YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2025; November Vol. 82 (No. 11): pp. 667–724.

Vojnosanitetski Pregled 2025 November Vol. 82 (No. 11): pp. 667–724.

Vol. 82, No.11, 2025

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
Ministry of Defence of the Republic of Serbia, University of Defence, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 82, br. 11, 2025

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720

Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu" indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

In November 2025, we mark 130 years since Wilhelm Conrad Röntgen (1845–1923) discovered a ray which he named the X-ray, thus indicating the unknown and mysterious nature of this type of radiation. For the revolutionary discovery of X-rays Röntgen received the Nobel Prize in Physics in 1901. The discovery of X-rays played a significant role in the development of multiple areas of medicine, including diagnostics and treatment. Despite the enormous advantages, potential health risks associated with X-rays have been recognized over time, underscoring the need for their cautious, controlled use in medicine.

U novembru 2025. godine obeležavamo 130 godina od kada je Vilhelm Konrad Rendgen (1845–1923) otkrio zrak koji je nazvao X-zrakom, označavajući ovim nazivom nepoznatu i zagonetnu prirodu ove vrste zračenja. Za revolucionarno otkriće X-zraka Rendgen je 1901. dobio Nobelovu nagradu za fiziku. Otkriće X-zraka imalo je značajnu ulogu u razvoju mnogih oblasti medicine, uključujući dijagnostiku i lečenje. Uprkos ogromnim prednostima, vremenom su prepoznati i potencijalni rizici za zdravlje koji su povezani sa X-zracima, što naglašava potrebu za njihovom opreznom i kontrolisanom upotrebom u medicini.

ORIGINAL ARTICLES

UDC: 616-008.9:616.348/.35-006.55
DOI: https://doi.org/10.2298/VSP250117070D

The association of metabolic syndrome with the characteristics of colorectal adenomas

Povezanost metaboličkog sindroma sa karakteristikama kolorektalnih adenoma

Dimitrije Damjanov*†, Željka Savić*†, Olgica Latinović Bošnjak*, Žarko Krnetić*, Vladimir Vračarić*, Nebojša Janjić*†, Božidar Dejanović*†

***University Clinical Center of Vojvodina, Clinic for Gastroenterology and Hepatology, Novi Sad, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia**

Abstract

Background/Aim. Metabolic syndrome (MetS) is associated with an increased risk of developing colorectal cancer (CRC). However, data on its relationship with colorectal adenomas (CRA), the primary precancerous lesions, remain unclear and limited in certain regions. The aim of this study was to examine the characteristics and distribution of CRA in patients with and without MetS. **Methods.** A cross-sectional study was conducted, including 80 patients with CRA, of whom 40 had MetS (MetS group), and 40 did not meet the criteria for MetS (control group). Demographic data, risk factors for CRC (smoking, alcohol consumption, and family history of CRC), protective factors (use of acetylsalicylic acid and nonsteroidal anti-inflammatory drugs, physical activity), and polyp characteristics (size, number, localization, and degree of advancement) were collected and compared between the two groups. The diagnosis of CRA was established by histological examination of polyp specimens retrieved during colonoscopy. The diagnosis of MetS was made if three or more of the following criteria were present: increased waist circumference (≥ 94 cm for males, or ≥ 80 cm for females); hypertriglyceridemia (≥ 1.7 mmol/L); reduced high-density lipoprotein cholesterol levels (< 1.0 mmol/L for males, or < 1.3 mmol/L for females); arterial hypertension (systolic blood pressure ≥ 130 mmHg, and/or diastolic blood pressure ≥ 85 mmHg); fasting hyperglycaemia (≥ 5.6 mmol/L). **Results.** The average age of the patients was 61 years. Males and females were equally present in both groups, as were all the common risk factors for CRA. There were no differences between the groups regarding adenoma size, number of detected adenomas, localization of adenomas, and the degree of histological advancement of the adenoma. **Conclusion.** No significant association was found between the presence of MetS and the characteristics and distribution of CRA in our study. Further studies with larger samples and biomarker analyses are needed to better understand the potential role of MetS-related factors in colorectal carcinogenesis.

Key words:
adenoma; colon; colorectal neoplasms; metabolic syndrome; polyps; risk factors.

Apstrakt

Uvod/Cilj. Metabolički sindrom (MetS) je povezan sa povećanim rizikom od razvoja kolorektalnog karcinoma (KRK). Međutim, podaci o njegovoj povezanosti sa kolorektalnim adenomima (KRA), primarnim prekanceroznim lezijama, ostaju nedovoljno jasni i ograničeni u određenim regionima. Cilj rada bio je da se ispitaju karakteristike i raspodela KRA kod bolesnika sa i bez MetS-a. **Metode.** Sprovedena je studija preseka koja je obuhvatila 80 bolesnika obolelih od KRA, od kojih je 40 imalo MetS (MetS grupa), dok 40 nije ispunjavalo kriterijume za MetS (kontrolna grupa). Prikupljeni su i upoređeni između dve grupe demografski podaci, faktori rizika za KRK (pušenje, konzumacija alkohola, porodična anamneza KRK), zaštitni faktori (upotreba acetilsalicilne kiseline i nesteroidnih antiinflamacijskih lekova, fizička aktivnost), kao i karakteristike polipa (veličina, broj, lokalizacija i stepen uznapredovalosti). Dijagnoza KRA postavljana je histološkom analizom uzoraka polipa dobijenih tokom kolonoskopije. Dijagnoza MetS postavljena je ukoliko su bila prisutna tri ili više sledećih kriterijuma: povećan obim struka (≥ 94 cm kod muškaraca ili ≥ 80 cm kod žena); hipertrigliceridemija (≥ 1,7 mmol/L); nizak nivo *high-density lipoprotein*–HDL holesterola (< 1,0 mmol/L kod muškaraca ili < 1,3 mmol/L kod žena); arterijska hipertenzija (sistolni krvni pritisak ≥ 130 mmHg i/ili dijastolni krvni pritisak ≥ 85 mmHg); hiperglikemija natašte (≥ 5,6 mmol/L). **Rezultati.** Prosečna starost bolesnika bila je 61 godina. Muškarci i žene bili su podjednako zastupljeni u obe grupe, kao i svi uobičajeni faktori rizika za KRA. Nije bilo razlika između grupa u

Correspondence to: Dimitrije Damjanov, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21 137 Novi Sad, Serbia. E-mail: dimitrije.damjanov@mf.uns.ac.rs

pogledu veličine adenoma, broja otkrivenih adenoma, lokalizacije adenoma i stepena histološke uznapredovalosti adenoma. **Zaključak.** Nije utvrđena značajna povezanost prisustva MetS sa karakteristikama i distribucijom KRA u našoj studiji. Neophodne su dalje studije sa većim uzorcima i analizom biomarkera kako bi se bolje razumela moguća uloga faktora povezanih sa MetS u kolorektalnoj karcinogenezi.

Ključne reči:
adenom; crevo, debelo; kolorektalne neoplazme; metabolički sindrom; polipi; faktori rizika.

Introduction

Colorectal cancer (CRC) is among the most preventable malignant diseases [1]. A key element in the carcinogenesis of most CRC cases is the polyp, a benign precancerous lesion [2]. The adenoma-carcinoma sequence is the most common pathway for the transformation of colorectal polyps into cancer [3]. The gradual progression of polyps into CRC over several years in the general population offers a valuable opportunity for early detection and removal [4, 5].

It is particularly important to identify risk factors for the development of colorectal adenomas (CRA), as this can aid in screening, risk modification, and prevention of CRC [6]. Risk factors for developing colorectal tumors include a family history of CRC or colorectal polyps, smoking, alcohol consumption, physical inactivity, and obesity [7, 8]. Additionally, research indicates that metabolic syndrome (MetS) is also a risk factor for CRC [9, 10]. The link between MetS and CRC is primarily attributed to insulin resistance and the role of insulin-like growth factor-1 [11, 12].

While some studies have demonstrated that MetS and its components are associated with the presence, multiplicity, and advancement of CRA [11–13], others have not confirmed these findings [14, 15]. Furthermore, most available data originate from East Asian and Western populations, whereas literature from Southeast Europe remains scarce. A prior regional study confirmed MetS as a predictor for CRA but found no associations with CRA characteristics [16].

The aim of our study was to examine differences in the characteristics and distribution of CRA between patients with and without MetS. Establishing such associations could potentially influence CRC screening strategies and post-polypectomy surveillance recommendations in clinical practice, particularly in populations with a high prevalence of MetS.

Methods

This cross-sectional study was conducted at the Department of Gastrointestinal Endoscopy of the University Clinical Center of Vojvodina, Novi Sad, Serbia. Individuals who underwent total colonoscopy and were diagnosed with CRA between February and July 2023 were invited to participate in an informative interview. Only individuals who voluntarily provided informed consent after an informational interview were enrolled in the study.

The study was approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-188, from October 27, 2022).

The study included 80 patients, divided into two groups. The first group included 40 consecutive patients aged 40 to 75 years with CRA who were diagnosed with MetS, i.e., the MetS group. The second group, i.e., the control group, consisted of 40 consecutive patients within the same age range who had CRA but did not meet the diagnostic criteria for MetS. Exclusion criteria included pregnancy, inadequate bowel preparation, CRC, inflammatory bowel disease, prior colon resection, insulin therapy, and the presence of hyperplastic polyps.

Colonoscopy was performed by gastroenterohepatologists and nurses with extensive experience in endoscopy. Data were extracted from medical records and included the following parameters: polyp size (measured using the diameter of open biopsy forceps), polyp morphology, number of polyps, and polyp distribution. Adenomas were classified as advanced if they met any of the following criteria: size ≥ 10 mm, presence of high-grade dysplasia, or presence of a villous component [17].

Anamnestic data for each participant from both the study and control groups were collected using a pre-designed questionnaire. Participants were interviewed and provided information on the following: age, gender, smoking status, alcohol consumption, family history of CRC, use of acetylsalicylic acid (ASA) and nonsteroidal anti-inflammatory drugs (NSAIDs), physical activity levels, elevated blood pressure (BP), diabetes mellitus, low high-density lipoprotein cholesterol (HDL-C), elevated triglycerides (TG), and medications used.

Venous blood samples of the participants were collected in the morning following a 12-hr fasting period. Glucose, TG, and HDL-C levels were measured using standard methods on an automated biochemical analyzer. Waist circumference was measured according to the World Health Organization guidelines [18], and BP was recorded using the Korotkoff method.

Participants were diagnosed with MetS if they fulfilled three or more of the following criteria: increased waist circumference (≥ 94 cm for males or ≥ 80 cm for females); elevated TG or use of TG-lowering medications (≥ 1.7 mmol/L); reduced HDL-C levels or use of HDL-C-raising medications (< 1.0 mmol/L for males or < 1.3 mmol/L for females); elevated BP or use of antihypertensives (systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg); fasting hyperglycemia or use of antihyperglycemic medications (≥ 5.6 mmol/L) [19].

Statistical analysis

Data were entered and analyzed using IBM SPSS statistical software, version 22.0. Descriptive statistical methods were applied to summarize the sample, including

measures of central tendency (mean), dispersion (standard deviation), and distribution range (minimum and maximum values). To address the research objectives and hypotheses, the Chi-square test was employed as a non-parametric method for comparing nominal-level data. In addition, the relationships between the variables of interest were evaluated using Pearson's linear correlation coefficient (a parametric method) and Spearman's rank correlation coefficient (a non-parametric method). The value of $p < 0.05$ was considered statistically significant.

Results

Demographic characteristics

The study included 80 patients with CRA. The sample was gender-balanced, with 40 males and 40 females. The average age of the patients was 60.76 years (standard deviation: 7.85). The distribution of MetS prevalence according to gender and age is provided (Table 1). The results of the independent samples test conducted to examine age differences between patients with MetS and those without MetS indicated a statistically significant difference, with older age observed in the group of patients with MetS.

Risk factors and protective factors for colorectal adenomas

Risk and protective factors for CRA in patients with MetS and in the control group were analyzed. When comparing patients with MetS to the control group, the results show that a statistically significant difference between these two groups exists only in the use of ASA, with more frequent use observed in patients with MetS (Table 2).

Table 1

Demographic data in the MetS and control groups

Parameters	Group		Test	*df*	*p*
	MetS	control			
Gender					
male	21 (52.5)	19 (47.5)	$\chi^2 = 0.20$	1	> 0.05
female	19 (47.5)	21 (52.5)			
Age, years	63.12 ± 6.70	58.40 ± 8.28	$t = 2.805$	78	**< 0.05**

MetS – metabolic syndrome.
All values are given as numbers (percentages) or mean ± standard deviation.
Bold value indicates statistical significance, $p < 0.05$.

Table 2

Risk and protective factors for CRA in the MetS and control groups

Parameters	Group		χ^2	*df*	*p*
	MetS	control			
Risk factors					
smoking status					
smokers	14 (35.0)	16 (40.0)			
ex-smokers	13 (32.5)	9 (22.5)			
non-smokers	13 (32.5)	15 (37.5)	1.003	2	> 0.05
Alcohol consumption					
yes	2 (5.0)	5 (12.5)			
no	38 (95.0)	35 (87.5)	1.409	1	> 0.05
Family history of CRC					
yes	8 (20.0)	13 (32.5)			
no	32 (80.0)	27 (67.5)	1.614	1	> 0.05
Protective factors					
ASA and/or NSAID use					
yes	12 (30.0)	4 (10.0)			
no	28 (70.0)	36 (90.0)	5.000	1	**< 0.05**
Physical activity					
yes	34 (85.0)	37 (92.5)			
no	6 (15.0)	3 (7.5)	1.127	1	> 0.05

CRA – colorectal adenomas; MetS – metabolic syndrome; CRC – colorectal cancer; ASA – acetylsalicylic acid; NSAID – nonsteroidal anti-inflammatory drug.
All values are given as numbers (percentages).
Bold value indicates statistical significance, $p < 0.05$.

Polyp characteristics in the total sample

The size of the polyp was ≥ 10 mm in the majority of cases. Sessile and pedunculated CRA were represented in relatively similar numbers. The majority of CRA were found in the distal colon, while fewer cases were observed in the proximal colon or in both parts of the colon. In most cases, only one CRA was present, while two or more adenomas were observed in a significantly smaller number of cases. In 57 cases, CRA were classified as advanced (Table 3).

Polyp size

There were no statistically significant differences in polyp size based on gender (χ^2 = 3.170, p > 0.05) or age (r = -0.006, p > 0.05). None of the risk or protective factors showed a statistically significant correlation with the size of CRA (smoking: r = 0.095, p > 0.05; alcohol: r = -0.030, p > 0 .05; family history: r = 0.045, p > 0.05; ASA: r = -0.112, p > 0.05; physical activity: r = -0.027, p > 0.05). No statistically significant differences in the size of CRA were observed between patients with MetS and the control group (Table 4).

Polyp morphology

There were no statistically significant differences in polyp morphology based on gender (χ^2 = 2.464, p > 0.05) or age (r = -0.067, p > 0.05). None of the risk or protective factors showed a statistically significant correlation with the

Table 3

Polyp characteristics in the total sample

Characteristics	n (%)
Polyp size	
≤ 5 mm	3 (3.8)
6–9 mm	24 (30.0)
≥ 10 mm	53 (66.3)
Polyp morphology	
sessile	37 (46.3)
pedunculated	43 (53.8)
Number of polyps	
1	60 (75.0)
2	18 (22.5)
≥ 3	2 (2.5)
Distribution of polyps	
proximal colon	11 (13.8)
distal colon	61 (76.3)
proximal and distal	8 (10.0)
Advanced adenoma	
yes	57 (71.3)
no	23 (28.7)

n – number.

Table 4

Polyp characteristics in the MetS and control groups

Characteristics	Group		χ^2	*df*	*p*
	MetS	control			
Polyp size					
≤ 5 mm	0 (0.0)	3 (7.5)			
6–9 mm	14 (35.0)	10 (25.0)			> 0.05
≥ 10 mm	26 (65.0)	27 (67.5)	3.686	2	
Polyp morphology					
sessile	19 (47.5)	18 (45.0)			> 0.05
pedunculated	21 (52.5)	22 (55.0)	0.050	1	
Number of polyps					
1	31 (77.5)	29 (72.5)			
2	8 (20.0)	10 (25.0)			> 0.05
≥ 3	1 (2.5)	1 (2.5)	0.289	2	
Distribution of polyps					
proximal	7 (17.5)	4 (10.0)			
distal	31 (77.5)	30 (75.0)			> 0.05
proximal and distal	2 (5.0)	6 (15.0)	2.835	2	
Advanced adenoma					
yes	27 (67.5)	30 (75.0)			> 0.05
no	13 (32.5)	10 (25.0)	0.549	1	

MetS – metabolic syndrome.
All values are given as numbers (percentages).

morphology of CRA (smoking: $r = 0.030$, $p > 0.05$; alcohol: $r = -0.068$, $p > 0.05$; family history: $r = 0.098$, $p > 0.05$; ASA: $r = -0.038$, $p > 0.05$; physical activity: $r = -0.013$, $p > 0.05$). No statistically significant differences in the morphology of CRA were observed between patients with MetS and the control group (Table 4).

Polyp number

There were no statistically significant differences in polyp number based on gender ($\chi^2 = 5.067$, $p > 0.05$) or age ($r = -0.010$, $p > 0.05$). None of the risk or protective factors showed a statistically significant correlation with the number of CRA (smoking: $r = 0.065$, $p > 0.05$; alcohol: $r = 0.159$, $p > 0.05$; family history: $r = 0.152$, $p > 0.05$; ASA: $r = -0.204$, $p > 0.05$; physical activity: $r = 0.185$, $p > 0.05$). No statistically significant differences in the number of CRA were observed between patients with MetS and the control group (Table 4).

Polyp distribution

There were no statistically significant differences in polyp localization based on gender ($\chi^2 = 3.621$, $p > 0.05$) or age ($r = -0.065$, $p > 0.05$). None of the risk or protective factors showed a statistically significant correlation with the localization of CRA (smoking: $r = -0.052$, $p > 0.05$; alcohol: $r = 0.024$, $p > 0.05$; family history: $r = -0.071$, $p > 0.05$; ASA: $r = 0.039$, $p > 0.05$; physical activity: $r = 0.217$, $p > 0.05$). No statistically significant differences in the localization of CRA were observed between patients with MetS and the control group (Table 4).

Polyp advancement

There were no statistically significant differences in polyp advancement based on gender ($\chi^2 = 1.526$, $p > 0.05$) or age ($r = 0.002$, $p > 0.05$). None of the risk or protective factors showed a statistically significant correlation with the advancement of CRA (smoking: $r = 0.154$, $p > 0.05$; alcohol: $r = -0.097$, $p > 0.05$; family history: $r = 0.128$, $p > 0.05$; ASA: $r = -0.166$, $p > 0.05$; physical activity: $r = 0.039$, $p > 0.05$). No statistically significant differences in the frequency of advanced CRA were observed between patients with MetS and the control group (Table 4).

Discussion

Malignant diseases represent a significant health problem for the population of Vojvodina. Tumors are the leading cause of morbidity and the second most frequent cause of death in this region (23.1% of cases) [20]. Globally, CRC is the third most frequently diagnosed malignant disease, and the second most common cause of malignancy-related mortality [21]. A similar situation is observed in Serbia [22]. The majority of CRC cases (60–65%) are sporadic, while 35–40% can be linked to hereditary predisposition for CRC [23].

Observing the population of patients with CRA in our region, based on our study results, if a significant difference in the characteristics and distribution of CRA between the MetS and control groups were to be established, the colonoscopy screening program, as well as the follow-up of patients after polypectomy, could be adapted according to the presence of MetS in individuals. This could influence morbidity and mortality associated with CRC. The central question of our research was: "Does the presence of MetS affect the size, number, localization, and histological characteristics of CRA?"

Age is one of the most dominant risk factors for CRA. With aging, the frequency, number, size, and degree of dysplasia of CRA increase [7, 24–27]. In our study, patients with MetS were statistically significantly older than patients who did not meet the criteria for MetS. This finding is expected, given that the prevalence of MetS increases with age [28]. The age of the patients did not statistically significantly correlate with the size, number, distribution, or degree of advancement of CRA, which contrasts with data reported in the literature. These discrepancies may be due to the limited sample size or population-specific differences.

Men have a 50% higher risk of having polyps larger than 10 mm and advanced polyps compared to women [29, 30]. Interestingly, the polyp detection rate is 5% higher in men than in women [31]. Contrary to the data from the literature, our study did not reveal statistically significant differences between men and women in terms of CRA size, localization, and number, nor in the prevalence of advanced polyps. This again may reflect sample size limitations or possible gender differences in health-seeking behavior and screening uptake.

The results of a 2021 meta-analysis showed that patients with MetS have a 1.39 times higher risk of having CRA compared to patients without MetS [32]. An important MetS-related factor responsible for the development of CRA is visceral obesity, given that visceral adipose tissue is hormonally active [33, 34]. In our study, no statistically significant difference was found in the distribution, number, size, or prevalence of advanced CRA in patients with MetS compared to those without MetS. This finding contrasts with previous research indicating that MetS significantly correlates with the presence of CRA at multiple locations [35], multiple CRA [36], larger CRA [37], and advanced CRA [13]. In a study by Trabulo et al. [14], participants with MetS had multiple CRA more frequently, but no differences were found in the size, distribution, or prevalence of advanced CRA compared to participants without MetS. Similarly, in a study by Milano et al. [15], MetS was significantly associated with the presence of colorectal polyps, but there were no differences in their size or number between participants with MetS and those without MetS. The discrepancy between our findings and those of other studies may be attributed to several factors. First, our sample size was relatively small. Second, the definition and measurement of MetS components, as well as the criteria for adenoma classification, vary across studies, complicating direct comparisons. Third, lifestyle and genetic factors specific to

our region could modulate the effect of MetS on colorectal neoplasia risk differently than in other populations.

When analyzing risk and protective factors for CRC in relation to MetS, our study found that patients with MetS were significantly more likely to use ASA and other NSAIDs. This is expected, as MetS patients often have cardiovascular or cerebrovascular diseases for which antiplatelet therapy is commonly indicated. However, none of the other commonly assessed risk or protective factors, including smoking, alcohol, physical activity, or family history of CRC, showed a statistically significant association with MetS in our cohort. Furthermore, none of these factors were associated with CRA characteristics such as size, morphology, number, distribution, or advancement.

These findings suggest that although MetS is an important systemic condition with established links to colorectal neoplasia, its influence on the phenotypic expression of CRA may vary depending on genetic, environmental, and lifestyle factors within specific populations. Given the inconsistency in the current literature, future studies in our region should aim for larger sample sizes and incorporate measurements of visceral fat and circulating adipokines such as adiponectin, leptin, and estrogen, which may play key roles in CRA pathogenesis [12, 33, 34].

The limitations of our study include a relatively small sample size and the use of a cross-sectional design.

Conclusion

Our study did not reveal a statistically significant difference in the sizes, number, distribution, and frequency of advanced colorectal adenomas between patients with and those without metabolic syndrome. The results of this research highlight the need for further research in our setting with larger sample sizes, incorporating the assessment of serum adipokine levels and evaluation of visceral obesity, in order to improve risk control for the development of colorectal adenomas and cancer.

R E F E R E N C E S

1. *Brenner H, Chen C.* The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. Br J Cancer 2018; 119(7): 785–92.
2. *Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB.* Colorectal cancer. The Lancet 2019; 394(10207): 1467–80.
3. *Sullivan BA, Noujaim M, Roper J.* Cause, Epidemiology, and Histology of Polyps and Pathways to Colorectal Cancer. Gastrointest Endosc Clin N Am 2022; 32(2): 177–94.
4. *Kanth P, Inadomi JM.* Screening and prevention of colorectal cancer. BMJ 2021; 374: n1855.
5. *Nguyen LH, Goel A, Chung DC.* Pathways of Colorectal Carcinogenesis. Gastroenterology 2020; 158(2): 291–302.
6. *Kastrinos F, Kupfer SS, Gupta S.* Colorectal Cancer Risk Assessment and Precision Approaches to Screening: Brave New World or Worlds Apart? Gastroenterology 2023; 164(5): 812–27.
7. *Sninsky JA, Shore BM, Lupu GV, Crockett SD.* Risk Factors for Colorectal Polyps and Cancer. Gastrointest Endosc Clin N Am 2022; 32(2): 195–213.
8. *Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE.* A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers (Basel) 2021; 13(9): 2025.
9. *Chen H, Zheng X, Zong X, Li Z, Li N, Hur J,* et al. Metabolic syndrome, metabolic comorbid conditions and risk of early-onset colorectal cancer. Gut 2021; 70(6): 1147–54.
10. *Shen X, Wang Y, Zhao R, Wan Q, Wu Y, Zhao L,* et al. Metabolic syndrome and the risk of colorectal cancer: a systematic review and meta-analysis. Int J Colorectal Dis 2021; 36(10): 2215–25.
11. *Uzunlulu M, Telci Caklili O, Oguz A.* Association between Metabolic Syndrome and Cancer. Ann Nutr Metab 2016; 68(3): 173–9.
12. *Mili N, Paschou SA, Goulis DG, Dimopoulos MA, Lambrinoudaki I, Psaltopoulou T.* Obesity, metabolic syndrome, and cancer: pathophysiological and therapeutic associations. Endocrine 2021; 74(3): 478–97.
13. *Kim BC, Shin A, Hong CW, Sohn DK, Han KS, Ryu KH,* et al. Association of colorectal adenoma with components of metabolic syndrome. Cancer Causes Control 2012; 23(5): 727–35.
14. *Trabulo D, Ribeiro S, Martins C, Teixeira C, Cardoso C, Mangualde J,* et al. Metabolic syndrome and colorectal neoplasms: An ominous association. World J Gastroenterol 2015; 21(17): 5320–7.
15. *Milano A, Bianco MA, Buri L, Cipolletta L, Grossi E, Rotondano G,* et al. Metabolic syndrome is a risk factor for colorectal adenoma and cancer: a study in a White population using the harmonized criteria. Therap Adv Gastroenterol 2019; 12: 1756284819867839.
16. *Damjanov D, Ičin T, Savić Ž, Janjić N, Nikolić S, Bošnjak OL,* et al. Visceral Fat Thickness, Serum Adiponectin, and Metabolic Syndrome in Patients with Colorectal Adenomas. J Pers Med 2024; 14(9): 1008.
17. *Gupta S, Lieberman D, Anderson JC, Burke CA, Dominitz JA, Kaltenbach T,* et al. Recommendations for Follow-Up After Colonoscopy and Polypectomy: A Consensus Update by the US Multi-Society Task Force on Colorectal Cancer. Am J Gastroenterol 2020; 115(3): 415–34.
18. *World Health Organization.* Waist Circumference and Waist-Hip Ratio [Internet]. Geneva: WHO; 2008 [cited 2023 Aug 26; accessed 2025 Aug 26]. Available from: https://iris.who.int/bitstream/handle/10665/44583/9789241501491_eng.pdf
19. *Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA,* et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120(16): 1640–5.
20. *Institute of Public Health of Vojvodina.* The state of health of the inhabitants of Vojvodina in 2019 [Internet]. Novi Sad: IZJZV; 2020 [cited 2023 Sep 7; accessed 2025 Aug 26]. Available from: http://www.izjzv.org.rs/?lng=lat&cir=0&link=4-21 (Serbian)
21. *Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I,* et al. Global cancer statistics 2022: GLOBOCAN estimates of

incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74(3): 229–63.
22. *Institute of Public Health of Serbia "Dr Milan Jovanović Batut"*. Malignant tumours in Republic of Serbia [Internet]. Beograd; 2022 [cited 2023 Sep 3; accessed 2025 Aug 26]. Available from: https://www.batut.org.rs/download/publikacije/MaligniTumoriuRepubliciSrbiji2020.pdf (Serbian)
23. *Keum N, Giovannucci E*. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol 2019; 16(12): 713–32.
24. *Peery AF, Crockett SD, Murphy CC, Lund JL, Dellon ES, Williams JL,* et al. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2018. Gastroenterology 2019; 156(1): 254–72.e11.
25. *Corley DA, Jensen CD, Marks AR, Zhao WK, de Boer J, Levin TR,* et al. Variation of adenoma prevalence by age, sex, race, and colon location in a large population: implications for screening and quality programs. Clin Gastroenterol Hepatol 2013; 11(2): 172–80.
26. *Heitman SJ, Ronksley PE, Hilsden RJ, Manns BJ, Rostom A, Hemmelgarn BR*. Prevalence of adenomas and colorectal cancer in average risk individuals: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2009; 7(12): 1272–8.
27. *O'Brien MJ, Winawer SJ, Zauber AG, Gottlieb LS, Sternberg SS, Diaz B,* et al. The National Polyp Study. Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. Gastroenterology 1990; 98(2): 371–9.
28. *Hirode G, Wong RJ*. Trends in the Prevalence of Metabolic Syndrome in the United States, 2011-2016. JAMA 2020; 323(24): 2526–8.
29. *McCashland TM, Brand R, Lyden E, de Garmo P; CORI Research Project*. Gender differences in colorectal polyps and tumors. Am J Gastroenterol 2001; 96(3): 882–6.
30. *Regula J, Rupinski M, Kraszewska E, Polkowski M, Pachlewski J, Orlowska J,* et al. Colonoscopy in colorectal-cancer screening for detection of advanced neoplasia. N Engl J Med 2006; 355(18): 1863–72.
31. *Rex DK, Schoenfeld PS, Cohen J, Pike IM, Adler DG, Fennerty BM,* et al. Quality indicators for colonoscopy. Am J Gastroenterol 2015; 110(1): 72–90.
32. *Wu H, Zhang J, Zhou B*. Metabolic syndrome and colorectal adenoma risk: A systematic review and meta analysis. Clin Res Hepatol Gastroenterol 2021; 45(5): 101749.
33. *Mendonça FM, de Sousa FR, Barbosa AL, Martins SC, Araújo RL, Soares R,* et al. Metabolic syndrome and risk of cancer: Which link? Metabolism. 2015; 64(2): 182–9.
34. *Keum N, Lee DH, Kim R, Greenwood DC, Giovannucci EL*. Visceral adiposity and colorectal adenomas: dose-response meta-analysis of observational studies. Ann Oncol 2015; 26(6): 1101–9.
35. *Chiu HM, Lin JT, Shun CT, Liang JT, Lee YC, Huang SP,* et al. Association of metabolic syndrome with proximal and synchronous colorectal neoplasm. Clin Gastroenterol Hepatol 2007; 5(2): 221–9.
36. *Kim JH, Lim YJ, Kim YH, Sung IK, Shim SG, Oh SO,* et al. Is metabolic syndrome a risk factor for colorectal adenoma? Cancer Epidemiol Biomarkers Prev 2007; 16(8): 1543–6.
37. *Morita T, Tabata S, Mineshita M, Mizoue T, Moore MA, Kono S*. The metabolic syndrome is associated with increased risk of colorectal adenoma development: the Self-Defense Forces health study. Asian Pac J Cancer Prev 2005; 6(4): 485–9.

Received on January 17, 2025
Revised on July 20, 2025
Revised on August 1, 2025
Accepted on August 20, 2025
Online First October 2025

ORIGINAL ARTICLE
UDC: 616.6-07
DOI: https://doi.org/10.2298/VSP250304071J

Urodynamic diagnosis of subvesical obstruction – significance of bladder outlet obstruction index and bladder contractility index

Urodinamska dijagnoza subvezikalne opstrukcije – značaj indeksa opstrukcije vrata mokraćne bešike i indeksa kontraktilnosti mokraćne bešike

Dimitrije Jeremić*†, Saša Vojinov*†, Stevan Stojanović*†, Ivan Levakov*†, Mladen Popov*†, Miloš Maletin*†, Ines Kalači†, Zoran Ružić‡, Tanja Lakić*§, Filip Dožić†, Dragan Grbić†

University of Novi Sad, *Faculty of Medicine, ‡Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia; University Clinical Center of Vojvodina, †Urology Clinic, §Center for Pathology and Histology, Novi Sad, Serbia

Abstract

Background/Aim. The bladder outlet obstruction (BOO) index (BOOI) is used during urodynamic testing to diagnose BOO. The bladder contractility index (BCI) is a urodynamic parameter used inconsistently. The aim of this study was to examine the correlation between BOOI and BCI. **Methods.** A retrospective study was conducted from 2021 to 2023, including 176 male patients. Using the *t*-test, analysis of variance, and correlation analysis, BOOI and BCI were analyzed. **Results.** High BOOI values (40–80) and weaker bladder contractility (BCI < 100), as potential causes of lower urinary tract symptoms (LUTS), coexisted in 11.37% of cases. A high BCI value (> 150) was associated with a significant number of patients (7.39%) with high BOOI values (> 40), acting as a compensatory mechanism that masked the true causes of LUTS. Patient groups with BCI < 100 and > 150 showed an inverse correlation with BOOI, as expected. Values of BOOI 20–39 and BCI 101–149 were considered a "gray zone". The correlation between $P_{det}Q_{max}$ and Q_{max} was not statistically significant (r = -0.2006), making BOO a factor that could influence this relationship. Additionally, the intraurethral catheter positioned during urodynamic testing significantly affected this correlation. As expected, a negative correlation was observed between Q_{max} and BOOI (r = -0.44841, $p < 0.001$), while BCI and Q_{max} had a positive linear correlation (R^2 = 0.2255, $p < 0.001$). The correlation between the two observed indices, BOOI and BCI, showed a positive linear correlation, presenting a physiological mechanism for BOO compensation (R^2 = 0.3292, $p < 0.001$). **Conclusion.** In combination with BCI, BOOI is sufficient for establishing a definitive diagnosis in the analyzed patient groups. It is recommended that BOOI, BCI, and Q_{max} always be used in combination. Q_{max}, as a measure mostly valued on uroflow, may be insufficient for diagnosis in unequivocal clinical cases.

Key words:
male; ureteral obstruction; urinary bladder; urination disorders.

Apstrakt

Uvod/Cilj. Indeks opstrukcije vrata mokraćne bešike [*bladder outlet obstruction* (*BOO*) *index* – BOOI] koristi se prilikom urodinamskog ispitivanja sa ciljem postavljanja dijagnoze BOO. Indeks kontraktilnosti mokraćne bešike (*bladder contractility index* – BCI) je urodinamski parametar koji se nekonzistentno koristi u praksi. Cilj rada bio je da se ispita korelacija između BOOI i BCI. **Metode.** Retrospektivna studija sprovedena je u periodu 2021–2023 godine i obuhvatila je 176 bolesnika muškog pola. Korišćenjem *t*-testa, analize varijanse i analize korelacije, analizirani su BOOI i BCI. **Rezultati.** Visoke vrednosti BOOI (40–80) i slabija kontraktilnost bešike (BCI < 100), kao mogući uzročnici simptoma od strane donjeg urinarnog trakta (*lower urinary tract symptoms* – LUTS) koegzistirale su u 11,37% slučajeva. Visoka vrednost BCI (> 150) povezana je sa značajnim brojem bolesnika (7,39%) sa visokim vrednostima BOOI (> 40), delujući kao kompenzatorni mehanizam koji prikriva prave uzroke LUTS. U grupama bolesnika sa BCI < 100 i > 150 pokazana je obrnuta korelacija sa BOOI, kao što je i očekivano. Vrednosti BOOI 20–39 i BCI 101–149 smatrane su „sivom zonom". Korelacija $P_{det}Q_{max}$ i Q_{max} nije pokazala statističku značajnost (r = -0,2006), što čini BOO faktorom koji može uticati na ovaj odnos. Pored ovoga, intrauretralni kateter postavljen tokom urodinamskog ispitivanja značajno je uticao na ovaj odnos. Kao što je očekivano, uočena je negativna korelacija između Q_{max} i BOOI (r = -0,44841, p < 0,001), dok su BCI i Q_{max}

Correspondence to: Dimitrije Jeremić, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: dimitrije.jeremic@mf.uns.ac.rs

imale pozitivnu linearnu korelaciju (R^2 = 0,2255, p < 0,001). Korelacija između dva ispitivana indeksa, BOOI i BCI, pokazala je pozitivnu linearnu povezanost, što ukazuje na postojanje fiziološkog mehanizma za kompenzaciju BOO (R^2 = 0,3292, p < 0,001). **Zaključak.** U kombinaciji sa BCI, BOOI je dovoljan za postavljanje definitivne dijagnoze u posmatranim grupama bolesnika. Preporuka je da se BOOI, BCI i Q_{max} uvek koriste u kombinaciji. Q_{max}, kao mera koja se najviše analizira prilikom urofloumetrije, može biti nedovoljna za dijagnozu u jasnim kliničkim slučajevima.

Ključne reči:
muškarci; ureter, opstrukcija; mokraćna bešika; mokrenje, poremećaji.

Introduction

The term lower urinary tract symptoms (LUTS) is generic and encompasses storage, voiding, and post-micturition symptoms, with a multifactorial etiology. Bladder outlet obstruction (BOO) refers to subvesical obstruction, also multifactorial in origin, and is defined by reduced urinary flow rate and increased detrusor pressure [1]. By its nature, BOO can be caused by a variety of causes, divided into two groups: anatomic and functional. Anatomic obstruction is mainly caused by benign prostatic enlargement (BPE), resulting in benign prostatic obstruction (BPO), bladder neck sclerosis, and urethral strictures. Functional BOO can be caused by detrusor-sphincter dyssynergia, functional obstruction of the bladder neck, and dysfunctional voiding. Histological diagnosis of benign prostatic hyperplasia (BPH) and consequent BPE/BPO results in elevation in detrusor pressure during voiding and clinically presents as a combination of obstructive and irritative symptoms of LUTS [2]. Detrusor underactivity (DU) has been defined by the International Continence Society (ICS) as a low detrusor pressure or short detrusor contraction resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span. It is a common finding in 11–40% of aging males [3]. Its clinical presentation is similar to BPO, manifesting as obstructive, irritative voiding symptoms or their combination, making it hard to distinguish BOO from DU. Urodynamic studies are not recommended as part of the routine diagnostic algorithm prior to operative treatment for BPO [4].

Although BOO index (BOOI) determines the degree of BOO, it is important to identify patients with DU in order to improve the results of de-obstructive surgery [3]. Since BOOI is widely used during urodynamics, it is essential to define parameters and urodynamic findings, such as bladder contractility (BC) index (BCI), which can help identify men without DU who will benefit most from operative de-obstructive surgery.

The aim of this study was to assess BOOI and BCI and their correlation. The analysis focused primarily on the relationship between BOOI and BCI, but also on the precise correlation between Q_{max} and $P_{det}Q_{max}$. Considering that DU may significantly complicate the evaluation of patients using uroflowmetry and urodynamics, correlations between BOOI/Q_{max} and BCI/Q_{max} were also examined.

Methods

This single-center, retrospective observational cohort study included 176 male patients in a three-year period from 2021 to 2023. The study was conducted at the University Clinical Center of Vojvodina, Novi Sad, Serbia, and it was approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-292, from August 26, 2024).

Inclusion criteria were as follows: patients aged between 50 and 80 years; age-adjusted prostate-specific antigen (PSA) < 2.6 ng/mL for patients aged 50–60 years and < 4 ng/mL for those older than 60 years; International Prostate Symptom Score (IPSS) ≤ 18; prostate volume between 40 and 80 mL; intravesical prostatic protrusion grade 0 or 1; residual urine < 100 mL; uroflowmetry Q_{max} < 10 mL/s; obstructive pattern of voiding on uroflowmetry; negative urine culture in previous 14 days; no prior medical treatment or treatment limited to phytotherapy or an alpha blocker (tamsulosin); completed urethrocystoscopy.

Exclusion criteria included: patients aged < 50 or > 80 years; PSA > 4 ng/mL; IPSS > 19; prostate volume < 40 or > 80 mL; residual urine > 100 mL; Q_{max} > 10 mL/s; normal voiding pattern on uroflowmetry; treatment with 5-alpha reductase inhibitors or previous prostate surgery; positive urine culture.

All patients underwent urodynamics analysis using the Medical Measurement System Software (MMS) Solar Blue, Enschede, the Netherlands. Patients were positioned supine, and all urodynamics recordings consisted of cystometry and pressure-flow study (filling and voiding phase). BOOI and BCI values were subsequently calculated.

Subvesical obstruction is defined by the BOOI on urodynamics, calculated using the equation: BOOI = $P_{det}Q_{max}$ - $2Q_{max}$, where P_{det} is detrusor pressure at peak flow rate and Q_{max} is the peak flow rate [5] (Table 1) (Figure 1).

Table 1
Subvesical obstruction defined by BOOI

BOOI	Inference
< 20	Unobstructed
20–40	Equivocal
> 40	Obstructed

BOOI – bladder outlet obstruction index; P_{det} – detrusor pressure at the peak flow rate; Q_{max} – peak flow rate; ICS – International Continence Society.
***Note*: BOOI = $P_{det}Q_{max}$ - $2Q_{max}$ according to ICS.**

BCI is a urodynamic parameter that represents the strength of BC, calculated using the formula: BCI = $P_{det}Q_{max}$

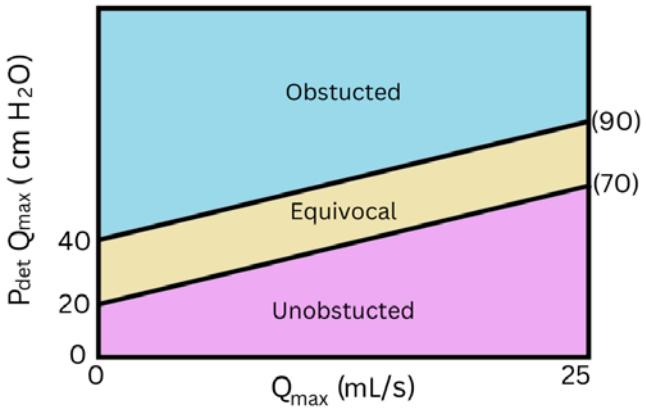

Fig. 1 – Presentation of the BOOI nomogram according to ICS. For abbreviations, see Table 1.

Table 2

Bladder contractility index (BCI) according to ICS

BCI	Contractility
> 150	Strong
100–150	Normal
< 150	Weak

***Note*: BCI is calculated as follows: BCI = $P_{det}Q_{max}$ + 5Q_{max}.**
For abbreviations, see Table 1.

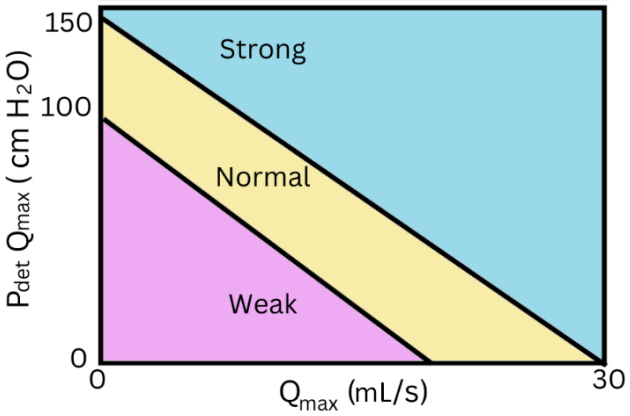

Fig. 2 – Presentation of bladder contractility normogram according to ICS. For abbreviations, see Table 1.

+ 5Q_{max} [5]. The results can be divided into three groups: strong, normal, and weak (Table 2) (Figure 2).

Statistical analysis

All statistical analyses were conducted using R Statistical Software (v4.1.2; R Core Team 2021). The independent samples *t*-test was applied to compare continuous variables between two independent groups, while analysis of variance (ANOVA) was employed for comparison across different patient categories based on BOO and BC. To assess associations between different urodynamic parameters—BOOI, BCI, $P_{det}Q_{max}$, and Q_{max}—correlation analysis was performed. The value of $p < 0.01$ was considered statistically significant.

Results

After the collection of data, patients were divided into nine groups according to values of BOOI and BCI (Figure 3).

High values of BOOI (> 40) could be accompanied by weak BC (BCI < 100) in 11.37% of patients (Group 9*), suggesting a possible cause of LUTS (Table 3). Strong BC (BCI > 150) was associated with BOOI > 40 in a significant number of patients (7.39%), showing that a strong BC might

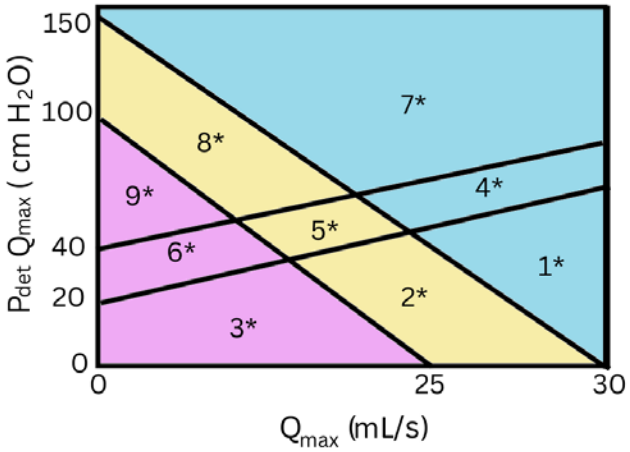

Fig. 3 – Composite nomogram permitting categorization of patients into nine zones based on the BOOI and BCI, with Q_{max} on the abscissa and $P_{det}Q_{max}$ on the ordinate. For abbreviations, see Tables 1 and 2.
***Note*: The asterisk (*) represents a legend for each group, and numbers from 1 to 9 represent groups of patients.**

Table 3

BOOI and BCI correlation

Parametres	BCI					
	≤ 100		101–149		≥ 150	
BOOI						
> 80	9*	0 (0)	8*	11 (6.25)	7*	10 (5.68)
60–80	9*	2 (1.14)	8*	7 (3.98)	7*	2 (1.14)
40–59	9*	18 (10.23)	8*	15 (8.52)	7*	1 (0.57)
20–39	6*	23 (13.07)	5*	17 (9.66)	4*	2 (1.14)
< 20	3*	40 (22.73)	2*	25 (14.21)	1*	3 (1.70)

For abbreviations, see Tables 1 and 2.
All values are given as numbers (percentages).
***Note*: The asterisk (*) represents a legend for each group, and numbers from 1 to 9 represent groups of patients.**

Table 4

Patients were divided into nine categories according to BOOI and BCI

Group	BOOI	BCI	Number of patients (%)
1*	< 20	≥ 150	3 (1.7)
2*	< 20	101–149	25 (14.21)
3*	< 20	≤ 100	40 (22.73)
4*	20–39	≥150	2 (1.14)
5*	20–39	101–149	17 (9.66)
6*	20–39	≤ 100	23 (13.07)
7*	> 40	≥150	13 (7.39)
8*	> 40	101–149	33 (18.73)
9*	> 40	≤ 100	20 (11.37)

For abbreviations, see Tables 1 and 2.
***Note*: The asterisk (*) represents a legend for each group, and numbers from 1 to 9 represent groups of patients.**

serve as a compensating mechanism of severe BPO (Group 7*). Group of patients with BOOI 20–39 and BCI 101–149 (Group 5*) could be considered "gray zone" depending on the level of other observed values for definitive clinical interpretation. This zone is particularly important in clinical decision-making and everyday practice. For better visualization of trends, patients with BOOI > 40 were further subdivided into three groups: BOOI 40–59, BOOI 60–80, and BOOI > 80, corresponding to Groups 9*, 8*, and 7* depending on BCI values. Among patients (6.25%) with BOOI > 80 and BCI values between 101–149, distinguishing whether LUTS was primarily due to BOO or BC became more challenging. As expected, groups with BCI < 100 and >150 showed an inverse correlation with BOOI.

Patients were divided into nine categories, ranging from Group 1* with no evident obstruction and preserved BC to Group 9* with evident obstruction and decreased contractility (Table 4).

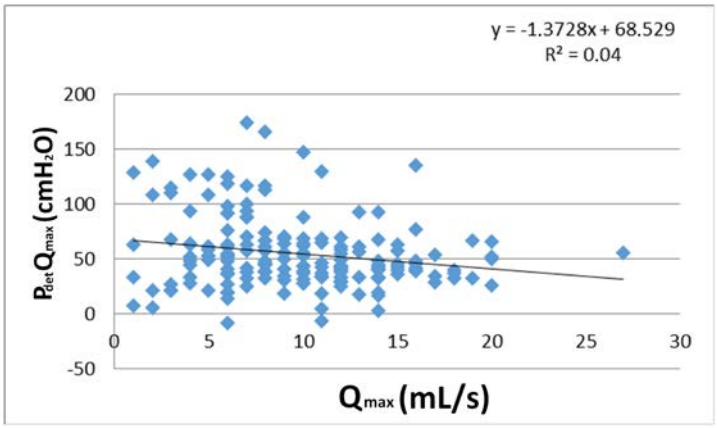

Fig. 4 – Q_{max} and $P_{det}Q_{max}$ correlation.
For abbreviations, see Table 1.

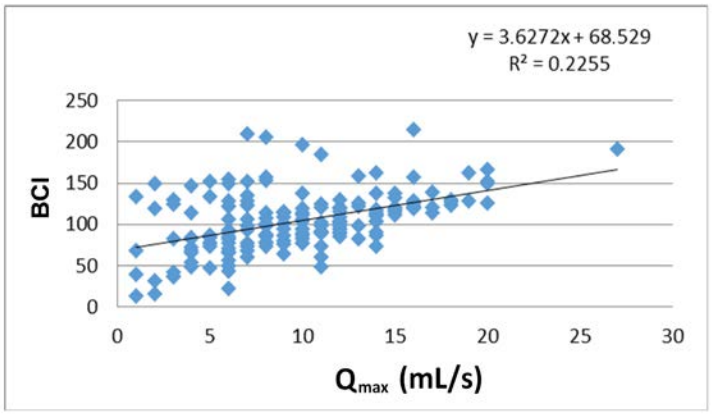

Fig. 5 – BCI and Q_{max} correlation.
For abbreviations, see Tables 1 and 2.

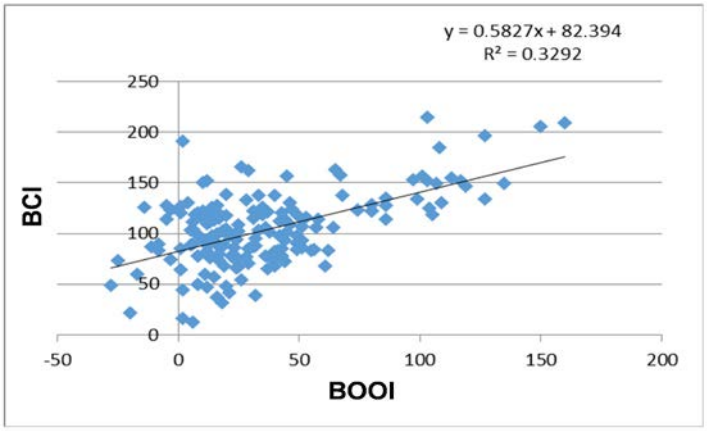

Fig. 6 – BOOI and BCI correlation.
For abbreviations, see Tables 1 and 2.

Analysis of $P_{det}Q_{max}$ and Q_{max} showed no statistically significant correlation (r = -0.2006) (Figure 4).

A slightly negative correlation could be observed between Q_{max} and BOOI (r = -0.44841, $p < 0.001$).

To assess the effect of BC on maximal flow, BCI and Q_{max} were analyzed, showing a positive correlation (R^2 = 0.2255, $p < 0.001$) (Figure 5).

A positive linear correlation between the observed indices BOOI and BCI was found (R^2 = 0.3292, $p < 0.001$) (Figure 6). Although there was linear correlation between all observed parameters, it was evident that this linear dependence was not strong enough to rely on a single parameter, emphasizing the need to assess both parameters routinely.

Discussion

DU is a frequent disorder affecting both male and female patients, causing significant morbidity and a reduction in quality of life. Its prevalence is surprisingly high, affecting 40.2% of male and 13.3% of female patients undergoing urodynamic studies for LUTS without any identifiable anatomical cause [6]. DU is defined as a contraction of reduced strength and/or duration, resulting in inadequate bladder emptying [7]. The most common symptoms related to DU include incomplete emptying, prolonged voiding, weak stream, hesitancy, and bladder hyposensitivity.

BOO, as defined by the ICS, is characterized as elevated detrusor pressure with reduced urine flow rate due to subvesical obstruction on urodynamic studies during the voiding phase. Subvesical obstruction is a polymorph in origin; the most frequent cause of subvesical obstruction in elderly males is caused by BPH, subsequent BPE, and resulting BPO [8].

The coexistence of BOO and DU presents a challenge for accurate diagnosis and treatment. Since DU has a high prevalence in concordance with BOO, it is important to identify patients with coexisting DU, making treatment options for BPH, as the most frequent cause of BOO, much more effective. Although some studies have investigated DU as a consequence of long-term BOO, no direct causal relationship has been established [9, 10]. On the other hand, long-term obstruction does lead to detrusor hypertrophy, changes in vasculature and innervation, possibly leading to detrusor overactivity and DU. Without treatment, long-term obstruction will lead to deterioration of detrusor function and decompensation. Recent studies, though limited, confirmed low levels of urothelial E-cadherin expression and increased expression of β3 and M3 in a group of patients with BOO and DU compared to patients with BOO and normal detrusor contractility [11]. The deposition of collagen in the detrusor may be a point at which DU persists despite de-obstructive treatment [12]. Transurethral resection of the prostate (TURP) and laser prostatectomy are recommended and effective treatment options for BPO unresponsive to medical therapy; however, unrecognized DU is a factor that can decrease the efficiency of operative treatment.

In our study, among the group of patients with BCI ≤ 149, it is evident that as the BOOI level decreases, the number of patients increases. This suggests that patients with weaker BC often do not have substantial subvesical obstruction, meaning that routine operative de-obstruction would not provide the expected improvement in voiding [13]. The group of patients with BCI 101–149 gives us a much sparser patient distribution. In a group of patients with BCI ≥ 150, the correlation was inverse to that seen in the BCI ≤ 149 group. This correlation may be a manifestation of activated bladder compensation mechanisms in order to overcome significant subvesical obstruction, eventually leading to possible bladder decompensation and obstructive consequences to the upper urinary tract [14, 15].

There is an ongoing debate regarding the exact mechanism of DU as a consequence of BOO, with hyperplasia and hypertrophy being the primary mechanisms. Further, myocyte damage may be provoked by reactive oxygen species, which damage muscle fibers, leading to the deposition of collagen, extracellular matrix remodeling, and ultimately, decompensation [14, 16]. In a study by Yang et al. [17] analyzing predictive factors for alleviation of LUTS symptoms after bipolar TURP, BCI and BOOI, among others, were identified as important predictors of the level of improvement following operative de-obstruction. A combination of BOOI and BCI is recommended in everyday use, as it enables the identification of patients who are most likely to benefit from surgery.

Q_{max} is the most valued and observed uroflowmetry parameter. According to the ICS, the Q_{max} cut-off of 10mL/s has a 47.0% sensitivity and a 70.0% specificity for diagnosing BOO [18]. A meta-analysis of 16 studies found that the Q_{max} value of 10 mL/s had a sensitivity and specificity of 68.3% and 70.5%, respectively, for the diagnosis of BOO [19]. $P_{det}Q_{max}$ represents intravesical pressure and is directly related to BC, but indirectly represents the degree of bladder outlet resistance (the more pronounced BOO, the higher the elevation in $P_{det}Q_{max}$). No statistically significant correlation was observed between $P_{det}Q_{max}$ and Q_{max}. In a study conducted by Tammela et al. [20], 216 patients in 11 centers were evaluated, and no statistically significant correlation was found between $P_{det}Q_{max}$ and Q_{max}.

BOOI, as a parameter of subvesical obstruction, was correlated to Q_{max}. When BC is normal, it is presumed that there is an inverse correlation: when BOOI is higher, a lower Q_{max} can be expected. Results of this study showed that Q_{max} and BOOI have a slight inverse correlation (r = -0.44841, $p < 0.001$). Since those two parameters should have a strong inverse correlation, we must presume that there is a contributing factor responsible for this weak interdependence. An additional factor that may lead to oscillation in BOOI and Q_{max} correlation is detrusor contractility and BCI. If we consider a high prevalence of DU, this may be a frequent and significant factor leading to a decrease in Q_{max}, despite lower values of BOOI. It is implied that BOOI, BCI, and Q_{max} are good prognostic factors of LUTS improvement after operative de-obstruction [21].

In order to assess the influence of BC on maximal flow, BCI and Q_{max} were correlated, and the analysis showed a statistically significant positive correlation (R^2 = 0.2255, $p < 0.001$). A positive correlation between higher Q_{max} and higher BCI is expected; however, this is not always observed in urodynamic analysis. The degree of BOO can influence and alter this correlation [22]. Van Dort et al. [23] found statistically significant differences in prostate size, as well as differences in urodynamic parameters between constrictive and compressive BOO. The findings of this study may provide the missing link, offering an elegant explanation for obstructive voiding difficulties in a subgroup of patients with small prostates.

Although a positive linear correlation between the observed parameters (BOOI and BCI) was found

($R^2 = 0.3292$, $p < 0.001$), it is evident that this linear dependence is insufficient for just one parameter to be observed and utilized. This emphasizes the need to assess both parameters regularly, especially in complicated, specific groups of patients with neurological LUTS [24, 25].

Conclusion

Patients with BCI ≤ 149 and BOOI in the unobstructed range would not have benefited from operative de-obstruction. In contrast, patients with BCI ≥ 150 showed an inverse correlation compared to the group with BCI ≤ 149. This is a group that would benefit the most from operative de-obstruction, possibly due to an activated bladder compensatory mechanism, but demanding in terms of diagnosis. As the bladder outlet and urethra are more than a rigid tube, elasticity and compression of surrounding tissue may contribute to the weak correlation between $P_{det}Q_{max}$ and Q_{max}. It is presumed that BOOI and Q_{max} should be in strong inverse correlation, but the results of this study showed that there is just a weak inverse correlation, bringing other factors into focus (BC and degree of BOO). BCI showed a positive correlation with values of Q_{max}. A positive linear correlation was also found between BOOI and BCI, but it is not sufficient to rely on a single parameter.

R E F E R E N C E S

1. *Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U,* et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 2002; 21(2): 167–78.
2. *Lee CL, Kuo HC.* Pathophysiology of benign prostate enlargement and lower urinary tract symptoms: Current concepts. Tzu Chi Med J 2017; 29(2): 79–83.
3. *Matsukawa Y, Naito Y, Ishida S, Matsuo K, Majima T, Gotoh M.* Two types of detrusor underactivity in men with nonneurogenic lower urinary tract symptoms. Neurourol Urodyn 2023; 42(1): 73–9.
4. *Drake MJ, Lewis AL, Young GJ, Abrams P, Blair PS, Chapple C,* et al. Diagnostic Assessment of Lower Urinary Tract Symptoms in Men Considering Prostate Surgery: A Noninferiority Randomised Controlled Trial of Urodynamics in 26 Hospitals. Eur Urol 2020; 78(5): 701–10.
5. *Abrams P.* Bladder outlet obstruction index, bladder contractility index and bladder voiding efficiency: three simple indices to define bladder voiding function. BJU Int 1999; 84(1): 14–5.
6. *Jeong SJ, Kim HJ, Lee YJ, Lee JK, Lee BK, Choo YM,* et al. Prevalence and Clinical Features of Detrusor Underactivity among Elderly with Lower Urinary Tract Symptoms: A Comparison between Men and Women. Korean J Urol 2012; 53(5): 342–8.
7. *Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U,* et al. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. Urology 2003; 61(1): 37–49.
8. *D'Ancona C, Haylen B, Oelke M, Abranches-Monteiro L, Arnold E, Goldman H,* et al. The International Continence Society (ICS) report on the terminology for adult male lower urinary tract and pelvic floor symptoms and dysfunction. Neurourol Urodyn 2019; 38(2): 433–77.
9. *Thomas AW, Cannon A, Bartlett E, Ellis-Jones J, Abrams P.* The natural history of lower urinary tract dysfunction in men: minimum 10-year urodynamic followup of transurethral resection of prostate for bladder outlet obstruction. J Urol 2005; 174(5): 1887–91.
10. *Al-Hayek S, Thomas A, Abrams P.* Natural history of detrusor contractility--minimum ten-year urodynamic follow-up in men with bladder outlet obstruction and those with detrusor. Scand J Urol Nephrol Suppl 2004; (215): 101–8.
11. *Jiang YH, Lee CL, Kuo HC.* Urothelial dysfunction, suburothelial inflammation and altered sensory protein expression in men with bladder outlet obstruction and various bladder dysfunctions: Correlation with urodynamics. J Urol 2016; 196(3): 831–7.
12. *Averbeck MA, De Lima NG, Motta GA, Beltrao LF, Abboud Filho NJ, Rigotti CP,* et al. Collagen content in the bladder of men with LUTS undergoing open prostatectomy: A pilot study. Neurourol Urodyn 2018; 37(3): 1088–94.
13. *Kim M, Jeong CW, Oh SJ.* Effect of preoperative urodynamic detrusor underactivity on transurethral surgery for benign prostatic hyperplasia: a systematic review and meta-analysis. J Urol 2018; 199(1): 237–44.
14. *Wang J, Ren L, Liu X, Liu J, Ling Q.* Underactive Bladder and Detrusor Underactivity: New Advances and Prospectives. Int J Mol Sci 2023; 24(21): 15517.
15. *Santos-Pereira M, Charrua A.* Understanding underactive bladder: A review of the contemporary literature. Porto Biomed J 2020; 5(4): e070.
16. *Kim SJ, Kim J, Na YG, Kim KH.* Irreversible Bladder Remodeling Induced by Fibrosis. Int Neurourol J 2021; 25(Suppl 1): S3–7.
17. *Yang J, Song H, Zhan H, Ding M, Luan T, Chen J,* et al. The influence of preoperative urodynamic parameters on clinical results in patients with benign prostatic hyperplasia after transurethral resection of the prostate. World J Urol 2023; 41(12): 3679–85.
18. *Reynard JM, Yang Q, Donovan JL, Peters TJ, Schafer W, de la Rosette JJ,* et al. The ICS-'BPH' Study: uroflowmetry, lower urinary tract symptoms and bladder outlet obstruction. Br J Urol 1998; 82(5): 619–23.
19. *Malde S, Nambiar AK, Umbach R, Lam TB, Bach T, Bachmann A,* et al. Systematic Review of the Performance of Noninvasive Tests in Diagnosing Bladder Outlet Obstruction in Men with Lower Urinary Tract Symptoms. Eur Urol 2017; 71(3): 391–402.
20. *Tammela TL, Schäfer W, Barrett DM, Abrams P, Hedlund H, Rollema HJ,* et al. Repeated pressure-flow studies in the evaluation of bladder outlet obstruction due to benign prostatic enlargement. Finasteride Urodynamics Study Group. Neurourol Urodyn 1999; 18(1): 17–24.
21. *El Khoury J, Hermieu N, Chesnel C, Xylinas E, Teng M, Ouzaid I,* et al. Primary bladder neck obstruction in men: The importance of urodynamic assessment and cystourethrography in measuring its severity. Neurourol Urodyn 2024; 43(4): 874–82.
22. *Rosier PFWM, Gammie A, Valdevenito JP, Speich J, Smith P, Sinha S.* ICS-SUFU standard: Theory, terms, and recommendations for pressure-flow studies performance, analysis, and reporting. Part 2: Analysis of PFS, reporting, and diagnosis. Neurourol Urodyn 2023; 42(8): 1603–27.
23. *Van Dort W, Rosier PFWM, van Steenbergen TRF, Geurts BJ, de Kort LMO.* Constrictive versus compressive bladder outflow obstruction in men: does it matter? Neurourol Urodyn 2024; 43(8): 2178–84.

24. *Marshall SJ, Wang D, Fung YC, Blaivas J*. Urodynamic findings that are most impactful for patients with neurogenic bladder and the literature that supports this. Curr Bladder Dysfunct Rep 2024; 19(2): 211–29.
25. *Croghan SM, Skolarikos A, Jack GS, Manecksha RP, Walsh MT, O'Brien FJ,* et al. Upper urinary tract pressures in endourology: a systematic review of range, variables and implications. BJU Int 2023; 131(3): 267–79.

Received on March 4, 2025
Revised on July 25, 2025
Accepted on August 6, 2025
Online First October 2025

ORIGINAL ARTICLE

UDC: 617.51:616.13-007.64-089-036.82
DOI: https://doi.org/10.2298/VSP250706074P

Impact of surgical timing on early functional and cognitive recovery after aneurysmal subarachnoid hemorrhage: the role of early rehabilitation

Uticaj vremena operacije na rani funkcionalni i kognitivni oporavak nakon aneurizmatskog subarahnoidnog krvarenja: uloga rane rehabilitacije

[1]Angelka Pešterac-Kujundžić*, [1]Nela Ilić[†‡], Vojislav Bogosavljević[†§], Andjela Milovanović[†‡], Sanja Tomanović Vujadinović[†‡], Una Nedeljković[†‡]

***Academy of Applied Studies, The College of Health Sciences, Belgrade, Serbia; [†]University of Belgrade, Faculty of Medicine, Belgrade, Serbia; University Clinical Center of Serbia, [‡]Center for Physical Medicine and Rehabilitation, [§]Clinic for Neurosurgery, Belgrade, Serbia**

[1]The two authors contributed equally to this study

Abstract

Background/Aim. Aneurysmal subarachnoid hemorrhage (aSAH) is a critical condition with significant functional and cognitive consequences. The optimal timing for surgical intervention remains controversial, particularly regarding early recovery. The aim of this study was to evaluate early functional and cognitive recovery in patients treated with early vs. delayed surgery, all of whom participated in a standardized early rehabilitation program. **Methods.** This prospective single-center cohort study included 114 patients who underwent surgery for ruptured intracranial aneurysms between November 2022 and November 2023. Patients were divided into two groups: the early surgery group, where the surgery was performed within three days after aneurysm rupture, and the delayed surgery group, where the surgery was performed more than three days after rupture. Functional status was assessed using the Functional Independence Measure (FIM) scale, and cognitive status was assessed using the Mini-Mental State Examination (MMSE). Both tests were administered at the start and end of the early rehabilitation program. Descriptive and inferential statistical methods (Wilcoxon signed-rank test, split-plot analysis of variance) were used for data analysis. **Results**. A statistically significant improvement in both functional and cognitive scores was achieved in both groups during the early rehabilitation program ($p < 0.001$). The greatest improvement was achieved in the FIM motor subscale. At discharge, the early surgery group achieved significantly higher FIM motor and total scores compared to the delayed surgery group ($p = 0.024$, $p = 0.037$, respectively). No statistically significant differences were found between the groups in MMSE or FIM cognitive scores. The severity of hemorrhage significantly affected changes in MMSE and FIM scores. Age, length of hospital stay, and the time period until rehabilitation initiation did not significantly influence patient recovery. **Conclusion.** Early surgical intervention for aSAH, combined with a standardized rehabilitation program, is associated with better early motor functional recovery compared to delayed surgery. Individualized rehabilitation strategies may be needed for patients who underwent delayed surgery.

Key words:
aneurysm, ruptured; convalescence; intracranial aneurysm; rehabilitation; subarachnoid hemorrhage; surgical procedures, operative.

Apstrakt

Uvod/Cilj. Subarahnoidno aneurizmatsko krvarenje (*aneurysmal subarachnoid hemorrhage* – aSAH) predstavlja kritično stanje sa značajnim funkcionalnim i kognitivnim posledicama. Pitanje optimalnog vremena za hiruršku intervenciju i dalje je kontroverzno, posebno u odnosu na rani opravak bolesnika. Cilj rada bio je da se ispita rani funkcionalni i kognitivni opravak kod bolesnika operisanih u ranom i odloženom terminu, uključenih u standardni rani rehabilitacioni program. **Metode.** Ova prospektivna kohortna studija jednog centra obuhvatila je 114 bolesnika operisanih zbog rupture intrakranijalne aneurizme u periodu od novembra 2022. do novembra 2023. godine. Bolesnici su podeljeni u dve grupe: rano operisane, kojima je operacija izvršena u roku od tri dana nakon rupture

Correspondence to: Una Nedeljković, University Clinical Center of Serbia, Center for Physical Medicine and Rehabilitation, Pasterova 2, 11 000 Belgrade, Serbia. E-mail: unaned@gmail.com

aneurizme, i odloženo operisane, kod kojih je operacija obavljena u terminu dužem od tri dana nakon rupture. Funkcionalni status procenjivan je upotrebom skale za procenu funkcionalne nezavisnosti (*Functional Independence Measure* – FIM), a kognitivni status upotrebom *Mini-Mental State Examination* (MMSE) testa. Oba testa primenjena su na početku i završetku ranog programa rehabilitacije. Metode deskriptivne i inferencijalne statistike (*Wilcoxon signed-rank test*, *split-plot analysis of variance*) korišćene su za analizu podataka. **Rezultati.** Tokom sprovođenja ranog rehabilitacionog programa u obe grupe je postignuto statistički značajno poboljšanje rezultata funkcionalnog i kognitivnog statusa ($p < 0{,}001$). Najveće poboljšanje postignuto je u FIM motornoj podskali. Na otpustu, rano operisani bolesnici postigli su značajno veće FIM motorne i ukupne rezultate u odnosu na odloženo operisane ($p = 0{,}024$, $p = 0{,}037$, redom). Nije bilo statistički značajne razlike između grupa u MMSE i FIM kognitivnim rezultatima. Stepen krvarenja značajno je uticao na promene u FIM i MMSE rezultatima. Godine starosti, dužina boravka u bolnici, kao i vremenski period do započinjanja rehabilitacije nisu značajno uticali na oporavak bolesnika. **Zaključak.** Rana hirurška intervencija za aSAH, udružena sa standardizovanim programom rehabilitacije, povezana je sa boljim ranim motornim funkcionalnim oporavkom bolesnika, u odnosu na odloženu operaciju. Za bolesnike operisane u odloženom periodu može biti potrebna primena individualizovanih strategija rehabilitacije.

Ključne reči:
aneurizma, ruptura; oporavak; aneurizma, intrakranijalna; rehabilitacija; krvarenje, subarahnoidno; hirurgija, operativne procedure.

Introduction

Aneurysmal subarachnoid hemorrhage (SAH) – aSAH represents a dramatic neurological event, with a global incidence of about 6.7 *per* 100,000 persons [1]. Approximately 85% of nontraumatic SAH result from ruptured aneurysm [2], with high early mortality rates [3]. Although the latest guidelines [4] recommend surgical treatment of ruptured intracranial aneurysm within 24 hrs, in clinical practice, many patients are operated on later due to delayed diagnosis and admission. Published data on the timing of surgery remain inconsistent. While some authors advocate for delayed surgery, others report better outcomes with early surgery, or no significant impact of timing at all [5–7].

Postoperative management is focused on reducing secondary complications, such as rebleeding, secondary ischemia, hydrocephalus, and other systemic complications [8–11]. Early rehabilitation, starting in intensive care and involving early mobilization, is crucial for preventing complications of prolonged bed rest [12–15]. Many studies have demonstrated that early rehabilitation is both safe and feasible [16–22], but data on early functional and cognitive recovery remain limited. The majority of existing research focuses on mid- and long-term outcomes [23–25], with limited evidence connecting early postoperative functional and cognitive status to later recovery.

Unlike most previous studies, which focus on outcomes assessed months or years after surgery, our study evaluates functional and cognitive status in the early postoperative period, immediately after completion of the initial rehabilitation program.

The aim of this study was to examine early functional and cognitive recovery in patients who underwent surgery in two different time periods (early and delayed surgery) and to identify factors that may be associated with these outcomes. We hypothesized that early surgical treatment, combined with an early rehabilitation program, would result in better early functional outcomes, while cognitive outcomes would not significantly differ between groups. As functional and cognitive status at discharge are critical for further management and planning of rehabilitation, our findings could be valuable for improving clinical protocols.

Methods

This prospective single-center cohort study included all patients treated at the Neurosurgery Clinic of the University Clinical Center of Serbia, Belgrade, Serbia, following surgical repair of a ruptured intracranial aneurysm from November 2022 to November 2023. This study was approved by the Ethics Committee of the University Clinical Center of Serbia (No. 1039/3, from October 26, 2022). Written informed consent was obtained from all participants.

A total of 114 patients were admitted for operative treatment of aSAH, with 46 patients in the early surgery (ES) group and 68 in the delayed surgery (DS) group, based on the neurosurgeon's decision. The ES group included patients who underwent intracranial aneurysm surgery within the first three days after rupture, while the DS group included patients operated on after more than three days from rupture. During hospitalization, three patients died (two in the DS and one in the ES group); their data were excluded from further analysis.

Exclusion criteria were previous SAH, brain injury, neurodegenerative disorder, brain tumors, or endovascular treatment of an aneurysm.

Data were collected from patients' medical records and included the following: age, gender, educational level, premorbid occupational status, relationship status, clinical severity of hemorrhage, complications, duration of inpatient rehabilitation (in days), time from aSAH to the start of rehabilitation, and total length of hospital stay.

The clinical status at hospital admission was assessed using the Hunt-Hess scale [26], which classifies the severity of aSAH into five grades, ranging from minimally symptomatic to coma. Grades IV and V are considered poor-grade SAH. The Functional Independence Measure (FIM) was administered at the beginning of the early rehabilitation program and

at hospital discharge. The FIM is a widely used and validated tool for assessing functional status in various neurological conditions [27]. It consists of 18 items, grouped into two subscales: motor and cognitive. The scale assesses six domains: self-care, continence, mobility, transfers, communication, and cognition. Each item is rated from 1 (total assistance) to 7 (complete independence). The maximum score is 91 for the motor subscale and 35 for the cognitive subscale.

The Mini-Mental State Examination (MMSE) [28] was used to assess cognitive impairment. This screening tool consists of 11 questions and evaluates orientation, attention, short-term memory, language skills, and visuospatial abilities. Each correct answer is awarded one point, with a maximum score of 30. Scores below 24 suggest cognitive impairment, classified as mild (19–23), moderate (10–18), or severe (0–9).

Early rehabilitation was initiated when patients met the inclusion criteria defined by the internal protocol of the University Clinical Center of Serbia, which required hemodynamic and respiratory stability, an intracranial pressure of less than 20 mmHg, and the absence of secondary neurological complications. The rehabilitation team consisted of a physical medicine and rehabilitation specialist, a neurosurgeon, a physical therapist, and nurses. The physical medicine and rehabilitation specialist set daily rehabilitation goals and determined the activity level after each morning assessment. The rehabilitation program was individualized, based on each patient's general health, neurological findings, and functional status, and included therapeutic exercises, gradual verticalization, and mobilization. Therapy was conducted twice a day, five days a week, and once on weekends if medically appropriate. If complications arose, the rehabilitation protocol was adjusted or temporarily discontinued.

Statistical analysis

Continuous variables were described using median (Me) and interquartile range (IQR), as well as arithmetic mean and standard deviation. Categorical variables were summarized as frequencies and percentages. Group differences were assessed using the independent samples *t*-test and Chi-square test, while changes in repeated measures were analyzed with the Wilcoxon signed-rank test. The effect of independent variables on change scores was evaluated using split-plot analysis of variance (SPANOVA). Statistical significance was set at $p \leq 0.05$. All analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

Results

The sociodemographic and clinical characteristics of the patients are presented in Table 1. There was a significant difference in age between the groups, with DS patients being significantly older than those in the ES group ($p = 0.01$). The DS group also had a significantly longer hospital length of stay and began early rehabilitation much later following aSAH ($p < 0.001$).

Table 1

Sociodemographic and basic clinical characteristics of patients according to surgical timing

Characteristics	Total (n = 114)	Surgery group		*p*-value
		early (n = 46)	delayed (n = 68)	
Gender				
male	41 (36.0)	19 (41.3)	22 (32.4)	0.329 [a]
female	73 (64.0)	27 (58.7)	46 (67.6)	
Age, years	53.56 ± 10.37	50.41 ± 10.05	55.69 ± 10.11	**0.008** [b]
Age categories				**0.013[a]**
27–45	28 (24.6)	16 (34.8)	12 (17.6)	
46–55	30 (26.3)	15 (32.6)	15 (22.1)	
>56	56 (49.1)	15 (32.6)	41 (60.3)	
Educational level				0.098 [a]
primary school	35 (30.7)	18 (39.1)	17 (25.0)	
high school	56 (49.1)	17 (37.0)	39 (57.4)	
college and university	23 (20.2)	11 (23.9)	12 (17.6)	
Employment				0.087 [a]
unemployed	33 (28.9)	10 (21.7)	23 (33.8)	
employed	68 (59.6)	33 (71.7)	35 (51.5)	
retired	13 (11.4)	3 (6.5)	10 (14.7)	
Relationship, marriage				0.606 [a]
yes	102 (89.5)	42 (91.3)	60 (88.2)	
no	12 (10.5)	4 (8.7)	8 (11.7)	
Hunt-Hess grade				0.992 [a]
I	17 (14.9)	7 (15.2)	10 (14.7)	
II	72 (63.2)	28 (60.9)	44 (64.7)	
III	18 (15.8)	8 (17.4)	10 (14.7)	
IV	2 (1.8)	1 (2.2)	1 (1.5)	
V	5 (4.4)	2 (4.3)	3 (4.4)	

Table 1 (continued)

Characteristics	Total (n = 114)	Surgery group		*p*-value
		early (n = 46)	delayed (n = 68)	
Intraventricular hemorrhage	8 (7)	2 (4.7)	6 (11.8)	0.282 [a]
Intracerebral hemorrhage	14 (12.3)	5 (11.6)	9 (17.6)	0.414 [a]
Ischemia	8 (7)	5 (11.6)	3 (5.9)	0.463 [a]
Hydrocephalus	3 (2.6)	1 (2.3)	3 (5.9)	0.622 [a]
Duration of rehabilitation, days	9.09 ± 6.63	8.13 ± 4.84	9.72 ± 7.56	0.215 [b]
Start of rehabilitation from an aneurysm rupture attack, days	13.27 ± 6.23	8.78 ± 3.13	16.24 ± 6.00	**< 0.001** [b]
Start of rehabilitation from an aneurysm rupture attack, days				**< 0.001** [a]
3–8	27 (23.7)	23(50.0)	4 (5.9)	
9–14	44 (38.6)	21 (45.7)	23 (33.8)	
15–20	33 (28.9)	2 (4.3)	31 (45.6)	
> 20	10 (8.8)	0 (0)	10 (14.7)	
LOS in hospital, days	22.39 ± 9.61	17.16 ± 5.69	25.91 ± 10.13	**< 0.001** [b]
LOS in hospital, days				**< 0.001** [a]
10–20	54 (47.4)	36 (78.3)	18 (26.5)	
21–30	46 (40.3)	8 (17.4)	38 (55.9)	
31–40	9 (7.9)	2 (4.3)	7 (10.3)	
> 40	5 (4.4)	0 (0)	5 (7.3)	

LOS – length of stay; n – number.
All values are given as numbers (percentages) or as mean ± standard deviation.
***Note*: [a] Chi-square test; [b] Independent samples *t*-test.**

Descriptive statistics for all outcome measures are shown in Table 2. The reliability [Cronbach alpha (α)] of the scales was satisfactory, ranging from 0.728 to 0.874. During the early rehabilitation period, scores on all tests improved significantly ($p < 0.001$), indicating enhancement of both functional and cognitive status among patients. The greatest improvement was observed in the FIM motor subscale, which almost doubled from baseline, as well as in the total FIM score. FIM cognitive scores also showed significant improvement during rehabilitation, although to a lesser extent compared to the motor domain. Initial MMSE scores indicated mild cognitive impairment, but these scores improved significantly by discharge. Due to aphasia, the MMSE could not be performed in 16 patients (9 in the ES group and 7 in the DS group).

We further examined whether differences existed between the groups in any of the outcome measures at the two time points (Table 3). Statistically significant differences were observed in FIM_2 motor scores ($p = 0.024$), with the ES group achieving substantially higher scores (Me = 73.00, IQR = 35.00) compared to the DS group (Me = 43.00, IQR = 52.00). Similarly, the ES group had significantly higher FIM_2 total scores at discharge (Me = 104.00, IQR = 50.00) than the DS group (Me = 70.00, IQR = 68.00; $p = 0.037$).

To determine the influence of various factors (group, gender, age categories, education level, employment status, relationship status, clinical grade of hemorrhage, time from aneurysm rupture to rehabilitation start, and length of hospital stay) on changes in outcome measures (FIM and MMSE),

Table 2

Descriptive statistics of functional and cognitive outcome measures at admission and discharge in all patients and by surgical timing group

Items	Min–Max	Median (IQR)	Mean ± SD	Skewness	Kurtosis	Shapiro-Wilk	α	*p*-value
MMSE_1	0–30	21.00 (18.00)	18.17 ± 10.91	-0.635	-0.985	0.854**	0.728	< 0.001
MMSE_2	0–30	25.00 (14.00)	20.09 ± 10.87	-1.020	-0.267	0.804**	0.874	
FIM_1motor	13–81	29.00 (46.00)	36.30 ± 23.39	0.556	-1.303	0.832**	0.819	< 0.001
FIM_2motor	13–91	60.50 (49.00)	51.63 ± 25.61	-0.270	-1.534	0.868**	0.865	
FIM_1cogn	5–35	27.00 (20.00)	23.71 ± 11.13	-0.512	-1.159	0.840**	0.807	< 0.001
FIM_2cogn	5–35	30.50 (17.00)	26.01 ± 10.41	-0.904	-0.544	0.802**	0.812	
FIM_1total	13–108	55.50 (61.00)	59.48 ± 32.04	0.228	-1.369	0.904**	0.825	< 0.001
FIM_2total	18–126	89.50 (62.00)	77.00 ± 34.30	-0.386	-1.363	0.879**	0.754	

Min–Max – minimum–maximum; IQR – interquartile range; SD – standard deviation; α – Cronbach alpha; MMSE_1 – baseline Mini-Mental State Examination; MMSE_2 – MMSE at discharge; FIM_1motor – baseline Functional Independence Measure, motor subscale; FIM_2motor – FIM motor subscale at discharge; FIM_1cogn – baseline FIM, cognitive subscale; FIM_2cogn – FIM cognitive subscale at discharge; FIM_1total – baseline FIM, total score; FIM_2total – FIM total score at discharge.
The Wilcoxon signed-rank test was performed.

we performed SPANOVA (Table 4). The effect of time was further analyzed across the two time intervals, revealing a statistically significant improvement in all outcome measures, with high partial eta-squared (η^2) values. The severity of hemorrhage was the only variable that had a significant effect on the change in MMSE scores (p = 0.008, partial η^2= 0.119). Group assignment (ES vs. DS) had a statistically significant effect on the change in FIM motor subscale scores (p = 0.056, partial η^2 = 0.033), with a greater improvement observed in the ES group (from Me = 35.00, IQR = 48.00 to Me = 72.50, IQR = 37.00; 107.14% increase), compared to the DS group (from Me = 24.00, IQR = 41.00 to Me = 43.00, IQR = 52.00; 79.16% increase). Severity of hemorrhage also significantly affected the change in FIM motor subscale scores (p = 0.037, partial η^2 = 0.091). No independent variables were found to significantly affect the change in FIM cognitive scores. Regarding the FIM total score, only the severity of hemorrhage was found to significantly influence change (p = 0.032, partial η^2 = 0.095).

Table 3

Comparison of functional and cognitive outcome scores between early and delayed surgery groups at admission and discharge

Items	Total (n = 114)	Surgery group		*p*-value[a]
		early (n = 46)	delayed (n = 68)	
MMSE_1*	21.00 (18.00)	23.00 (13.00)	19.00 (19.00)	0.189
MMSE_2*	25.00 (14.00)	27.00 (12.00)	22.00 (16.00)	0.197
FIM_1motor	29.00 (46.00)	46.00 (48.00)	24.00 (46.00)	0.158
FIM_1cogn	27.00 (20.00)	34.00 (16.00)	24.00 (21.00)	0.226
FIM_1total	55.50 (61.00)	71.00 (60.00)	48.00 (63.00)	0.178
FIM_2motor	60.50 (49.00)	73.00 (35.00)	43.00 (52.00)	**0.024**
FIM_2cogn	30.50 (17.00)	35.00 (13.00)	28.00 (19.00)	0.132
FIM_2total	89.50 (62.00)	104.00 (50.00)	70.00 (68.00)	**0.037**

n – number. For other abbreviations, see Table 2.
All values are given as median (interquartile range). [a]Mann-Whitney *U* test was used.
***Note*: *Testing of MMSE was performed on 98 patients (37 in the early surgery group and 61 in the delayed surgery group) due to aphasia.**

Table 4

Effects of clinical and demographic variables on changes in functional and cognitive outcomes (SPANOVA results)

Variables	Wilks' Lambda	*F*	*p*-value	partial η^2
MMSE	0.890	13.659	0.000	0.110
MMSE x group		0.998	0.320	0.009
MMSE x gender		1.841	0.178	0.016
MMSE x age		0.171	0.843	0.003
MMSE x education		0.135	0.874	0.002
MMSE x employment		0.714	0.493	0.019
MMSE x relationship status		0.013	0.910	0.000
MMSE x Hunt-Hess grade		3.626	**0.008**	0.119
MMSE x rehab from attack		1.008	0-393	0.034
MMSE x LOS		0.374	0.772	0.013
FIMmotor	0.559	86.812	0.000	0.441
FIMmotor x group		3.740	**0.056**	0.033
FIMmotor x gender		3.316	0.071	0.030
FIMmotor x age		2.007	0.140	0.038
FIMmotor x education		0.773	0.464	0.014
FIMmotor x employment		1.510	0.228	0.039
FIMmotor x relationship status		0.382	0.539	0.005
FIMmotor x Hunt-Hess grade		2.658	**0.037**	0.091
FIMmotor x MMSE		0.934	2.487	0.065
FIMmotor x rehab from attack		1.074	0.365	0.037
FIM motor x LOS		0.568	0.638	0.020
FIMcogn	0.797	27.685	0.000	0.203
FIMcogn x group		1.305	0.256	0.012
FIMcogn x gender		1.959	0.165	0.018
FIMcogn x age		0.262	0.770	0.005
FIMcogn x education		2.017	0.138	0.036
FIMcogn x employment		0.327	0.722	0.009

Table 4 (continued)

Variables	Wilks' Lambda	*F*	*p*-value	partial η^2
FIMcogn x relationship status		0.092	0.762	0.001
FIMcogn x Hunt-Hess grade		2.120	0.083	0.075
FIMcogn x MMSE		0.858	0.775	0.001
FIMcogn x rehab from attack		0.398	0.755	0.014
FIMcogn x LOS		0.444	0.722	0.016
FIMtotal	0.533	95.341	0.000	0.467
FIMtotal x group		3.361	0.069	0.030
FIMtotal x gender		3.387	0.068	0.030
FIMtotal x age		1.059	0.351	0.020
FIMtotal x education		1.141	0.323	0.021
FIMtotal x employment		1.045	0.357	0.027
FIMtotal x relationship status		0.163	0.687	0.002
FIMtotal x Hunt-Hess grade		2.749	**0.032**	0.095
FIMtotal x MMSE		0.961	0.238	0.238
FIM x rehab from attack		0.925	0.432	0.032
FIMtotal x LOS		0.506	0.679	0.018

SPANOVA – split-plot analysis of variance; η^2 – eta-squared; rehab from attack – start of rehabilitation from aneurysm rupture. For other abbreviations, see Tables 1 and 2.

Discussion

The general characteristics of our patient sample are comparable to those reported in other studies [6, 29, 30]. In comparison to large United States-based studies on surgical timing [6], our sample had a higher proportion of patients operated on after 72 hrs (over half in our cohort versus one-fourth in the United States data). This likely reflects differences in healthcare organization and the limited availability of diagnostic tools and specialized neurosurgical centers in our setting. The treating neurosurgeon made the decision regarding the timing of surgery, and we did not influence this process. There were no differences between groups in education or marital status, suggesting these factors did not impact the timing of hospital admission.

Baseline FIM scores in our cohort were higher than those reported by Saciri and Kos [31], although discharge scores were similar. This suggests that FIM gain during early rehabilitation was smaller in our patients. However, our rehabilitation period averaged only nine days, compared to 21 days in the previous study, resulting in more than double the FIM gain *per* day of rehabilitation. This accelerated gain could be attributed to the intensity of our rehabilitation program or to natural recovery processes, which may be more pronounced in the early post-surgical period. Some research supports the notion that early gains in rehabilitation are greater than later gains [32, 33], highlighting the potential benefits of starting rehabilitation as soon as possible. Studies by Olkowski et al. [19, 34] using rehabilitation protocols similar to ours also demonstrated better functional outcomes at discharge for patients included in early rehabilitation. However, their outcome measures were not directly comparable to ours.

A notable finding in our study was the marked discrepancy between the FIM motor and cognitive domains. FIM motor scores were significantly lower (31.8% of the maximum at the beginning of rehabilitation, rising to 66.0% at discharge) than FIM cognitive scores (77.0% at baseline, 87.0% at discharge). The FIM cognitive domain may lack sensitivity for detecting subtle cognitive changes in certain neurological patients [35], which could limit its usefulness for tracking cognitive recovery in this context. Only one published study reported separate FIM motor and cognitive data in the early postoperative period [24], and it included only patients with pre-existing neurological deficits. It is therefore unclear whether the motor-cognitive discrepancy we observed is specific to our cohort or represents a broader phenomenon.

MMSE was used in our study and has been validated as a reliable measure of cognitive status in similar populations. Consistent with previous findings [31, 36], our patients exhibited cognitive impairment at baseline, although scores were slightly higher than those reported elsewhere. There is evidence that physical rehabilitation, even without specific cognitive interventions, can improve cognitive function [37–39], likely through mechanisms of neuroplasticity and spontaneous brain recovery [40].

Apart from age, which was higher in the DS group, sociodemographic characteristics did not differ significantly between groups. Previous research identified age as a predictor of functional outcome [41, 42]. However, our data did not confirm this effect on the change in outcome measures. The most notable differences between groups were observed in FIM motor and FIM total scores at discharge. Lower initial scores in the DS group may reflect a higher complication burden or longer preoperative hospitalization. At discharge, FIM motor scores and FIM motor gain were almost twice as high in the ES group, indicating a different trajectory of functional recovery. As cognitive scores did not differ significantly between groups, it appears that the difference in total FIM scores was driven primarily by motor outcomes. This is further supported by our analysis, which found that group assignment had a significant influence on changes in FIM motor scores. Additionally, Hunt-Hess grade significantly affected changes in both FIM motor and total scores, as well as MMSE results, in line with prior reports [41, 43, 44]. However,

we found no differences in hemorrhage severity between groups, so this variable is unlikely to explain the group differences in functional outcome.

The duration of rehabilitation was similar in both groups, which could partially account for the lower FIM scores in the DS group. Since age, timing of rehabilitation onset, and hospital length of stay did not significantly affect FIM change, we suggest that the relatively short duration of acute rehabilitation may have limited recovery, particularly for the DS group. Unfortunately, in our institution, rehabilitation is constrained by the acute care hospital setting, with no dedicated subacute rehabilitation ward. Most patients (66.6%) were discharged home (82.0% in the ES group and 58.5% in the DS group), emphasizing the need for tailored rehabilitation management, especially for those in the DS group.

The main limitations of this study were its single-center design and the relatively small sample size. As rehabilitation strategies are highly dependent on institutional protocols and surgical decision-making, our results largely reflect local clinical practice. Nevertheless, because a large proportion of our patients underwent delayed surgery, these data contribute valuable insights into the early functional and cognitive recovery trajectories of this specific group. Our findings are consistent with previous research indicating that patients operated on in the delayed period have worse early outcomes [5], even when early rehabilitation is provided. We focused exclusively on the acute postoperative period and cannot comment on long-term recovery trajectories. Further studies are needed to assess the impact of early rehabilitation gains on long-term outcomes and to define optimal rehabilitation strategies.

Conclusion

Our findings demonstrate that patients who underwent delayed surgical treatment for aneurysmal subarachnoid hemorrhage exhibited poorer early motor functional recovery compared to those who were operated on acutely, despite participation in the same early rehabilitation program. Factors such as older age, longer hospital stay, and later initiation of rehabilitation did not show a significant effect on motor functional recovery in this cohort. These results suggest that a standardized early rehabilitation program may not be equally effective for all patient groups and highlight the potential need for tailored rehabilitation strategies for patients undergoing delayed surgical intervention.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

1. *Hughes JD, Bond KM, Mekary RA, Dewan MC, Rattani A, Baticulon R,* et al. Estimating the global incidence of aneurysmal subarachnoid hemorrhage: a systematic review for central nervous system vascular lesions and meta-analysis of ruptured aneurysms. World Neurosurg 2018; 115: 430–47.e7.
2. *Van Gijn J, Rinkel GJ.* Subarachnoid haemorrhage: diagnosis, causes and management. Brain 2001; 124(Pt 2): 249–78.
3. *Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V.* Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol 2009; 8(4): 355–69.
4. *Hoh BL, Ko NU, Amin-Hanjani S, Chou SH-Y, Cruz-Flores S, Dangayach NS,* et al. 2023 Guideline for the management of patients with aneurysmal subarachnoid hemorrhage: a guideline from the American Heart Association/American Stroke Association. Stroke 2023; 54(7): e314–70. Erratum in: Stroke 2023; 54(12): e516.
5. *Nieuwkamp DJ, de Gans K, Algra A, Albrecht KW, Boomstra S, Brouwers PJ,* et al. Timing of aneurysm surgery in subarachnoid haemorrhage – an observational study in The Netherlands. Acta Neurochir 2005; 147(8): 815–21.
6. *Siddiq F, Chaudhry SA, Tummala RP, Suri MF, Qureshi AI.* Factors and outcomes associated with early and delayed aneurysm treatment in subarachnoid hemorrhage patients in the United States. Neurosurgery 2012; 71(3): 670–8.
7. *Yao Z, Hu X, Ma L, You C, He M.* Timing of surgery for aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis. Int J Surg 2017; 48: 266–74.
8. *Fujii M, Yan J, Rolland WB, Soejima Y, Caner B, Zhang JH.* Early brain injury, an evolving frontier in subarachnoid hemorrhage research. Transl Stroke Res 2013; 4(4): 432–46.
9. *Teo M, Guilfoyle MR, Turner C, Kirkpatrick PJ; STASH Collaborators.* What factors determine treatment outcome in aneurysmal subarachnoid hemorrhage in the modern era? A post hoc STASH analysis. World Neurosurg 2017; 105: 270–81.
10. *Zhao B, Tan X, Zhao Y, Cao Y, Wu J, Zhong M,* et al. Variation in patient characteristics and outcomes between early and delayed surgery in poor-grade aneurysmal subarachnoid hemorrhage. Neurosurgery 2016; 78(2): 224–31.
11. *Chen S, Li Q, Wu H, Krafft PR, Wang Z, Zhang JH.* The harmful effects of subarachnoid hemorrhage on extracerebral organs. Biomed Res Int 2014; 2014: 858496.
12. *Brower RG.* Consequences of bed rest. Crit Care Med 2009; 37(10 Suppl): S422–8.
13. *Jang MH, Shin MJ, Shin YB.* Pulmonary and physical rehabilitation in critically ill patients. Acute Crit Care 2019; 34(1): 1–13.
14. *Needham DM.* Mobilizing patients in the intensive care unit: improving neuromuscular weakness and physical function. JAMA 2008; 300(14): 1685–90.
15. *Vorona S, Sabatini U, Al-Maqbali S, Bertoni M, Dres M, Bissett B,* et al. Inspiratory muscle rehabilitation in critically ill adults: a systematic review and meta-analysis. Ann Am Thorac Soc 2018; 15(6): 735–44.
16. *Ma Z, Wang Q, Liu M.* Early versus delayed mobilisation for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev 2013; 2013(5): CD008346.
17. *Karic T, Røe C, Nordenmark TH, Becker F, Sorteberg W, Sorteberg A.* Effect of early mobilization and rehabilitation on complications in aneurysmal subarachnoid hemorrhage. J Neurosurg 2017; 126(2): 518–26.
18. *Morello A, Spinello A, Staartjes VE, Bue EL, Garbossa D, Germans MR,* et al. Early versus delayed mobilization after aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis of efficacy and safety. Neurosurg Focus 2023; 55(6): E11.
19. *Olkowski BF, Devine MA, Slotnick LE, Veznedaroglu E, Liebman KM, Arcaro ML,* et al. Safety and feasibility of an early mobilization program for patients with aneurysmal subarachnoid hemorrhage. Phys Ther 2013; 93(2): 208–15.
20. *Takara H, Kohatsu Y, Suzuki S, Satoh S, Abe Y, Miyazato S,* et al. Initiating mobilization is not associated with symptomatic cer-

ebral vasospasm in patients with aneurysmal subarachnoid hemorrhage: a retrospective multicenter case-control study. Phys Ther Res 2022; 25(3): 134–42.
21. *Yang X, Cao L, Zhang T, Qu X, Chen W, Cheng W,* et al. More is less: effect of ICF-based early progressive mobilization on severe aneurysmal subarachnoid hemorrhage in the NICU. Front Neurol 2022; 13: 951071.
22. *Young B, Moyer M, Pino W, Kung D, Zager E, Kumar MA.* Safety and feasibility of early mobilization in patients with subarachnoid hemorrhage and external ventricular drain. Neurocrit Care 2019; 31(1): 88–96.
23. *Dorhout Mees SM, Molyneux AJ, Kerr RS, Algra A, Rinkel GJ.* Timing of aneurysm treatment after subarachnoid hemorrhage: relationship with delayed cerebral ischemia and poor outcome. Stroke 2012; 43(8): 2126–9.
24. *Kara B, Yozbatiran N, Arda MN.* Functional results of physiotherapy programme on patients with aneurysmal subarachnoid hemorrhage. Turk Neurosurg 2007; 17(2): 83–90.
25. *O'Dell MW, Watanabe TK, De Roos ST, Kager C.* Functional outcome after inpatient rehabilitation in persons with subarachnoid hemorrhage. Arch Phys Med Rehabil 2002; 83(5): 678–82.
26. *Hunt WE, Hess RM.* Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968; 28(1): 14–20.
27. *Dodds TA, Martin DP, Stolov WC, Deyo RA.* A validation of the functional independence measurement and its performance among rehabilitation inpatients. Arch Phys Med Rehabil 1993; 74(5): 531–6.
28. *Folstein MF, Folstein SE, McHugh PR.* "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12(3): 189–98.
29. *De Rooij NK, Linn FH, van der Plas JA, Algra A, Rinkel GJ.* Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and time trends. J Neurol Neurosurg Psychiatry 2007; 78(12): 1365–72.
30. *Harrison CH, Taquet M, Harrison PJ, Watkinson PJ, Rowland MJ.* Sex and age effects on risk of non-traumatic subarachnoid hemorrhage: retrospective cohort study of 124,234 cases using electronic health records. J Stroke Cerebrovasc Dis 2023; 32(8): 107196.
31. *Saciri BM, Kos N.* Aneurysmal subarachnoid haemorrhage: outcomes of early rehabilitation after surgical repair of ruptured intracranial aneurysms. J Neurol Neurosurg Psychiatry 2002; 72(3): 334–7.
32. *León-Carrión J, Machuca-Murga F, Solís-Marcos I, León-Domínguez U, Domínguez-Morales MR.* The sooner patients begin neurorehabilitation, the better their functional outcome. Brain Inj 2013; 27(10): 1119–23.
33. *Tepas JJ 3rd, Leaphart CL, Pieper P, Beaulieu CL, Spierre LR, Tuten JD,* et al. The effect of delay in rehabilitation on outcome of severe traumatic brain injury. J Pediatr Surg 2009; 44(2): 368–72.
34. *Olkowski BF, Binning MJ, Sanfillippo G, Arcaro ML, Slotnick LE, Veznedaroglu E,* et al. Early mobilization in aneurysmal subarachnoid hemorrhage accelerates recovery and reduces length of stay. J Acute Care Phys Ther 2015; 6(2): 47–55.
35. *Van der Putten JJ, Hobart JC, Freeman JA, Thompson AJ.* Measuring change in disability after inpatient rehabilitation: comparison of the responsiveness of the Barthel index and the Functional Independence Measure. J Neurol Neurosurg Psychiatry 1999; 66(4): 480–4.
36. *Milovanovic A, Grujicic D, Bogosavljevic V, Jokovic M, Mujovic N, Markovic IP.* Efficacy of early rehabilitation after surgical repair of acute aneurysmal subarachnoid hemorrhage: outcomes after verticalization on days 2-5 versus day 12 post-bleeding. Turk Neurosurg 2017; 27(6): 867–73.
37. *Shimamura N, Matsuda N, Satou J, Nakano T, Ohkuma H.* Early ambulation produces favorable outcome and nondemential state in aneurysmal subarachnoid hemorrhage patients older than 70 years of age. World Neurosurg 2014; 81(2): 330–4.
38. *Varuges JA, Mazlan M.* Rehabilitation characteristics and outcomes of adults with traumatic brain injury: a retrospective study in UMMC, a tertiary centre in Klang Valley. Med J Malaysia 2023; 78(2): 190–6.
39. *Pushko OO.* The influence of active rehabilitation on the recovery of cognitive and psychoemotional disorders after ischemic stroke. Wiad Lek 2021; 74(8): 1910–6.
40. *Stillman CM, Cohen J, Lehman ME, Erickson KI.* Mediators of physical activity on neurocognitive function: a review at multiple levels of analysis. Front Hum Neurosci 2016; 10: 626.
41. *Karic T, Røe C, Nordenmark TH, Becker F, Sorteberg A.* Impact of early mobilization and rehabilitation on global functional outcome one year after aneurysmal subarachnoid hemorrhage. J Rehabil Med 2016; 48(8): 676–82.
42. *Takemoto Y, Hasegawa Y, Hashiguchi A, Moroki K, Tokuda H, Mukasa A.* Predictors for functional outcome in patients with aneurysmal subarachnoid hemorrhage who completed in-hospital rehabilitation in a single institution. J Stroke Cerebrovasc Dis 2019; 28(7): 1943–50.
43. *Brooks FA, Ughwanogho U, Henderson GV, Black-Schaffer R, Sorond FA, Tan CO.* The link between cerebrovascular hemodynamics and rehabilitation outcomes after aneurysmal subarachnoid hemorrhage. Am J Phys Med Rehabil 2018; 97(5): 309–15.
44. *Dombovy ML, Drew-Cates J, Serdans R.* Recovery and rehabilitation following subarachnoid haemorrhage. Part I: Outcome after inpatient rehabilitation. Brain Inj 1998; 12(6): 443–54.

Received on July 6, 2025
Accepted on September 3, 2025
Online First October 2025

ORIGINAL ARTICLE

UDC: 340.6:[616-091.5:616-036.88-02
DOI: https://doi.org/10.2298/VSP240815073M

Characteristics of violent deaths in the autopsy material of the Pathology and Forensic Medicine Institute of the Military Medical Academy in Belgrade

Karakteristike nasilne smrti u obdukcionom materijalu Instituta za patologiju i sudsku medicinu Vojnomedicinske akademije u Beogradu

Nadica Marinković*†, Nataša Perković Vukčević†‡, Ivan Aleksić*†

Military Medical Academy, *Pathology and Forensic Medicine Institute, ‡National Poison Control Center, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Violent death is caused by external factors. The aim of this study was to determine certain characteristics of violent deaths in the autopsy material of the Pathology and Forensic Medicine Institute of the Military Medical Academy in Belgrade, Serbia. **Methods.** A retrospective study of autopsy reports from forensic autopsies performed from 2010 until 2019 at the Pathology and Forensic Medicine Institute identified certain characteristics of violent death. **Results.** Out of 2,763 forensic autopsies performed during that period, violent death was established in 43.68% of the deceased. The majority of those who died violently were men (73.73%). Violent death was most common in people aged 21–40 years (34.96%). The most dominant cause of violent death was mechanical injuries, followed by chemical and asphyxiation injuries, while physical injuries were the rarest. In 59.32% of violent death cases, toxicological analysis of blood and urine samples showed individual or combined presence of alcohol, drugs, and illegal substances. **Conclusion.** The presented results may be significant for taking measures that will prevent and reduce the number of violent deaths.

Key words:
autopsy; death; forensic medicine; poisoning; serbia; suicide; wounds and injuries.

Apstrakt

Uvod/Cilj. Nasilna smrt nastaje delovanjem spoljašnjih činilaca. Cilj rada bio je da se utvrde određene karakteristike nasilnih smrti na obdukcionom materijalu na Institutu za patologiju i sudsku medicinu Vojnomedicinske akademije u Beogradu, Srbija. **Metode.** Retrospektivnom studijom obdukcionih nalaza sudskomedicinskih obdukcija koji su obavljeni u periodu od 2010. do 2019. godine na Institutu za patologiju i sudsku medicinu, utvrđene su određene karakteristike nasilne smrti. **Rezultati.** Od 2 763 sudskomedicinskih obdukcija u tom periodu, nasilna smrt je ustanovljena kod 43,68% obdukovanih. Najveći broj umrlih nasilnom smrću bili su muškarci (73,73%). Nasilna smrt je bila najčešća kod osoba starosti 21–40 godina (34,96%). Najdominantniji uzrok nasilne smrti bile su mehaničke povrede, zatim hemijske i asfiktične, dok su fizičke povrede bile najređe. U 59,32% slučajeva nasilnih smrti toksikološkom analizom uzoraka krvi i urina ustanovljeno je pojedinačno ili kombinovano prisustvo prisustvo alkohola, lekova i nedozvoljenih sredstava. **Zaključak.** Prikazani rezultati mogu biti značajni za preduzimanje mera koje će sprečiti i smanjiti broj nasilnih smrti.

Ključne reči:
autopsija; smrt; medicina, sudska; trovanje; srbija; samoubistvo; povrede.

Introduction

At the Pathology and Forensic Medicine Institute (PFMI) of the Military Medical Academy (MMA) in Belgrade, Serbia, forensic autopsies are performed based on the order of the Public Prosecutor's Office in Belgrade, but also the public prosecutor's offices throughout Serbia, on patients who were transported to the MMA after suffering injuries and treated there until their death. A public prosecutor's office issues an order to perform the autopsy in case of violent death, but also in case of sudden, suspicious, and unclear death, which may be natural or violent. Violent deaths may

Correspondence to: Nadica Marinković, Military Medical Academy, Pathology and Forensic Medicine Institute, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: nadicamarinkovic@yahoo.com

be caused by injuries from all groups of standard classification, and may be homicidal, suicidal, or accidental by their origin. In forensic terminology, the concepts of manner—natural vs. violent—as well as the classification of violent deaths into accidental, suicidal, and homicidal, were defined by Prof. Milovan Milovanović in the first half of the 20th century. After the autopsy and based on the police investigation report, in some cases, it is possible to determine which of the three types of death has occurred. Such determination is sometimes impossible, and the judiciary ultimately renders a final decision [1, 2]. From 2002 to 2019, the number of deaths in Serbia decreased by 1.29%. The number of violent deaths *per* 100,000 residents of Serbia dropped from 52.3% to 40.9% from 2002 until 2019. The percentage of violent deaths was 3.82% in 2002 and 2.79% of the total number of deaths in Serbia in 2019 [3]. Violent death occurs as a result of external factors, and it is possible to reduce the number of deaths by pointing out certain factors that lead to such deaths.

The aim of this study was to determine certain characteristics of violent deaths in the autopsy material of PFMI of MMA in Belgrade, and thus indicate the need for the development of certain preventive measures designed to reduce the number of violent deaths.

Methods

The autopsy reports from the autopsies performed at PFMI of MMA in Belgrade from 2010 until 2019 were analyzed. Additionally, toxicological analyses of blood and urine samples collected during these autopsies were conducted by the Department of Toxicological Chemistry of the MMA, and were reviewed alongside police reports submitted to PFMI before the autopsy. The study was approved by the Ethics Committee of MMA (No. 56/2024, from June 10, 2024).

The toxicological analyses of blood, urine samples, and stomach content, aimed at detecting the presence of alcohol, drugs, and psychoactive substances, were conducted using several methods: gas chromatography with a flame ionization detector (head space technique), liquid chromatography with ultraviolet (UV) spectrum detection and comparison of UV spectrum retention time with the UV spectra toxicology library, and liquid chromatography with mass spectrometry and comparison of mass spectrum with the standard spectrum.

Age representation was determined by grouping individuals into five categories based on their years of life: 0–20, 21–40, 41–60, 61–80, and 81 years or older.

The results were processed by applying descriptive statistics: average, minimum, maximum, and percentage.

Results

A total of 2,763 forensic autopsies were performed at PFMI of MMA from 2010 to 2019. Violent death was confirmed in 1,207 (43.68%) of autopsied victims. The highest number of violent deaths, 121, occurred in September, and the lowest, 79, in May. The majority of victims of violent deaths, 890 (73.73%), were male. The highest number of victims of violent death (34.96%) were aged 21–40 years (Figure 1). The mean age of violent death victims was 52.12 years. The youngest victim of violent death was a male newborn, while the oldest male victim was 96 years old, and the oldest female victim was 92 years old.

In the majority of cases, 488 (40.43%), the death was a result of mechanical injuries. The leading cause of injuries was blunt force trauma caused by mechanical tools in 330

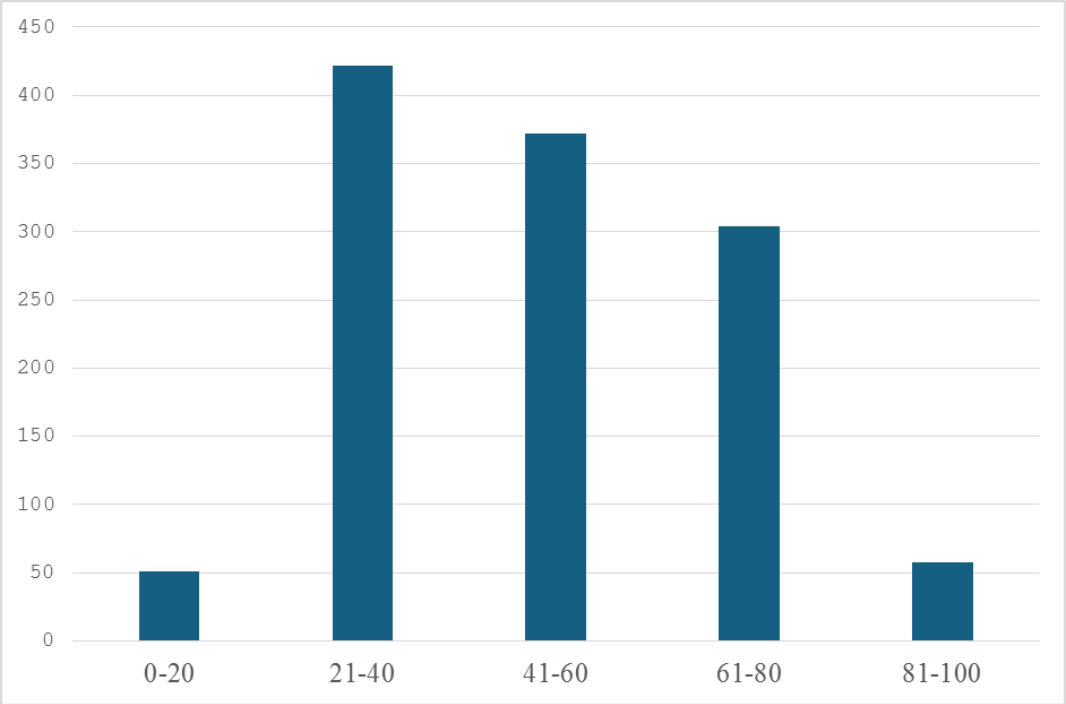

Fig. 1 – Number of violent death victims by age (years).

(27.34%) cases (Table 1). These injuries often occur in road traffic accidents, at work while operating machines, or can be caused by physical attack, falling at the same level, or falling from a height.

Table 1

Distribution of types of injuries

Types of injuries	n
Mechanical	
Blunt force trauma	330
falling from a height	150
falling at the same level	73
traffic accidents	83
trauma at work	12
trauma by another person	12
Firearms, explosives	115
gunshot wounds	98
explosive injuries	17
Spikes and blades	43
Total	488
Asphyxial	
hanging	217
drowning	33
manual or ligature strangulation	6
mors e bolo	20
aspiration of vomit or other contents	16
Total	292
Chemical	
heroin	164
cocaine	4
MDMA	3
methadone	11
alcohol	41
drug	89
pesticide	7
corrosive agent	52
mushrooms	3
Total	374
Physical	
burns	23
carbon monoxide poisoning	19
electrocution	7
freezing	4
Total	53

MDMA – 3,4-methylenedioxymethamphetamine; n – number.

Mechanical injuries caused by blunt force in traffic accidents resulted in fatalities in 83 cases (6.88% out of all 1,207 violent deaths). The pedestrians injured in an impact with a passenger vehicle and train account for 5.21% of the total number of violent deaths, but also the largest portion of fatalities in traffic accidents, i.e., 55.42%. Pedestrians killed in an impact with a passenger vehicle account for 35 (42.17%) of all traffic accident victims, and such deaths were all accidental. The average age of pedestrian victims was 55.98 years, with a predominance of males (71.43%). Among all the victims of traffic accidents, 11 (13.25%) were pedestrians injured by a train. All cases involved suicides of males with an average age of 54.27 years. The fatally injured drivers, front seat and back seat passengers in passenger cars, buses, or motorcycle riders account for 28 (33.74%) of all fatalities in traffic accidents. All injuries were accidental, with the average age of victims being 56.93 years, and with a predominance of males with 78.57%. There were 9 fatally injured persons (10.84% of all fatalities in traffic accidents) in airplane or helicopter accidents. The average age of victims of air traffic accidents was 38.11 years. The youngest victim in this study was a 5-day-old newborn, and there was only one female victim.

The mechanical blunt force traumas at work were all accidental and accounted for 12 (0.99%) cases of violent deaths, with the average age of victims being 47.67 years, all of them male.

Blunt force homicide, caused by a mechanical tool swung by another person, was determined as the cause of death in 12 (0.99%) cases. The average age of victims was 55.33 years, with a slight predominance of males (58.33%).

Mechanical blunt force trauma due to accidental falls at the same level led to fatalities in 73 (6.05%) cases of all cases of violent deaths. All deaths were accidental, with the average age of victims being 69.15 years, and with a higher percentage of males, 73.60%.

Mechanical blunt force trauma from falls from a height was identified in 150 (12.43%) cases of violent deaths, accounting for 30.74% of all deaths caused by mechanical blunt force trauma (Figure 2). The average age was 47.92 years, with the youngest victim being 15 years old and the oldest victim being 91 years old. Males account for 61.33% of all the victims of falls from a height. In 8 (5.33%) cases, the accidents occurred due to falls from scaffolding or windows during work activities, 68 (45.34%) were suicides, and all other cases, 74 (49.33%) of them, were of undetermined origin by the time of conclusion of the court proceedings.

In cases of mechanical blunt force injuries, head injuries were the most common, occurring in 187 (56.67%) cases. The cause of death was identified as damage to vital brain centers (contusions, haemorrhages, fractures). Thoracic injuries were identified in 83 (25.15%) cases, abdominal injuries in 7 (2.12%), and multiple injuries and injuries to the entire body in 53 (16.06%) cases.

Mechanical injuries caused by firearms were identified in 98 (8.12%) cases, with a predominance of male victims at 94.90%. The mean age of firearms victims was 52.92 years. The youngest victim who died from a gunshot wound was 16, while the oldest was 87 years old. Suicidal intentions in cases of gun-related deaths were identified in 89 (90.82%) cases, all of them men. In nine cases, there was murder with a firearm, and the victims were women in 66.67%. In 79 (80.61%) cases, gunshot wounds were localized on the head. Explosive injuries with the destruction of entire body parts were found in 17 (1.41%) cases of violent death victims, with the predominance of men at 88.24% and the mean age of victims was 44.38 years. The majority of deaths caused by explosive injuries were suicides in 12 (70.59%) cases, accidents in 3 (17.65%), and homicides in 2 (11.76%) cases.

Mechanical injuries caused by spikes and blades of mechanical tools were identified in 43 (3.56%) cases, with a predominance of men at 74.41%. The average age of victims injured by spikes and blades of mechanical tools was 52.74

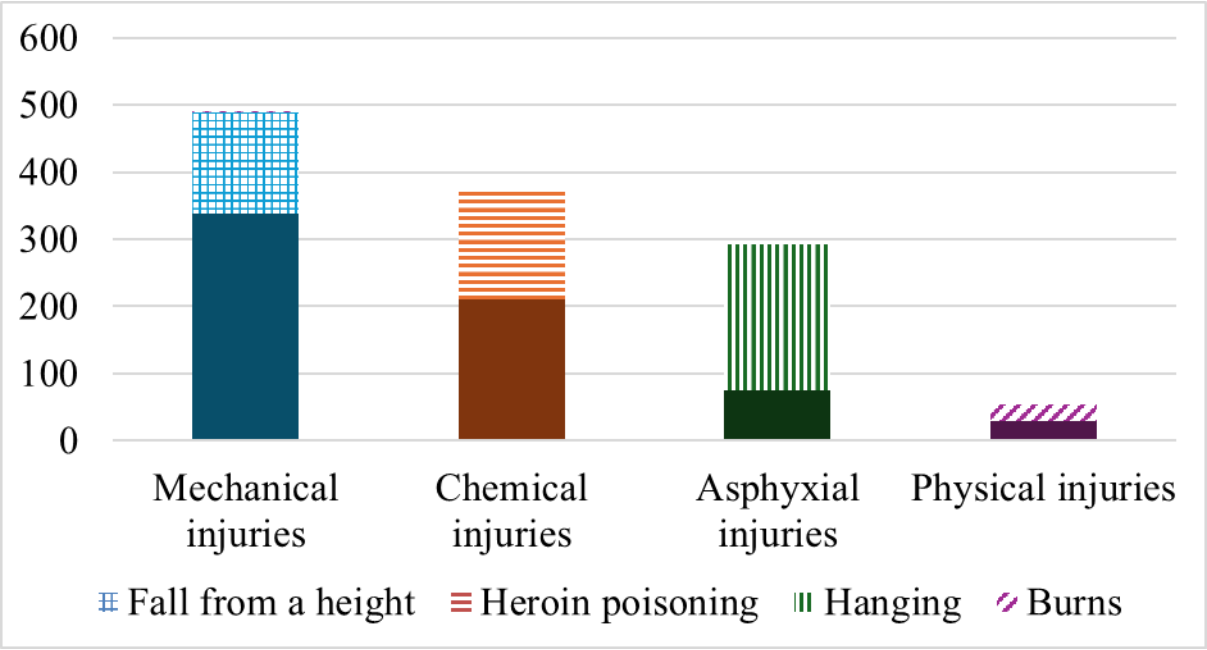

Fig. 2 – Number of lethal injuries by type.

years, with the youngest victim being 25 years old and the oldest 92 years old. The death was classified as homicide in 10 (23.36%) cases, with equal representation of male and female victims. Suicides caused by spikes or blades of mechanical tools were identified in 33 (76.74%) cases, with a predominance of men at 84.88%. The most frequent locations of injuries were in the thorax region (48.84%), neck (20.93%), limbs (18.60%), and abdomen (11.63%).

The chemical and toxicological analyses of blood and urine samples taken during the autopsy were performed in 1,003 (83.10%) cases, and in 716 (59.32%) cases, a positive toxicological result was found for the presence of alcohol, drugs, and illegal substances, individually or in combination. In 287 (23.78%) cases, no tested substances were found. In 204 (16.90%) cases of all violent deaths, toxicological analyses were not performed either due to instructions from the prosecutor's office or because of the specific duration of survival and treatment at the hospital after suffering the injury.

Chemical injuries were found to be the cause of death in 374 (30.99%) autopsied cases, with the dominance of heroin poisoning in 164 cases, 13.59% out of all violent deaths or 43.85% of all deaths due to chemical injuries (Figure 2). The average age of those whose cause of death was heroin overdose was 33.51 years, with the youngest victim being 22 and the oldest 47 years of age. Here, there is also a noticeable predominance of males (86.58%). Methadone poisoning was found in 11 (0.91%) cases, cocaine in 4 (0.33%), and 3,4-methylenedioxymethamphetamine (MDMA) in 3 (0.25%) cases of all violent death cases, and all cases were predominated by men. There were no cases of heroin, cocaine, methadone, or MDMA poisoning with the elements that would indicate the homicidal origin of poisoning; most victims were long-term addicts, and the answer to the question of whether it was a suicide or an accident would be given by the judicial authorities. Ethanol and methanol poisoning were found to be the cause of death in 41 (3.40%) cases of violent deaths. The average age of victims of alcohol poisoning was 45.34 years, with the predominance of men (87.80%). The ethanol concentration in cases of poisoning was from 3.87 to 6.58 mg/mL, while the methanol concentration was from 0.21 to 2.41 mg/mL. Drug poisoning was found in 89 (7.37%) cases, with the predominance of psychotropic drugs from the group of benzodiazepines, antidepressants, and antiepileptics in 85 (95.50%) cases, and the poisoning with antiarrhythmics in 4 (4.50%) cases. Toxicological analyses showed that the number of drugs found in body fluid samples of those who died due to drug poisoning ranged from 1 to a maximum of 13, and the mean value of the number of drugs was 3.73. Women (64.05%) are more often the victims of drug poisoning compared to men, with the average age of victims being 49.57 years. Corrosive agent poisoning (hydrochloric or acetic acid) was found in 52 (4.31%) cases, with the predominance of women (59.61%) (Table 1). The mean age of autopsied victims of corrosive agent poisoning was 73.00 years. The youngest victim was 35 years old, and the oldest was 88 years old. Pesticide poisoning was found in 7 (0.58%) cases, and mushroom poisoning in 3 (0.25%) cases, with a predominance of males. Alcohol, pesticide, and mushroom poisonings were accidental, while poisonings with corrosive agents were all of suicidal origin. Drug poisonings were suicidal in 70 (78.65%) cases and of undetermined origin in 19 (21.35%) cases.

Asphyxial injuries were the cause of violent deaths in 292 (24.19%) cases. The majority of deaths, 223 cases (18.48% out of all violent deaths), were caused by strangulation, accounting for 76.37% of all deaths caused by asphyxiation. Hanging accounted for the largest portion, 217 (17.98% of all violent death cases or 74.31% of the total number of deaths caused by asphyxiation) (Figure 2). The

average age of hanging victims was 50.74 years, with a strong predominance of men (78.80%). The youngest victim of hanging was 17 years old, while the oldest was 94. All cases of hanging were committed with suicidal intentions. Tightening the noose or hands around the neck is of homicidal origin and was found to be the cause of death in 6 (0.50%) cases of all violent death cases. The mean age of these victims was 53.5 years, with equal representation of both genders. Suffocation was the leading cause of death in 69 (5.71%) cases or 23.63% of all violent deaths due to asphyxiation. Drowning was identified in 33 (2.73%) cases, with the average age of victims being 48.61 years. Accidental drowning was found in 7 (21.21%) cases, while other cases of drowning were of suicidal origin. Choking on a piece of food (*mors e bolo*) accounted for 20 (1.65%) cases of all violent deaths, with a mean age of 56.69 years. Aspiration of vomit or other contents was the cause of death in 16 (1.33%) cases of all violent deaths, and the average age was 46.77 years (Table 1). Cases of choking on a piece of food and aspiration of vomit were accidental. All forms of suffocative asphyxiation are also dominated by men (71.01%).

Physical injuries were identified as the cause of death in 53 (4.39%) cases (Figure 2). Death due to fire was confirmed in 42 cases, namely 19 (1.57%) cases of carbon monoxide poisoning and 23 (1.91%) cases of third-degree burns. Exposure to fires was also predominated by men (69.04%) compared to women. The average age of fire victims was 60.19 years. Electrocution was found in 7 (0.58%) cases of all violent death cases, all in males with an average age of 37.68 years. Freezing led to a fatal outcome in 4 (0.33%) cases of violent deaths (Table 1). The average age of victims of freezing to death was 69.8 years, and all victims were men. All physical injuries were accidental.

The monthly frequency of fatal injuries, the body parts where such injuries were inflicted, and the results of toxicological analyses of body fluid samples taken during autopsies were analyzed for the three most common types of injuries leading to violent death, namely asphyxiation injuries by hanging (17.98%), chemical injuries from heroin poisoning (13.59%), and mechanical blunt force injuries due to a fall from a height (12.43%).

Cases of hanging were the most prevalent in July (14.75%) and the least in February (4.60%). The largest number of hangings occurred in the victim's apartment (59.91%), then, at the auxiliary building and the yard of the house where the victim lived (28.11%), in public spaces (7.83%), at hospitals (2.77%), and workplaces (1.38%). In 193 hanging cases, toxicological analyses were performed. The blood and urine samples in 107 (54.44%) cases revealed the presence of alcohol, drugs, or illegal substances, either isolated or combined. In 24 (12.43%) hanging cases, the toxicological analysis of blood and urine samples showed only the presence of various types of medications. The presence of alcohol was detected in 53 (24.46%) cases, with methanol content in addition to ethanol found in 28 of those cases. The combination of drugs and alcohol was found in 25 (12.95%) cases. Among the drugs found, in isolation or combined with alcohol, the most frequent were the benzodiazepines in 35 (16.13%) cases, painkillers and antipyretics in 14 (6.45%) cases, and antiepileptics in 9 (4.15%) cases. Antipsychotics, antidepressants, and cardiotropic drugs are less frequent. A combination of alcohol, drugs, and heroin metabolites was found in four cases, while narcotic analgesic methadone was found in one case.

Heroin overdoses were most prevalent in February (14.02%) and the least prevalent in July (3.04%). Death most often occurred in the victim's apartment (59.15%), in public spaces (32.93%), in someone else's apartment (7.32%), and, in one case, in prison (0.60%). Only heroin and its metabolites were found in 4.89% of cases, heroin and alcohol in 11.58%, heroin and psychotropic drugs in 37.80%, and heroin, alcohol, and psychotropic drugs in 45.73% of cases. When it comes to psychotropic substances, the most frequent were benzodiazepines (62.80%), antiepileptics (3.04%), and antidepressants (0.61%). In 34 (20.73%) cases of heroin overdoses, syringes and powdery substances were found next to the body during the investigation. Among all these causes, heroin, opium alkaloids (noscapine, papaverine, codeine) were found, and even paracetamol in five cases. No other toxic substance was found in other submitted samples.

Deaths caused by mechanical injuries due to falls from a height are most frequent in July (13.6%) and the least frequent in December (5.6%). In 62.40% of cases, it was a fall from the victim's apartment, in 17.60% a fall from the roof or floor of a building where the victim did not live, 8.80% a fall from a hospital window during hospitalization, 8.80% a fall from a bridge, and in 2.40% of cases, it was a fall from the workplace window. In 61 (49.19%) out of 124 cases of falls from a height for which toxicological analyses of blood and urine samples were performed, the isolated or combined presence of alcohol, drugs, or illegal substances was found. In 20 (16.13%) cases, the presence of various drugs was found, and the presence of only alcohol was found in 15 (12.10%) cases (in 14 cases, the presence of methanol was found in combination with ethanol). The presence of alcohol in combination with various drugs was found in 21 (16.93%) cases. Among the drugs found, the most frequent were benzodiazepines in 17 (11.33%) cases, painkillers and antipyretics in 10 (6.66%) cases, and antipsychotics in 6 (4.00%) cases. Antidepressants and antiepileptics have a somewhat lower degree of representation. The illegal substances were found in 5 (4.03%) cases (heroin and heroin metabolites in combination with alcohol and drugs in 2 cases, cocaine in 1 case, and MDMA in 2 cases).

Discussion

Violent deaths in our study were far more frequent among men, which coincides with the research of other authors [4–6]. The authors from Finland claim that from the second decade of life, violent deaths are three to five times more frequent among men than among women [7]. In our study, all cases of violent deaths caused by mechanical blunt force trauma at the workplace, train collisions involving pedestrians, and physical injuries from electric shock and frostbite

occurred in male victims. Women dominate only in cases of corrosive agent poisoning and drug poisoning, as also claimed by other authors [8].

Violent deaths are the most frequent among people of the most productive age, i.e., from 21 to 40 years of age. Among most other authors, the age distribution is also dominant during this period of life, or somewhat outside this window [4, 6, 9]. In our study, victims of heroin overdose were the youngest, while corrosive agent poisoning victims were the oldest.

Mechanical injuries were the most frequent cause of death in our study. The results of other studies also show the predominance of mechanical blunt force trauma [4, 10]. Blunt force injuries were the result of traffic accidents in more than 60% of cases [11]. Traffic accidents led to a fatal outcome in 58.51% of forensic autopsies [6]. A study conducted in our region, in Northern Macedonia, has identified road traffic accidents as the leading cause of death [12]. The report on the state of road traffic safety in Serbia states that, from 2010 to 2019, the average number of fatalities in road traffic accidents in Serbia was 613 [13]. A smaller share of road traffic accident victims in our study is explained by the different jurisdictions of the prosecutor's offices that submit the bodies for autopsy, since most traffic fatalities are referred to other institutions for forensic autopsy.

In our study, the most common type of violent death from the group of mechanical injuries was blunt force trauma caused by a fall from a height. Other authors state that in the 30–39 age group, deaths most often result from gunshot wounds, falls from a height, road traffic accidents, and hanging [6]. In Slovakia, suicide by jumping from a height is, after hanging, the second most chosen method of ending life [14]. In contrast, results from a study conducted in Tunisia differ significantly, showing that deaths due to falls from a height were predominantly accidental and suicidal in only 13.5% of cases, with the majority of victims not having confirmed psychiatric diseases [15]. Another study, however, reported that 63% of deaths from falls from a height were suicides, and 32% of these victims had been treated for psychiatric disorders [16].

In our study, gunshot injuries ranked fourth in frequency, while in countries with a freer firearms market, such as the United States of America (USA), the percentage of gunshot injuries is 11.4% higher than in other highly developed countries [17]. A study of homicide cases also indicates the predominance of gunshot wounds autopsied in South Carolina, where in only 18% of cases the injuries were mechanical blunt force traumas or injuries inflicted by blade or asphyxiation [18]. Injuries to the head are more frequent in suicides caused by firearms, while injuries to the thorax are more frequent in homicide cases [19].

Among the victims of homicides in our study caused by mechanical injuries inflicted by a spike or a blade, both men and women are equally represented, while a retrospective study by the authors from Tunisia showed that even in cases of homicides with a blade or a spike, the victims are dominated by men, with thoracic injuries being the most common [20].

Deaths caused by chemical injuries account for 30.99% of violent deaths in our study. Such a high prevalence of chemical injuries is influenced by the fact that the National Poison Control Center is located within the MMA, so the patients from Serbia with suspected poisoning are hospitalized at the MMA. After death, they are autopsied at the PFMI of MMA based on the order of the competent prosecutor's office. Heroin, as a means of poisoning among all types of illegal substances, is the most represented in our study, and the victims of heroin poisoning have the lowest average age. Research by authors from Norway shows an increase in the age of victims of opioid poisoning from 33 to 43 years of age, a decrease in the number of heroin poisonings, and an increase in the number of poisonings by different prescription drugs [9]. Other authors point out the constant number of deaths due to heroin overdose and an increase in the number of prescription opioid overdoses [21]. A study conducted in Türkiye showed that the majority of suicides (86.4%) were committed by drug poisoning [22]. In cases of heroin poisoning, the authors state that, in addition to heroin, other substances were also found (3.23%); in cases without heroin, combinations of four to five substances were found, and in our study, in cases of drug poisoning, one to thirteen substances were found [23]. The analyses of the contents of the supplied syringes and substances found next to the bodies of the autopsied victims during the investigation did not prove the presence of toxic adulterants, while other authors report the presence of xylazine in 2% of cases [24].

The type of substances used for poisoning depends on local ecological and economic factors, so the methods of poisoning vary across different countries. Cases of pesticide poisoning in our study were rare, and other authors have also emphasized a decline in the number of pesticide poisonings in the past 20 years [25]. In contrast, the authors from India reported the highest number of pesticide poisoning cases at 44%, while among the drugs that were used less frequently, benzodiazepines were the most common [26]. The study conducted in Nepal reported 49.12% of unknown substance poisoning cases, followed by 38.10% of organophosphate and 12.70% of rodenticide poisoning cases [27].

In our study, injuries caused by asphyxiation were predominantly due to hanging. Other authors report that the total percentage of deaths by asphyxiation is 15.7%. Of that number, 41.8% are cases of hanging, most frequently among men, while homicide by manual strangulation or use of a noose makes up a smaller percentage of all deaths by asphyxiation, which is 2.3% [28]. Based on a retrospective study of autopsies performed in Poland, the authors found that one in six autopsies was due to asphyxiation injuries, with hanging being the most common cause [29]. Research on suicides among young people aged 15–19 years in Serbia, as well as research by authors from Slovakia, showed that the most common method of committing suicide was by hanging [14, 30]. Similarly, hanging has been reported as the most common cause of death among the survivors of the attack on the World Trade Center, but also among the homeless people in the USA [31, 32]. Research by authors from Greece on the violent deaths of people over 65 years of age showed that the

most common cause of death was asphyxiation due to drowning. When it comes to suicides, the leading cause of death was hanging, while in cases of homicide, the leading cause of death was suffocation [33]. The most common method of committing suicide in Brazil among men was by hanging at their own home, which coincides with our results [34].

Research in Poland showed that after hanging, the most common methods of suicide were falling from a height and poisoning, with male predominance, where all suicides were more common in urban areas [35].

The use of alcohol, drugs, and illegal substances is considered a risk factor for both perpetrating and experiencing violence. According to authors who analyzed numerous studies of substance abuse among patients whose injuries were the result of violence, alcohol was detected in 13–66% of cases, while illegal substances were found in 37% of hospitalized patients [36]. In our study on violent deaths, toxicological analyses showed that benzodiazepines were the most frequently detected drugs. Other authors also report the most frequent presence of benzodiazepines in cases of opioid overdose in 48% of cases, hanging in cases of suicide in 13% of cases, and suicide using a firearm in 17% of cases [37]. In the autopsied cases of violent deaths in the Republic of South Africa, the presence of alcohol was found in 41% of cases, illegal substances in 61%, and more than one substance in 49% of cases [38]. A retrospective analysis of urine screening in hospitalized trauma patients in rural parts of Virginia found substances in 58% of cases, most commonly opiates, alcohol, benzodiazepines, and cannabis [39]. The toxicological analysis of the suicides in Berlin found the tricyclic antidepressants in 48.1% and alcohol in 37.2% of suicide cases [40]. A study on the presence of alcohol in body fluids taken during forensic autopsies in the Canary Islands showed that 31.8% of cases were positive for ethanol [41]. Suicides with a positive toxicological result in a study conducted in Spain, dominated by alcohol, cocaine, and benzodiazepines, were found mostly in cases of hanging [42]. In Washington, the toxicological analyses performed on suicidal violent death victims were dominated by ethanol, antidepressants, opioids, and benzodiazepines [43]. The authors point out that 27.6% of blood samples of violent death victims in Brazil were positive for ethanol [5]. Research on suicides by hanging in Poland showed the presence of alcohol in more than half of the samples, which shows that alcohol is a significant suicidal factor [44]. Research in Norway showed that 35.7% of patients who were treated for various injuries had the presence of psychoactive substances, mostly alcohol, in 23% of cases [45]. Blood alcohol concentration was found in 55% of the autopsied victims of violent deaths in Lithuania and in 57.1% of cases of violent deaths in the USA [46, 47]. The authors report that more than a quarter of gun violence victims had consumed alcohol prior to death. For prevention, they recommend enforcing penalties for carrying firearms while intoxicated and prohibiting firearm possession for individuals convicted of driving under the influence [48].

Conclusion

Violent deaths in the Pathology and Forensic Medicine Institute of the Military Medical Academy in Belgrade, Serbia, are most common among people aged 21–40 years. The most frequent causes of violent deaths were falls from a height among mechanical injuries, hanging among asphyxial injuries, and heroin poisoning among chemical injuries. In more than half of violent deaths, toxicological analyses revealed the presence of alcohol, drugs, and/or illegal substances, which may be a contributing factor in the occurrence of violent death. The number of violent deaths can be reduced by stricter control and punishment of non-compliance with the rules for dispensing psychotropic drugs, strict control and punishment of driving under the influence of alcohol and psychostimulants, as well as broader access to timely psychological and psychiatric support.

REFERENCES

1. *Marinković N.* Forensic medicine. Belgrade: Medija Centar Odbrana; 2018.
2. *Tasić M.* Forensic medicine. Novi Sad: Zmaj; 2006. p. 531.
3. *Statistical Office of the Republic of Serbia.* Statistical Yearbook of the Republic of Serbia 2020 [Internet]. Belgrade: Statistical Office of the Republic of Serbia; 2020 [cited 2025 Sept 1]. Available from: https://publikacije.stat.gov.rs/G2020/pdf/G20202053.pdf
4. *Zhang S, Wang W, Wei M, Luo Y, Long W, Li L,* et al. Forensic characteristics of 4866 violent injury cases in Sichuan Province China. Sci Rep 2023; 13(1): 5959.
5. *Gonçalves REM, de Carvalho Ponce J, Leyton V.* Alcohol Consumption and Violent Deaths in the City of Sao Paulo in 2015. Subst Use Misuse 2020; 55(11): 1875–80.
6. *Ossei PPS, Ayibor WG, Agagli BM, Aninkora OK, Fuseini G, Oduro-Manu G,* et al. Profile of unnatural mortalities in Northern part of Ghana; a forensic-based autopsy study. J Forensic Leg Med 2019; 65: 137–42.
7. *Junno JA, Pakanen L, Oura P.* Unnatural-cause mortality patterns of Northern Finnish men and women diverge in adolescence - A 53-year follow-up. Prev Med Rep 2021; 22: 101337.
8. *Khan M, Khurram M, Raza S.* Gender based differences in patients of poisoning managed at a Medical Unit. J Pak Med Assoc 2019; 69(7): 1025–8.
9. *Edvardson HME, Clausen T.* Opioid related deaths in Norway in 2000-2019. Drug Alcohol Depend 2022; 232: 109281.
10. *Paigham AM, Ataye AW.* Study of the mechanical causes of death in fatalities referred to the Department of Forensic Medicine. Int J Sci Res 2020; 9(9): 617–22.
11. *Timsinha S, Parajuli SR.* Mechanical Injury among Medicolegal Cases in the Department of Emergency in a Tertiary Care Centre: A Descriptive Cross-sectional Study. JNMA J Nepal Med Assoc 2022; 60(256): 1000–3.
12. *Bujaroska Perkovikj M, Anastasova L, Stankov A, Zhivikj Z, Poposka V, Petrusevska-Tozi L.* The role of alcohol and patterns of alcohol - related death in Republic of North Macedonia within the period 2007-2020. Forensic Sci Med Pathol 2023; 20(3): 933–40.
13. *Road Traffic Safety Agency.* Statistical report on the state of road traffic safety in the Republic of Serbia in 2019 [Internet]. Belgrade: Road Traffic Safety Agency; 2020 [cited 2025 Sept 1]. Available from: https://www.abs.gov.rs/static/uploads/1446

0_izvestaj-o-stanju-bezbednosti-saobracaja-u-republici-srbiji-u-2019.-godini.pdf (Serbian)
14. *Šidlo ŠJ, Kováč KV, Očko OP, Mikuláš ML, Šikuta ŠJ.* Unusual mechanism of injury in a case of suicide jump from height. Soud Lek 2019; 64(1): 2–4.
15. *Chelly S, Mtira A, Gharesellaoui S, Hassine M, Jedidi M, Mahjoub M,* et al. Fatal falls from great height in Sousse (Tunisia): Study of 141 medicolegal autopsy cases. Tunis Med 2023; 101(11): 800–4.
16. *Zdarílek M, Očko P, Šikuta J, Nižnanský Ľ, Šidlo J.* Addictive substance in fatal cases of fall/jump from height. Soud Lek 2017; 62(2): 14–7. (Czech)
17. *Grinshteyn E, Hemenway D.* Violent death rates in the US compared to those of the other high income countries, 2015. Prev Med 2019; 123: 20–6.
18. *Sullivan C, Presnell SE.* Non-firearm - related homicides at the Medical University of South Carolina, 2013-2018. Am J Forensic Pathol 2011; 43(2): 110–6.
19. *Negin J, Bell J, Ivancic L, Alpers P, Nassar N.* Gun violence in Australia, 2002-2016: a cohort study. Med J Aust 2021; 215(9): 414–20.
20. *Belghith M, Ben Khelil M, Marchand E, Banasr A, Hamdoun M.* Homicidal sharp force cases: An 11-year autopsy based study. J Forensic Leg Med 2022; 88: 102347.
21. *Roxburgh A, Hall WD, Dobbins T, Gisev N, Burns L, Pearson S,* et al. Trends in heroin and pharmaceutical opioid overdose deaths in Australia. Drug Alcohol Depend 2017; 179: 291–8.
22. *Aktas N, Gulacti U, Lok U, Aydin I, Borta T, Celik M.* Characteristics of the traumatic forensic cases admitted to emergency department and errors in the forensic report writing. Bull Emerg Trauma 2018; 6(1): 64–70.
23. *Brådvik L, Berglund M, Frank A, Lindgren A, Löwenhielm P.* Number of addictive substances used related to increased risk of unnatural death: A combined medico-legal and case-record study. BMC Psychiatry 2009; 9: 48.
24. *Johnson J, Pizzicato L, Johnson C, Viner K.* Increasing presence of xylazine in heroin and/or fentanyl deaths, Philadelphia, Pennsylvania, 2010-2019. Inj Prev 2021; 27(4): 395–8.
25. *Albano GD, Malta G, La Spina C, Riftorito A, Provenzano V, Triolo V,* et al. Toxicological findings of self-poisoning suicidal deaths: a systematic review by countries. Toxics 2022; 10(11): 654.
26. *Samaria S, Pandit V, Akhade S, Biswal S, Kannauje PK.* Clinical and epidemiological study of poisoning cases presenting to the Emergency Department of a Tertiary Care Center in Central India. Cureus 2024; 16(1): e52368.
27. *Khan AS, Pandey A, Pandey A.* Poisoning among autopsies conducted in the department of Forensic Medicine and Toxicology in a Tertiary Care Centre. JNMA J Nepal Med Assoc 2023; 61(264): 639–42.
28. *Azmak D.* Asphyxial deaths: a retrospective study and review of the literature. Am J Forensic Med Pathol 2006; 27(2): 13–44.
29. *Trnka J, Gesicki M, Suslo R, Siuta J, Drobnik J, Pirogowicz I.* Deaths as results of violent asphyxia in autopsy reports. Adv Exp Med Biol 2013; 788: 413–6.
30. *Lazarević KK, Dolićanin ZĆ, Stojanović MM, Bogdanović DČ, Milićević SR.* Violent deaths among adolescents in Serbia: past, present and future. Centr Eur J Public Health 2021; 29(4): 279–83.
31. *Seil K, Takemoto E, Farfel MR, Huynh M, Li J.* Exploratory case study of suicide among a sample of 9/11 survivors. Int J Environ Res Public Health 2021; 19(1): 57.
32. *Kleinman R, Morris N.* Suicide, homicide, and other violent deaths among people experiencing homelessness in the United States: A cross-sectional study. Pub Health Rep 2023; 138(2): 309–14.
33. *Nikitopulu T, Moraitis K, Tsellou M, Stefanidou-Loutsidou M, Spiliopoulou C, Papadodima S.* Violent deaths among elderly in Attica, Greece: A 5-year survey (2011-2015). J Forensic Leg Med 2019; 65: 75–80.
34. *Roza TH, Marchionatti LE, Gosmann NP, de Canto GC, Machado PV, Massuda R,* et al. Characteristics of death by suicide in postmortem studies in Brazil: A systematic review and meta-analysis. Suicide Life Threat Behav 2023; 53(6): 1086–107.
35. *Karnecki K, Gos T, Steiner J, Mańkowski D, Kaliszan M.* Epidemiology of suicide in the Tri-City metropolitan area in Poland in 2010-2019. Eur Arch Psychiatry Clin Neurosci 2023; 273(4): 911–20.
36. *Lau G, Ang JY, Kim N, Gabbe BJ, Mitra B, Dietze PM,* et al. Prevalence of alcohol and other drug use in patients presenting to hospital for violence related injuries: a systematic review. Trauma Violence Abuse 2024; 25(1): 306–26.
37. *Ghosh T, Bol K, Butler M, Gabella B, Kingcade A, Kaplan G,* et al. Epidemiologic assessment of benzodiazepine exposure among suicide deaths in Colorado, 2015-2017. BMC Public Health 2020; 20(1): 1149.
38. *Auckloo MBKM, Davies BB.* Post-mortem toxicology in violent fatalities in Cape Town, South Africa: A preliminary investigation. J Forensic Leg Med 2019; 63: 18–25.
39. *Mansoor K, De Souza Concalves B, Lakhani HV, Tashani M, Jones SE, Sodhi K,* et al. Prevalence of substance abuse among trauma patients in rural West Virginia. Cureus 2023; 15(3): e36468.
40. *Mathling M, Krumbiegel F, Hartwig S, Parr MK, Tsokos M.* Toxicological findings in suicides–frequency of antidepressant and antipsychotic substances. Forensic Sci Med Pathol 2019; 15(1): 23–30.
41. *Almeida-González M, Luzardo OP, Boada LD, Zaragoza E, Meilán MJ, Zumbado M,* et al. Ethanol levels in legally autopsied subjects (2016-2017): Update of data and epidemiological implications in relation to violent deaths n Canary Islands (Spain). J Forensic Leg Med 2019; 68: 101868.
42. *Collados-Ros A, Torres-Sánchez C, Pérez-Cárceles MD, Luna A, Legaz I.* Suicidal Behavior and its relationship with postmortem forensic toxicological findings. Toxics 2022; 10(6): 319.
43. *Cuchara B, Diaz FJ.* An 8-year retrospective study on suicides in Washington. Am J Forensic Med Pathol 2020; 41(1): 18–26.
44. *Lasota D, Pawlowski W, Krajewski P, Staniszewska A, Goniewicz K, Czerski R,* et al. Alcohol intoxication and suicide by hanging in Poland. Alcohol Alcohol 2020; 55(3): 278–83.
45. *Wilson T, Wisborg T, Vindenes V, Jamt RG, Furuhaugen N, Bogstrand ST.* Psychoactive substances have major impact on injuries in rural arctic Norway - A prospective observation study. Acta Anaesthesiol Scand 2021; 65(6): 824–33.
46. *Miščikienė L, Štelemėkas M, Petkevičienė J, Rehm J, Lange S, Trišauskė J.* The prevalence of alcohol-related deaths in autopsies performed in Lithuania between 2017 and 2020: a cross-sectional study. Eur J Public Health 2024; 34(5): 979–85.
47. *Greene N, Tomedi LE, Cox ME, Mello E, Esser MB.* Alcohol testing and alcohol involvement among violent deaths by state, 2014-2016. Prev Med 2021; 148: 106527.
48. *Branas CC, Han S, Wiebe DJ.* Alcohol Use and Firearm Violence. Epidemiol Rev 2016; 38(1): 32–45.

Received on August 15, 2024
Revised on August 27, 2025
Accepted on September 3, 2025
Online First October 2025

ORIGINAL ARTICLE

UDC: 616.65-006-037
DOI: https://doi.org/10.2298/VSP250315072V

Peritumoral infiltration of CD3 lymphocytes makes a difference in prostate cancer

Peritumorska infiltracija CD3 limfocita pravi razliku kod karcinoma prostate

Milka Vještica*†, Sandra Trivunić-Dajko‡, Božana Babić†§, Saša Jungić *†, Jelena Berendika*†

University Clinical Center of the Republic of Srpska, *Clinic for Oncology, §Department of Pathology, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; †University of Banja Luka, Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; ‡University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. Prostate cancer (PC) is the second most common malignancy in men, and the fifth leading cause of cancer death. The most important prognostic parameters are tumor, node, and metastasis (TNM) status, initial prostate-specific antigen (PSA), Gleason score, and grade group. However, these parameters are insufficient for accurate prediction of the course of the disease. The aim of this study was to examine the prognostic and predictive value of $CD3^+$ T lymphocyte infiltration, including the $CD8^+$ T-cell subset, in PC tissue across various disease stages. **Methods.** A prospective cohort study included 90 newly diagnosed patients with PC, divided into three groups [30 with medium/high-risk localized disease (Group A), 30 with locally advanced disease (Group B), and 30 with metastatic disease (Group C)]. The patients were followed for 120 months (10 years). The difference in the density of $CD3^+$ and $CD8^+$ T lymphocytes was analyzed in the tumor tissue (TT) and at the invasive margin (IM). The influence of infiltration of $CD3^+$ T and $CD8^+$ T lymphocytes on progression-free survival (PFS) and overall survival (OS) in patients with PC was analyzed. Lymphocytic infiltration was quantified at the area of greatest density using an optical Leica microscope at ×200 magnification and photographed with a Leica camera. The field of view was 0.226377 mm^2. Lymphocytes were then manually counted with the aid of commercially available software Aperio 12.0. Correlations with clinical parameters, PFS, and OS were analyzed using receiver operating characteristic (ROC) curves, Kaplan-Meier survival analysis, and correlation coefficients. **Results.** High peritumoral infiltration of $CD3^+$ lymphocytes was an independent predictor of shorter PFS and OS (log-rank $p < 0.001$). $CD3^+$ IM infiltration correlated significantly with initial PSA, nadir PSA, and perineural invasion ($p < 0.05$), while there was no correlation with Gleason score, International Society of Urological Pathology (ISUP) grade, or age. Thresholds of $CD3^+$ IM predictive of cancer-specific mortality [area under the curve (AUC) = 0.681, $p = 0.0017$], OS (AUC = 0.634, $p = 0.0173$), and PFS (AUC = 0.657, $p = 0.0062$) were identified using ROC analysis. No significant prognostic value was found for $CD3^+$ T lymphocyte infiltration in the TT, nor for $CD8^+$ T lymphocyte infiltration in either TT or at the IM. **Conclusion.** $CD3^+$ T lymphocyte infiltration at IM is a significant prognostic and predictive biomarker in PC, indicating tumor aggressiveness and potential influence on treatment response. These findings support the integration of immune profiling into routine histopathological evaluation to refine risk stratification and guide personalized treatment strategies in patients with PC.

Key words:
biomarkers; progression-free survival; prostatic neoplasms; survival; t-lymphocytes.

Apstrakt

Uvod/Cilj. Karcinom prostate (KP) je drugi po učestalosti karcinom kod muškaraca i peti vodeći uzrok smrtnosti od karcinoma. Najvažniji prognostički parametri su stadijum tumora, limfnih čvorova i metastaza (*tumor, node, metastasis* – TNM), početni nivo prostata-specifičnog antigena (PSA), Gleason-ov skor i gradus grupa tumora. Međutim, ovi parametri su nedovoljni za precizno predviđanje toka bolesti. Cilj rada bio je da se ispita prognostička i prediktivna vrednost infiltracije $CD3^+$ T limfocita, uključujući $CD8^+$ T subpopulaciju, u tkivu KP kroz različite stadijume bolesti. **Metode.** Prospektivna kohortna studija obuhvatila je 90 novodijagnostikovanih obolelih od KP podeljenih u tri grupe [30 sa lokalizovanom bolešću srednjeg/visokog rizika (Grupa A), 30 sa lokalno uznapredovalom bolešću (Grupa B) i 30 sa metastatskom bolešću (Grupa C)]. Bolesnici su praćeni 120 meseci (10 godina). Razlika u gustini $CD3^+$ i $CD8^+$ T limfocita analizirana je u tumorskom tkivu (TT) i na invazivnoj margini (IM). Analiziran je uticaj infiltracije $CD3^+$

Correspondence to: MilkaVještica, University Clinical Center of the Republic of Srpska, Clinic for Oncology, St. 12 beba, 78 000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina. E-mail: milka.vjestica@kc-bl.com

T i CD8+ T limfocita na preživljavanje bez progresije bolesti (*progression-free survival* – PFS) i ukupno preživljavanje (*overall survival* – OS) obolelih od KP. Infiltracija limfocita kvantifikovana je na području najveće gustine korišćenjem optičkog Leica mikroskopa pri uvećanju od ×200 i fotografisana Leica kamerom. Veličina vidnog polja iznosila je 0,226377 mm². Limfociti su zatim ručno prebrojani uz pomoć komercijalno dostupnog softvera Aperio 12.0. Korelacije sa kliničkim parametrima, PFS i OS analizirane su korišćenjem *receiver operating characteristic* – ROC krive, Kaplan-Meier analize preživljavanja i koeficijenata korelacije. **Rezultati.** Visoka peritumorska infiltracija CD3+ limfocita bila je nezavisni prediktor kraćeg PFS i OS (*log-rank* $p < 0,001$). Infiltracija CD3+ T limfocita u IM značajno je korelirala sa početnim vrednostima PSA, najnižim vrednostima (nadir) PSA i perineuralnom invazijom ($p < 0,05$), dok nije bilo korelacije sa Gleasonovim skorom, gradusom *International Society of Urological Pathology* – ISUP ili starošću bolesnika. Granične vrednosti CD3+ IM koje predviđaju smrtnost zbog karcinoma [*area under the curve* (AUC) = 0,681, $p = 0,0017$], OS (AUC = 0,634, $p = 0,0173$) i PFS (AUC = 0,657, $p = 0,0062$) identifikovane su ROC analizom. Nije pronađena značajna prognostička vrednost za infiltraciju CD3+ T limfocita unutar TT, niti za infiltraciju CD8+ T limfocita u TT ni na IM. **Zaključak.** Infiltracija CD3+ T limfocita na IM predstavlja značajan prognostički i prediktivni biomarker kod KP, koji odražava agresivnost tumora i potencijalno utiče na terapsijki odgovor. Ovi nalazi podržavaju integraciju imunskog profilisanja u rutinsku histopatološku evaluaciju u cilju poboljšanja procene rizika i vođenja personalizovanih terapijskih strategija kod obolelih od KP.

Ključne reči:
biomarkeri; preživljavanje, bez progresije; prostata, neoplazme; preživljavanje; limfociti t.

Introduction

Prostate cancer (PC) is the second most common cancer among men worldwide and the fifth leading cause of cancer death [1]. Patients with PC can present in very different forms of the disease, ranging from localized hormone-sensitive forms to metastatic castration-resistant forms. The most important prognostic parameters include tumor, node, and metastasis (TNM) status, initial prostate-specific antigen (PSA) level, Gleason score (GS), and grade group. However, these parameters are insufficient for an accurate prediction of the course of the disease. New biomarkers are needed to improve risk stratification and enable more personalized treatment [2]. The role of the local immune response is widely discussed. In recent years, particular emphasis has been placed on the tumor microenvironment as a significant factor that can either inhibit or promote tumor growth. Tumor-infiltrating lymphocytes (TILs), arising as part of the inflammatory response to malignant cells, represent an important component of the antitumor defense. Their location, function, and abundance provide valuable insights into the nature and strength of the immune response against cancer [3]. For a long time, PC was considered an immunologically "cold" cancer. However, new research challenges this view, demonstrating that PC has a highly heterogeneous immune environment, with the immune landscape playing a significant role in determining the disease's prognosis. The immune system can potentially play dual roles, either promoting or suppressing tumor progression and metastasis [4]. Additionally, the localization of TILs at the invasive margin (IM) and within the tumor tissue (TT) plays an important role in tumor biology and patient outcomes [5]. During carcinogenesis, immune cells, including T and B lymphocytes, accumulate in prostate tissue [6]. T lymphocytes, all of which express CD3, are essential for immune surveillance, with CD8+ T cells playing a key role in antitumor immunity.

The aim of this study was to examine the prognostic and predictive value of CD3+ and CD8+ T lymphocyte infiltration in PC tissue across various disease stages by analyzing differences in their density.

Methods

The study was initiated as a prospective cohort study, including 90 patients newly diagnosed with adenocarcinoma of the prostate [30 with medium/high-risk localized disease (Group A), 30 with locally advanced disease (Group B), and 30 with metastatic disease (Group C)], between January 1, 2013, and January 1, 2015. The patients were followed for disease progression and survival over the subsequent 120 months (10 years). Demographic and clinical data were obtained from medical records, while histopathological data for all cases were reviewed and registered by two experienced pathologists (BB and MB). The tumors were graded according to the International Society of Urological Pathology (ISUP) grading system and GS. Patients were classified into risk groups according to the European Association of Urology recommendations [7]. Intermediate-risk patients were defined as those with tumor category cT2b or GS 7 (ISUP grade 2/3), or PSA levels between 10 ng/mL and 20 ng/mL. High-risk patients were defined as those with tumor category cT2c or GS ≥ 8 (ISUP grade 4/5) or PSA levels >10–20 ng/mL. Locally advanced patients are defined as those with tumor category cT3-T4 or N1, regardless of GS, ISUP grade, or PSA level [7]. The patients with metastatic disease had one or more metastases confirmed by one of the imaging methods (computed tomography, magnetic resonance, positron emission tomography/computed tomography, etc.).

Following the diagnostic workup, the disease stage was determined in each patient. All cases were subsequently reviewed by a multidisciplinary oncology board, which developed individualized treatment plans.

Initial therapy for all patients consisted of androgen deprivation therapy (ADT), achieved either through the administration of luteinizing hormone-releasing hormone analogs and antiandrogens (bicalutamide) or *via* bilateral

orchiectomy. In patients with localized and locally advanced PC, ADT was administered for a duration of two to three years. Patients with metastatic disease received continuous ADT for the remainder of their clinical course.

After six months of ADT, patients with localized and locally advanced disease were treated with definitive external beam radiotherapy to the prostate. In patients with metastatic disease, palliative radiotherapy was administered to bone lesions as clinically indicated.

Of the total number of patients, 95.6% underwent hormonal therapy under the oncology board's recommendations, 98.9% received the planned radiotherapy, and 92.4% completed the full course of the prescribed treatment.

Patients who experienced disease progression were re-evaluated by the multidisciplinary oncology board, and subsequent management was guided by current oncological guidelines and drug availability. Systemic treatments most frequently included docetaxel, abiraterone, and other standard therapies for advanced PC. Subsequent therapies administered after disease progression did not affect progression-free survival (PFS) but may have influenced overall survival (OS).

Pathology

Tissue samples obtained from the primary biopsy (before any treatment) of the prostate were used to analyze lymphocytic infiltration. Histopathological and immunohistochemical analyses were performed on tissue samples obtained by needle biopsy (88.9%) and transurethral resection of the prostate (TURP) (11.1%). Multiple 3 mm sections were cut using a microtome, mounted on glass slides, and sealed with paraffin. The antibodies used in this study were as follows: CD3 [mouse monoclonal anti-human CD3, Clone 4B11 – Agilent (Dako), Cat. No. K800221-2] and CD8 [rabbit polyclonal anti-human CD8, Agilent (Dako), Cat. No. K800221-2].

Tissue samples were washed in tris-buffered saline (TBS) for 3 × 3 min, and the sections were incubated with EnVision™ FLEX+ Mouse (Linker) or EnVision™ FLEX+ Rabbit (Linker), depending on whether mouse or rabbit antibody was used, for 15 min at room temperature in a humidified chamber. The sections were washed in 0.05M TBS, pH 7.6, for 3 × 3 min and incubated with EnVision™ FLEX/horseradish peroxidase detection reagent for 20 min at room temperature in a humidified chamber. After another wash in 0.05M TBS, pH 7.6, for 3 × 3 min, the sections were incubated in 3,3′-diaminobenzidine solution for 5–10 min at room temperature, rinsed in running water, contrasted with Mayer's hematoxylin for 1 min, and washed additionally in water.

After that, the tissue sections were dehydrated through a series of ethanol solutions of increasing concentrations (70%, 96%, and 100%), immersed in xylene, and then mounted with a suitable synthetic resin (DPX or Neo-Mount) and a coverslip.

If the target antigen was present, a chromogen precipitate appeared at the site, showing brown color in contrast to the surrounding tissue, which was blue. All slides were examined under a Leica optical microscope at ×200 magnification (10 × 20) and photographed using a Leica camera, with a field of view measuring 548.98 × 412.36 μm (0.226377 mm²). The counting of lymphocytes in the field of view was performed using the commercially available software Aperio 12.0. Counting was done by manually marking each stained lymphocyte with a marker, and the software automatically counted the marked lymphocytes. T lymphocytic infiltration (including total $CD3^+$ T cells and the $CD8^+$ T-cell subset) was counted at the greatest density within TT and at IM. The area of the IM is defined as 0.5 mm within the tumor and 1 mm surrounding the TT. Necrotic regions were excluded from the evaluation.

Ethical statement

The study was approved by the Ethics Committee of the University Clinical Center of the Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina (No. 01-9-546-2/18, from November 2, 2018).

Statistical analysis

The Kolmogorov-Smirnov test was done to estimate deviation from the normal distribution. Since most variables were not normally distributed, nonparametric statistics (Spearman's coefficient of rank correlation – rho) were also used. All the tests were two-sided, and a $p < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) analysis was applied to assess the cut-off point for $CD3^+$ infiltration at the IM ($CD3^+$ IM value) in relation to cancer-specific death (yes/no). The same analysis was used to evaluate OS and PFS relative to $CD3^+$ IM status (low vs. high lymphocytic infiltration). The area under the curve (AUC) was calculated to estimate the discriminatory power of lymphocytic infiltration without considering the specificity and sensitivity parameters. Kaplan-Meier survival analysis was performed to estimate patient survival probabilities, considering OS, PFS, and survival time. Progression status (yes/no) was used as the endpoint, with $CD3^+$ IM status as the grouping variable. Statistical analysis was done using MedCalc version 22.0.2. (MedCalc Software, Mariakerke, Belgium) and Paleontological Statistics (PAST) version 4.17 software [8] and Statistics Kingdom software [9].

Results

An overview of the demographic, clinical, and histopathological characteristics is presented in Table 1.

In our study, patients ranged in age from 55 to 90 years, with a mean age of 69.59 ± 7.03 years. Among them, 68.9% were aged 65 or above. The pathohistological diagnosis was established from biopsy tissue samples in 88.9% of cases, and from TURP specimens in 11.1%. Patients with localized,

Table 1

Demographic, clinical, and histopathological characteristics of the patients

Parameter	Value
Age, years	
< 65	28 (31.1)
65–80	54 (60.0)
> 80	8 (8.9)
Type of biopsy	
needle biopsy	80 (88.9)
TURP	10 (11.1)
Stage of disease	
localized (T1N0M0, T2N0M0)	30 (33.3)
local advanced (T3N0M0, T4N0M0)	30 (33.3)
metastatic (TxN1M0, TxNxM1)	30 (33.3)
Initial PSA, ng/mL	
< 10	17 (18.9)
10–20	13 (14.4)
> 20	60 (66.7)
Grade group	
I	8 (8.8)
II	13 (14.4)
III	12 (13.3)
IV	19 (21.1)
V	38 (42.2)
Gleason score	
6	8 (8.8)
7	25 (27.7)
8	19 (21.1)
9	29 (32.2)
10	9 (10.0)
Perineural invasion	
yes	58 (64.4)
no	32 (35.6)
Nadir PSA, ng/mL	
< 0.5	67 (74.4)
0.5–4	12 (13.3)
> 4	11 (12.2)
Nadir testosterone, ng/mL	
> 0.5	2 (2.2)
0.5–0.2	10 (11.1)
< 0.2	78 (86.7)
Progression disease	
yes	46 (51.1)
no	44 (48.9)
PFS, months	68.94 ± 43.37 (5 to 120)
Death caused by cancer	
yes	34 (37.8)
no	56 (62.2)
OS, months	81.26 ± 39.49 (11 to 120)

TURP – transurethral resection of the prostate; PSA – prostate-specific antigen; PFS – progression-free survival; OS – overall survival.
Values are presented as numbers (percentages), except for PFS and OS, which are shown as mean ± standard deviation (range).

locally advanced, and metastatic disease were equally represented. Initially, 66.7% of patients had highly elevated PSA values > 20 ng/mL. High-grade groups (IV and V) and high GSs (8–10) were present in 63.3% of patients. Perineural invasion was observed in 64.4% of patients. ADT resulted in PSA nadir levels below 0.5 ng/mL in 74.4% of patients and testosterone nadir levels below 0.2 ng/mL in 86.7% of patients. During the ten-year follow-up period, disease progression occurred in 51.1% of patients, while death caused by carcinoma was recorded in 37.8% of patients. Demographic and cytopathological characteristics according to groups are presented in Table 2.

Figure 1 shows the difference between low (Figure 1A, 1B) and high (Figure 1C, 1D) $CD3^+$ T-cell infiltration at the IM of PC, before and after manual marking and counting of $CD3^+$ cells.

Table 2

Demographic, clinical, and histopathological characteristics according to groups

Parameter	Mean	Median	SD	Min–Max
Age, years				
A	70.47	70.5	7.45	55–90
B	69.93	69	6.69	58–80
C	68.37	67	6.99	56–84
total	69.59	68	7.03	55–90
$CD3^+$ IM*				
A	81.07	38.5	91.64	7–357
B	136.07	77.5	188.33	10–790
C	179.53	128	216.72	3–1,017
total	132.22	75	176.75	3–1,017
$CD3^+$ TT*				
A	285.9	218	308.46	19–1,190
B	244.6	148	286.31	15–1,312
C	294.87	231.5	250.21	5–1,091
total	275.12	194.5	280.35	5–1,312
$CD8^+$ IM*				
A	35.5	23	26.84	6–114
B	31.33	25	28.93	0–114
C	54.63	38	52.5	3–251
total	40.49	27	38.86	0–251
$CD8^+$ TT*				
A	105.97	76	114.79	5–539
B	84.53	52.5	117.56	0–571
C	106	62.5	103.3	0–402
total	98.83	61.5	111.25	0–571
Gleason score				
A	7.07	7	0.91	6–9
B	8.43	9	1.01	7–10
C	8.7	9	0.88	7–10
total	8.07	8	1.17	6–10
ISUP grading				
A	2.5	2	1.31	1–5
B	4.2	5	1.03	2–5
C	4.5	5	0.78	2–5
total	3.73	4	1.37	1–5
Initial PSA				
1	14.6	10.27	10.11	4.8–52
2	49.03	29.5	59.78	3.6–311
3	1,023.95	268.25	2,224.28	2.8–9,824
total	362.53	27.25	1,354.5	2.8–9,824
PFS				
A	104.93	120	28.98	27–120
B	76.77	74.5	34.39	21–120
C	25.13	18.5	19	5–73
total	60.03	60	34.17	5–120

Table 2 (continued)

Parameter	Mean	Median	SD	Min–Max
OS				
A	109.03	120	26.26	27–120
B	89.04	98.5	33.59	21–120
C	45.33	42	27.68	11–120
total	81.26	87.5	39.49	11–120

$CD3^+$ IM – number of $CD3^+$ T lymphocytes at the invasive margin (IM); $CD3^+$ TT – number of $CD3^+$ T lymphocytes in the tumor tissue (TT); $CD8^+$ IM – number of $CD8^+$ T lymphocytes at the IM; $CD8^+$ TT – number of $CD8^+$ T lymphocytes in the TT; ISUP – International Society of Urological Pathology; Group A – medium/high-risk localized disease (30 patients); Group B – locally advanced disease (30 patients); Group C – metastatic disease (30 patients); SD – standard deviation; Min – minimum; Max – maximum.
For other abbreviations, see Table 1.
***Note*: *$CD3^+$ and $CD8^+$ values represent the number of T lymphocytes *per* field of view (0.226377 mm^2).**

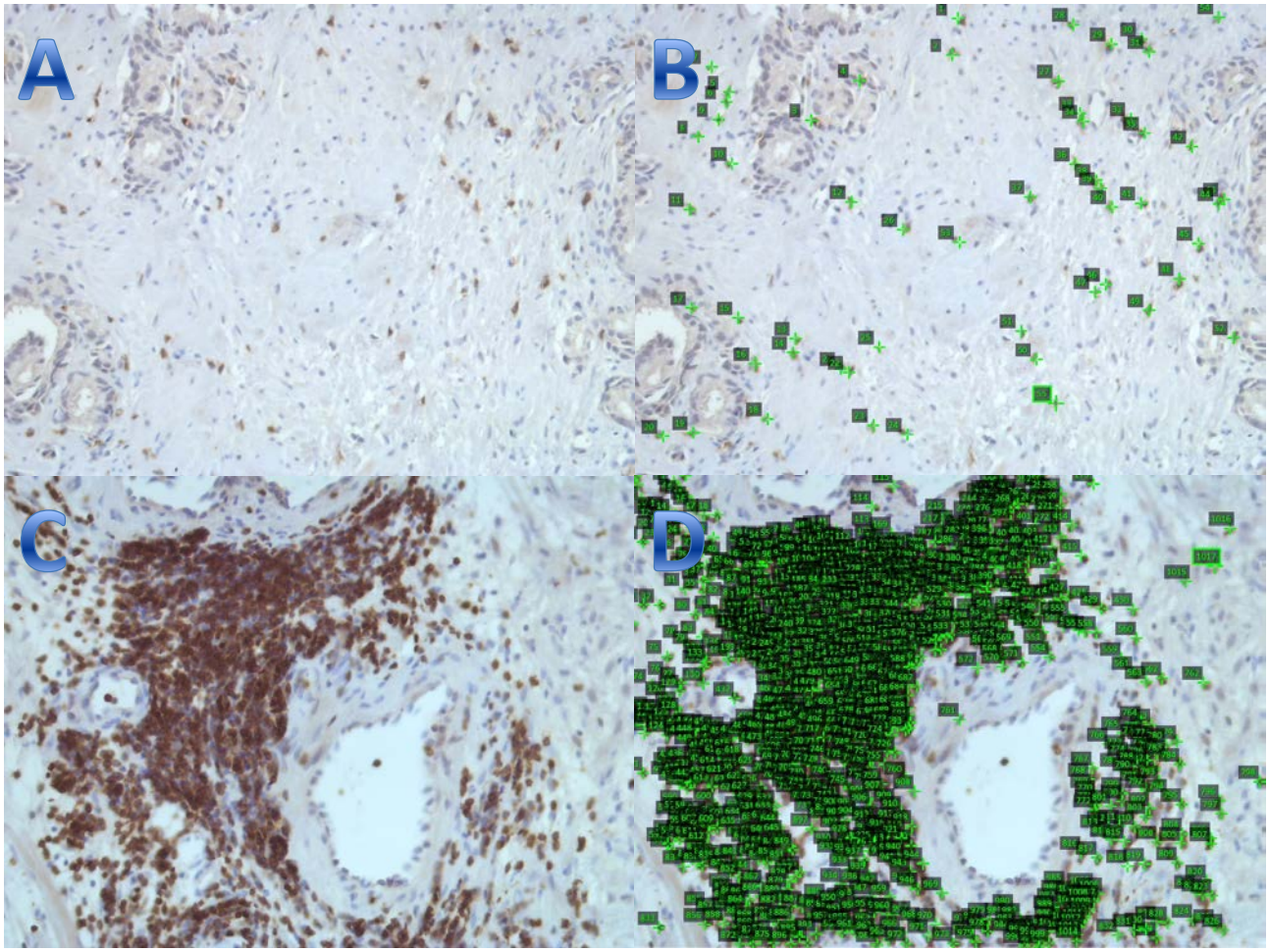

Fig. 1 – Immunohistochemical detection and analysis of $CD3^+$ T lymphocytes infiltration at the invasive margin in prostate cancer tissue. Low lymphocytic infiltration before (A) and after (B) manual marking and quantification of $CD3^+$ T lymphocytes. High $CD3^+$ T lymphocytes infiltration before (C) and after (D) manual marking and quantification. $CD3^+$ T cells were detected using 3,3′-diaminobenzidine immunostaining (brown), with hematoxylin counterstaining (blue). Images, captured at ×200 magnification, were analyzed using Aperio 12.0 software (field of view size: 0.226377 mm^2).

Differences in the number of $CD3^+$ T lymphocytes at the IM were analyzed among three groups (A, B, and C) of PC patients (Figure 2). Significant differences in lymphocytic infiltration were observed between different disease stages (groups), particularly when comparing localized/locally advanced disease with metastatic PC.

The results of the correlation coefficient among observed parameters showed statistically significant correlations between $CD3^+$ IM and PFS, as well as $CD3^+$ IM and OS (Table 3), indicating that higher $CD3^+$ IM values were associated with shorter PFS and OS. There is also a correlation between the number of $CD3^+$ IM T lymphocytes and disease stage, perineural infiltration, initial PSA, and nadir PSA. In contrast, no correlation was found between the number of $CD3^+$ IM T lymphocytes and GS or ISUP grade. No statistically significant correlations with PFS or OS were found for $CD3^+$ infiltration in TT, nor for $CD8^+$ T lymphocyte infiltration at either the TT or IM (Table 3).

The ROC analysis was performed to evaluate whether the $CD3^+$ IM value has discriminatory potential with respect to cancer-specific mortality. The result of the ROC analysis indicated statistically significant differentiation reliability [AUC = 0.681, standard error (SE) = 0.0577, 95% confidence interval (CI): 0.575 to 0.776, $p = 0.0017$], where a cut-off value > 73 was observed for maximum sensitivity of 72.73 and specificity of 63.17 (Figure 3).

This result suggests that $CD3^+$ IM values above 73 are predictive of cancer-related death with relatively high specificity and sensitivity. We defined two groups based on the cut-off value of $CD3^+$ IM: low lymphocytic infiltration and high lymphocytic infiltration. The ROC analysis was performed using OS and PFS as criteria.

When OS was used as the criterion, ROC analyses showed statistically significant discriminatory power (AUC = 0.634, SE = 0.0563, 95% CI: 0.526 to 0.733, $p = 0.0173$). A cut-off value ≤ 54 was observed for maximum sensitivity of 46.67 and specificity of 80.00 (Figure 4). In case of PFS, the same analyses also showed statistically significant discriminatory power (AUC = 0.657, SE = 0.0574, 95% CI: 0.550 to 0.754, $p = 0.0062$). The cut-off value ≤ 34 was identified for a maximum sensitivity of 48.89 and a specificity of 84.44 (Figure 5).

The results of the Kaplan-Meier analysis showed that when the survival time was defined as PFS, and progression status (yes/no) was used as the endpoint, $CD3^+$ IM status (low vs. high lymphocytic infiltration) was a significant indicator of PFS. The difference between the groups was statistically significant (log-rank test: $\chi^2 = 11.75$, $p = 0.0006$) (Figure 6). A total of 15 patients (8 in the low lymphocyte infiltration group and 7 in the high lymphocyte infiltration group) who died of other causes without prior disease progression were excluded from the analysis. The average PFS for patients with low lymphocytic infiltration was 81.16 months, and for the group with high lymphocytic infiltration was 56.73 months. In patients with low lymphocytic infiltration, 120 months without progression was reached by 64.4% of patients. During the same period, among the patients with high lymphocytic infiltration, 33.3% remained progression-free for 120 months.

The same result was obtained when OS was considered as survival time (log-rank test – $\chi 2 = 13.429588$, $p = 0.000024787$) (Figure 7). Patients who died due to another cause (15 patients) were excluded from the analysis. The average OS for patients with low lymphocytic

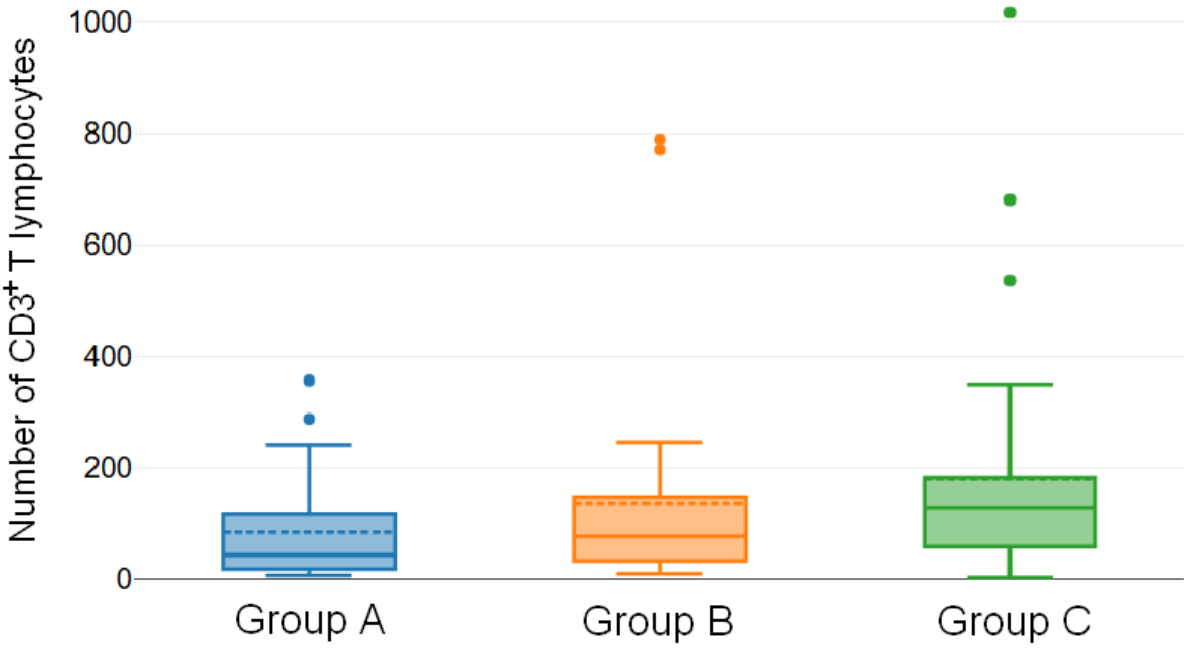

Fig. 2 – Differences in the number of $CD3^+$ T lymphocytes at the invasive margin according to disease stage. Group A – medium/high-risk localized disease; Group B – locally advanced disease; Group C – metastatic disease.

Table 3

Correlation coefficients between T lymphocyte infiltration and patient clinical/pathological data

Parameters	$CD3^+$ IM	$CD3^+$ TT	$CD8^+$ IM	$CD8^+$ TT
PFS	ρ = -0.2216 95% CI: -0.4145 to -0.0097 *p* = 0.03579*	ρ = -0.1049 95% CI: -0.3086 to 0.1079 *p* = 0.325	ρ = - 0.115 95% CI: -0.31791 to 0.09792 *p* = 0.2804	ρ = -0.01874 95% CI: -0.2278 to 0.1919 *p* = 0.8608
OS	ρ = -0.2146 95% CI: -0.4083 to-0.002522 *p* = 0.04221*	ρ = -0.1044 95% CI: -0.3081 to 0.1084 *p* = 0.3274	ρ = -0.1602 95% CI: -0.3592 to 0.0528 *p* = 0.1315	ρ = -0.02125 95% CI: -0.2301 to 0.1895 *p* = 0.8424
Stage of disease	ρ = 0.3036 95% CI: 0.09528 to 0.4864 *p* = 0.003627*	ρ = 0.06312 95% CI: -0.149 to 0.2696 *p* = 0.5933	ρ = 0.1787 95% CI: -0.03412 to 0.376 *p* = 0.09198	ρ = 0.01493 95% CI: -0.1956 to 0.2241 *p* = 0.8889
Age	ρ = -0.033 95% CI: -0.2414 to 0.1781 *p* = 0.7569	ρ = -0.1093 95% CI: -0.3126 to 0.1036 *p* = 0.305	ρ = -0.1026 95% CI: -0.3064 to 0.1102 *p* = 0.3359	ρ = -0.05281 95% CI: -0.26 to 0.159 *p* = 0.621
Gleason score	ρ = 0.1197 95% CI: -0.09326 to 0.3222 *p* = 0.2611	ρ = -0.003977 95% CI: -0.2137 to 0.2061 *p* = 0.9703	ρ = -0.06511 95% CI: -0.2715 to 0.147 *p* = 0.5421	ρ = -0.01861 95% CI: -0.2276 to 0.1921 *p* = 0.8618
ISUP grading	ρ = 0.1302 95% CI: -0.08283 to 0.3318 *p* = 0.2213	ρ = 0.01553 95% CI: -0.195 to 0.2247 *p* = 0.8845	ρ = -0.03539 95% CI: -0.2435 to 0.1759 *p* = 0.7406	ρ = -0.01146 95% CI: -0.2208 to 0.1989 *p* = 0.9147
Perineural infiltration	ρ = 0.2994 95% CI: 0,09079 to 0.4828 *p* = 0.004152*	ρ = 0.2399 95% CI: 0.02856 to 0.4307 *p* = 0.02276*	ρ = 0.09921 95% CI: -0.1136 to 0.3033 *p* = 0.3522	ρ = 0.1251 95% CI: -0.08791to 0.3272 *p* = 0.2401
Initial PSA	ρ = 0.2517 95% CI: 0.04082 to 0.4412 *p* = 0.01669*	ρ = 0.08614 95% CI: -0.1264 to 0.2911 *p* = 0.4195	ρ = 0.1267 95% CI: -0.0863 to 0.3286 *p* = 0.234	ρ = 0.01447 95% CI: -0.196 to 0.2237 *p* = 0.8923
Nadir PSA	ρ = 0.2825 95% CI: 0.07299 to 0.4681 *p* = 0.006982*	ρ = 0.1188 95% CI: -0.09415 to 0.3214 *p* = 0.2647	ρ = 0.1595 95% CI: -0.05347 to 0.3586 *p* = 0.1332	ρ = 0.02123 95% CI: -0.1895 to 0.2301 *p* = 0.8425
Nadir testosterone	ρ = 0.0616 95% CI: -0.1504 to 0.2682 *p* = 0.5641	ρ = 0.02959; 95% CI: -0.1815 to 0.238 *p* = 0.7819	ρ = -0.07021 95% CI: -0.2072 to 0.2072 *p* = 0.5108	ρ = -0.03583 95% CI: -0.244 to 0.1754 *p* = 0.7374

CI – confidence interval.
For other abbreviations, see Tables 1 and 2.
Statistical test, Spearman's coefficient of rank correlation (rho), was used.
***Note*: *statistically significant value.**

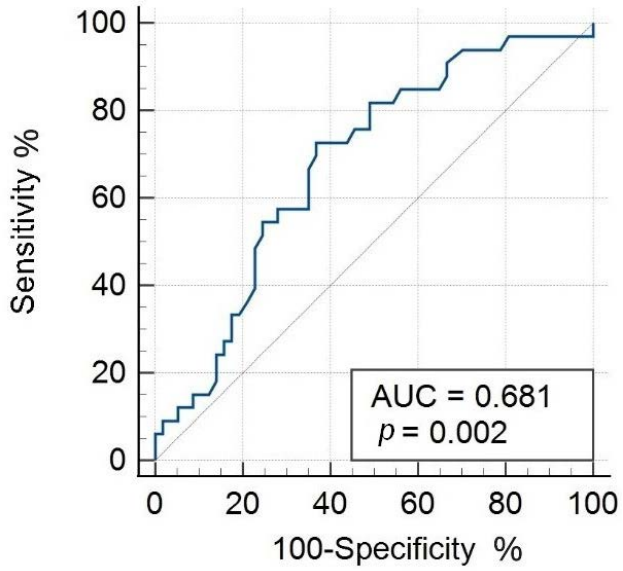

Fig. 3 – Receiver operating characteristic curve for CD3⁺ T infiltration at the invasive margin of prostate cancer tissue. AUC – area under the curve.

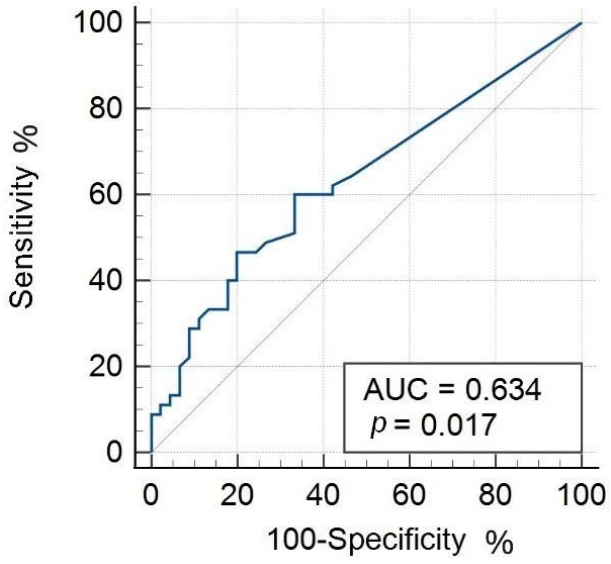

Fig. 4 – Receiver operating characteristic curve for overall survival. AUC – area under the curve.

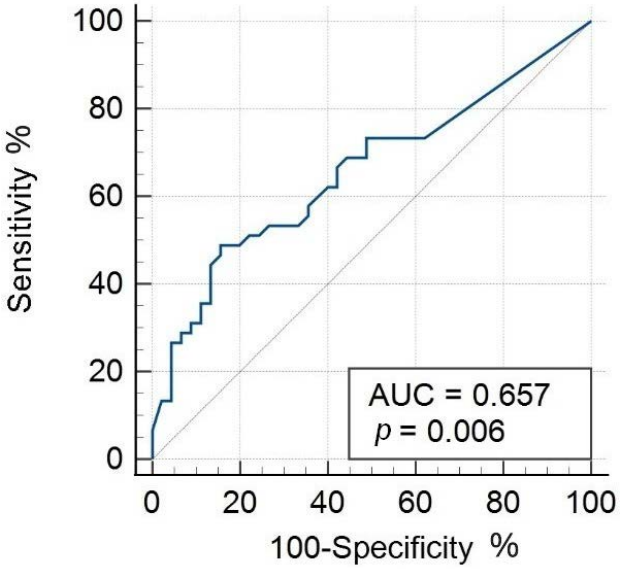

Fig. 5 – Receiver operating characteristic curve for progression-free survival. AUC – area under the curve.

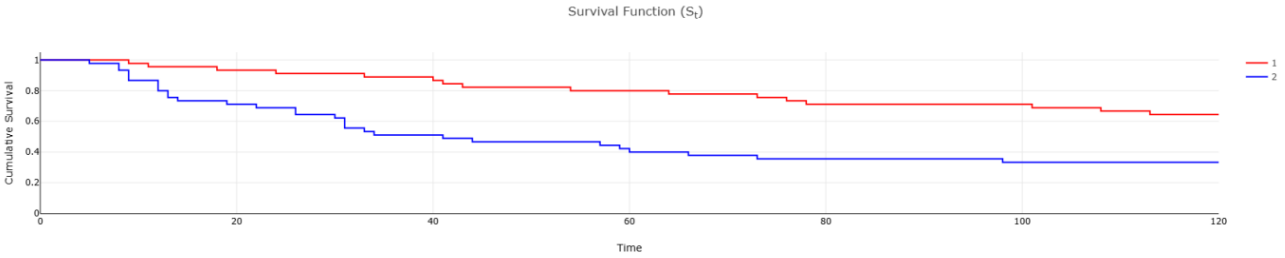

Fig. 6 – Kaplan-Meier analysis of progression-free survival based on CD3⁺ T-cell infiltration at the invasive margin (IM).
***Note*: Patients were grouped according to CD3⁺ IM status (low vs. high), with progression status (yes/no) used as the event endpoint. The red and blue lines indicate groups with low and high lymphocyte infiltration, respectively.**

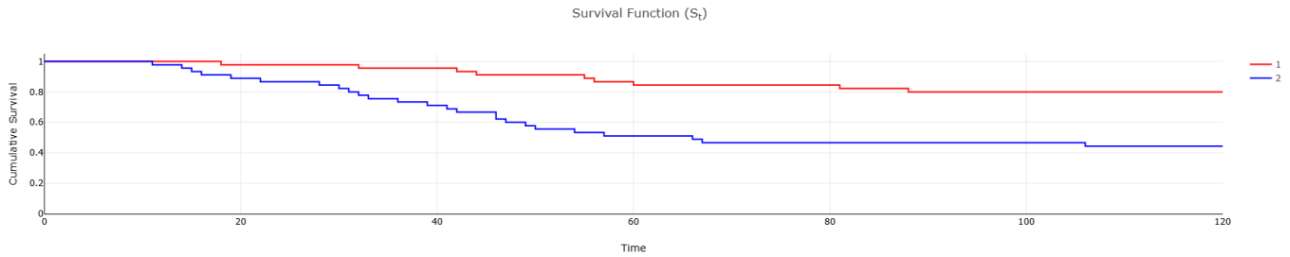

Fig. 7 – Kaplan-Meier analysis of overall survival based on CD3⁺ T-cell infiltration at the invasive margin (IM). ***Note*****: Patients were grouped according to CD3⁺ IM status (low vs. high), with progression status (yes/no) used as the event endpoint. The red and blue lines indicate groups with low and high lymphocyte infiltration, respectively.**

infiltration was 91.36 months, and 71.16 months for patients with high lymphocytic infiltration. In a group of patients who had low lymphocytic infiltration, 120 months of OS were reached by 80% of patients. During the same period, among patients with high lymphocytic infiltration, 44% achieved a total survival of 120 months.

Discussion

For a long time, PC was considered an immunologically "cold" tumor. In recent years, however, TILs, particularly T lymphocytes, have become the focus of intense investigation. In our study of 90 patients with localized, locally advanced, and metastatic PC, where all lymphocytes were counted in the areas of highest density (within TT and at IM), we found that lymphocyte infiltration was not abundant. Based on our findings, we agree that PC is not an immune cell-enriched tumor, showing only limited TIL infiltration, as supported by the literature [10].

Regarding CD8⁺ T cells, our analysis did not find a significant association between CD8⁺ T cell infiltration in TT or at IM and clinical outcomes such as PFS or OS. This aligns with the results reported by Davidsson et al. [11], who found no correlation between CD4⁺ and CD8⁺ T cell infiltration and survival in a case-control study. Conversely, our findings contradict those of Yang et al. [12], who reported CD8⁺ T infiltration as a favorable prognostic factor for OS. Such discrepancies may be attributed to differences in patient populations, sample types, treatment regimens, or TIL quantification methods.

However, the situation differs in relation to CD3⁺ T lymphocytes. The results of correlation analyses among observed parameters showed statistically significant associations between CD3⁺ IM and PFS, as well as CD3⁺ IM and OS, in such a way that higher values of CD3⁺ IM corresponded to lower values of PFS and OS. In our research, we proved that CD3⁺ T lymphocytic infiltration on IM (peritumoral tissue) makes a statistically significant difference in PFS and OS, which correlates with the findings of Andersen et al. [2] who indicated that different immune cell infiltration patterns in stroma (peritumoral) and epithelium (intratumorally) have a complex biological role for the development and/or progression of PC. Similar results were reported by Kärjä et al. [13], who found that low TIL expression was associated with local disease and indicated a favorable clinical behavior. Richardsen et al. [14] confirm that a high density of CD3⁺ T lymphocytes in tumor cell areas and tumor stromal areas of PC correlated with metastatic disease. In more than 3,000 patients, Flammiger et al. [15] investigated the prognostic effects of TILs in PC. They found that patients with very low and very high densities of intratumoral CD3⁺ T cells had a significantly shorter recurrence period (measured as an increase in PSA level), unlike patients with intermediate numbers of T cells. However, they did not investigate the influence of different subtypes of T lymphocytes on the clinical outcome. Long-term study Molina et al. [16] showed that increased infiltration of CD3⁺ T lymphocytes correlated with a worse prognosis of PC, but CD8⁺ T lymphocytes had no impact on long-term OS. In our study, we found that infiltration of CD3⁺ T lymphocytes at IM is an independent predictor of shorter PFS and OS, and such patients require a more intensive therapeutic approach. For CD3⁺ T cells in TT and CD8⁺ T cells in TT and at IM, we did not find a statistically significant correlation with PFS and OS. In our study, no correlation was found between lymphocytic infiltration and patient age, in contrast to the findings reported by Soliman et al. [3]. However, a correlation was confirmed between CD3⁺ T lymphocytes at IM and both the initial PSA and nadir PSA values, as well as with perineural invasion. These findings suggest that CD3⁺ T lymphocytes at IM may play a role not only in the immune response but also in the biological behavior and aggressiveness of the tumor. The absence of correlation with patient age indicates that lymphocytic infiltration is more closely associated with tumor-related factors than with host age-related immune variability.

Limitations

This study has several limitations. The relatively small sample size (n = 90) and single-center design may limit the generalizability of results. Tissue samples for immunohistochemical analysis were obtained *via* needle biopsy and TURP, unlike many other studies that used radical prostatectomy specimens, which may better represent the entire tumor microenvironment. This difference in sampling could affect the assessment of lymphocytic infiltration. Clinical TNM staging was determined based on physical examination and imaging,

without pathological confirmation, which may affect staging accuracy. Only $CD3^+$ and $CD8^+$ markers were analyzed, without broader immune profiling. Furthermore, infiltration was evaluated only at baseline, with no longitudinal follow-up of the immune response during treatment. All patients were initially treated with hormonal suppression and radiotherapy, as these were the only available treatment options 11 to 13 years ago when their therapy began. Today, treatment strategies would likely differ, which may limit the generalizability of our findings to current clinical practice. Lastly, while the impact on PFS was analyzed, post-progression therapies may have influenced OS outcomes.

Conclusion

In our research, we demonstrated that peritumoral infiltration of $CD3^+$ lymphocytes is an independent predictor of shorter PFS and OS in PC patients. This infiltration also shows significant correlation with both prognostic and predictive factors, including initial PSA levels, perineural invasion, and the achievement of maximal disease regression, as indicated by nadir PSA.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I,* et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74(3): 229–63.
2. *Andersen LB, Nørgaard M, Rasmussen M, Fredsøe J, Borre M, Ulhøi BP,* et al. Immune cell analyses of the tumor microenvironment in prostate cancer highlight infiltrating regulatory T cells and macrophages as adverse prognostic factors. J Pathol 2021; 255(2): 155–65.
3. *Soliman SAM, Yassen NN, Abdelaal SE, Shabana ME.* Expression of CD3+ and CD8+ tumor-infiltrating lymphocytes in prostatic carcinoma: immunohistochemical study and image analysis. Egypt J Pathol 2024; 44(2): 207–14.
4. *Ness N, Andersen S, Valkov A, Nordby Y, Donnem T, Al-Saad S,* et al. Infiltration of CD8+ lymphocytes is an independent prognostic factor of biochemical failure-free survival in prostate cancer. Prostate 2014; 74(14): 1452–61.
5. *Zafar MM, Rauf Z, Sohail A, Khan AR, Obaidullah M, Khan SH,* et al. Detection of tumour infiltrating lymphocytes in CD3 and CD8 stained histopathological images using a two-phase deep CNN. Photodiagnosis Photodyn Ther 2022; 37: 102676.
6. *Esmail RSEN, Kamal A, Shabana M.* Immunoscore As A Predictor Of Disease Recurrences And Patientsâ€™ Survival in Colon Cancer: A Clinicopathologic Study. Open Access Maced J Med Sci 2020; 8(E): 143–9.
7. *Lam TBL, MacLennan S, Willemse PM, Mason MD, Plass K, Shepherd R,* et al. EAU-EANM-ESTRO-ESUR-SIOG Prostate Cancer Guideline Panel Consensus Statements for Deferred Treatment with Curative Intent for Localised Prostate Cancer from an International Collaborative Study (DETECTIVE Study). Eur Urol 2019; 76(6): 790–813.
8. *Hammer Ø, Harper DAT, Ryan PD.* PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 2001; 4(1): 1–9.
9. *Statistics Kingdom.* Correlation Coefficient Calculator [Internet]. Melbourne: Statistics Kingdom [cited 2025 Jun 23; accessed 2025 Aug 28]. Available from: https://www.statskingdom.com/correlation-calculator.html
10. *Vilaça M, Correia Pinto J, Magalhães H, Reis F, Mesquita A.* Tumor-Infiltrating Lymphocytes in Localized Prostate Cancer: Do They Play an Important Role? Cureus 2023; 15(1): e34007.
11. *Davidsson S, Ohlson AL, Andersson SO, Fall K, Meisner A, Fiorentino M,* et al. CD4 helper T cells, CD8 cytotoxic T cells, and FOXP3(+) regulatory T cells with respect to lethal prostate cancer. Mod Pathol 2013; 26(3): 448–55.
12. *Yang Y, Attwood K, Bshara W, Mohler JL, Guru K, Xu B,* et al. High intratumoral CD8+ T-cell infiltration is associated with improved survival in prostate cancer patients undergoing radical prostatectomy. Prostate 2021; 81(1): 20–8.
13. *Kärjä V, Aaltomaa S, Lipponen P, Isotalo T, Talja M, Mokka R.* Tumour-infiltrating lymphocytes: A prognostic factor of PSA-free survival in patients with local prostate carcinoma treated by radical prostatectomy. Anticancer Res 2005; 25(6C): 4435–8.
14. *Richardsen E, Uglehus RD, Due J, Busch C, Busund LT.* The prognostic impact of M-CSF, CSF-1 receptor, CD68 and CD3 in prostatic carcinoma. Histopathology 2008; 53(1): 30–8.
15. *Flammiger A, Bayer F, Cirugeda-Kühnert A, Huland H, Tennstedt P, Simon R,* et al. Intratumoral T but not B lymphocytes are related to clinical outcome in prostate cancer. APMIS 2012; 120(11): 901–8.
16. *Molina OE, LaRue H, Simonyan D, Hovington H, Vittrant B, Têtu B,* et al. Regulatory and memory T lymphocytes infiltrating prostate tumors predict long term clinical outcomes. Front Immunol 2024; 15: 1372837.

Received on March 15, 2025
Revised on July 15, 2025
Revised on August 19, 2025
Accepted on September 3, 2025
Online First October 2025

CASE REPORT

UDC: 616-053.2:616.831-006
DOI: https://doi.org/10.2298/VSP250214063S

Initially disseminated pediatric high-grade midline glioma without H3 K27M alteration – a case report and literature review

Inicijalno diseminovani pedijatrijski visokogradusni gliom srednje linije bez H3 K27M alteracije – prikaz bolesnika i pregled literature

Dragana Stanić Tišma*†, Danica Grujičić†‡, Aleksandar Kostić‡, Marija Denčić Fekete†, Predrag Filipović*, Marija Popović-Vuković*†, Jelena Bokun*†, Marina Nikitović*†

***Institute of Oncology and Radiology of Serbia, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ‡University Clinical Center of Serbia, Clinic for Neurosurgery, Belgrade, Serbia**

Abstract

Introduction. Pediatric high-grade gliomas (HGGs) constitute an extremely heterogeneous group of highly aggressive brain tumors. While leptomeningeal dissemination is commonly observed in patients through the course of the disease, cases with initial dissemination are rare. **Case report.** This paper reports the case of a 12-year-old boy diagnosed with an initially disseminated HGGs with midline localization. Despite surgical intervention and a multidisciplinary treatment approach involving craniospinal radiotherapy and chemotherapy, the patient experienced rapid neurological deterioration and disease progression, and ultimately succumbed to the disease 13 months after diagnosis. In contrast to the vast majority of similar pediatric cases documented in the literature, our patient exhibited an absence of H3 K27M alteration. To our knowledge, this is a unique presentation of a midline HGG with leptomeningeal cranial and spinal dissemination at diagnosis without the expected molecular pattern typically associated with such cases. **Conclusion.** This case highlights that, whether disseminated or not, pediatric HGGs have similarly poor survival outcomes with no effective treatments. It also underscores the widespread challenge of incomplete molecular profiling in these tumors. This emphasizes the urgent need for a comprehensive molecular analysis of these tumors worldwide to advance diagnosis and guide the development of personalized therapy.

Key words:
brain neoplasms; glioma; neoplasm metastasis; pediatrics; survival.

Apstrakt

Uvod. Pedijatrijski gliomi visokog gradusa (*high-grade gliomas* – HGG) čine izuzetno heterogenu grupu visoko agresivnih tumora mozga. Dok se leptomeningealna diseminacija obično opaža kod bolesnika tokom bolesti, slučajevi sa inicijalnom diseminacijom su retki. **Prikaz bolesnika.** Pririkazan je slučaj 12-godišnjeg dečaka sa dijagnozom inicijalno diseminovanog HGG srednje lokalizacije. Uprkos hirurškoj intervenciji i multidisciplinarnom pristupu lečenja koji je uključivao kraniospinalnu radioterapiju i hemioterapiju, bolesnik je doživeo brzo neurološko pogoršanje i progresiju bolesti i na kraju je podlegao bolesti 13 meseci nakon dijagnoze. Za razliku od velike većine sličnih pedijatrijskih slučajeva dokumentovanih u literaturi, naš bolesnik je pokazao odsustvo H3 K27M alteracije. Prema našim saznanjima, ovo je jedinstvena prezentacija HGG srednje lokalizacije sa leptomeningealnom kranijalnom i spinalnom diseminacijom pri postavljanju dijagnoze bez očekivanog molekularnog obrasca koji je tipično povezan s takvim slučajevima. **Zaključak.** Ovim prikazom slučaja ističe se da su ishodi preživljavanja pedijatrijskih bolesnika sa HGG, bez obzira da li su diseminovani ili ne, podjednako loši, uz odsustvo efikasnih terapijskih opcija. Takođe, ukazuje na veliki problem nepotpune molekularne karakterizacije ovih tumora. Ovi podaci dodatno naglašavaju hitnu potrebu sveobuhvatne molekularne analize ovih tumora širom sveta, u cilju unapređenja dijagnostike i razvoja personalizovanih terapijskih pristupa.

Ključne reči:
mozak, neoplazme; gliom; neoplazme, metastaze; pedijatrija; preživljavanje.

Correspondence to: Dragana Stanić Tišma, Institute of Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia. E-mail: dragana221@gmail.com, dragana.stanic@ncrc.ac.rs

Introduction

Pediatric high-grade gliomas (HGGs) constitute up to 20% of pediatric tumors of the central nervous system (CNS) [1]. These tumors' propensity for rapid progression complicates their management, resulting in an unfavorable prognosis. HGGs remain the leading cause of pediatric brain tumor death. The current standard of care is maximal resection of the tumor, followed by radiochemotherapy. Surgical resection, whenever feasible, provides the most significant overall survival (OS) benefit [2].

Pediatric HGGs are an extremely heterogeneous group of highly aggressive brain tumors. After years of molecular and clinical research, H3 K27M-altered diffuse midline glioma, H3 G34-mutant diffuse hemispheric glioma, and diffuse pediatric-type HGG, H3-wildtype and IDH-wildtype, became distinct entities of the pediatric HGG group in the new 5th edition of the World Health Organization (WHO) 2021 CNS tumor classification [3].

Diffuse midline gliomas (DMGs) can arise from midline structures, such as the brainstem, cerebellum and cerebellar peduncles, thalamus, hypothalamus, pineal region, spinal cord, third ventricle, and the ganglio-capsular region. The majority, around 70–80% of pediatric DMGs, possess a lysine-to-methionine mutation at position 27 of histone 3.1, 3.2, or 3.3, which forms a pathological histone mutation that causes derepression of polycomb repressive complex 2 (PRC2) [4]. PRC2 is an enzyme complex that maintains gene transcriptional repression and plays an essential role in the maintenance of cellular identity as well as normal organismal development [5]. These molecular changes partly determine the intrinsic nature of the tumor, its invasive growth, and its unresponsiveness to systemic therapy (which is also caused by the blood-brain barrier). Considering all of the above, patients with H3 K27M-altered DMGs have a rather dismal prognosis despite the advances in therapy, with a two-year survival of less than 10% [6]. The survival of patients with H3 G34-mutant diffuse hemispheric glioma and diffuse pediatric-type HGG (H3-wildtype and IDH-wildtype) is slightly better than that of H3 K27-altered tumors. However, overall prognosis unfortunately remains poor, with two-year OS rates of approximately 20–30% for the former and 15–25% for the latter [7–8].

The current standard treatment for DMGs is focal radiotherapy, which may provide temporary symptom relief and radiographic stabilization but does not significantly alter the disease's poor prognosis. Although various systemic agents and targeted therapies are under investigation, none have yet demonstrated a proven benefit in improving outcomes [9].

DMGs (especially pontine gliomas) are known to metastasize along the neuraxis [10–12]. In literature, leptomeningeal dissemination occurred in 17–56% of patients prior to their death [13–15]. However, at the time of diagnosis, leptomeningeal dissemination is rare, with reported incidence rates ranging from 3% to 18% [15, 16]. Extraneural metastases from DMGs are very rare, typically localized in the bones, and are usually observed after surgical interventions, which are thought to disrupt the blood-brain barrier [17, 18]. Furthermore, in some cases, dissemination to the peritoneal cavity *via* shunt was observed [19]. Most data on DMG dissemination patterns come from isolated case reports or small series, with no large-scale systematic studies on dissemination across specific molecular subtypes. The majority of tumors described in the literature with dissemination and aggressive behavior, whether metastasizing within or outside the CNS, are associated with the H3 K27M mutation [18–22]. Since initially disseminated DMGs are exceptionally rare, there are no consensus guidelines for treatment.

The national referral centers for treating childhood brain tumors in Serbia are the University Clinical Center of Serbia, Clinic for Neurosurgery, Belgrade, Serbia, and the Institute of Oncology and Radiology of Serbia, Belgrade. These hospitals admit any pediatric patient suspected of having a brain tumor to confirm the diagnosis and devise a treatment strategy through a common multidisciplinary team. A case of a child was presented with initially disseminated HGG with midline localization, without H3 K27M alteration.

Case report

A 12-year-old boy presented to the local pediatrician with vertigo and instability while walking. According to the parents, the symptoms had manifested several days prior to admission. Neurological examination revealed discrete divergent strabismus. Nystagmus was present during gaze to the left. Romberg's test, as well as the tandem gait test, was positive. The rest of the examination was uneventful.

Initially, a head computed tomography (CT) was performed and revealed multiple tumor lesions in the brain, an expansive lesion in the middle portion of the left cerebellar hemisphere, the entire pons expanded, measuring 30 mm in its anterior-posterior diameter and cerebellar tonsils descending 5 mm below the foramen magnum, and another lesion in the area of the anterior genus of the corpus callosum that spread contralaterally for about 5 mm (Figure 1).

The patient underwent neurosurgical intervention in the following days. Maximal reduction of the tumor in the posterior cranial *fossa* was performed. The postoperative recovery period was without any complications.

Histopathology revealed a high-grade glial tumor, composed of poorly differentiated astrocytes (Figure 2).

Immunohistochemically (IHC), tumor cells variably expressed INI1, S-100, GFAP, OLIG2, CD56, synaptophysin, MAP2, and p35. H3K27me3 staining showed mostly retained nuclear expression, while epithelial membrane antigen (EMA) and IDH1 p.R132H were negative. ATRX was deemed inconclusive due to inconsistent staining and the absence of a positive internal control. A high proliferation rate was confirmed with the Ki-67 proliferation index, focally approaching 30% (Figure 3). Real-time polymerase chain reaction for the *BRAF* gene mutation did not show any presence. In summary, the diagnosis of pediatric HGG, not otherwise specified, was made by our experienced neuropathologist, with a remark

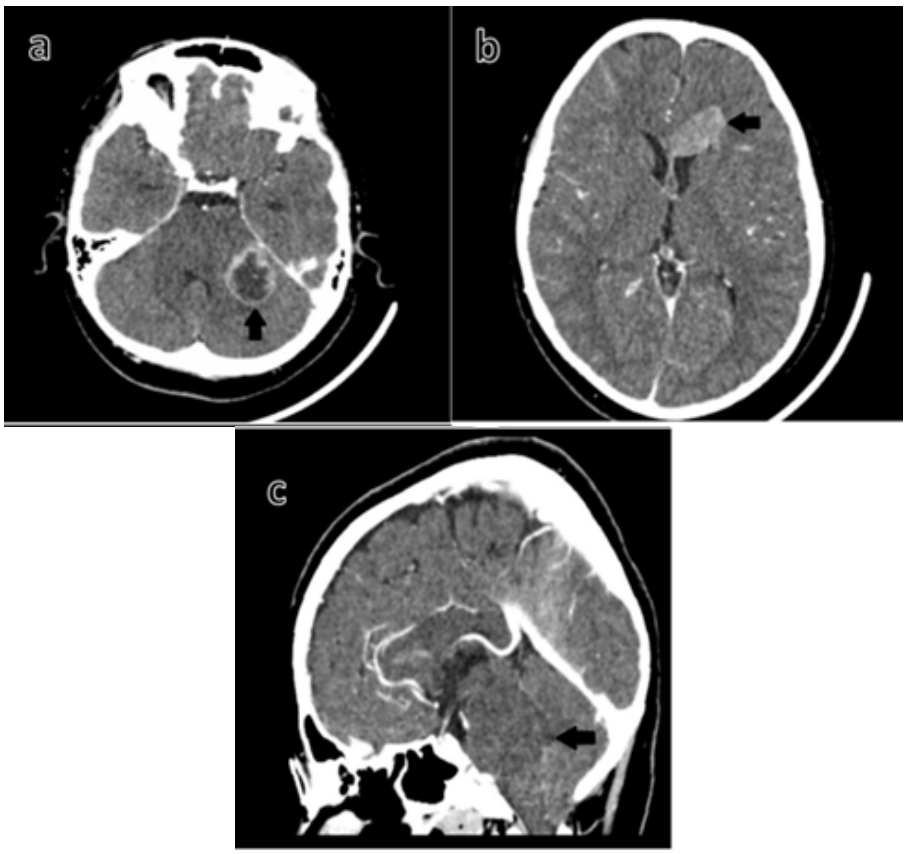

Fig. 1 – Initial computed tomography imaging. a) An expansive heterogeneous lesion with ring postcontrast enhancement in the middle level of the left cerebellar hemisphere. The lesion infiltrates the left cerebellar peduncle (black arrow). b) A hyperdense lesion with irregular edges and postcontrast enhancement at the level of the genus/anterior portion of the left corpus callosum (black arrow). c) The pons exhibits significant expansion (black arrow).

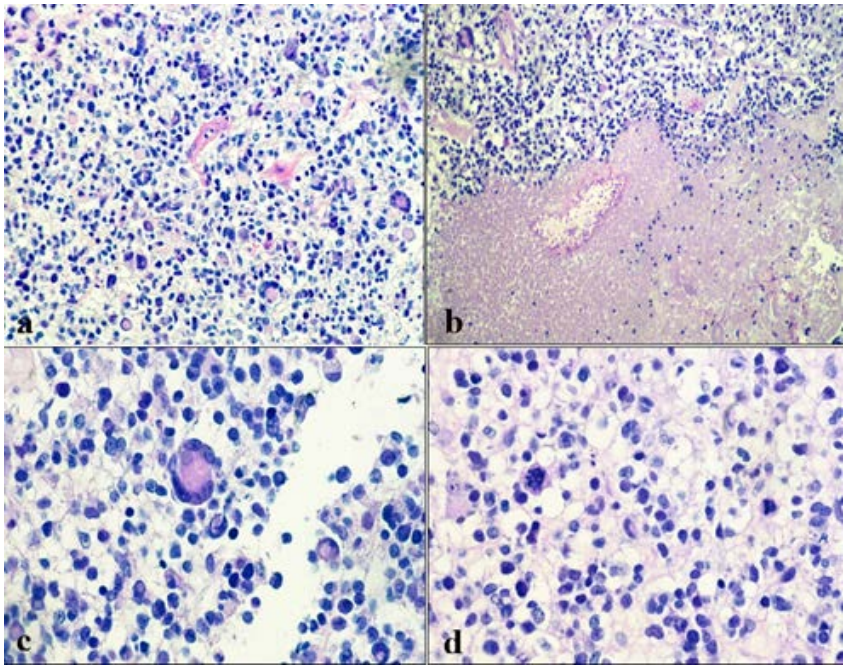

Fig. 2 – High-grade diffuse pediatric glioma, not otherwise specified (hematoxylin and eosin staining). a) Hypercellular tumor composed of pleomorphic glial cells with hyperchromatic nuclei and indistinct nucleoli (×10). b) Foci of non-palisading necrosis (×10). c) Multinuclear giant tumor cell within a sheet of smaller cells (×20). d) Conspicuous mitotic activity with atypical mitosis (×20).

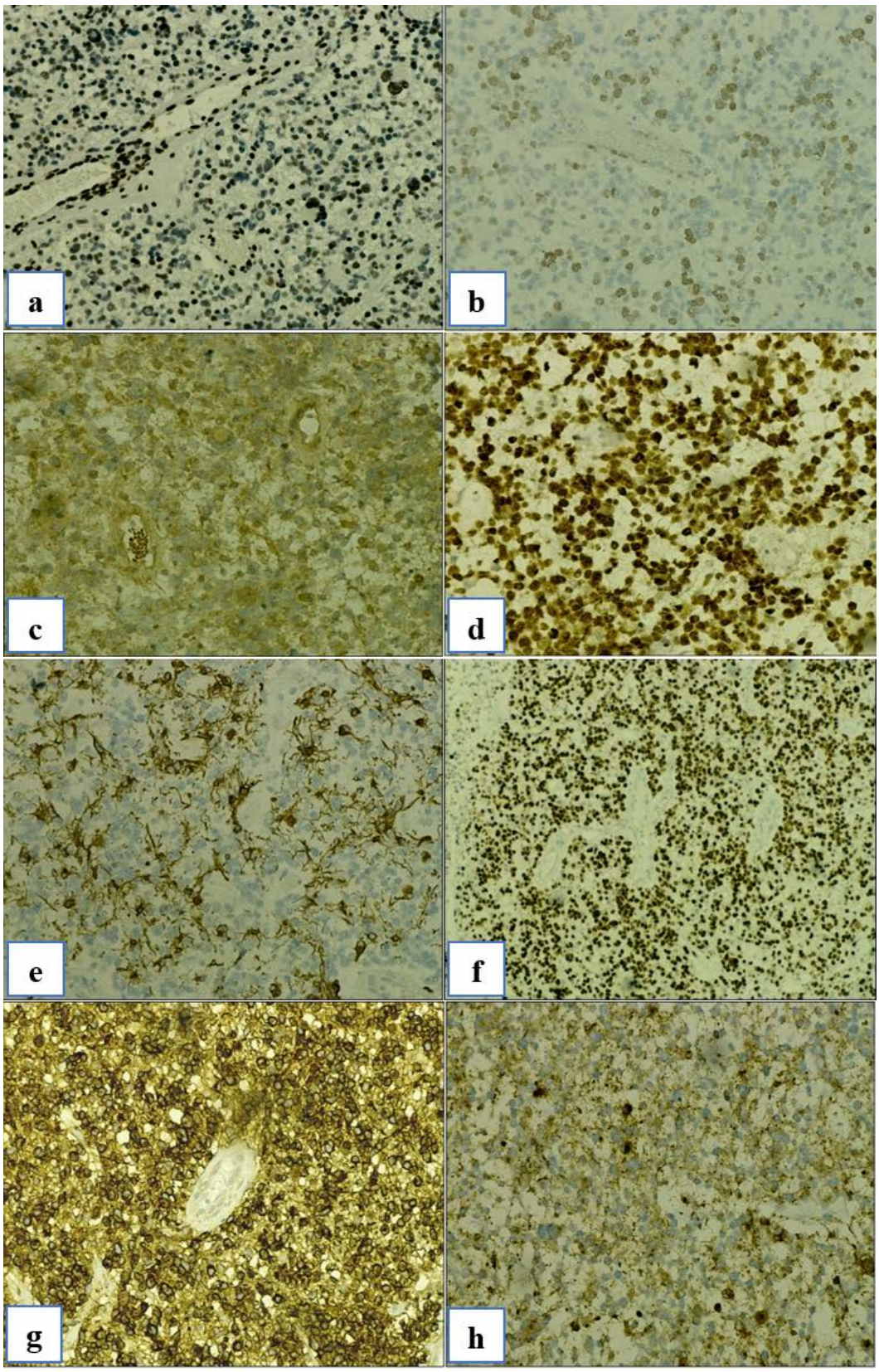

Fig. 3 – High-grade diffuse pediatric glioma, not otherwise specified (immunohistochemical studies): a) focal GFAP immunoreactivity; b) diffuse OLIG2 immunoreactivity; c) IDH1 immunonegativity; d) diffuse p53 immunoexpression; e) H3K27me3 mostly retained nuclear expression, giant multinucleated tumor cells with retained nuclear expression; f) high Ki-67 proliferation index; g) diffuse MAP2 immunoexpression; h) synaptophysin staining in rare tumor cells.

***Note*: All images were captured at ×20 magnification, except f), which was at ×10.**

that further molecular analysis should be done in order to closely define this tumor entity.

The patient was presented to the multidisciplinary tumor board, which indicated treatment continuation with radiotherapy and chemotherapy with temozolomide, according to the HGG protocols. Given the absence of consensus guidelines for disseminated cases, we based our decision on the best available evidence and clinical judgment.

Shortly after the tumor board presentation, the patient's condition deteriorated with the development of dysphagia, dysarthria, and headaches. Neurological examination demonstrated hemiparesis on the left side as well as the presence of bulbar symptomatology. At this time, the child could not walk.

Due to the neurological deterioration, magnetic resonance imaging (MRI) of the head and entire spine was performed. This MRI, performed three weeks after the surgery, showed significant growth of the left frontal lesion with a contralateral propagation and signs of perifocal edema. Diffuse tumor infiltration of the pons was also present, appearing T2 hyperintense. In addition, a smaller lesion was visible on the T1 post-contrast images within the postoperative cavity in the left cerebellar hemisphere, with residual tumor adherent to the cavity wall. The tumor lesions showed an elevation of perfusion as well as a restriction of diffusion. The findings from the spine imaging strongly indicated the presence of leptomeningeal dissemination (three nodules/drop metastases were revealed at the level of the 10^{th} and 11^{th} thoracic vertebral bodies – Th10 and Th11) (Figure 4).

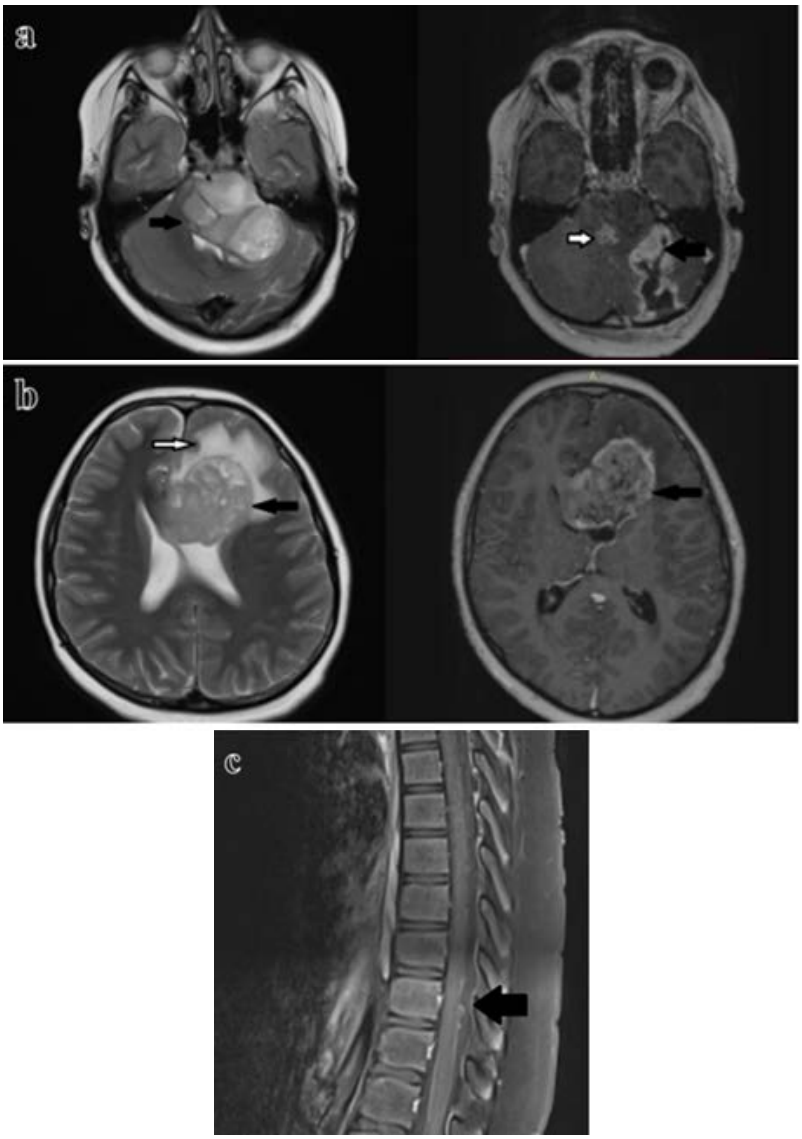

Fig. 4 – Postoperative brain and spine magnetic resonance imaging. a) A postoperative cavity with the presence of a residual tumor that is adherent to the cavity wall (black arrow, right). Diffuse infiltration of the pons (black arrow, left) with a demarked T1 postcontrast lesion (white arrow, right). b) Significant progression of the frontal lesion (black arrows, left and right) with contralateral propagation and infiltration of the anterior portion of the corpus callosum (white arrow, left). c) On T1 postcontrast images, three nodules at the level of Th10 and Th11 vertebral bodies are highly suspicious for leptomeningeal dissemination (black arrow).

Radiotherapy was initiated with concomitant chemotherapy using temozolomide (75 mg/m^2 daily). The patient was planned for craniospinal irradiation with 36 Gy in 20 fractions, followed by a sequential boost of 18 Gy in 10 fractions to the intracranial areas of the disease (the frontal lesion and posterior cranial *fossa*), for a total dose of 54 Gy. After five fractions, the treatment was complicated by grade 2 neutropenia (according to the Common Terminology Criteria for Adverse Events v.5.0), which required granulocyte-colony stimulating factor administration, and grade 2 thrombocytopenia, which required platelet transfusion. Due to hematological toxicity, concomitant temozolomide was discontinued. Radiotherapy was completed without interruptions.

Evaluation of the disease was performed six weeks after. MRI of the head and spine revealed volumetric regression of the tumor lesion located frontally, while other intracranial lesions were stationary. Regarding spinal dissemination, a new nodule, with postcontrast enhancement, appeared at the level of Th4 (Figure 5).

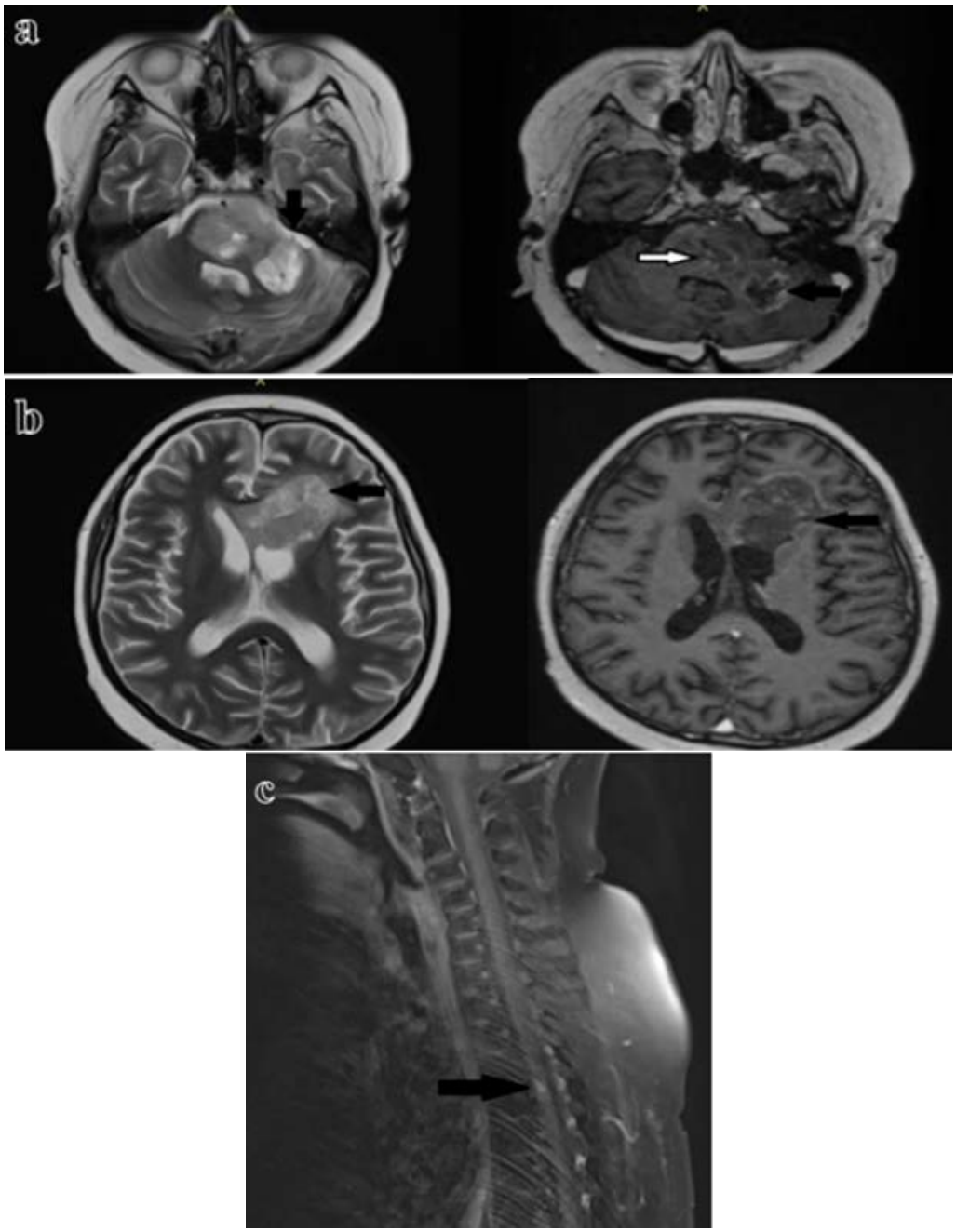

Fig. 5 – Brain and spine magnetic resonance imaging 6 weeks after radiotherapy. a) Retracted postoperative cavity with a still present postcontrast enhancement of the cavity wall (black arrow, right). Diffuse tumor infiltration within the pons persists (black arrow, left) with a similar postcontrast-enhanced lesion diameter (white arrow, right). b) A frontal lesion with still persistent contralateral spread with volumetric regression (black arrows, left and right). c) A new postcontrast-enhanced nodule appeared at the level of the Th4 vertebral body (black arrow).

Treatment was continued with adjuvant monthly temozolomide (200 mg/m^2 daily on days 1–5 of each 28-day cycle). The neurological status of the patient deteriorated. He developed paraplegia and was completely bedridden with episodes of vomiting and headaches. After four cycles of adjuvant temozolomide, MRI of the head and spine demonstrated further volumetric regression of the frontal lesion, but a small new lesion appeared caudally, while the lesions in the posterior *fossa* and spine were unchanged.

Adjuvant treatment was continued for up to eight cycles of adjuvant temozolomide. During this period, the patient occasionally developed grade 3 neutropenia, which required administration of granulocyte-colony stimulating factor. MRI evaluation showed significant progression of the intracranial disease: the frontal lesion expanded in all directions, the pontine lesion also progressed, and the dominant progression was downward to the medulla oblongata (Figure 6). Radiologic progression was followed by further neurological deterioration, including somnolence, immobility, and dyspnea. The patient died 13 months after the diagnosis.

Discussion

Over a 16-year period, from January 2007 to December 2022, 58 patients aged 0–18 years with newly diagnosed HGGs and 37 with diffuse intrinsic pontine gliomas (DIPG) were treated at the Institute of Oncology and Radiology of Serbia. Of these, 11 (11.6%) patients developed metastases. In 10 (10.5%) patients, metastases occurred after initial treatment, while in 1 (1.1%) patient metastases were present at diagnosis – the case described here.

To support the analysis and contextualization of this case, we performed a focused narrative literature review. The search was conducted using PubMed and Google Scholar, employing keywords such as “pediatric high-grade glioma,” “diffuse midline glioma,” “H3K27M,” “H3-wildtype,” “leptomeningeal dissemination,” and “CNS metastases.” Studies included were English language publications involving pediatric patients, with a focus on case reports, retrospective series, and review articles published up to December 2024. Priority was given to literature describing

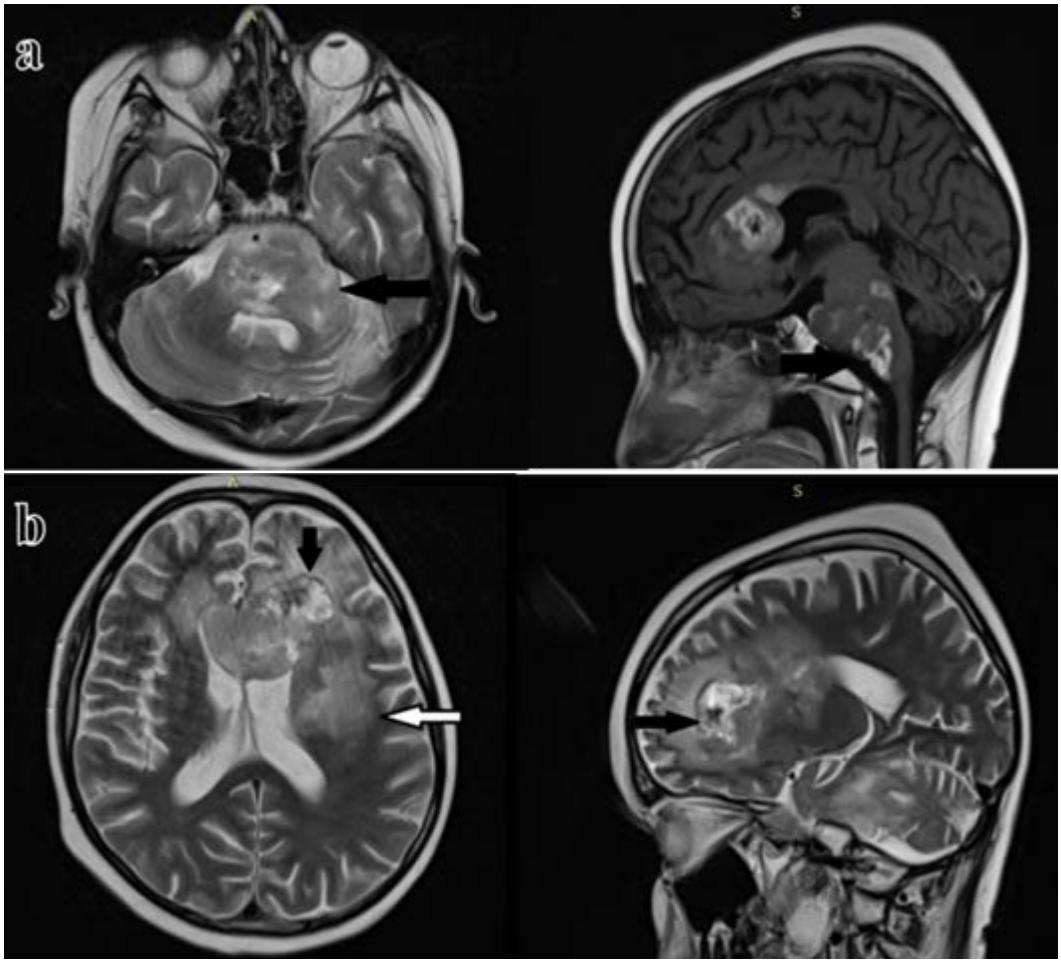

Fig. 6 – Brain magnetic resonance imaging after 8 cycles of adjuvant temozolomide. a) A marked progression of pontine infiltration in all directions (black arrow, left), but the dominant is caudal into the medulla oblongata (black arrow, right). b) Lesion localized frontally in marked progression with infiltration of the rostrum, anterior portion of the corpus callosum (black arrow, left), left corona radiata, and centrum semiovale (white arrow, left) with marked perifocal edema (black arrow, right).

disseminated DMG cases, their molecular characteristics, diagnostic approaches, and treatment outcomes. This review aimed to highlight patterns of dissemination, clinical management strategies, and prognostic differences relevant to our case.

In the largest series so far, the German group reported a 3.1% incidence of primary [16] and 17% of secondary dissemination [14] in patients with HGGs and DIPG included in the HIT-GBM studies. We found a lower incidence of primary and secondary tumor dissemination in our patients, most probably due to the low frequency of completely performed CNS staging initially and during follow-up. The MRI of the brain was conducted routinely in most of our patients, but the MRI of the spine, as well as cerebrospinal fluid cytology, were not a part of the examination in patients with HGGs and DIPG until recent years, so there is a high possibility that some cases with dissemination were not diagnosed initially and subsequently during the course of the disease. In a study by Sethi et al. [15], a high incidence of leptomeningeal dissemination was detected in their cohort of pediatric DIPG patients with close prospective neuraxis MRI surveillance (56% of patients, 18.7% initially). They also found dissemination in two of their patients on autopsy, without previous detection on imaging. Buczkowicz et al. [23] discovered that 38.6% of the patients with DIPG had leptomeningeal spread at autopsy. Recognizing the high potential for leptomeningeal dissemination in HGGs with midline localization, we have successfully integrated routine craniospinal MRI into standard protocols in Serbia for both initial diagnosis and follow-up examinations in pediatric patients with these tumors and all HGGs. Whenever possible, we additionally perform a cytological examination of cerebrospinal fluid.

In the past, surgical intervention for DIPG was primarily focused on treating hydrocephalus, and biopsies were uncommon due to the tumor's location. However, in the current molecular era, obtaining a definitive pathological diagnosis of pediatric HGGs and identifying molecular targets has become increasingly important for research and treatment planning. Biopsies, although limited in their prognostic yield, are necessary for accurate diagnosis. In a case described by Navarro et al. [24], a dural biopsy targeting the lumbar region provided a diagnostic result for an H3K27M-positive spinal lesion, although previously conducted stereotactic and open biopsies of the intracranial midline lesion were inconclusive. The morbidity associated with biopsies in certain locations may delay treatment, suggesting the need for earlier consideration of dural biopsies in patients with leptomeningeal spread. Liquid biopsy, using circulating tumor deoxyribonucleic acid in cerebrospinal fluid, is being explored as a non-invasive alternative that could potentially lead to earlier and more specific diagnoses [25]. A maximal reduction of the tumor in the posterior cranial *fossa* was conducted in our patient, which yielded a histopathological diagnosis. However, most of our patients with pontine lesions do not undergo any form of biopsy procedure. Therefore, we are eagerly waiting for the validation and implementation of liquid biopsy techniques as a diagnostic tool for these tumors. Meanwhile, based on the findings of Nazarian et al. [22] and Hoffman et al. [26], where similar mutational arrangements were observed across all disease sites, a biopsy of the metastatic sites could be considered in patients with dissemination as an alternative.

Most data on HGG dissemination patterns come from isolated case reports or small series, with no large-scale systematic studies on dissemination across specific molecular subtypes. In contrast to our case, the vast majority of midline HGGs described in the literature with dissemination and aggressive behavior in children, whether metastasizing within or outside the CNS, are associated with the H3 K27M mutation [18–22, 24, 27–35]. Some authors reported extracranial metastases of hemispheric H3.3G34R-mutant tumors in children [21, 36, 37]. There is a case of a child with leptomeningeal dissemination and poor prognosis without *H3F3A* mutation reported by Japanese authors [38]. However, the localization of this H3-wildtype and IDH-wildtype primary tumor was the frontal lobe. In contrast, Aghajan et al. [39] reported a case of a pediatric patient with an atypical anaplastic astrocytoma in the cerebellum and diffuse leptomeningeal spread that achieved long-term survival. Molecular analysis revealed unique genetic alterations, highlighting the heterogeneity of pediatric HGGs. Notably, the absence of H3 K27M and IDH mutations, *EGFR*, *p53*, *ATRX*, and *BRAF V600E*, in this case, suggests the existence of different molecular subtypes within pediatric HGGs, with a potential for a good prognosis. In our patient, H3K27me3 staining showed mostly retained nuclear expression, suggesting the absence of H3 K27M alteration. To our knowledge, this is a unique presentation of a midline HGG with leptomeningeal cranial and spinal dissemination at diagnosis without the expected molecular pattern typically associated with such cases. Unfortunately, the limited resources in Serbia, a middle-income country, pose significant constraints on our ability to conduct further investigations into tumor subtypes, perform additional IHC staining, and carry out molecular testing, which is essential for a more comprehensive characterization of this tumor.

Limited availability of diagnostic testing poses a significant challenge in accurately diagnosing CNS tumors, particularly in light of the evolving WHO 2016 and 2021 classification systems. This report highlights the difficulties encountered in low and middle-income countries (LMICs) due to the lack of modern equipment and resources for molecular analysis. These limitations significantly hinder precise diagnosis, especially for CNS tumors in children, and given that the vast majority of children live in LMICs, this represents a substantial problem. Addressing these challenges requires global collaboration and investment in accessible diagnostic technologies for LMICs, ensuring equitable access to accurate diagnosis and guiding optimal treatment for children worldwide.

The question of whether craniospinal irradiation is indicated in patients with disseminated HGGs was raised by Müller et al. [40]. The findings from their paper on craniospinal irradiation with concurrent temozolomide for primary

metastatic pediatric HGGs or DIPG indicate that this treatment approach, although feasible, is associated with limited efficacy and severe myelotoxicity. The study reported disease progression in all patients and a median OS of just over seven months. Similarly, our presented case of a 12-year-old boy with pediatric midline HGG and leptomeningeal dissemination demonstrates the challenges in managing such aggressive tumors. Our patient exhibited progression right after craniospinal radiotherapy treatment with a new lesion on the MRI of the spine at the level of Th4. Moreover, due to myelotoxicity, concomitant temozolomide was discontinued in our patient after five fractions of radiotherapy. Despite the implementation of craniospinal radiotherapy and chemotherapy, the patient experienced rapid neurological deterioration and disease progression, and eventually succumbed to the disease 13 months after the diagnosis. Benesch et al. [16] reported a median OS of 1.5 years in patients with primary disseminated HGGs treated by local radiotherapy and chemotherapy, without statistically significant differences in OS between the group with and without dissemination. So far, no form of treatment has resulted in OS benefit in disseminated HGGs, whether it was local or craniospinal radiotherapy or the addition of various chemotherapy and immunotherapy agents [15, 16, 23, 41]. These findings highlight the urgent need for more effective treatment strategies that can improve outcomes in pediatric HGGs with or without metastatic spread. Further prospective studies are warranted to develop more effective treatments for HGGs in children.

Conclusion

Pediatric high-grade gliomas, particularly diffuse midline gliomas, remain formidable entities with limited treatment options and poor prognoses. Regardless of whether they present as focal or disseminated, these tumors are associated with equally poor overall survival, and no current treatment has demonstrated a meaningful improvement in patient outcomes. Future efforts should prioritize comprehensive molecular analysis worldwide to refine our understanding of different tumor entities and guide the development of personalized treatment strategies.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C,* et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015-2019. Neuro Oncol 2022; 24(Suppl 5): v1–95.
2. *Pollack IF, Agnihotri S, Broniscer A.* Childhood brain tumors: current management, biological insights, and future directions. J NeurosurgPediatr 2019; 23(3): 261–73.
3. *Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D,* et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol 2021; 23(8): 1231–51.
4. *Louis DN, Giannini C, Capper D, Paulus W, Figarella-Branger D, Lopes MB,* et al. cIMPACT-NOW update 2: diagnostic clarifications for diffuse midline glioma, H3 K27M-mutant and diffuse astrocytoma/anaplastic astrocytoma, IDH-mutant. Acta Neuropathol 2018; 135(4): 639–42.
5. *Yang Y, Li G.* Post-translational modifications of PRC2: signals directing its activity. Epigenetics Chromatin 2020; 13(1): 47.
6. *Chatwin HV, Cruz Cruz J, Green AL.* Pediatric high-grade glioma: moving toward subtype-specific multimodal therapy. FEBS J 2021; 288(21): 6127–41.
7. *Crowell C, Mata-Mbemba D, Bennett J, Matheson K, Mackley M, Perreault S,* et al. Systematic review of diffuse hemispheric glioma, H3 G34-mutant: Outcomes and associated clinical factors. Neurooncol Adv 2022; 4(1): vdac133.
8. *Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR,* et al. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. Cancer Cell 2017; 32: 520–37.e5.
9. *Al Sharie S, Abu Laban D, Al-Hussaini M.* Decoding Diffuse Midline Gliomas: A Comprehensive Review of Pathogenesis, Diagnosis and Treatment. Cancers (Basel) 2023; 15(19): 4869.
10. *Gururangan S, McLaughlin CA, Brashears J, Watral MA, Provenzale J, Coleman RE,* et al. Incidence and patterns of neuraxis metastases in children with diffuse pontine glioma. J Neurooncol 2006; 77(2): 207–12.
11. *Tarnaris A, Edwards RJ, Lowis SP, Pople IK.* Atypical external hydrocephalus with visual failure due to occult leptomeningeal dissemination of a pontine glioma. Case report. J Neurosurg 2005; 102(2 Suppl): 224–7.
12. *Kanai R,* Tasaka *M, Sejima H, Uchida N, Nakano A, Akiyama Y,* et al. Brain stem glioblastoma with multiple large cyst formation and leptomeningeal dissemination in a 4-year-old girl. Brain Dev 2005; 27(1): 58–61.
13. *Donahue B, Allen J, Siffert J, Rosovsky M, Pinto R.* Patterns of recurrence in brain stem gliomas: evidence for craniospinal dissemination. Int J Radiat Oncol Biol Phys 1998; 40(3): 677–80.
14. *Wagner S, Benesch M, Berthold F, Gnekow AK, Rutkowski S, Sträter R,* et al. Secondary dissemination in children with high-grade malignant gliomas and diffuse intrinsic pontine gliomas. Br J Cancer 2006; 95(8): 991–7.
15. *Sethi R, Allen J, Donahue B, Karajannis M, Gardner S, Wisoff J,* et al. Prospective neuraxis MRI surveillance reveals a high risk of leptomeningeal dissemination in diffuse intrinsic pontine glioma. J Neurooncol 2011; 102(1): 121–7.
16. *Benesch M, Wagner S, Berthold F, Wolff JE.* Primary dissemination of high-grade gliomas in children: experiences from four studies of the Pediatric Oncology and Hematology Society of the German Language Group (GPOH). J Neurooncol 2005; 72(2): 179–83.
17. *Bhatt NS, Houser K, Belongia M, Ellison DW, Foy A, Jarzembowski J,* et al. Diffuse Midline Glioma With Osseous Metastases at Diagnosis: A Case Report. J Pediatr Hematol Oncol 2020; 42(7): e673–6.
18. *Lazow MA, Leach JL, Trout AT, Breneman JC, Fouladi M, Fuller C.* Extraneural Metastases of Diffuse Midline Glioma, H3 K27M-Mutant at Diagnosis: Case Report, Review of the Literature, and Identifying Targetable Alterations. J Pediatr Hematol Oncol 2022; 44(2): e597–604.

19. *Stephens S, Tollesson G, Robertson T, Campbell R.* Diffuse midline glioma metastasis to the peritoneal cavity via ventriculoperitoneal shunt: Case report and review of literature. J Clin Neurosci 2019; 67: 288–93.
20. *Lu VM, Brown DA, Daniels DJ.* Rare Diffuse Intrinsic Pontine Glioma Metastasis Throughout the Brain and Spine. World Neurosurg 2020; 140: 301–2.
21. *Mohiuddin S, Maraka S, Usman Baig M, Gupta S, Muzzafar T, Valyi-Nagy T,* et al. Case series of diffuse extraneural metastasis in H3F3A mutant high-grade gliomas: Clinical, molecular phenotype and literature review. J Clin Neurosci 2021; 89: 405–11. Erratum in: J Clin Neurosci 2021; 93: 286.
22. *Nazarian J, Mason GE, Ho CY, Panditharatna E, Kambhampati M, Vezina LG,* et al. Histological and molecular analysis of a progressive diffuse intrinsic pontine glioma and synchronous metastatic lesions: a case report. Oncotarget 2016; 7(27): 42837–42.
23. *Buczkowicz P, Bartels U, Bouffet E, Becher O, Hawkins C.* Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: diagnostic and therapeutic implications. Acta Neuropathol 2014; 128(4): 573–81.
24. *Navarro RE, Golub D, Hill T, McQuinn MW, William C, Zagzag D,* et al. Pediatric midline H3K27M-mutant tumor with disseminated leptomeningeal disease and glioneuronal features: case report and literature review. Childs Nerv Syst 2021; 37(7): 2347–56.
25. *Pan Y, Long W, Liu Q.* Current Advances and Future Perspectives of Cerebrospinal Fluid Biopsy in Midline Brain Malignancies. Curr Treat Options Oncol 2019; 20(12): 88.
26. *Hoffman LM, DeWire M, Ryall S, Buczkowicz P, Leach J, Miles L,* et al. Spatial genomic heterogeneity in diffuse intrinsic pontine and midline high-grade glioma: implications for diagnostic biopsy and targeted therapeutics. Acta Neuropathol Commun 2016; 4: 1. Erratum in: Acta Neuropathol Commun 2016; 4: 13.
27. *Dyson K, Rivera-Zengotita M, Kresak J, Weaver K, Stover B, Fort J,* et al. FGFR1 N546K and H3F3A K27M mutations in a diffuse leptomeningeal tumour with glial and neuronal markers. Histopathology 2016; 69(4): 704–7.
28. *Nambirajan A, Suri V, Kedia S, Goyal K, Malgulwar PB, Khanna G,* et al. Paediatric diffuse leptomeningeal tumor with glial and neuronal differentiation harbouring chromosome 1p/19q co-deletion and H3.3 K27M mutation: unusual molecular profile and its therapeutic implications. Brain Tumor Pathol 2018; 35(3): 186–91.
29. *Paul M, Crawford J, Elster J.* HGG-08. Bone metastases in pediatric high-grade glioma: a case series and review of the literature. Neuro Oncol 2018; 20(Suppl 2): i90.
30. *Handis C, Tanrıkulu B, Danyeli AE, Özek MM.* Spinal intramedullary H3K27M mutant glioma with vertebral metastasis: a case report. Childs Nerv Syst 2021; 37(12): 3933–7.
31. *Silva MA, Mirchia K, Chamyan G, Maher O, Wang S, Niazi T.* Disseminated diffuse midline glioma associated with poorly differentiated orbital lesion and metastases in an 8-year-old girl: case report and literature review. Childs Nerv Syst 2022; 38(10): 2005–10.
32. *Fomchenko EI, Erson-Omay EZ, Kundishora AJ, Hong CS, Daniel AA, Allocco A,* et al. Genomic alterations underlying spinal metastases in pediatric H3K27M-mutant pineal parenchymal tumor of intermediate differentiation: case report. J Neurosurg Pediatr 2019; 25(2): 121–30.
33. *Song D, Xu D, Gao Q, Hu P, Guo F.* Intracranial Metastases Originating From Pediatric Primary Spinal Cord Glioblastoma Multiforme: A Case Report and Literature Review. Front Oncol 2020; 10: 99.
34. *Al Sharie S, Talafha M, Abu Laban D, Al Awabdeh T, Al-Mousa A, Al-Masri N,* et al. H3 K27M-mutant diffuse midline glioma with osseous metastases: A case report and a literature review. Clin Neuropathol 2022; 41(6): 263–70.
35. *Gelder CL, Hawkins C, Zapotocky M, Dirks P, Bartels U, Bouffet E.* Diffuse intrinsic pontine glioma ventricular peritoneal shunt metastasis: a case report and literature review. Childs Nerv Syst 2019; 35(5): 861–4.
36. *Kay MD, Pariury HE, Perry A, Winegar BA, Kuo PH.* Extracranial Metastases From Glioblastoma With Primitive Neuronal Components on FDG PET/CT. Clin Nucl Med 2020; 45(3): e162–4.
37. *Jethanandani A, Gule-Monroe MK, Chen M, Johnson JM.* Extraneural Metastases From a High-Grade Glioma (HGG) With an H3F3A G34R Mutation. Front Oncol 2019; 9: 373.
38. *Kinoshita T, Yano H, Nakayama N, Suzui N, Iida T, Endo S,* et al. Pediatric Giant Cell Glioblastoma Presenting with Intracranial Dissemination at Diagnosis: A Case Report. NMC Case Rep J 2021; 8(1): 151–7.
39. *Aghajan Y, Malicki DM, Levy ML, Crawford JR.* Atypical anaplastic astrocytoma with unique molecular features and diffuse leptomeningeal spread in a child with long-term survival. BMJ Case Rep 2019; 12(2): e228153.
40. *Müller K, Schlamann A, Guckenberger M, Warmuth-Metz M, Glück A, Pietschmann S,* et al. Craniospinal irradiation with concurrent temozolomide for primary metastatic pediatric high-grade or diffuse intrinsic pontine gliomas. A first report from the GPOH-HIT-HGG Study Group. Strahlenther Onkol 2014; 190(4): 377–81.
41. *Müller K, Schlamann A, Seidel C, Warmuth-Metz M, Christiansen H, Vordermark D,* et al. Craniospinal irradiation with concurrent temozolomide and nimotuzumab in a child with primary metastatic diffuse intrinsic pontine glioma. A compassionate use treatment. Strahlenther Onkol 2013; 189(8): 693–6.

Received on February 14, 2025
Revised on June 13, 2025
Revised on July 12, 2025
Accepted on July 23, 2025
Online First September 2025

INSTRUCTIONS TO THE AUTHORS

The VSP publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system **(http://aseestant.ceon.rs/index.php)**, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that make them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The manuscripts submitted to the VSP pass in-house and external peer review. All authors pay "Article Processing Charge" for coverage all editing and publishing expenses. Domestic authors pay 5,000 RSD, and those from aboard 150 euros. The editing and publishing fee is required for substantive editing, facts and references validations, copy editing, and publishing online and in print by editorial staff of the Journal. No additional fees, other than stated above, are required even if an author who already paid the fee would have more articles accepted for publishing in the year when fee was paid. All authors who pay this fee may, if want, receive printed version of the Journal in year when fee is payed. Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure. The requirement about paying "Article Processing Charge" does not apply to reviewers, members of the Editorial Board and the Publisher's Council of the Journal, young researchers and students, as well as any of the subscribers of the Journal.

The VSP publishes: **editorials, original articles**, **short communications**, **reviews/meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

The *Vojnosanitetski pregled* journal fully complies with section II.A.4 of the ICMJE recommendations (update January 2025, **https://www.icmje.org/icmje-recommendations.pdf**), which describes authors' responsibilities and journal requirements regarding the use of artificial intelligence (AI)-assisted technologies in manuscript preparation.

Vojnosanitetski pregled adopts the following ICMJE recommendations on AI-assisted technologies in scientific publications:

- Chatbots cannot be authors;
- Authors should be transparent when AI-assisted technologies are used and provide information about how they were used;
- Authors are responsible for the work performed by AI-assisted technologies in their paper (including the accuracy of what is presented and the absence of plagiarism) and for appropriate attribution of all sources (including the material produced by the AI-assisted technologies).

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**). The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ‖, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al.* Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper left corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and acronyms

Authors are encouraged to use abbreviations and acronyms in the manuscript in the following manner: abbreviations and acronyms must be defined the first time they are used in the text consistently throughout the whole manuscript, tables, and graphics; abbreviations should be used only for terms that appear more than three times in text; abbreviations should be sparingly used.

An alphabetical list of all abbreviations used in the paper, followed by their full definitions, should be provided on submission.

Detailed Instructions are available at the web site:
www.vsp.mod.gov.rs

UPUTSTVO AUTORIMA

VSP objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“**(http://aseestant.ceon.rs/index.php)** neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu svakog od autora rada potpisanu od svih autora, treba skenirati i poslati uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Rukopisi pristigli u Redakciju časopisa podležu internoj i eksternoj recenziji. Svi autori dužni su da plate “Article Processing Charge” za pokriće troškova jezičke, stručne i tehničke obrade rukopisa, kao i njegovog objavljivanja. Domaći autori plaćaju iznos od 5 000 dinara, a inostrani 150 eura. Dodatna plaćanja nisu predviđena čak i u slučaju da autor koji je već prethodno platio traženi iznos, ima više prihvaćenih radova za objavljivanje u godini u kojoj je izvršio uplatu. Svi autori koji su platili “Article Processing Charge” mogu, ukoliko žele, dobijati štampanu verziju časopisa tokom godine u kojoj je izvršena uplata. Plaćanje ovog iznosa ne garantuje prihvatanje rukopisa za objavljivanje i ne utiče na ishod recenzije.

Od obaveze plaćanja pokrića navedenih troškova oslobođeni su recenzenti, članovi Uređivačkog odbora i Izdavačkog saveta VSP, studenti i mladi istraživači, kao i pretplatnici časopisa.

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Časopis Vojnosanitetski pregled se u potpunosti pridržava odeljka II.A.4 preporuka ICMJE (ažurirano januara 2025, **https://www.icmje.org/icmje-recommendations.pdf**), koji opisuje odgovornosti autora i zahteve časopisa u vezi sa korišćenjem tehnologija asistiranih veštačkom inteligencijom (artificial intelligence – AI) u pripremi rukopisa.

Vojnosanitetski pregled usvaja sledeće ICMJE preporuke o tehnologijama asistiranih AI-om u naučnim publikacijama:

• Četbotovi ne mogu biti autori;
• Autori treba da budu transparentni kada se koriste tehnologije asistirane AI-om i da pruže informacije o tome kako su ih koristili;
• Autori su odgovorni za deo teksta napisan od strane tehnologije asistirane AI-om u svom radu (uključujući tačnost onoga što je predstavljeno i odsustvo plagijata) i za odgovarajuće navođenje svih izvora (uključujući materijal dobijen korišćenjem tehnologija asistiranih AI-om).

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu:
www.vsp.mod.gov.rs