained roots, and bone cysts [1]. Tooth impaction is described as a tooth that has not erupted into its designated position in the dental arch due to malposition or insufficient space [2], with a fully formed root (closed dental papilla) [3, 4]. Mutations in the parathyroid hormone 1 receptor (PTHR1) contribute to primary eruption failure [5]. The retained roots become "forgotten" in the jaws, presenting as dental pain and a cystic lesion [6].

The appropriate local anesthesia method for this purpose is the so-called "mandibular nerve anesthesia," even though it is an inaccurate term according to Malamed [7]. This is the technique of local direct conduction anesthesia for the inferior alveolar nerve block (IANB), as outlined by Halsted, and the lingual nerve, along with the buccal nerve when needed. However, there are indications of failure in up to 20% of cases, or even higher [8, 9].

Sillanpää et al. [10] introduced the mylohyoid nerve mandibular anesthesia (MMA), achieving anesthesia of the first molar in 21% of cases. Later, Altug et al. [11] administered 1 mL of 4% articaine HCl with epinephrine 1:200,000 for a sublingual distal injection into the distal root of the first molar in an effort to attain anesthesia of the mylohyoid nerve, achieving local anesthesia in half of the subjects. Clark et al. [12] reported very limited anesthetic effectiveness for mandibular premolars using MMA. Surprisingly, a recent report [13] emerged regarding the use of 4% articaine with a 1:100,000 adrenaline mixture along with dexamethasone, with the intent of achieving MMA [14], which demonstrated an anesthetic success rate identical to 2% lidocaine with adrenaline 1:100,000 for Halsted's IANB.

The purpose of this research was to present the efficacy of MMA as a primary mandibular anesthesia, utilizing 4% articaine with epinephrine and the addition of dexamethasone, for the dentoalveolar surgery of impacted mandibular second permanent premolar.

CASE REPORT

The patient's full verbal and signed consent was obtained for all diagnostic, anesthetic, and surgical procedures in our department (Informed Consent), which were in accordance with the Helsinki Declaration. We received approval from the ethics boards of our institutions for the following treatment as well.

Anesthesia variables and protocol

The surgery was performed under local anesthesia by modified mylohyoid nerve anesthesia. The measured variables were as follows: onset time – the time measured from the moment of injection to the first sign of anesthesia effectiveness represented by numbness on the ipsilateral hemitongue and lip; the patient's intraoperative pain was measured with a visual analog scale (VAS); the effectiveness of administered anesthesia was considered successful if the

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measurements were "no pain up to 4 mm, or a pain rating of up to 44 mm," which is considered mild pain [14, 15]; duration of the anesthesia – the time from onset to the cessation of lip and tongue numbness.

An aseptic plastic 5 mL syringe (Nipro syringe, Shanghai International Holding Corp. GmbH (Europe), Hamburg, Germany) served as a container, which consisted of 3.5 mL of 4% articaine with 1:100,000 adrenaline (Artinibsa, 40 mg/mL + 0.01 mg/mL adrenaline; Inibsa Dental S.L.U., Barcelona, Spain) along with 0.5 mL of dexamethasone (4 mg/1 mL), and a sterile needle 21 G x 1½ ", 0.8 x 40 mm (Nipro needle, Nipro Europe N.V., Zaventem, Belgium).

A 51-year-old Caucasian female patient was examined because of a recurring left dentoalveolar abscess (2–3 times), in the region of the missing lower left second premolar and molars, over the previous six months. Physical examination showed a restriction when opening the mouth (trismus), with two middle fingers barely fitting interincisally. Medical history revealed that the patient had undergone gallstone abdominal surgery 6 months before the episodes of dentoalveolar abscesses.

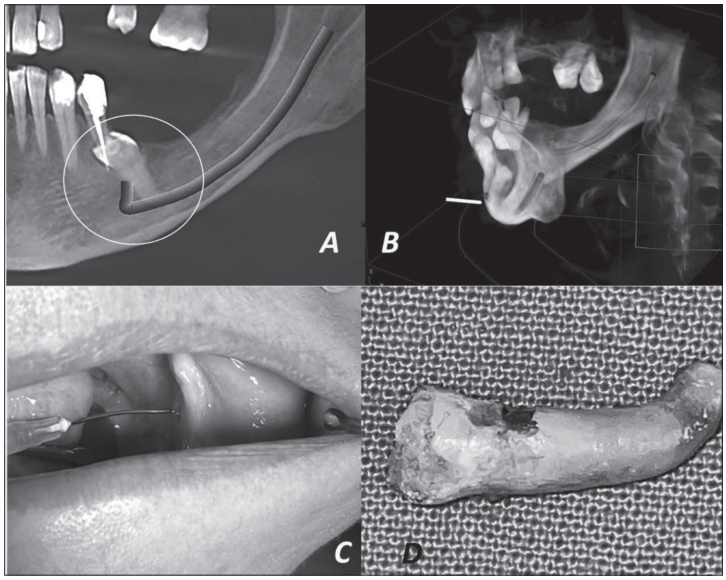

Figure 1. A) Cone-beam computed tomography (CBCT) imaging of the left mandibular side, showing the impacted second premolar (marked with a white circle); B) CBCT shows a "hook-like" angulation of the root apex (indicated by the white arrow); C) intraoral positioning of the needle for mylohyoid nerve mandibular anesthesia; D) extracted lower second premolar in one piece, with the root tip as a "hook"

The X-ray examination, including cone-beam computed tomography (CT) (Sirona Galileos 3D Cone Beam Scanner®) was performed. The 3D image revealed that the second premolar was completely embedded in the mandibular bone, with the middle part of the root and the crown being lingually oriented. The procedure of MMA started with an angulated needle (107°), which was sublingually injected through the sublingual mucosa into the projection of the site of missing the third lower left molar. The anesthetic maneuver was performed below the attachment line of the mylohyoid muscle on the mandible, reaching a depth of approximately 15 mm. After a negative aspiration test was obtained, approximately 3.5 mL of the anesthetic solution (articaine + dexamethasone) was slowly deposited over 10–15 seconds. The remaining ≈ 0.5 mL of anesthetic solution was buccally injected. With scalpel #15 from the imaginary position of missing tooth #36 (distally), through a sulcular lingual incision encompassing teeth #34 and #33 (medially), a lingual mucoperiosteal flap was elevated. With the use of a sterile round carbide-steel burr (No. 167–141, Meisinger HM, Neuss, Germany), the crown of impacted #35 was exposed. The successful extraction of impacted tooth #35 was performed (Figure 1 A–D). The elevated mucoperiosteal flap was sutured with single interrupted nonabsorbable sutures (Silk USP 4/0 EP 2, SMI AG St.Vith, Belgium). The measured postoperative anesthesia variables were as follows: onset time of the hemitongue and lip, 1 minute and 2 minutes, respectively; the measured patient's intraoperative pain was 8 mm (VAS); duration of the anesthesia was 270 minutes. The operation time was 30 minutes. The sutures were removed on the eighth postoperative day, with an uneventful postoperative course.

Ethics: The patient has been treated in accordance with the Helsinki Declaration. Informed consent was obtained from the patient before the operation, and for the following treatment the Ethics Board approvals of our institutions were received (No. 14/16-2019-1 EO dated 28th November 2023, and No. 12-16502/2-6 dated December 21, 2023) as well.

DISCUSSION

Goldberg et al. [9] in their study of IANB found that the ranges of anesthetic success were as follows: Halsted's technique, 25–62%; Gow-Gates technique, 16–44%; and the Vazirani–Akinosi technique, 13–50%. Failure of Halsted's technique includes sensitivity of mandibular teeth, such as the molars [16], and the mandibular nerve particularly sensitizes the premolar, canine, and incisor teeth via the accessory foramina on the lingual mandibular plate, with an incidence of 60% [17].

We used MMA, which is categorized by the VAS scale as "no pain – mild pain." [13] There is evidence that separately used local anesthesia for lingual infiltration with 1.8 mL of 2% lidocaine with 1:100,000 adrenaline, as additional local anesthesia to the standard Halsted's IANB, with 3.6 mL of 2% lidocaine with 1:100,000 epinephrine, showed no contributive effect in increasing anesthetic success for mandibular posterior teeth [18, 19, 20].

The second reason is the presence of nutrient foramina on the lingual side of the mandible, which are "weak anatomical spots," through which articaine can pass into the bone and diffuse into the IAN [21]. The third reason is the intensification of articaine anesthesia's pharmacokinetic properties by adding dexamethasone. The fourth reason is the possibility that the articaine and dexamethasone

anesthetic mixture is sufficient for anesthesia success [22]. Stojanović et al. [23] significantly prolonged the anesthetic duration (592.5 minutes) of 0.75% ropivacaine with the addition of dexamethasone for Halsted's IANB. Directly mixing dexamethasone with anesthetic for perioperative analgesia provides neural painful signal transmission in nociceptive C-fibers and blocks ectopic neuronal discharge [23]. In the presented cases, the VAS scores were 8 mm and 28 mm [14], respectively, indicating anesthetic success in providing perioperative analgesia (no pain up to 4 mm, or mild pain up to 44 mm on the VAS) [24], and prolonging the duration of the anesthesia to 270 minutes.

Other authors who studied the anesthetic efficacy of 4% articaine *vs*. 2% lidocaine during the surgical removal of the third molar found that the duration of the anesthetic effect of articaine was 231 minutes, and that of lidocaine was 174 minutes, with Halsted's technique [24, 25]. It is proven that the concentration of articaine in the alveolus of the extracted tooth is 100 times higher than that in systemic circulation [26]. Articaine is an amide anesthetic, which has a combination of an ester group and a thiophene ring; the thiophene ring enables articaine's very high lipophilicity and rapid diffusion through the bone into nerve cells by the biochemical mechanism of chemically induced intramolecular hydrogen bonding of bone tissue [24]. Pathak et al. [27] also found that dexamethasone convincingly prolongs the duration of anesthesia through blocking vasodilatation induced by bradykinin. Dexamethasone added perineurally to the mylohyoid nerve and upon penetration of the lingual plate of the mandible to the inferior alveolar nerve could act as a neuroprotective drug, which is proven in clinical practice [27].

The U.S. Food and Drug Administration recommendation specifies a maximum recommended dose of 7 mg/kg (11.9 mL) of 4% articaine per visit, without an established absolute maximum; nonetheless, there is a maximum limit of 11 cartridges of 4% articaine with 1:100,000 epinephrine [28, 29, 30].

In conclusion, the modified mylohyoid nerve anesthesia may have the capacity to act as the main and sole local anesthesia, or as additional anesthesia for the surgery of impacted mandibular second permanent premolars.

Conflict of interest: None declared.

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Модификована анестезија милохиоидног нерва применом 4% артикаина са адреналином и дексаметазоном у мандибуларној дентоалвеоларној хирургији

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САЖЕТАК

Увод Употреба модификоване анестезије милохиоидног нерва у дентоалвеоларној хирургији је ретка.

Приказ болесника Приказана је 51-годишња пацијенткиња са хроничном упалом и болом у пределу недостајућег доњег левог другог преткутњака и кутњака. Конусном компјутеризованом томографијом мандибуле утврђена је лингвална импакција мандибуларног сталног другог преткутњака са леве стране. Апликовано је 4 *ml* анестетичког раствора састављеног од 3,5 *ml* 4% артикаина са 1 : 100.000 адреналина и 0,5 *ml* дексаметазона. Ефикасност модификоване анестезије милохиоидног нерва у смањењу интраоперативног бола процењена је помоћу визуелне аналогне скале (*VAS,* изражена у *mm*), која је забележила вредност од 8 *mm*, док је трајање анестезије износило 270 минута.

Закључак Модификована анестезија милохиоидног нерва може се користити као примарна анестезија или као додатна опција код неуспешне Халстедове анестезије.

Кључне речи: милохиоидни нерв; анестезија; артикаин; дексаметазон; дентоалвеоларна хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Suspected Lyme-associated peripheral facial palsy in an adolescent with pre-existing sensorineural hearing loss – a diagnostic challenge

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SUMMARY

Introduction Lyme neuroborreliosis (LNB) represents a neurological manifestation of the infection caused by *Borrelia burgdorferi sensu lato*. According to established European diagnostic criteria, confirmed LNB requires cerebrospinal fluid pleocytosis and evidence of intrathecal antibody synthesis; however, these criteria are not always fulfilled in clinical practice, which may lead to diagnostic uncertainty.

Case report A 15-year-old girl presented with acute right-sided peripheral facial palsy. Serological testing demonstrated positive IgM and IgG antibodies to *Borrelia burgdorferi sensu lato*. Cerebrospinal fluid analysis was not performed, precluding confirmation of neuroborreliosis. Brain magnetic resonance imaging (MRI) excluded central causes of facial palsy. The patient received empirical antibiotic therapy in combination with corticosteroids, followed by partial clinical recovery over time. Pre-existing sensorineural hearing loss and incidental MRI findings were considered unrelated to the acute presentation.

Conclusion This case highlights the limitations of attributing peripheral facial palsy to Lyme disease based solely on serological findings. In the absence of cerebrospinal fluid analysis, diagnostic uncertainty remains, emphasizing the need for cautious interpretation of laboratory results and thorough consideration of alternative etiologies.

Keywords: neuroborreliosis; Lyme disease; peripheral facial paralysis; adolescents; rehabilitation

INTRODUCTION

Lyme neuroborreliosis (LNB) represents a neurological manifestation of infection caused by *Borrelia burgdorferi sensu lato* and, according to established European Federation of Neurological Societies criteria, is defined by a compatible clinical presentation in combination with cerebrospinal fluid (CSF) pleocytosis, and evidence of intrathecal antibody synthesis [1]. These criteria are essential for confirming the diagnosis and distinguishing LNB from other neurological conditions.

Lyme borreliosis is the most common vector-borne disease in Europe, with a substantial and increasing public health burden. Recent epidemiological data indicate that an average 132,000 cases are reported annually across Europe, with neurological involvement occurring in a subset of patients [2, 3]. Among these, peripheral facial nerve palsy represents one of the most frequent clinical manifestations, particularly in pediatric populations [1, 4]. However, the presence of facial palsy alone is not sufficient to establish a diagnosis of LNB without appropriate CSF findings [1].

In clinical practice, the diagnostic evaluation of patients presenting with peripheral facial palsy may be challenging, particularly in regions with higher background seroprevalence of *Borrelia* exposure [5, 6]. Positive serological findings may reflect previous contact with the pathogen rather than active infection, which limits their specificity in the absence of confirmatory CSF analysis [1]. Consequently, attributing facial nerve palsy to Lyme disease based solely on serology may lead to overdiagnosis and misinterpretation of causality.

Although Lyme disease has been associated with a wide spectrum of neurological manifestations, isolated peripheral facial palsy remains a typical presentation, whereas involvement of additional cranial nerves or atypical features requires careful evaluation [1, 7]. Concurrent findings, such as sensorineural hearing loss, should be interpreted with caution, as clinical and laboratory findings in Lyme disease may be nonspecific and seropositivity does not necessarily indicate causality; therefore, such findings may represent pre-existing or unrelated conditions rather than manifestations of acute infection [3, 7].

This case report describes an adolescent presenting with peripheral facial palsy and positive *Borrelia* serology in a setting where CSF analysis was not performed. The aim of this report is not to demonstrate confirmed neuroborreliosis, but to highlight the diagnostic uncertainty and limitations associated with interpreting serological findings in the absence of CSF confirmation, as well as the importance of considering alternative etiologies.

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CASE REPORT

A 15-year-old previously healthy female from a suburban region of Belgrade was admitted to the pediatric cardiology department due to palpitations and intermittent chest discomfort. Initial evaluation, including Holter monitoring, revealed frequent ventricular extrasystoles without structural abnormalities or hemodynamic compromise. Further cardiological assessment, including ergospirometry, and repeat Holter electrocardiogram monitoring, showed no significant abnormalities. Routine laboratory analysis, including a metabolic panel and C-reactive protein, was within normal limits.

During hospitalization, on the first day after admission, the patient developed acute right-sided facial weakness, characterized by progressive inability to fully close the right eye and facial asymmetry (House–Brackmann grade IV) [8]. She denied fever, headache, recent infection, head trauma, rash, otalgia, vertigo, or known tick exposure. Past medical history was notable only for febrile seizures in early childhood, with no recurrence after the age of five. Immunizations were up to date, and family history was negative for neurological or autoimmune diseases.

Neurological examination revealed a peripheral right facial nerve palsy, including loss of forehead wrinkling, lagophthalmos, flattening of the nasolabial fold, and drooping of the mouth angle. Motor strength, sensory examination, cerebellar function, gait, and deep tendon reflexes were normal. Otoscopic examination showed no signs of middle ear pathology.

Audiological evaluation was performed as part of the assessment of cranial nerve function. Pure-tone audiometry demonstrated bilateral moderate-to-severe high-frequency sensorineural hearing loss (right ear: 2 kHz = 60 dB, 4 kHz = 80 dB; left ear: 2 kHz = 60 dB, 4 kHz = 65 dB), consistent with previously documented congenital hearing impairment. Tympanometry was type A bilaterally. Acoustic reflexes were reduced at 500 Hz on the right and absent at higher frequencies bilaterally.

Ophthalmological examination revealed conjunctival hyperemia and early signs of exposure keratopathy, and treatment with artificial tears and lubricating gel was initiated.

Laboratory and diagnostic evaluation for infectious and autoimmune causes included complete blood count, inflammatory markers, and serological testing. Serological analysis for *Borrelia burgdorferi sensu lato* was performed using a line blot assay and demonstrated the presence of specific IgM and IgG antibodies. CSF analysis was not performed.

Brain magnetic resonance imaging and magnetic resonance angiography showed no evidence of central nervous system pathology. An incidental choroidal fissure cyst without clinical significance was identified (Figure 1).

The patient was treated with intravenous ceftriaxone, followed by oral doxycycline (100 mg twice daily for seven days), in combination with corticosteroid therapy. Supportive management included artificial tears and physical therapy.

Clinical improvement was observed during hospitalization, with partial recovery of facial movements and improved eyelid closure. No systemic complications were noted.

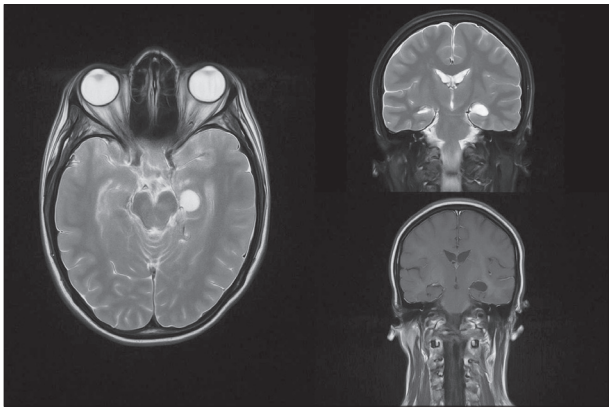

Figure 1. Magnetic resonance imaging diagnostics for exclusion of central facial palsy – cyst of the choroidal fissure; the patient was admitted to a pediatric day hospital and received intravenous ceftriaxone, corticosteroids with gradual tapering, gastroprotection, structured facial physiotherapy including electrostimulation of the right facial nerve and facial exercises, and continuous ocular lubrication; coagulation analysis, including von Willebrand factor antigen and factor XIII activity, were within normal limits; cardiac evaluation showed persistent but hemodynamically insignificant ventricular extrasystoles

After 20 days of hospitalization, the patient was discharged in stable condition with mild residual facial asymmetry. Outpatient management included continuation of doxycycline therapy, physiotherapy, and scheduled follow-up with neurology, otorhinolaryngology, ophthalmology, and cardiology.

DISCUSSION

Peripheral facial palsy represents a common clinical presentation in pediatric patients and may be associated with Lyme disease; however, it is not specific for LNB. According to established European criteria, confirmed neuroborreliosis requires CSF pleocytosis and evidence of intrathecal antibody synthesis [1, 9, 10]. In the absence of CSF analysis, the present case can only be classified as suspected Lyme-associated facial palsy rather than confirmed neuroborreliosis. This distinction is essential to avoid overinterpretation of clinical and laboratory findings.

The initial diagnostic consideration in this case included the possibility of broader cranial nerve involvement due to the coexistence of facial palsy and sensorineural hearing loss [3, 7]. However, audiological findings were consistent with previously documented congenital impairment, and no additional neurological deficits were identified. Therefore, the clinical presentation was ultimately limited to isolated peripheral facial palsy. This highlights the importance of careful interpretation of concurrent findings, particularly when pre-existing conditions may confound the clinical picture.

The interpretation of *Borrelia* serology requires particular caution. Positive IgM and IgG antibodies may reflect previous exposure rather than active infection, especially in regions with higher background seroprevalence. Importantly, seropositivity alone is not sufficient to

establish a diagnosis of active Lyme disease in any clinical form [7, 11, 12]. In the absence of CSF analysis, neither neuroborreliosis nor Lyme-associated cranial neuritis can be confirmed. While lumbar puncture may be deferred in selected pediatric cases, its role remains central when neuroborreliosis is considered. Furthermore, alternative and more common causes of peripheral facial palsy, such as idiopathic (Bell's palsy) or viral etiologies, should be carefully considered, particularly in cases with inconclusive diagnostic findings [5, 13].

Current guidelines recommend oral doxycycline or intravenous ceftriaxone for the treatment of Lyme-associated peripheral facial palsy, typically administered for 14–21 days [7, 9, 14]. In the present case, a sequential regimen of intravenous ceftriaxone followed by oral doxycycline, in combination with corticosteroids, was used. This approach does not represent a standard treatment strategy and likely reflects the diagnostic uncertainty at the time of clinical decision-making. The use of corticosteroids in suspected Lyme-associated facial palsy remains controversial, with limited and conflicting evidence regarding their benefit, particularly in the absence of confirmed neuroborreliosis [15].

Supportive management, including eye protection and physical therapy, was implemented during hospitalization [16]. However, given the limited detail regarding the type and duration of rehabilitation, its contribution to clinical recovery cannot be clearly established [17].

This case also underscores the importance of aligning reported findings with documented clinical data. Follow-up neurological and audiological outcomes should be interpreted cautiously and, where relevant, clearly presented within the case description to support subsequent discussion [1, 7].

In conclusion, this case illustrates the diagnostic challenges in evaluating peripheral facial palsy in the context of positive *Borrelia* serology. The absence of CSF analysis precludes confirmation of neuroborreliosis, emphasizing the limitations of serological testing alone. Careful adherence to established diagnostic criteria, critical interpretation of laboratory findings, and consideration of alternative etiologies are essential to avoid overstated causal associations.

Ethics: Written informed consent was obtained from the patient's parents for treatment and publication of this case report. No identifiable patient information is included in this case report, and all data and images have been fully anonymized.

Conflict of interest: None declared.

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Суспектна периферна фацијална парализа удружена са лајмском болешћу код адолесцента са претходно дијагностикованим сензоринеуралним губитком слуха – дијагностички изазов

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САЖЕТАК

Увод Лајмска неуроборелиоза представља неуролошку манифестацију инфекције изазване бактеријом *Borrelia burgdorferi sensu lato*. Према утврђеним европским дијагностичким критеријумима, потврђена лајмска неуроборелиоза захтева плеоцитозу у цереброспиналној течности и доказе о интратекалној синтези антитела; међутим, ови критеријуми се не испуњавају увек у клиничкој пракси, што може довести до дијагностичке несигурности.

Приказ болесника Девојчица од 15 година примљена је због акутне периферне парализе десне стране лица. Серолошко тестирање је показало присуство позитивних *IgM* и *IgG* антитела на *Borrelia burgdorferi sensu lato*. Анализа цереброспиналне течности није извршена, чиме је потврда неуроборелиозе онемогућена. Магнетна резонанца мозга искључила је централне узроке парализе лица. Болесница је примила емпиријску антибиотску терапију у комбинацији са кортикостероидима, након чега је временом постигнуто делимично клиничко побољшање. Претходно дијагностикован сензоринеурални губитак слуха и инцидентални налази магнетне резонанце сматрани су неповезаним са акутном презентацијом.

Закључак Овај случај истиче ограничења приписивања периферне парализе лица лајмској болести на основу само серолошких налаза. У одсуству анализе цереброспиналне течности, дијагностичка несигурност остаје, наглашавајући потребу за опрезном интерпретацијом лабораторијских резултата и детаљним разматрањем алтернативних етиологија.

Кључне речи: неуроборелиоза; лајмска болест; периферна парализа фацијалиса; адолесцент; рехабилитација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Treatment for massive conjunctival malignant melanoma – analysis of two cases

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SUMMARY

Introduction Conjunctival malignant melanoma (CMM) is a highly malignant tumor. Due to its low incidence, atypical early clinical manifestations, and diverse pathological forms, it is easily misdiagnosed or missed in clinical practice, along with extremely poor prognosis. This study aims to report the treatment outcomes of two cases of massive malignant melanoma of CMM to provide insights into clinical diagnosis and treatment strategies.

Outlines of cases Two cases of massive malignant melanoma of the CMM are reported. Both patients presented to our hospital due to black masses in the eyelid, which were diagnosed as malignant melanoma upon examination. After tumor resection, combined treatment with oral administration of temozolomide capsules and intravenous injection of cisplatin was performed, and no recurrence was observed during one year follow-up.

Conclusion This study suggests that although CMM is rare and has subtle early symptoms, timely diagnosis, thorough surgical resection combined with personalized adjuvant therapy (including chemotherapy, targeted, and immunotherapy) can improve patient prognosis. Enhancing awareness among clinicians and patients, and implementing genetic testing-guided precision treatment, are key to improving therapeutic outcomes.

Keywords: malignant melanoma; CMM; chemotherapy; case report

INTRODUCTION

Conjunctival melanoma (CM) is a rare and highly aggressive malignant conjunctival tumor. Its clinical manifestations are easily confused with other ocular diseases, leading to frequent misdiagnosis or neglect. This can subsequently result in severe visual impairment, a significant decline in quality of life, and even death due to distant metastasis. The incidence of this disease is relatively higher among individuals with fair skin. The overall incidence rate is approximately 0.46 cases per 1,000,000 people per year, accounting for about 0.25% of melanomas in all body sites and about 5% of all ocular melanomas [1]. CM is the second most common malignant conjunctival tumor after conjunctival squamous cell carcinoma [2]. Its origin can be traced back to the basal melanocytes in the conjunctival epithelium. According to research statistics, about 70% of CM cases develop from conjunctival melanocytic intraepithelial neoplasia or primary acquired melanosis with atypia, while the remaining cases may arise from malignant transformation of pre-existing nevi or spontaneously [3].

At present, there is no standardized treatment protocol for CM. Commonly used therapeutic approaches in clinical practice include surgical resection with or without adjunctive cryotherapy, topical chemotherapy (such as mitomycin C, 5-fluorouracil, or interferon α-2b), brachytherapy, proton beam radiotherapy, or external photon irradiation. For cases with severe local tissue invasion in the advanced stage, radical orbital exenteration is required [4–7]. However, these treatment methods are associated with a high incidence of postoperative complications and a tumor recurrence rate ranging from 33% to 45% [8, 9]. Therefore, patients need lifelong follow-up monitoring. Although significant progress has been made in the targeted and immunotherapy of cutaneous melanoma in recent years, the relevant research data on applying similar therapeutic strategies (such as anti-*BRAF*, anti-MEK, anti-PDL1, etc.) to the treatment of CM are promising but extremely limited, mostly derived from single-patient cases or small case series of patients with no surgical opportunity before surgery or advanced disease [10, 11, 12].

Given the rarity of CM, its propensity for misdiagnosis, and the limitations of current treatment options, this study aims to provide a detailed analysis of two cases of conjunctival malignant melanoma (CMM) to explore the diagnostic and therapeutic processes. It is hoped that this study will offer clinical physicians more precise diagnostic criteria and more effective therapeutic strategies as a reference, while also providing new ideas and directions for future research on the treatment of CMM.

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REPORTS OF CASES

Patient profile

Patient 1, a 52-year-old man, was admitted to Tangshan People's Hospital on December 4, 2021, due to a black mass on the left palpebral margin. One week prior, a flat black mass was incidentally found on the left palpebral margin and conjunctiva, with ulceration and bleeding but no pain. Orbital computed tomography (CT) showed a thickened, elevated lesion on the left conjunctiva, with no distant metastasis. He had no significant medical history and no abnormalities on full-body examination. Ophthalmic examination revealed diffuse, flat, elevated black masses in the upper palpebral margin, 2/3 of the middle and outer palpebral conjunctiva, central fornix conjunctiva, and upper bulbar conjunctiva of the left eye, with a significant mass in the outer 1/3 of the palpebral margin and scab coverage (Figure 1).

Patient 2, a 74-year-old male, was admitted to our hospital's Ophthalmology Department on May 6, 2020, for a black mass in the right conjunctiva present for 1.5 years. The mass was discovered 1.5 years ago, smooth, painless, and without ulceration or bleeding. He underwent surgical excision at another hospital (details unknown) but received no further diagnosis or treatment. A year ago, the mass recurred and a new mass appeared in the lower fornix of the right eye, which rapidly grew and bled repeatedly after ulceration over the past two months. The ophthalmic examination of the right eye revealed adhesions at the upper and lower eyelid fissures, and a poorly demarcated, purplish-black, elevated mass measuring 1.2 cm × 0.8 cm × 0.6 cm was observed in the central lower fornix (Figure 2).

Ocular color Doppler ultrasound revealed a 1.04 cm × 0.64 cm hypoechoic nodule in the lower eyelid of the right eye with abundant blood flow signals (Figure 3A). Malignant melanoma was suspected in both patients. Orbital CT showed lesions in the lower fornix of the right eye (Figure 3B).

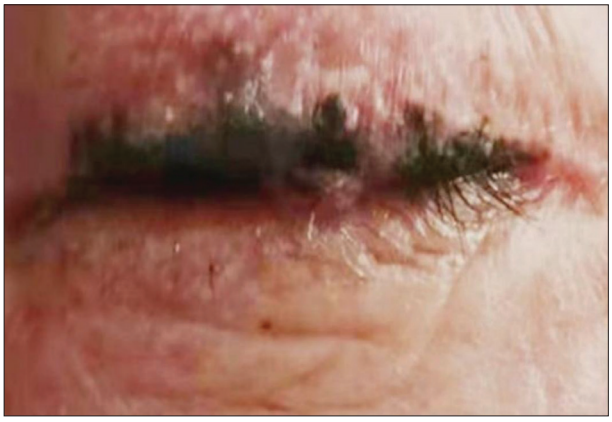

Figure 1. Patient 1: pre-treatment appearance of palpebral margin of malignant melanoma

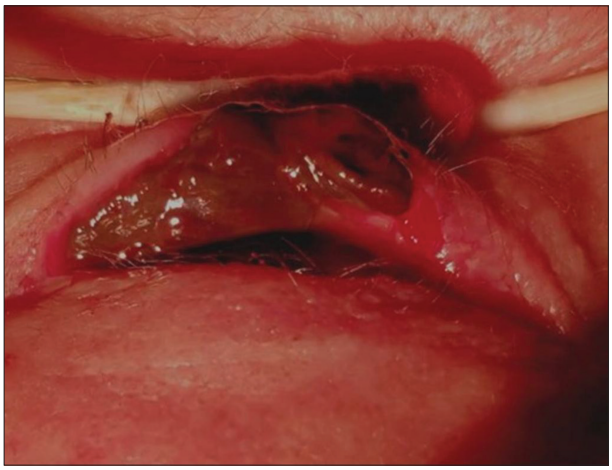

Figure 2. Patient 2: pre-treatment appearance of palpebral margin of malignant melanoma

Surgical methods

After comprehensive imaging assessments, including CT scans of the head, orbit, and chest, and color Doppler ultrasound of the neck, abdomen, pelvis, and urinary system, patient 1 underwent extended resection of the left palpebral margin and CMM, along with blepharoplasty, conjunctival sac reconstruction, and lateral canthoplasty under general anesthesia on December 8, 2021. During the surgery, part of the mass was removed. The patient's oral hard palate mucosa was taken to replace the upper eyelid tarsal plate, with the lower edge of the hard palate mucosa implanted in the lower tarsal sulcus. To reconstruct the damaged tissues, the patient's buccal mucosa (inner cheek lining) was grafted to replace the fornix (the fold between the eyelid and the eyeball) and the upper bulbar conjunctiva (the white part of the eye). This graft helped form a new conjunctival sac to restore normal eye function. Additionally, the lower tarsal plate (the supportive structure of the lower eyelid) was repositioned to repair the defect in the lower palpebral margin, ensuring both functional and aesthetic restoration of the eyelid. Meanwhile, the patient's buccal mucosa was taken to replace the fornix and upper bulbar conjunctiva and form the conjunctival sac, followed by transferring the lower tarsal plate to repair the lower palpebral margin defect. After the lateral canthal angle was formed, the skin tissue defect of the eyelid was covered with a skin flap, completing the eyelid reconstruction and plasty.

Patient 2 underwent extended resection of the right upper palpebral CMM, along with blepharoplasty and resection of the conjunctival mass in the lower fornix of the right eye under general anesthesia on May 18, 2020. The entire layer of the upper eyelid and lateral canthus was removed. The hard palate mucosa was harvested to replace the tarsal plate, with its lower edge implanted in the lower tarsal sulcus. After forming the lateral canthal angle, the eyelid skin defect was covered with a skin flap, completing the reconstruction.

Immunohistochemical results showed: Melan-A (-), Ki-67 (+, 60%), CK (-), HMB45 (+), S-100 (+), Vimentin (+). Genetic testing revealed a *BRAF* V600E mutation, indicating that targeted therapy with *BRAF* inhibitors would be appropriate for this patient. This mutation is significant as it guides the therapeutic approach and helps tailor

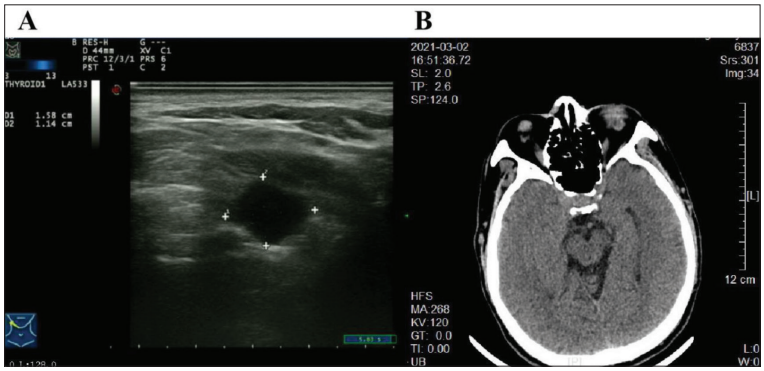

Figure 3. Ultrasound image analysis and computed tomography (CT) scan; A – abundant blood flow signals observed in ocular nodules via conventional ultrasound; B – orbital CT scan showed a lesion in the right lower fornix

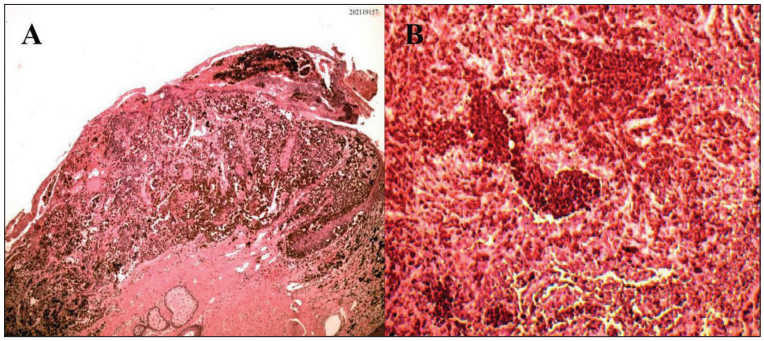

Figure 4. Postoperative histological findings (H&E staining); A – patient 1: postoperative pathological diagnosis: *lentigo maligna* (melanoma) of the conjunctival malignant melanoma; B – patient 2: postoperative pathological examination: malignant melanoma, nodular type; scale = 100μm

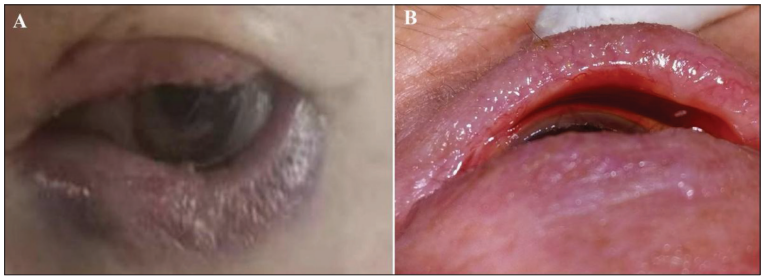

Figure 5. A – healing status of patient 1 three months after conjunctival reconstruction surgery; B – postoperative healing of the right lower fornix in patient 2

personalized treatment strategies, which are crucial for managing CMM.

Pathological and immunohistochemical results

Patient 1: Postoperative pathological diagnosis revealed malignant melanoma of CMM, with 2 mitoses/mm² (Figure 4A). The tumor measured 2.2 cm × 1 cm in maximum area and < 2 mm in maximum thickness. Ulceration and necrosis were present on the mucosal surface, with submucosal invasion. No vascular or nerve invasion was observed, and the lateral resection margin and base were tumor-free, indicating complete excision. No abnormal lymph nodes or metastatic lesions were found. Immunohistochemical analysis confirmed HMB45 positivity, and genetic testing identified a *BRAF* p.V600E mutation, which may influence treatment strategies.

Patient 2: Postoperative pathological examination revealed nodular type malignant melanoma in the vertical growth phase, with 10 mitoses/mm² and no obvious ulceration (Figure 4B). Immunohistochemical examination: Melan-A (-), Ki-60 (+, 60%), CK (-), HMB45 (+), S-100 (+), Vimentin (+). The tumor was nodular with peripheral fibrous tissue (pT3b), no obvious vascular tumor thrombus, and no tumor-infiltrating lymphocytes.

Postoperative chemotherapy regimen

Both patients received a combination systemic therapy regimen consisting of toripalimab and cisplatin. Specifically, toripalimab injection at a dose of 189 mg was administered via intravenous infusion once every two weeks for a total of 12 cycles. Concurrently, cisplatin injection at a dose of 40 mg per day was administered via intravenous infusion for three consecutive days per month, with this cycle repeated every month for a total of six cycles. Three months post-surgery, the eyelid fissure incision, transplanted hard palate mucosa, and buccal mucosa all survived in patient 1, with the formation of a conjunctival sac without adhesion or stenosis.

Meanwhile, the size and movement of the left eyelid move were normal (Figure 5A). As of the most recent follow-up, one year and five months after treatment, the patient remains in good physical condition with no evidence of metastases.

Patient 2 showed no recurrence of purple-black elevated mass in the upper conjunctiva and bulbar conjunctiva of the right eye three months post-surgery, with a smooth surface (Figure 5B). After one year of follow-up post-treatment, the patient was in good physical condition without any abnormal lesions.

Ethics: This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Tangshan People's Hospital. Written informed consent was obtained from the participant.

DISCUSSION

A 52-year-old male patient presented with a black mass on the left eyelid margin, which initially had no ulceration but later showed signs of bleeding. The incidence of this disease increases with age, particularly in individuals over 60, who account for more than 50% of cases, while those under 20 represent only 1%. The disease is more common in elderly males and individuals of Caucasian descent

[13]. The two cases reported in this paper were both male patients, aged 52 and 74 years, respectively. CMM typically occurs unilaterally, with irregular borders and visible pigmentation. It often originates from acquired primary malignant melanoma or, in some cases, from conjunctival nevus pigmentosus. Clinically, it manifests as a slowly enlarging mass on the conjunctiva, with significant variation in initial size, sometimes as small as a grain of rice. Patients may experience occasional eye redness or a foreign body sensation, but usually no vision loss. Some masses may also present with bleeding or exudation [3].

The two cases in this study developed acquired primary malignant melanoma with a history ranging from one week to 1.5 years. Patient 2 discovered a black mass on the right conjunctiva 1.5 years prior to treatment-seeking, which recurred a year ago and rapidly grew over the previous two months, causing repeated bleeding after ulceration.

BRAF gene single-base mutations are the most common genetic variations in malignant melanoma and are a key target for targeted therapy. In China, the *BRAF* mutation rate in malignant melanoma is approximately 25.5%, with 89.1% being the *BRAF* (V600E) mutation [14]. Other rare mutations include V600K, V600R, and V600D [15]. Domestic studies have reported *BRAF* V600E and *c-Kit* mutation rates of 7.7% [16]. In this study, one case of *BRAF* V600E mutation was detected, while no *c-Kit* gene mutation was found.

A 74-year-old male discovered a black mass on the right conjunctiva, present for 1.5 years prior to diagnosis. Initially smooth and non-ulcerated, the mass later grew rapidly and bled recurrently. CMM is rare and morphologically complex, making diagnosis, especially of non-pigmented variants, particularly challenging. It should be differentiated from the following diseases: Conjunctival nevus pigmentosus [17]: commonly found in the bulbar conjunctiva and limbus of the eyelid fissure, it is a benign neuroectodermal tumor. It has a smooth surface, uniform black color, and no ulceration or exudation. The immunohistochemical Ki-67 proliferation index is usually < 10%; p53 and cyclin D1 may show diffuse strong positivity, increasing with disease progression. Melan A can be expressed in both benign nevus cells and malignant melanoma, but its expression in malignant melanoma may decrease with increasing tumor thickness, reduced disease-free survival, and increased mortality. Poorly differentiated squamous cell carcinoma [18]: the most common conjunctival malignancy, it often occurs at the junction of the palpebral margin skin and conjunctiva, the limbus in the eyelid fissure area, and the lacrimal caruncle in the medial canthus, with UV exposure as a key trigger. Some malignant melanomas can mimic this carcinoma with prominent nucleoli. P40, P63, or CK5/6 are positive markers for poorly differentiated squamous cell carcinoma, while HMB-45, MelanA, and S-100 are negative. Poorly differentiated sarcoma [19]: malignant melanoma cells can be spindle-shaped or polygonal, with some having abundant pink cytoplasm and deviated nuclei, resembling rhabdomyosarcoma. Rhabdomyosarcoma cells are positive for myogenin and MyoD1, while S-100, MelanA, and HMB-45 are negative. Tumor cells can also appear in bundles, spindle-shaped or cigar-shaped, resembling leiomyosarcoma. Leiomyosarcoma cells are positive for desmin and SMA, while S-100, MelanA, and HMB-45 are negative. All malignant melanomas are negative for myogenic markers.

Due to minimal early visual impact, some patients overlook CMM, delaying treatment. Improving prognosis and survival depends on understanding the clinical and pathological features of CMM and raising patient awareness for early treatment.

In this study, both patients underwent extensive tumor resection combined with postoperative systemic chemotherapy of toripalimab and cisplatin, achieving favorable local control with no signs of recurrence or metastasis during the short-term follow-up (1–1.5 years). This outcome shows positive significance when compared with larger cohort studies. An international multicenter study involving 288 CMM patients reported that despite receiving surgery combined with adjuvant therapies (such as local chemotherapy or brachytherapy), the cumulative five-year and 10-year local recurrence rates were as high as 19.3% and 36.9%, respectively [20]. The absence of recurrence in our cases within a relatively short follow-up period suggests that the surgical resection combined with immunochemotherapy regimen we adopted may provide additional benefits in controlling local recurrence. Currently, the treatment of CMM is becoming more diversified, and the therapeutic choices in our cases can be cross-referenced with recent literature advances. For patients with high-risk factors after surgical excision, local adjuvant therapy remains a standard option. Apart from the systemic treatment used in our cases, local agents (such as mitomycin C or interferon α-2b) and adjuvant brachytherapy (e.g., iodine-125 plaque) are commonly employed and effective modalities [21]. Particularly for lesions involving the sclera, postoperative adjuvant iodine-125 plaque radiotherapy has demonstrated favorable tumor control rates and potential for vision preservation in medium-term follow-up [22]. Meanwhile, immune checkpoint inhibitors (such as anti-PD-1 agents) have shown promise in the treatment of advanced CMM [23]. The use of toripalimab in this study was based on its established efficacy in melanoma treatment [24], and experience with systemic immunotherapy as postoperative adjuvant therapy for CMM remains limited. The favorable outcome in our cases provides preliminary clinical support for this strategy. Furthermore, the *BRAF* V600E mutation detected in patient 2 offers a critical direction for potential salvage therapy in the future. For advanced melanoma with *BRAF* mutations, combination therapy with *BRAF*/MEK inhibitors has become the standard. Although the incidence of this mutation is relatively low in CMM, once present, targeted therapy represents an important systemic treatment option.

The two cases of CMM reported in this study, despite differences in clinical presentation and disease duration, both achieved good local control and short-term recurrence-free survival through comprehensive imaging evaluation, wide surgical excision, and combined postoperative

systemic chemotherapy. This offers the following insights for the management of similar clinical cases: CMM often presents as a painless pigmented lesion in its early stages, easily overlooked by patients. Clinicians should maintain a high index of suspicion for any new or changing pigmented lesions in the conjunctival region, particularly in middle-aged to elderly males and individuals of Caucasian descent. It is recommended to utilize imaging studies such as orbital CT and color Doppler ultrasound to assess the lesion extent and vascularity, and to perform early pathological biopsy with immunohistochemistry (e.g., HMB45, S-100, Melan-A) for definitive diagnosis. Surgical excision remains the cornerstone for localized disease, aiming for histologically complete resection (negative margins). For extensive or recurrent lesions, wider excision combined with eyelid reconstruction and conjunctival sac formation should be considered. Both patients in this study received postoperative systemic therapy with toripalimab combined with cisplatin, highlighting the potential value of combined immunotherapy and chemotherapy in the adjuvant setting. Furthermore, the detection of a *BRAF* V600E mutation in patient 2 suggests that such patients may benefit from *BRAF* inhibitor-targeted therapy. Therefore, we recommend genetic testing (e.g., for *BRAF*, *c-Kit*, NRAS) for all confirmed patients to guide subsequent targeted or immunotherapy choices. CMM carries a high risk of recurrence and metastasis, necessitating lifelong regular follow-up including ocular examination and systemic imaging. The treatment team should involve ocular oncologists, pathologists, medical oncologists, and radiation oncologists to collaboratively develop and adjust treatment plans for comprehensive disease management.

In summary, the management of CMM should emphasize a comprehensive strategy of early diagnosis, prompt and complete surgical excision, personalized adjuvant therapy, and long-term follow-up. Further prospective studies are needed to establish the efficacy and safety of standardized chemotherapy, targeted, and immunotherapy regimens for this rare malignancy.

Conflict of interest: None declared.

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Лечење масивног малигног меланома конјунктиве – анализа два случаја

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САЖЕТАК

Увод Малигни меланом конјунктиве представља изразито малигни тумор. Због ниске инциденције, атипичних раних клиничких манифестација и разноврсних патолошких облика, у клиничкој пракси се лако погрешно дијагностикује или превиди, а прогноза је изузетно неповољна. Циљ ове студије је приказ исхода лечења два случаја масивног малигног меланома конјунктиве, ради пружања увида у клиничку дијагностику и терапијске стратегије.

Приказ болесника Приказана су два случаја масивног малигног меланома конјунктиве. Оба болесника јавила су се у нашу установу због црних тумефакција на очном капку, које су након испитивања дијагностиковане као малигни меланом. Након хируршке ресекције тумора примењена је комбинована терапија периоралном применом темозоломида и интравенском применом цисплатина. Током једногодишњег периода праћења није забележен рецидив.

Закључак Иако је малигни меланом конјунктиве редак и у раној фази може имати суптилне симптоме, благовремена дијагноза, темељна хируршка ресекција у комбинацији са индивидуализованом адјувантном терапијом (укључујући хемиотерапију, циљану терапију и имунотерапију) могу побољшати прогнозу болесника. Повећање свести код клиничара и болесника, као и примена прецизне терапије вођене генетским тестирањем, кључни су за унапређење терапијских исхода.

Кључне речи: малигни меланом; малигни меланом конјунктиве; хемиотерапија; приказ случаја

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

New promising repurposed drugs and vaccines anticancer treatment possibilities – review of the current reports

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SUMMARY
The potential of repurposing already approved and registered non-oncological drugs and vaccines for the development of new anticancer treatments is presented. A large number of registered non-oncological drugs and vaccines modulate key cancer-related processes (such as neoangiogenesis, apoptosis, necroptosis, ferroptosis, autophagy, and aerobic glycolysis) by targeting the same upstream or downstream molecular biomarkers and signaling pathways, in the same anticancer direction. Beyond individual agents, combinations of repurposed drugs and vaccines are of greater interest for investigation, as they may reveal synergistic anticancer effects. Some repurposed drugs and vaccines are outlined for less toxic, more efficient, and affordable cancer therapies, with potential for further clinical investigations and possible impact on official clinical treatment guidelines. This review aims to present current reports of drugs and vaccines repurposed for anticancer treatment.
Keywords: cancer treatment; repurposed drugs; vaccines

INTRODUCTION

The standard oncological treatment regimen, as defined in the regularly updated Clinical Practice Guidelines and implemented globally, typically includes surgery, radiation therapy, chemotherapy, hormone therapy, targeted therapy, immunotherapy, photodynamic and laser therapy, cryotherapy, thermal therapy, stem cell therapy, and nanomedicine-based therapies. Cancer management involves expert diagnosis, treatment planning, symptom management, supportive care, and addressing complications related to cancer or its treatments, such as those arising during radiotherapy or chemotherapy. Drug and vaccine repurposing in cancer treatment explores the potential of previously approved, affordable, and less toxic or less expensive pharmaceuticals as new weapons in the fight against cancer [1, 2].

DRUG REPURPOSING

Many pharmaceuticals, originally developed for non-cancer conditions, now demonstrate significant potential as cost-effective and less toxic supportive therapies in oncology. Examples of such medications include metformin, ivermectin, chloroquine, itraconazole, some antibiotics, ribavirin, propranolol, simvastatin, fenofibrate, acetylsalicylic acid, diclofenac, celecoxib, omeprazole, caffeine, disulfiram, ursodeoxycholic acid (UDCA), 2-deoxyglucose, melatonin, vitamin D3, lithium, haloperidol, COVID-19 vaccine, etc. Selected newer examples of drug repurposing for cancer therapy are presented in Table 1. Clinical trials are currently underway with some of these repurposed medications, which have shown encouraging anticancer effects [1, 2].

A large number of the aforementioned registered non-oncological drugs modulate key cancer-associated processes (proliferation, neoangiogenesis, aerobic glycolysis, multidrug resistance, apoptosis, necroptosis, pyroptosis, ferroptosis, autophagy) by acting in the same anticancer direction on overlapping molecular targets / biomarkers, such as (abbreviations used: tumor promoter (P), tumor suppressor (S); effects on other targets: stimulation /, inhibition \):

1) Transcription factors: NF-κB a (P – tumor promoter), p65 (Rel A) (P), Integrins (P), STATs (P), Nrf2 (S – tumor suppressor), Nanog (P), KLF4 (P), Sox2 (P), Oct4 (P), JNKs (P), HES1, K13, MUC2 (P), VDR – vitamin D receptor (S), Hippo \ YAP (P), TCFs (P), LEFs (P);

2) Cell cycle proteins: Cyclin D_1 (P), CDKs (P), p21 (S), p27 (S), Ki-67 (P), PCNA (P), Akt = PKB (P), mTOR (P), EGFR (P), Raf (P), RAS (P), MAPK (P), PI3K (P);

3) Cell signaling cascade proteins: MDR (MDPRs, MRP1–7, P-gp tyrosine kinase) (P), IκB (P), Hedgehog (P), ABC (P), PKC (P, S), JAKs (P), Wnt / β-catenin (P), ILs (IL1, IL6, IL8, IL10, IL17) (P), IGF-1 (P), PTEN (S), Eps8 (P);

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Table 1. Selected newer examples of drug repurposing for cancer therapy

Pharm. class / drug name	Type of cancer cells tested (in order of incidence) as promising new therapeutic indication															
	Breast	Lung	Colon	Prostate	Melanoma	Leukemia	Liver	Pancreatic	Ovarian	Kidney	Endocrine	Brain	Glioblastoma	Neuroblas-toma	Cervical	Various / other cancer
Anti-diabetics																
Metformin	+	+	+	+				+							+	
Gliclazide	+	+	+	+				+							+	
Antihelmintics																
Ivermectin	+		+	+				+								
Flubendazole	+	+	+		+	+	+							+		
Anti-malarial																
Chloroquine	+						+	+					+			
Anti-fungal																
Itraconazole		+		+												+
Clotrimazole	+	+	+													
Antibiotics																
Doxycycline			+	+												+
Minocycline	+								+				+			
Ciprofloxacin			+	+		+				+						+
Antiviral																
Ritonavir	+					+		+	+							
Nelfinavir	+	+							+							
Acyclovir	+												+			
Ribavirin	+	+				+			+							
Cidofovir													+			+
Beta-blockers																
Propranolol	+	+	+	+	+	+		+	+					+		+
Carvedilol	+	+		+	+	+							+			
Antihyperlipidemics																
Simvastatin	+	+		+												+
Fenofibrate	+	+														
Angiotensin receptor blockers																
Losartan	+	+			+			+	+							+
Candesartan			+	+			+			+						
ACE inhibitors																
Captopril			+	+			+			+						
Enalapril			+													
Direct vasodilators																
Minoxidil	+								+							
Hydralazine	+			+					+							+
Potassium K^+ channel inhibitors																
Glipalamide	+	+			+											+
Verapamil	+	+		+		+		+						+		
Calcium channel blockers																
Mibefradil	+					+							+			+
Nifedipine	+		+													
Immunosuppressive																
Rapamycin	+															+
Nonsteroidal anti-inflammatory drugs (NSAID)																
Acetylsalicylic acid	+		+	+			+	+	+							+
Diclofenac	+		+	+			+	+	+				+			+
Celecoxib	+	+	+	+					+							+
Etoricoxib	+	+	+					+								
Antipsychotic																
Haloperidol								+					+			
Anti-epileptic																
Lamotrigine	+											+				
Mood stabilizer																
Lithium		+		+		+					+			+		+

4) Apoptosis and/or necroptosis associated proteins: Bcl-2 (P), Bax (S), Cytochrome C (S), PARPs (P), p53 (S), p63 (S), p73 (P, S), caspase 3, 8, 9, 12 (S), p38MAPK (P), Mcl-1 (P);

5) Death receptors on the cell surface: FAS (S), TRAIL (P, S);

6) Autophagy (ATG) related proteins: ATG (ATG5, ATG9A, ATG12, ATG13) (P, S), LC3 (S), Beclin1 (P, S), p62 (P), PAK1 (P);

7) Antioxidant defense proteins and other targets / markers: ROS (P, S), COX-2 (PTG S2) (P), iNOS / NO (P), TNF-α (P), PGE2 (P);

8) Angiogenesis and tumor microenvironment-associated proteins: CD31 (= PECAM1) (P), CD34 (P), HIFs (HIF-1α, HIF-1β, HIF-2α, HIF-2β) (P), VEGF / VEGFR (P), MEK / ERK (P), CXC (P), CXCL12 (= SDF1) (P), CXCR4 (P), FAK (= PTK2) (P);

9) Cellular metabolism and metabolic reprogramming associated proteins: GLUTs (1–13) (P), TLRs (1–24) (P);

10) Embryonic development-associated proteins: TGF-β / SMAD (P);

11) Membrane receptors: PANX1 (P), P2X4 (P), P2X7 (P);

12) Membrane-bound proteins (transcription factors) shuttling between the cytoplasm and nucleus: Notch (P);

13) Oncogene addiction targets: PTK (P), MYC (C-, N-, L-) (P), cABL (P).

Also, the aforementioned drugs modulate various signaling pathways in the same anticancer direction. A significant proportion of anticancer signaling pathways are functionally linked to NF-κB signaling, either upstream, downstream, or directly [3] (symbols used for effects on other targets: stimulation /, inhibition \): IκB (IKK)/NF-κB/STAT3\apoptosis; EGFR/RAS/Raf/p38/MAPK/MEK/ERK/Akt/mTOR/NF-κB/P-gp (MDPR); PI3K/Akt/JNK/IκBα/NF-κB/P-gp; TGF-α/PI3K/Akt/mTOR/HIF/IκBα/NF-κB/HIF/VEGF; PI3K/PKC/NF-κB…; STAT3/PI3K/Akt/…/NF-κB…; p38/MAPK/PI3K/Akt/…/NF-κB …; p38/MAPK/JNK/ERK/Akt/IκBα/NF-κB…; TNF/IκB (IKKα, IKKα, IKKγ = NEMO)/NF-κB…; IL1/IκB/NF-κB…; TLRs/IκB/NF-κB…; TLRs/IL1/IκB/NF-κB…; TLR4/TNF-α, IL1α/IκB/NF-κB…; TLRs/p38/MAPK/PI3K/Akt/…/NF-κB…; TLRs/p38/MAPK/ERK/Akt/IκBα/NF-κB…; PANX1/ILs/NF-κB…; P2X4, P2X7/ROS/…/NF-κB. Non-oncological agents such as mebendazole, deoxycholic acid, and folinic acid stimulate NF-κB, a key cellular signaling pathway that promotes cell survival and proliferation while inhibiting apoptosis, thereby fostering pro-tumorigenic processes.

Combining chemically distinct non-oncologic agents targeting the same anticancer pathways can enhance efficacy without increasing toxicity, potentially offering synergistic anticancer regimens. The overlap in anticancer targets justifies parallel rescue and dose–response experiments.

Among antidiabetic medications, metformin may demonstrate anticancer properties, potentially inhibiting cancer cell proliferation, promoting apoptosis, and increasing cancer cell sensitivity to chemotherapy. Further studies are crucial to clarify the mechanisms of anticancer effects and assess the clinical efficacy and safety of antidiabetic drugs [4].

Our experimental findings *in vivo* demonstrated that metformin administered alone [5] or in combination with one of the following agents: caffeine [6], disulfiram [7, 8], nitroglycerin [9, 10, 11], itraconazole [10, 11], or diclofenac [11, 12] exerted significant anticancer effects against fibrosarcoma in hamsters, without inducing observable toxicity. Importantly, the administered doses were equivalent to standard therapeutic doses used in humans. Anticancer effects of examined dual drug combinations were validated through dose-response experiments and synergism was determined by Combination Index (CI) analysis (CI < 1). The hypothesis that the examined combinations act synergistically against cancer cells through NF-κB inhibition was successfully tested in rescue experiments by adding an NF-κB stimulator, such as mebendazole or deoxycholic acid, to these combinations in order to abrogate the anticancer effects and 'rescue' tumor growth. Therefore, the use of metformin in combination with caffeine, disulfiram, nitroglycerin, itraconazole, or diclofenac may represent a promising and safe adjuvant anticancer approach, warranting further clinical investigation [6–12]. We anticipate that the therapeutic efficacy of metformin, when combined with anticancer therapies, will exceed that of the same therapies administered without metformin. It is our expectation that such strategies will provide a more effective and safer treatment approach in clinical practice, while also guiding future research into the potential of metformin in cancer therapy [13].

In a clinical study of patients with advanced cervical cancer using fluoroazomycin arabinoside, metformin decreased tumor hypoxia and improved the cervical cancer response to radiation [14].

An *in vitro* investigation on repurposed pharmacological agents revealed that the combination of disulfiram and copper gluconate displayed a marked cytotoxic effect against glioblastoma stem cells [15].

A recent *in vitro* study repositioned propranolol, a non-selective beta-blocker commonly used in the treatment of various cardiovascular conditions, as a promising and cost-effective therapeutic option for colorectal cancer treatment, identifying T cells as its primary target [16]. Oral administration of propranolol has been shown to delay tumor progression and improve survival rates of tumor-bearing mice in fibrosarcoma and colon cancer models. Propranolol inhibited tumor angiogenesis and promoted T cell infiltration. A recent study identified propranolol as an immunomodulatory agent, suggesting its potential to enhance immunotherapies with checkpoint inhibitors in patients with soft tissue sarcoma, and possibly in other cancer types [17].

An *in vitro* study demonstrated that propranolol (non-selective β1/2-adrenergic receptor blocker) and selective β2-adrenergic receptor blockers induced apoptosis in human colorectal carcinoma HCT116 cells following radiation treatment. Furthermore, β2-adrenergic receptor blockade markedly inhibited tumor growth *in vivo* in nude mice bearing HCT116 colorectal cancer xenografts, suggesting its potential use as an adjuvant strategy to enhance clinical outcomes of colorectal cancer after radiotherapy [18].

A recently identified novel class of ciprofloxacin–amino acid conjugates has demonstrated significant potency against human breast, colon, and lung cancer cell lines. These results indicate that the proper modification of ciprofloxacin could lead to the development of promising anticancer agents through appropriate derivatization in easy synthetic processes [19].

Itraconazole demonstrated promising properties as an anticancer agent and may be a potent adjuvant to immunotherapy for endometrial cancer [20]. Antifungal drug itraconazole *in vitro* effectively inhibited the proliferation and invasion of Ishikawa cells by inducing apoptosis. *In vivo* experiments on tumor-bearing mice further confirmed its synergistic potential in combination with immune checkpoint inhibitors: tumor volume and weight were significantly reduced [20].

A recent study highlighted the anticancer effects of antihistamine loratadine on lung adenocarcinoma cells, both *in vitro* and *in vivo*. Loratadine inhibited cell proliferation, increased autophagy and apoptotic cell death. In the mouse model, loratadine reduced tumor growth and angiogenesis, while promoting autophagy and apoptosis. These findings suggest that loratadine may serve as a potential therapeutic agent for inhibiting lung adenocarcinoma progression, warranting further investigation [21]. A retrospective study involving 4522 patients with lung cancer during the 2006–2018 period demonstrated a positive correlation between loratadine administration and improved survival outcomes, with a dose-dependent effect. Higher loratadine uptake was linked to better survival rates in lung cancer patients. Additionally, lung cancer mortality showed a dose-dependent reduction as the cumulative use of loratadine increased [22].

The results of a seven-year retrospective study that included 734 patients with immunogenic tumors revealed that patients treated with cationic amphiphilic antihistamines desloratadine, cyproheptadine, and ebastine exhibited significantly improved median overall survival and progression-free survival, along with a nearly 50% reduction in the risk of all-cause mortality [23].

A retrospective analysis demonstrated that melanoma and lung cancer patients who received H1-antihistamines during immunotherapy treatment showed significantly better clinical outcomes, including improved survival rates. These results strongly suggest the potential benefit of using antihistamines in cancer patients with allergies and elevated plasma histamine levels [24].

An *in vitro* study conducted on HeLa cells revealed novel mechanisms of action for azelastine hydrochloride, a drug commonly used in antiallergic treatment. The results highlighted the anti-proliferative, cytotoxic, autophagic, and apoptotic effects of azelastine on HeLa cells, suggesting its potential for future application in cancer therapy [25].

A recent *in vitro* study demonstrated that acetylsalicylic acid produced apoptosis in human colon cancer cell line HT29 in a manner that is dependent on its concentration [26].

A three-year, double-blind, randomized, placebo-controlled trial was conducted with patients diagnosed with rectal and colon cancer. A total of 314 patients were administered acetylsalicylic acid, while 312 received a placebo. The results indicated that acetylsalicylic acid significantly reduced the incidence of cancer recurrence compared to the placebo group [27].

Dysregulation of estrogen receptor alpha, found in about 70% of breast cancers, is key to the disease initiation and progression. A recent in vitro study suggests that acetylsalicylic acid may be a potential therapeutic agent for targeting estrogen receptor alpha, particularly in breast cancers resistant to tamoxifen [28].

The cytotoxic effects of the novel nano Chitosan-Paracetamol composite were evaluated using the human colon cancer cell line HCT-29. The findings highlighted the potent biological activity of the nano composite, effectively inhibiting the proliferation of colon cancer cells [29].

Recent *in vitro* and *in vivo* studies on mouse models of adenocarcinoma, hepatocellular carcinoma, and breast cancer revealed that high doses of vitamin C (ascorbic acid), when combined with oncolytic adenoviruses, produced notable synergistic antitumor effects, including a significant increase in the number of T cells [30].

Experiments *in vitro* on renal carcinoma cell lines and *in vivo* on a mouse xenograft model demonstrated that vitamin C treatment enhanced the efficacy of immunotherapy and significantly increased the intratumoral infiltration of T cells, suggesting a potential role of vitamin C in anticancer therapy [31].

The combination of vitamin D and sericin demonstrated significant anticancer effects at low doses against human non-small cell lung cancer cells. *In vitro* findings revealed a strong correlation with *in silico* analyses, highlighting a significant enhancement of apoptosis in lung cancer cells [32].

A recent study demonstrated that magnetothermodynamic therapy, utilizing a novel vitamin K3 nanoparticle complex with copper, zinc, and ferrite, exhibited anticancer effects both *in vitro* and *in vivo* against lung adenocarcinoma. Complete tumor elimination was achieved *in vivo* within 30 days [33]. The synergistic therapy based on vitamin K3 nanoparticles showed significant anticancer effects by inducing apoptosis, causing tumor regression *via* reactive oxygen species (ROS) generation, inhibiting cell proliferation, and reducing metastasis [33].

A randomized controlled trial (2017–2021) involving 101 patients with hepatocellular carcinoma demonstrated that the combination of vitamin K and transarterial chemoembolization exhibited significant anticancer effects when compared to transarterial chemoembolization alone [34]. The results demonstrated that vitamin K supplementation enhanced the anticancer effects of transarterial chemoembolization by reducing the levels of des-γ-carboxy prothrombin, a well-known factor involved in tumor growth and angiogenesis, which is produced in hepatocellular carcinoma under hypoxia induced by transarterial chemoembolization [34].

Lithium, primarily utilized in the medical treatment of psychiatric disorders, exhibits certain anticancer properties, functioning through mechanisms such as apoptosis,

autophagy, cell cycle arrest, while also inhibiting proliferation, invasion and metastasis [35]. Lithium has shown anticancer activity *in vitro* against various types of cancer, including myeloma, neuroblastoma, glioblastoma, skin cancers, gastrointestinal cancers, breast cancer, hepatocellular carcinoma, lung cancer, nasopharyngeal cancer, pancreatic cancer, prostate cancer, colon cancer, and lymphatic tissue cancers. Clinical trials indicate that lithium may enhance anticancer effects when used alongside standard therapies in leukemia, small cell lung carcinoma, thyroid cancer, prostate cancer, and neuroendocrine tumors [36].

An *in vivo* study conducted on a subcutaneous tumor xenograft model of colorectal carcinoma demonstrated that treatment with the probiotic strain *Lactobacillus plantarum* L168 significantly reduced tumor volume in tumor-bearing mice [37]. Results demonstrated that *Lactobacillus plantarum* L168 and its metabolite, indole-3-lactic acid, contributed to preliminary activation of $CD8^+$ T cell immunity against tumor growth and improved the activity of tumor-infiltrating $CD8^+$ T cells by lowering their cholesterol levels [37].

In a preclinical mouse melanoma model, monotherapy with the probiotic strain *Bacteroides fragilis* BF839 led to significant tumor growth inhibition. When combined with anti-PD-1 antibody, it demonstrated a synergistic effect, promoting tumor regression. Furthermore, oral administration of BF839 notably enhanced the efficacy of immune checkpoint inhibitors in patients with advanced solid tumors, particularly in the long-term adjuvant treatment cohort, which showed significantly improved overall survival compared to those receiving short-term adjuvant therapy [38]. Using the probiotic BF839 to modulate gut microbiota could present a novel approach to improving the effectiveness of immune checkpoint inhibitors, particularly in long-term adjuvant tumor treatment [38].

VACCINES

Evidence supporting oncologic applications has been documented for 15 licensed vaccines. Ten of these: BCG, influenza, diphtheria, pneumococcus, tetanus, human papillomavirus (HPV), measles, smallpox, varicella-zoster and typhoid vaccines already have completed or ongoing clinical evaluations in cancer settings [39]. Among the remaining vaccines, pertussis, yellow fever, and rotavirus show preclinical activity that justifies structured clinical testing, while mechanistic data for the cholera vaccine, together with observational findings in colorectal cancer, also support further translational work. Several observations are of particular relevance: intravesical typhoid vaccine outperformed BCG in a preclinical bladder cancer model; perioperative influenza vaccination may counteract surgery-induced NK-cell suppression; intratumoral measles vaccine has produced objective responses in cutaneous T cell lymphoma; and HPV vaccination has elicited responses in cutaneous squamous cell carcinoma. Across these examples, vaccines act by initiating or enhancing anti-tumor immune activity, with outcomes influenced not only by their immunobiological properties but also by administration strategies and their integration with other (immuno)therapeutic modalities, which warrant closer examination in future studies [39]. In contrast, available evidence for hepatitis B and mumps (excluding the measles component) remains limited.

Many vaccines, including the COVID-19 vaccine, may selectively target tumor-associated antigens without affecting healthy cells. Identifying anticancer effects in vaccines already approved for other indications could significantly reduce both the time and cost of developing new oncology treatments. Protective efficacy of a live-attenuated SARS-CoV-2 vaccine was tested in Syrian golden hamsters and in mice [40]. Fourteen days after challenge, sera from immunized hamsters and mice had high antibody levels and neutralizing capacity. Immunization protected hamsters and mice against the virus eliciting neutralizing antibodies and T cell responses. Spatial (local) host gene expression near virus-infected sites of the top 100 genes in cancer-related pathways (mean pathway activity score) such as MAPK, JAK-STAT, TGF-β and TNF-α correlated with the immune response [40].

Recent experiments in tumor-bearing mice demonstrated that SARS-CoV-2 mRNA vaccines sensitized tumors to immune checkpoint blockade. Administration of these vaccines within 100 days of starting checkpoint inhibitors correlated with improved median and three-year overall survival across multiple large retrospective patient groups, including patients with immune non-responsive tumors. These findings indicated that clinically available mRNA vaccines targeting non-tumor antigens acted as potent immune modulators, enhancing tumor responsiveness to checkpoint inhibition [41].

Research in anticancer treatment includes vaccines, not only well-known ones, like BCG, but also personalized ones (based on an individual's specific characteristics). Immunization with an anticancer vaccine would represent a dream come true. Tailored mRNA cancer vaccine therapy (modified for specific populations or groups) represents an innovative approach aimed at targeting existing tumors in patients. An mRNA vaccine is engineered to elicit an mRNA-mediated immune response that specifically targets cancer cells. As of 2024, several mRNA cancer vaccines are undergoing clinical trials [42].

Personalized mRNA cancer vaccines constitute an advanced therapeutic modality that mobilizes the immune system to target malignant cells via tumor-specific antigens. As malignancies evolve, they accrue somatic mutations that generate neoantigens absent in normal tissues. Because these neoantigens are patient-specific, they offer both an opportunity for precision immunotherapy and a challenge in vaccine development. Clinically, personalized mRNA vaccines have shown promising outcomes: for example, in gastrointestinal cancers they elicited neoantigen-specific T cell responses and reduced tumor burden [43], and in late-stage pancreatic ductal adenocarcinoma and in non-small-cell lung cancer induced tumor size reduction in combination with pembrolizumab [44]. Despite this promise, several impediments remain: the manufacturing workflows are laborious and expensive; mRNA delivery,

stability and expression *in vivo* require further optimization. To address these issues, investigators are refining antigen-screening methodologies and exploring novel delivery platforms. Looking ahead, mRNA tumor vaccines are poised to become integral components of multimodal cancer therapy, complementing surgery, cytotoxic chemotherapy, radiotherapy and immune checkpoint inhibitors. They offer the potential to expand therapeutic options, improve survival and enhance quality of life for cancer patients. With continued technological maturation and clinical validation, personalized mRNA cancer vaccines may become a powerful tool in oncology [45].

New research suggests that oncolytic herpes simplex virus can be repurposed as a cancer vaccine by removing a virulence gene. Oncolytic viruses cause direct lysis of tumor cells, resulting in the release of soluble antigens, danger signals, and type I interferons that stimulate antitumor immune responses. A synergistic effect was observed between a modified oncolytic herpes simplex virus and a phosphoinositide 3-kinase (PI3K) inhibitor, resulting in suppression of tumor growth, prolonged survival, and the induction of robust antitumor immunity in a mouse model of ovarian cancer [46].

CONCLUSION

The fight against cancer can be strengthened through diverse strategies, including drug repurposing, anticancer agents, and vaccines. In alignment with the principle of '*Primum non nocere*', all the aforementioned therapeutic approaches and treatments possess the potential to improve current oncological practices. In conclusion, repurposing approved non-oncological drugs and vaccines offers a promising strategy for enhancing anticancer treatment outcomes, particularly as adjunct therapies to improve efficacy and as preventive measures to reduce the risk of relapse, thus warranting further clinical research to optimize their therapeutic potential. We hope that this review will serve as a valuable reference for advancing the clinical application of the anticancer effects of repurposed drugs and vaccines, and contribute to the accelerated development of combination therapies involving repurposed pharmaceuticals for cancer treatment.

Ethics: The authors declare that the article was written in accordance with the ethical standards of the journal and of institutions for each author included

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Conflict of interest: None declared.

Abbreviations

ABC - ATP-Binding Cassette
Akt - Protein Kinase B (PKB)
ATG (5, 9A, 12, 13) - Autophagy-Related Genes
Bax - B-Cell Lymphoma 2-Associated X Protein
Bcl-2 - B-Cell Lymphoma 2
Beclin1 - Autophagy Related 1
cABL - cytoplasmic Abelson tyrosine kinase
Caspase 3, 8, 9, 12 - Cysteine-Asparatic Proteases
CD31 (PECAM1) - Platelet Endothelial Cell Adhesion Molecule 1
CD34 - Cluster of Differentiation 34, a protein marking hematopoietic stem cells
CDKs - Cyclin-Dependent Kinases
COX-2 (PTGS2) - Cyclooxygenase-2
CXC - CXC chemokines, a family of small signaling proteins, CXC motif refers to the sequence of cysteine residues in the protein, Cys-X-Cys arrangement, where X is amino acid
CXCL12 (SDF1) - C-X-C Motif Chemokine Ligand 12, (known as Stromal-Derived Factor 1, SDF1)
CXCR4 - C-X-C Motif Chemokine Receptor 4
Cyclin D1 - a crucial cell cycle protein, drives cell cycle progression by activating CDKs
Cytochrome C –electron-transporting protein crucial for cellular respiration and apoptosis
EGFR - Epidermal Growth Factor Receptor
MEK - Mitogen-Activated Protein Kinase
mTOR - Mechanistic Target of Rapamycin
MUC2 - Mucin 2
MYC (C-, N-, L-) - Myelocytomatosis Oncogene (C-, N-, L- Types)
Nanog - Homeobox Transcription Factor Nanog
NF-κB - Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
Notch - Notch Receptor Signaling Pathway (in development and tissue homeostasis)
Nrf2 - Nuclear Factor Erythroid 2-Related Factor 2
Oct4 - Octamer-Binding Transcription Factor 4
p21 - Cyclin-Dependent Kinase Inhibitor 1A
p27 - Cyclin-Dependent Kinase Inhibitor 1B
P2X4, P2X7- P2X Purinoceptor (4, 7)
p38 - Mitogen-Activated Protein Kinase p38
p53 - Tumor Protein P53
p62 - Sequestosome 1 Protein
p63 - Tumor Protein p63, a transcription factor, a member of the p53 family of tumor-suppressor proteins
p65 (Rel A) - p65 Subunit of NF-κB (RelA)
p73 - Tumor Protein p73
PAK1 - p21-Activated Kinase 1
PANX1 - Pannexin 1
PARPs - Poly(ADP-Ribose) Polymerases

Eps8 - Epidermal Growth Factor Receptor Pathway Substrate 8
ERK - Extracellular Signal-Regulated Kinase
FAK (PTK2) - Focal Adhesion Kinase
FAS - Fatty Acid Synthase
GLUTs (1-13) - Glucose Transporters
Hedgehog - Hedgehog Signaling Pathway
HES1 - Hairy and Enhancer of Split 1 (transcription factor)
HIFs (HIF-1α, HIF-1β, HIF-2α, HIF-2β) - Hypoxia-Inducible Factors (1α, 1β, 2α, 2β)
Hippo - Hippo Signaling Pathway (in cell growth)
IGF-1 - Insulin-Like Growth Factor 1
IL1β - Interleukin 1 Beta
ILs (IL1, IL6, IL8, IL10, IL17) - Interleukins
iNOS - Inducible Nitric Oxide Synthase
IκB (IKK) - Inhibitor of κB, regulates NF-κB
IKKα, IKKβ, IKKγ (= NEMO) - IκB Kinase Subunits (α, β, γ)
IκBα - Inhibitor of κB Alpha, protein that regulates the Nuclear Factor kappa B (NF-κB) pathway
JAK - Janus Kinase
JNK - c-Jun N-terminal Kinase
K13 - Kinesin Family Member 13
KLF4 - Krüppel-Like Factor 4
Ki-67 - Antigen for Monoclonal Antibody Ki-67
LC3 - Microtubule-Associated Protein 1 Light Chain 3
LEFs - Lymphoid Enhancer-Binding Factors
MAPK - Mitogen-Activated Protein Kinase
Mcl-1 - Myeloid Cell Leukemia-1
MDPRs - Multi-Drug Resistance Proteins
MDR - Multi-Drug Resistance
PCNA - Proliferating Cell Nuclear Antigen
PGE2 - Prostaglandin E2
P-gp - P-Glycoprotein (permeability glycoprotein, also known as ABCB1), a drug efflux pump
PI3K - Phosphoinositide 3-Kinase
PKC - Protein Kinase C
PTEN - Phosphatase and Tensin Homolog
PTK - Protein Tyrosine Kinase
Raf - Rapidly Accelerated Fibrosarcoma Kinase
RAS - Rat Sarcoma Viral Oncogene Homolog
ROS - Reactive Oxygen Species
SMAD - SMAD Family of Proteins (involved in TGF-β signaling)
Sox2 - SRY (Sex-Determining Region Y)-Box Transcription Factor 2
STATs - Signal Transducers and Activators of Transcription
TCFs - T-Cell Factors (transcription factors in Wnt signaling)
TGF-α, TGF-β - Transforming Growth Factor (α, β)
TLRs - Toll-Like Receptors
TNF - Tumor Necrosis Factor
TNF-α - Tumor Necrosis Factor Alpha
TRAIL - TNF-Related Apoptosis-Inducing Ligand
VDR - Vitamin D Receptor
VEGF - Vascular Endothelial Growth Factor
VEGFR - Vascular Endothelial Growth Factor Receptor
Wnt - Wingless-Int Family
YAP - Yes-Associated Protein (in Hippo signaling)
β-catenin - Beta-Catenin (Cadherin-Associated Protein), in Wnt signaling pathway and cell adhesion

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Нове обећавајуће антиканцерске терапије пренамењеним лековима и вакцинама – преглед најновијих истраживања

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САЖЕТАК

У раду се приказује потенцијал пренамењених већ одобрених и регистрованих неонколошких лекова и вакцина за развој нових антиканцерских терапија. Велики број регистрованих неонколошких лекова и вакцина модулише кључне процесе повезане са канцером (као што су неоангиогенеза, апоптоза, некроптоза, фероптоза, аутофагија и аеробна гликолиза), делујући на исте усходне или нисходне молекуларне мете, биомаркере и сигналне путеве, у истом антиканцерском правцу. Осим појединачних, за испитивање су много интересантније комбинације пренамењених лекова и вакцина где може бити откривено синергистичко антиканцерско деловање. Неки пренамењени лекови и вакцине се истичу као потенцијални кандидати за мање токсичне, ефикасније и приступачније терапије канцера, са потенцијалом за даља клиничка истраживања и могућим утицајем на званичне клиничке смернице лечења. Овај преглед има за циљ да представи резултате најновијих истраживања неонколошких лекова и вакцина пренамењених за лечење канцера.

Кључне речи: антиканцерска терапија; пренамењени лекови; вакцине

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

The language of medicine today: English as the new Latin – benefits and challenges

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SUMMARY
The language of medicine constitutes a specialized register characterized by precision, distinctive functional elements, and historical continuity. Rooted in Latin and Greek, medical terminology has long served as the foundation of stable cross-linguistic communication. For centuries, Latin functioned as the lingua franca of medical education, scholarship, and clinical practice, before gradually being replaced by vernacular languages. After World War II, English emerged as the dominant language of medicine, supported by the geopolitical influence of Anglophone countries, the rise of international organizations, and the globalization of medical publishing and education.
The aim of this paper is to critically examine the establishment of English as the new Latin in global medical communication, highlighting both the benefits and challenges of this phenomenon. The primary benefits include universality of communication, standardized terminology and education, facilitated access to scientific literature, international collaboration, efficiency in crisis situations, as well as increased visibility and impact of scholarly research. Conversely, the challenges entail linguistic inequality, obstacles for non-native speakers, loss of linguistic and cultural diversity, bias in research dissemination, and limited accessibility for patients.
Undoubtedly, medical English has become the lingua franca of the international health care community in the 21st century. Yet concerted efforts are required to ensure professional inclusivity, preserve linguistic diversity, and establish a balance between the principles of efficiency and equity in future global medical communication.
Keywords: medical English; the Latin language; health care communication; history of medicine

INTRODUCTION

The language of medicine represents a specific functional professional register [1]. Among its defining features are a branching semantic structure encompassing a broad general terminological field and narrow sub-terminological fields, as well as a balanced parallelism in the use of both oral and written discourse. When we add to this the requirement that health care professionals must use this language of science and professional practice with great precision in communication among themselves, with patients, and with the wider public, the importance of mastering it becomes even more evident. Medical languages currently in use across the world, including medical English, are fundamentally rooted in Latin, with a substantial portion of vocabulary derived from Greek. These Greco-Latin elements continue to shape the morphology and semantics of medical terminology, ensuring stability and universality across linguistic boundaries [2]. For centuries, Latin served as the lingua franca of communication within the medical community, both at the scientific and clinical diagnostic levels. Over time, however, its role was gradually assumed by English. One of the aims of this paper is to examine the timing and reasons for this transition.

More specifically, the aim of this paper – and at the same time its principal significance – is to provide a critical reflection on the benefits and challenges associated with the establishment of English as the dominant language of today's global medical community. To this end, the study employs a diachronic method, a descriptive-comparative approach, and explanatory analysis. These methods were employed to present a succinct historical account of the evolution of the professional language under consideration, to delineate and compare the principal reference sources that have addressed this subject, and to provide an analytical exposition of the key arguments advanced by both perspectives.

HISTORICAL BACKGROUND: LATIN AS THE LANGUAGE OF MEDICINE

There are numerous scholarly studies that examine the historical perspective of Latin in the field of medicine. These works address the role of Latin in medieval and Renaissance medicine, its use in medical education, prescriptions, and scholarly writings, the shift from Latin to vernacular languages, as well as the enduring legacy of Greek and Latin roots in medical terminology.

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According to Marečková et al. [3], Latin functioned as the language of medical treatises, university lectures, and diagnostic discourse for centuries, thereby ensuring a high degree of uniformity across Europe's medical community. It was equally important in education and practice – medical curricula in European universities were largely based on Latin texts, while physicians and pharmacists relied on Latin prescriptions to guarantee precision and universality [4, 5].

Beginning in the 18th and 19th centuries, Latin gradually lost its exclusive status as the language of medicine. The rise of national identities, the expansion of vernacular scientific publishing, and the democratization of education contributed to the adoption of local languages in medical discourse. Medical treatises and textbooks increasingly appeared in German, French, and other European vernaculars, reflecting cultural shifts and the need for broader accessibility [3]. This transition marked a turning point in the linguistic history of medicine, as Latin ceased to be the sole medium of scholarly and clinical communication. Despite this shift, the legacy of Latin – together with Greek – remains deeply embedded in medical terminology. The majority of anatomical, pathological, and pharmacological terms are Greco-Latin hybrids, combining Greek roots with Latin morphological structures. This hybrid system continues to provide precision, universality, and semantic stability across languages [2, 6].

THE RISE OF ENGLISH IN MEDICAL COMMUNICATION

During the 19th and early 20th centuries, the linguistic landscape of medical science underwent significant changes. Scholarly communication was multilingual, with German, French, and English used almost equally. As noted by Baethge [7], during the interwar period (1920s–1930s → 1920s–1930s), German held a dominant position in clinical research and pathology, while French was particularly influential in tropical medicine and neurology. European colonial expansion spread Western biomedical practices worldwide, embedding English alongside French in colonial medical institutions [8].

The linguistic balance shifted dramatically after World War II. The decline of German and French influence coincided with the growing geopolitical and scientific dominance of the United States and the United Kingdom. As a result, English gradually displaced other languages and became the principal medium of international medical communication [9]. Keller [8] observes that the subsequent growth of multinational health organizations, international medical education, and globalized research networks further reinforced English as the default language of medicine. In short, by the late 20th century, it became the lingua franca of international medical communication, effectively replacing the earlier role of Latin.

At present, English dominates medical publishing and international professional exchange. Leading scientific journals, including *The Lancet, New England Journal of Medicine,* and *BMJ*, require submissions in English, while major databases such as PubMed and the Cochrane Library index primarily English-language content [7]. While this ensures global accessibility, it also highlights issues of modern medical colonialism [10] as well as linguistic inequality for non-English-speaking researchers [11]. Beyond publishing, English is the language of international medical conferences, professional associations, and collaborative research networks. Global organizations such as the World Health Organization and the World Medical Association conduct their congresses and issue guidelines in English, thereby enabling communication across borders and facilitating multinational cooperation [12, 13]. In university programs and international training initiatives, English has likewise become the default medium of instruction, preparing future physicians to work in diverse, multinational environments [14].

BENEFITS OF ENGLISH AS THE NEW LATIN

It can be stated with certainty that there are numerous benefits to the use of English as the medium of communication within the international medical community. The establishment of English as the new Latin has provided medicine with a unified linguistic framework that ensures clarity, accessibility, and collaboration across borders. Standardized medical terminology enables health care professionals worldwide to communicate with precision and without ambiguity, facilitating the exchange of scientific and clinical opinions, practices, and achievements. This linguistic uniformity reduces the risk of misinterpretation, strengthens the accuracy of clinical documentation and guidelines, and ultimately contributes to safer and more reliable patient care, particularly for individuals who seek treatment outside their home countries [3].

As noted earlier, the dominance of English in medical publishing has further reinforced its role as the global language of medicine. According to the findings of Baethge [7], "analyses of MEDLINE records show that between 1995 and 2009, English articles accounted for 87–90% of PubMed entries, rising to over 93% by 2010–2014, more than 95% by 2015–2019, and exceeding 97% in 2020–2023." Publishing in English therefore guarantees greater visibility, citation frequency, and recognition, allowing important findings to reach the widest possible audience and accelerating the dissemination of innovations across the global medical community [15].

English also plays a decisive role in education. The widespread availability of textbooks, teaching materials, and online resources in English has transformed medical education into an international enterprise. Students and young physicians from diverse linguistic backgrounds can access the same authoritative sources, ensuring uniformity in training and knowledge acquisition. International medical schools and exchange programs increasingly adopt English as the language of instruction, preparing graduates for practice and facilitating their mobility in multinational environments [14]. In this way, English contributes to the development of a global medical workforce equipped to meet the challenges of modern health care.

Equally important is the role of English in fostering collaboration. Multinational clinical trials, international guidelines, and large-scale research projects rely on English as their working language, enabling diverse teams to coordinate effectively and share results with transparency. Worldwide medical societies and regulatory bodies organize their congresses and circulate recommendations in English, thereby establishing a common linguistic framework for cross-border collaboration [12, 13]. This linguistic standardization strengthens global networks, supports evidence-based practice, and enhances the efficiency of collaborative problem-solving in health care [12, 13].

Finally, English simplifies communication among international companies engaged in biomedical activities. Legal, financial, and professional interactions are facilitated when a single language is employed, reducing administrative complexity and ensuring smoother cooperation in areas such as pharmaceutical development, medical technology, and global health initiatives.

Taken together, these benefits demonstrate why English has assumed the role once held by Latin: it provides medicine with a common linguistic foundation that ensures clarity, accessibility, visibility, and collaboration. By unifying medical communication across publishing, education, clinical practice, and international cooperation, English has become indispensable to the advancement of global health.

CHALLENGES IN GLOBAL MEDICAL COMMUNICATION

On the other hand, there are challenges and drawbacks associated with the status of English as a lingua franca. Relying on a single language for international communication creates barriers that affect equity, diversity, and the dissemination of knowledge.

One major concern is the language barrier. Researchers who are not native speakers of English often encounter obstacles when preparing manuscripts or presenting at international forums. These difficulties extend beyond vocabulary to include rhetorical conventions and stylistic norms that may disadvantage otherwise high-quality work [11].

Closely connected to this is the issue of inequality. Mastery of English increasingly serves as a filter for professional advancement, determining who gains access to prestigious journals, collaborative projects, and career opportunities. This dynamic reinforces existing hierarchies, privileging scholars from Anglophone countries while limiting visibility for others [11, 16].

The predominance of English also contributes to a loss of diversity. Local medical traditions and indigenous knowledge systems risk being overshadowed when research is conducted and published exclusively in English. This reduction in linguistic plurality narrows the epistemological base of medicine and may hinder culturally sensitive approaches to health care [9].

Another challenge is bias in research dissemination. Non-English studies are frequently underrepresented in indexing services and systematic reviews, despite their relevance and quality. This selective visibility distorts the global evidence base, privileging English-language findings while sidelining important regional perspectives [15].

Finally, the dominance of English has profound educational implications. In countries such as Serbia, and across Eastern Europe, medical students face the dual responsibility of mastering complex scientific content while simultaneously acquiring advanced English skills. This dual burden places them at a disadvantage compared to peers in Anglophone contexts.

Taken together, these challenges underscore the paradox of English as the new Latin: while it facilitates global communication, it also risks excluding voices and perspectives essential to the richness of medical science. Addressing these issues requires deliberate efforts to promote linguistic inclusivity, diversify publication practices, and recognize the value of multilingual contributions to global health.

CONCLUSION

In summary, medical English today stands as the modern equivalent of Latin, functioning as the universal language of health care. Its advantages are clear: a unified linguistic framework that ensures precision, accessibility, and international collaboration; enhanced visibility and dissemination of research; standardized education and training; and strengthened global networks that support evidence-based practice. On the other hand, its dominance also brings challenges: language barriers for non-native speakers; inequalities in career advancement; loss of linguistic and cultural diversity; bias in research dissemination; and additional burdens on medical education in non-English-speaking countries.

Future research should explore several important directions. One question is whether English will continue to dominate or whether multilingualism may gain ground in global medical communication. Another promising area is the role of translation technologies and artificial intelligence in bridging language gaps, potentially reducing inequities in publishing and collaboration. Scholars should also examine strategies to preserve linguistic diversity in medicine, ensuring that local traditions and non-English contributions remain visible and valued. Finally, ethical considerations must be addressed, particularly the need for inclusivity and fairness in global medical discourse.

Taken together, these reflections highlight both the indispensability of English and the necessity of conscious efforts to mitigate its drawbacks. By balancing efficiency with inclusivity, the medical community can ensure that global communication remains both effective and equitable.

Ethics: The authors declare that the article was written in accordance with the ethical standards of the journal and of institutions for each author included.

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Језик медицине данас: енглески као нови латински – предности и изазови

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САЖЕТАК

Језик медицине је специјализовани језички регистар који се одликује прецизношћу, дистинктивним функционалним елементима и историјским континуитетом. Укорењена у латинском и грчком језику, медицинска терминологија основ је стабилне међујезичке комуникације. Током векова, латински је служио као лингва франка медицинског образовања, научног рада и клиничке праксе, пре него што је постепено замењен вернакуларима. Након Другог светског рата, енглески је постао доминантни језик медицине, потпомогнут геополитичким утицајем англофоних земаља, успоном међународних организација и глобализацијом медицинског издаваштва и образовања.

Циљ овога рада јесте да критички сагледа успостављање енглеског језика као новог латинског у глобалној медицинској комуникацији, истичући притом предности и изазове ове појаве. Главне предности су: универзалност комуникације, стандардизованост терминологије и образовања, лак приступ научној литератури, међународна сарадња, ефикасност у кризним ситуацијама, као и значајна видљивост и утицај стручног истраживања. Насупрот томе, изазови су: језичка неједнакост, препреке за неизворне говорнике, губитак лингвистичке и културолошке разноликости, пристрасност у дисеминацији истраживања, ограничена доступност пацијентима.

Без сумње се може закључити да је медицински енглески лингва франка међународне здравствене заједнице у 21. веку. Међутим, потребни су заједнички напори како би се обезбедила професионална инклузивност, очувала језичка разноликост и успоставила равнотежа између начела ефикасности и правичности у будућој глобалној медицинској комуникацији.

Кључне речи: медицински енглески; латински језик; комуникација у здравству; историја медицине

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарише. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, коментари по позиви, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant – Cross Check* (*iThenticate*). Радови код којих се докаже плагијаризам/ аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (https://www.nlm.nih.gov/mesh/meshhome.html)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.

Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4* (*flv*). У првом кадру филма мора се навести: у

наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике" у CA се објављују фотографије, цртежи, схеме и графикони. Слике се означавају арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видео-приказа у е-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *https://www.nlm.*

nih.gov/bsd/uniform_requirements.html. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs/en/submission-letter/SubmissionLetterForm2023.pdf*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА И НАКНАДЕ ЗА ОБРАДУ И ОБЈАВЉИВАЊЕ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 9 Статута Друштва) у години у којој рад предају на разматрање.

Следеће накнаде су обавезне како би рад био прегледан, обрађен и потенцијално објављен у *Српском архиву за целокупно лекарство*:

- накнада за преглед сваког примљеног рада домаћих аутора: 6.000 динара по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада домаћих аутора: 12.000 динара по раду;
- накнада за преглед сваког примљеног рада страних аутора: 75 евра (или 9000 динара) по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада страних аутора: 150 евра (или 18000 динара) по раду.

Накнаде се плаћају пре прегледања, односно пре објављивања рада. Радови за које нису плаћене накнаде неће бити прегледани, односно објављени.

Треба напоменути да уплата накнаде за преглед рада није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и накнаду за преглед чланка, као доказ о уплатама. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за преглед чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

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