YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2026; January Vol. 83 (No. 1): pp. 1–66.

Vojnosanitetski Pregled 2026 January Vol. 83 (No. 1): pp. 1–66.

Vol. 83, No.1, 2026

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
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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. 83, br. 1, 2026

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
Open Access
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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

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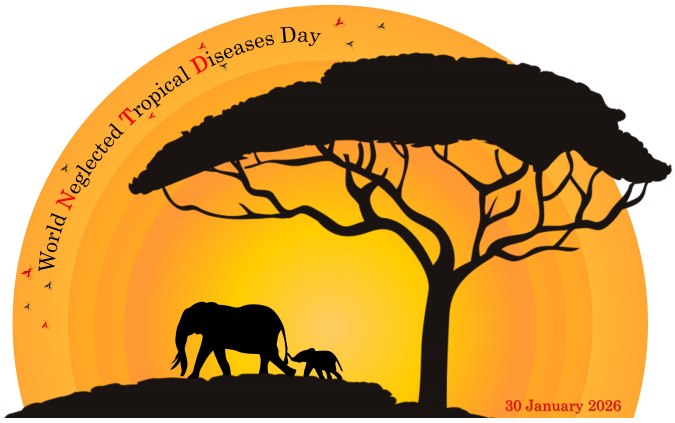

World Neglected Tropical Diseases (NTD) Day is observed annually on 30 January. More than one billion people worldwide live with NTDs. On the occasion of World NTD Day, the World Health Organization invites all communities to unite, act, and eliminate these diseases. This year's theme, "Unite, Act, Eliminate," highlights the importance of global cooperation among health institutions and international organizations in the fight against NTDs and their complete elimination.

Svetski dan zanemarenih tropskih bolesti (ZTB) obeležava se svake godine 30. januara. Više od jedne milijarde ljudi širom sveta živi sa ZTB. Povodom Svetskog dana ZTB, Svetska zdravstvena organizacija poziva sve zajednice da se ujedine, deluju i iskorene ove bolesti. Ovogodišnja tema „Ujedinimo se, delujmo, eliminišimo" ističe važnost globalne saradnje zdravstvenih institucija i međunarodnih organizacija u borbi protiv ZTB i njihovoj potpunoj eliminaciji.

E D I T O R I A L

UDC: 655.41
DOI: https://doi.org/10.2298/VSP2601005V

Vojnosanitetski pregled in 2026 – new challenges, same dreams

Vojnosanitetski pregled u 2026 – novi izazovi, isti snovi

Dragana Vučević

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In line with long-established practice, the editorial published in the first issue of each volume of *Vojnosanitetski pregled* (VSP) provides a comprehensive overview of the activities of the Editorial Board (EB) and Editorial Office (EO) over the past year and outlines plans for the year ahead. The analysis includes both manuscripts submitted in 2025 and articles published during the same period.

Consistent with previous years, the manuscripts submitted in 2025 covered a broad range of research topics in clinical and experimental medicine, dentistry, and pharmacy. In 2025, a total of 277 manuscripts were received, slightly fewer than in 2024 (15 manuscripts fewer). Analyzing the period from 2020 to 2025, excluding 2023 when 226 manuscripts were submitted, it can be seen that the number of manuscripts received in 2025 remains comparable to the levels observed in 2020, 2021, and 2022, with 280, 278, and 265 manuscripts, respectively. As expected, Original Articles represented the largest category of submissions (205 or 74.0%), followed by Case Reports (32 or 11.6%) (Table 1).

The analysis of authors' institutional affiliations showed that 88.8% of submissions came from civilian health and academic institutions, whereas 11.2% were affiliated with the University of Defence in Belgrade, Serbia. Among the manuscripts submitted by civilian institutions, the proportion involving international authors increased from 46.5% in 2024 to 62.9% in 2025. We are particularly encouraged by the participation of international authors, with submissions coming from 10 countries across both our region and more distant parts of the world: Bosnia and Herzegovina, Bulgaria, China, India, Iran, Montenegro, Pakistan, Saudi Arabia, Slovenia, and Turkey (listed in alphabetical order). We sincerely appreciate the trust these authors have placed in our journal, with particular acknowledgment of submissions from China and Turkey, which accounted for the largest proportions of international

Table 1

Categories and the number of manuscripts submitted to the *Vojnosanitetski Pregled* in 2025

Category	n (%)
Original Article	205 (74.0)
Case Report	32 (11.6)
Current Topic	17 (6.1)
General Review	5 (1.8)
History of Medicine	5 (1.8)
Short Communication	5 (1.8)
Meta-analysis	4 (1.4)
Editorial	2 (0.7)
Letter to the Editor	1 (0.4)
Scientific Meeting Report	1 (0.4)
Total	277 (100)

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contributions (China 30.6%, Turkey 12.9%). What is particularly encouraging is that the proportion of papers submitted by authors from abroad has shown a steady increase over the past few years, reflecting the growing interest of the international scientific community in our journal. We hope this trend will continue in the future, contributing further to the international visibility and recognition of VSP.

During the pre-review stage, 55.5% of manuscripts were declined, while 44.5% progressed to the peer-review process. The proportion of manuscripts rejected at the pre-review stage increased slightly compared with 49.1% in 2024, reflecting the application of more rigorous editorial criteria. Among those that underwent the peer-review process, up to December 31, 2025, 44.8% were accepted for publication after the required revisions, and 21.0% were rejected. The remaining 34.2% are still under review. These percentages are consistent with those reported in the previous year.

The total number of articles undergoing the DOI assignment process in 2025 (including those submitted prior to 2025) was 84, slightly lower than in 2024 (92 articles). Extended review periods, longer than desired, directly affected the time to decision on manuscript status (accepted or rejected) and contributed to the lower number of articles published in 2025. The average time from submission to acceptance (after two positive reviews) or rejection (after two negative reviews) for manuscripts submitted during 2025 was 86 and 113 days, respectively. The average time to receive a DOI for manuscripts submitted and accepted for publication during 2025 was 137 days.

One factor contributing to the lower number of manuscripts entering the DOI assignment process and the reduced number of publications in 2025 was the withdrawal of manuscripts by authors after receiving conditionally positive or positive peer reviews. This behavior likely reflects attempts to submit manuscripts, whose quality was substantially improved by the suggestions of the VSP reviewers, to journals with a higher impact factor (IF). Addressing this issue will be a priority for the EB to ensure that VSP's peer-review resources are used to the fullest benefit of VSP.

In 2025, a total of 92 articles were published, including one Scientific Meeting Report (Table 2), slightly fewer than the 102 articles published in 2024. One of the factors contributing to this decrease may be a higher rejection rate at the pre-review stage, as well as the previously mentioned phenomenon of manuscript withdrawal after authors received conditionally positive or positive peer reviews. During 2025, a total of eight manuscripts were withdrawn at this stage.

As in previous years, the majority of published articles fell into the categories of Original Articles (66 or 71.7%) and Case Reports (15 or 16.3%), with numbers almost identical to those in 2024.

Considering the authors' affiliations, an analysis of the published articles showed that the majority of authors were from civilian institutions, both domestic and international (81.6%), of which approximately 36.2% were from abroad, followed by authors from the University of Defence in Belgrade (18.0%). These proportions were roughly the same as in 2024. However, the number of published papers by foreign authors increased by 13.7% compared to 2024. This increase was expected, reflecting both the higher influx of submissions from international authors and the greater number of their manuscripts entering the peer-review process.

We sincerely thank all authors who have chosen VSP to present their research findings, with special acknowledgment to our international contributors. Your work is essential in promoting the journal's visibility and impact. We look forward to your continued contributions in further strengthening VSP's role within the scientific community.

According to the Center for Evaluation in Education and Science database, which provides full-text access to articles published in VSP, a total of 38,147 full-text downloads were recorded in 2025, of which 2,036 corresponded to articles published in 2025. The article with the highest number of downloads (86) was *Association between C-reactive protein-albumin-lymphocyte (CALLY) index and cerebral edema in acute ischemic stroke patients* (DOI: https://doi.org/10.2298/VSP240625086O).

According to the EBSCO database, VSP articles were accessed over 54,370 times in 2025 by 2,003 different universities, colleges, libraries, and other

Table 2

Categories and the number of articles published in the *Vojnosanitetski Pregled* in 2025

Category	n (%)
Original Article	66 (71.7)
Case Report	15 (16.3)
History of Medicine	3 (3.3)
Meta-analysis	2 (2.2)
Editorial	2 (2.2)
General Review	1 (1.1)
Current Topic	1 (1.1)
Short Communication	1 (1.1)
Scientific Meeting Report	1 (1.1)
Total	92 (100)

institutions worldwide. Pukyong National University (South Korea) and Ajman University (United Arab Emirates) ranked first and second, with 7,406 and 5,833 downloads, respectively. Notably, the Max Planck Institute in Heidelberg (Germany), one of Europe's leading scientific institutions, increased its number of downloads from our journal from 1,475 in the previous year to 5,141 in 2025. VSP articles published in 2025 were downloaded 1,937 times, representing a twofold decrease compared with the previous year. The observed reduction may be explained, at least partially, by the smaller number of articles published in 2025. The article with the highest number of downloads (92) was *Deficits in naming and auditory comprehension of terms in individuals with vascular dementia* (DOI: https://doi.org/10.2298/VSP240406081V). As noted above, article downloads in 2025 were lower than in 2024, which is a cause for concern. Nevertheless, it is encouraging that VSP articles were read a total of 4,372 times, representing an increase of more than 440 compared with the previous year, when 3,930 reads were recorded, and provides strong motivation for further improvements in the journal's accessibility and visibility.

The IF of VSP for 2024 remained unchanged from the previous year (2023) at 0.2. As increasing the IF is one of the journal's key goals, the EB has continued to apply a rigorous selection process for manuscripts entering the review phase. The focus is on papers that, due to their quality and significance, can attract the attention of the scientific community and therefore have a high potential for citations. Achieving this goal requires the continuous and active engagement of all members of the EB. Equally important is the careful selection of reviewers who are experts in their respective fields and willing to dedicate the necessary time and expertise. As an encouraging sign that the EB is moving in the right direction, we note the increase in the 5-year IF from 0.2 to 0.3, reported in July 2025.

In 2025, the contributions of 222 reviewers (Table 3) were invaluable to our work, and we are deeply grateful to all of them. We would like to give special recognition to Professors Čvorović Ljiljana, Dedić Gordana, Đukanović Ljubica, Kezić Aleksandra, Lepić Milan, Mikić Dragan, Nožić Darko, Rasulić Lukas, Resan Mirko, Stanojević Ivan, Šuljagić Vesna, Tomić Aleksandar, and Vidaković Aleksandra (listed in alphabetical order), each of whom reviewed at least three manuscripts in 2025.

Table 3

Reviewers of the *Vojnosanitetski pregled* in 2025

Abbasoglu Osman	Ćupurdija Vojislav	Gajić Selena	Jovanović Bogdan
Aleksandrić Srdjan		Gašić Jugoslav	Jovanović Milan
Aleksić Aleksandra	Damjanov Ivan	Golubović Jagoš	Jovanović Simić Jelena
Asmis Lars	Davidović Lazar	Grubović Rastvorčeva Rada	Jovanović Predrag
Atanasijević Igor	de Carvalho Dornelas Bruno	Guzina Golac Nataša	Jovanović Vanja
			Jovičić Žikica
Babić Srdjan	Dedić Gordana	Horvat Olga	Jović Zoran
Babić Uroš	Demetriades Andreas	Hossein Rezvani Mohammad	
Bajčetić Milica	Dimić Andreja	Hoveyan Julieta	Kačar Aleksandra
Bajkin Branislav	Dimitrijević Zorica		Kareem Mohammed Ahmed
Baljošević Ivan	Dinčić Evica		Karličić Vukoica
Bančević Vladimir	Dinić Miroslav	Ignjatović Mile	Kezić Aleksandra
Basta Nikolić Marijana	Dragić Milorad	Ilić Jugoslav	Knežević-Ušaj Slavica
Baščarević Vladimir	Dragović Tamara	Ilić Tanja	Kopitović Ivan
Biočanin Vladimir	Drobac Milan	Ilić Tihomir	Kostić Milena
Bižić Marta		Ivanov Milan	Kostić Smiljana
Bodrožić Jelena	Djokić Jelena		Kovačević Božidar
Bojović Marko	Djordjević Dragan	Jahić Azra	Kozomara Ružica
Bošković Aneta	Djordjević Olivera	Jakšić Vesna	Krasić Dragan
Brajušković Goran	Djukanović Ljubica	Janković Slobodan	Krejović Sanja
Brković Voin	Djukanović Marija	Janković Srdja	Kuzmanović Miloš
Bubalo Marija	Djukić Slobodanka	Janković Veličković Ljubinka	
Budić Ivana	Djurić Ivica	Jauković Ljiljana	
	Djurić Predrag	Jianu Dragos Catalin	Lance Marcus D.
Cancrini Fabiana		Jocić Miodrag	Lazarević Simović Snežana
Cvetković Gordana	Eri Živka	Jotić Ana	Lepić Milan
		Jovančević Ljiljana	Liu Qiquan
Čanadanović Vladimir	Filipović Nenad	Jovanikić Olivera	Lučić Miloš
Čvorović Ljiljana	Filipović Tamara	Jovanović Ankica	Lukić Milica

Table 3 (continued)

Mandić Rajčević Stefan	Nastasović Tijana	Radulović Nataša	Stojimirović Biljana
Manojlović Nebojša	Nešković Mihailo	Rajević Milica	Stojković Goran
Marić Nebojša	Nešković Vojislava	Rakić Mia	Stojšić Mirjana
Marinković Dejan	Nikolić Aleksandra	Ramdaniati Sri	
Marjanović Ivan	Nikolić Jakoba Nataša	Ranković Aleksandar	Šarac Sanja
Marković Aleksa	Nikolić Ratko Jelena	Rasulić Lukas	Šuljagić Vesna
Matić Milan	Nikolić Ljubiša	Rašić Dejan	Šurbatović Maja
Matijašević Jovan	Ninković Milica	Rašković Aleksandar	
Matijević Stevo	Novaković Nenad	Ratinac Miloš	Tamamyan Gevorg
Mavija Milka	Novković Mirjana	Resan Mirko	Tanasković Slobodan
Mijajlović Milija	Nožić Darko	Ristić Andjelka	Tepšić Ostojić Vesna
Mijatović Jovin Vesna		Ristivojević Bojan	Tiodorović Danica
Mikić Dragan	Obradović Radmila	Roganović Branka	Todorović Ljubomir
Milanović Borko	Obradović Slobodan	Rondović Goran	Todorović Zoran
Milenković Branislava			Tomić Aleksandar
Milijašević Boris	Paripović Dušan	Samardžić Janko	Trbojević Stanković Jasna
Milivojević Vladimir	Pasternak Janko	Savić Slobodan	Trifunović Miroljub
Milojević Andjela	Pavić Ognjen	Slović Živana	
Milosavljević Marko	Pavličević Goran	Sotirović Jelena	Vasović Dolika
Milosavljević Miloš	Pavlović Sonja	Sovtić Aleksandar	Veličković Radovanović Radmila
Milošević Nikola	Pejčić Ana	Spasojević Brankica	Vezmar Kovačević Sandra
Milovanović Dragan	Petković Djurić Danijela	Spiroski Dejan	Vidaković Aleksandra
Milovanović Zagorka	Petković Stojan	Srzentić Snežana	Vlahović Dmitar
Miljić Predrag	Petronijević Nataša	Stamenković Dragoslav	Vujašković Mirjana
Minić Predrag	Petrović Vladimir	Stanić Tišma Dragana	Vuković Jelena
Mirković Ljiljana	Pham Khanh Trinh Quoc	Stankov Karmen	
Mirković Nikola	Polovina Snežana	Stanković Ivan	Zdravković Nebojša
Mirošević Nikica	Poulia Kalliopi Anna	Stanković Nebojša	Zeba Snježana
Mišović Miroslav		Stanković Nikola	Zlatanović Dragan
Mladenović Zorica	Radević Svetlana	Stanojević Ivan	Zlatanović Petar
Momčilović Antić Ana	Radić Milica	Stepanović Aleksandar	
	Radoičić Dragan	Stepić Nenad	
Nale Djordje	Radosavljević Aleksandra	Stevanović Dejan	Živković Slavoljub

Considering VSP's current IF and the fact that authors naturally tend to submit their most significant research to higher-impact journals, it is understandable that VSP may not always be their first choice. Therefore, it is the responsibility of the EB to encourage and motivate domestic authors to submit their most important research to VSP, helping to sustain and strengthen the journal's position on the SCI list. Members of the EB can, by recommending experts in relevant fields who respect the deadlines for manuscript evaluation, contribute to shortening the time to a final decision on manuscripts and also increase authors' interest in VSP. We would like to warmly thank all editors who, during 2025, made extra efforts to bring such experts onto VSP's reviewer team.

During 2025, the EO faced organizational challenges, as a result of which the preparation of manuscripts that, following two positive reviews, were accepted for publication and entered the DOI assignment process was slower than planned. Nevertheless, the planned publication schedule was fully maintained, and all 12 issues of the journal were published on a monthly basis. I would like to express my sincere appreciation to the members of the EO, whose dedication and professional commitment ensured the timely publication of each issue.

We also gratefully acknowledge the continued support of the Publisher's Advisory Board of VSP, whose willingness to assist the editorial team and the EO has been of great importance.

Entering the new year provides an opportunity to express our sincere and heartfelt gratitude to all our collaborators who, although not members of the EB or the EO, consistently support the journal through their professionalism, dedication, and long-standing commitment. We extend our special thanks to Ms. Aleksandra Kužet (National Library of Serbia, Department of Scientific Information) and Mr. Nebojša Krstić (Neolibris DOO) for their invaluable and ongoing contributions to the journal. Their support in 2025, as well as throughout previous years, has been an important source of stability, reliability, and reassurance for us.

Last year, we were not able to accomplish everything we had planned. Therefore, we see 2026 as a new opportunity to continue what we have started. Our established plans continue to guide us, while our ultimate

goal remains the improvement of the journal's quality, relevance, and reputation within the scientific community.

At the beginning of the new year, we wish all authors, readers, reviewers, editors, and collaborators good health, personal happiness, and professional success. We look forward to continuing our collaboration and hope that 2026 will bring even greater achievements and satisfaction for all of us.

GENERAL REVIEW

UDC: 616.15-006.04-08:[576.3:615.38
DOI: https://doi.org/10.2298/VSP250822002T

Stem cell transplantation – an overview of clinic-based data and practice in the era of new drugs

Transplantacija matičnih ćelija – pregled podataka iz kliničke prakse u eri novih lekova

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Abstract

Hematopoietic stem cell transplantation (HSCT) has been the therapy of choice for treating some hematologic malignancies and selected non-malignant disorders for decades. With the introduction of novel immunotherapeutic and cell-mediated approaches, the role of autologous HSCT (auto-HSCT) and allogeneic HSCT (allo-HSCT) should be redefined. Auto-HSCT remains the standard of treatment for multiple myeloma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma. The use of novel agents, including proteasome inhibitors, immunomodulatory drugs, monoclonal and bispecific antibodies, enhances the intensity and efficacy of the therapeutic response and opens debate on an optimized timing for HSCT. Allo-HSCT represents the most effective type of adoptive immunotherapy, ensuring complete and long-term hematopoietic reconstitution, often accompanied by the graft-versus-leukemia effect. It remains the main curative treatment for acute leukemias, high-risk myelodysplastic and myeloproliferative syndromes, and severe aplastic anemia. Improvements in stem cell (SC) donor selection, *ex vivo* manipulations of harvested cells, and graft engineering with superior immune monitoring have broadened and expanded their applicability, while improving safety and clinical outcome. Despite rapid progress in cellular and other immunotherapies, HSCT continues to play an essential role in the treatment of numerous hematologic disorders. A combination of HSCT with novel drugs and other immunotherapies offers the potential for personalized and safer treatment with long-term positive clinical outcomes, ensuring that HSCT remains a highly relevant method in modern medicine. The aim of this review was to summarize current biological concepts of SCs, as well as important advances in the rapidly developing fields of SC research, and to determine the place and efficacy of HSCT nowadays, in the era of new therapeutic approaches and agents.

Keywords:
allografts; cryopreservation; hematologic diseases; immunotherapy; multiple myeloma; stem cells; transplantation, autologous.

Apstrakt

Transplantacija hematopoetskih matičnih ćelija (*hematopoietic stem cell transplantion* – HSCT) već decenijama predstavlja terapiju izbora u lečenju pojedinih hematoloških maligniteta i nekih nemalignih poremećaja. Uvođenjem novih imunoterapijskih i ćelijama-posredovanih pristupa trebalo bi da bude redefinisana uloga autologne HSCT (auto-HSCT) i alogene HSCT (alo-HSCT). Auto-HSCT i dalje ostaje standard u lečenju multiplog mijeloma, Hočkinovog limfoma i ne-Hočkinovog limfoma. Primena novih medikamenata, uključujući inhibitore proteazoma, imunomodulacijske lekove, monoklonska i bispecifična antitela, povećava intenzitet i efikasnost terapijskog odgovora na HSCT i otvara raspravu o optimalnom vremenu za primenu HSCT. Alo-HSCT predstavlja

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najefikasniji oblik adoptivne imunoterapije, obezbeđujući kompletnu i dugotrajnu hematopoetsku rekonstituciju, neretko praćenu efektom "kalem protiv leukemije" (*graft-versus-leukemia*). Ona ostaje primarni kurativni vid lečenja akutnih leukemija, visoko-rizičnih mijelodisplastičnih i mijeloproliferativnih sindroma, kao i teške aplastične anemije. Poboljšanja u izboru donora matičnih ćelija (*stem cell* – SC), *ex vivo* manipulaciji prikupljenih ćelija, kao i inženjering grafta uz napredni imunski monitoring, proširila su i unapredila primenljivost ove terapije uz istovremeno povećanje bezbednosti i poboljšanje kliničkog ishoda. Uprkos brzom napretku u oblasti ćelijama posredovane i drugim agensima posredovane imunoterapije, HSCT i dalje ima ključnu ulogu u lečenju pojedinih hematoloških poremećaja. Kombinacija HSCT sa novim lekovima i drugim vidovima imunoterapije pruža mogućnost personalizovanog i bezbednijeg lečenja sa dugotrajnim povoljnim kliničkim ishodima, i obezbeđuje da HSCT ostane visoko relevantan metod u savremenoj medicini. Cilj ovog rada bio je da se sumiraju trenutni biološki koncepti SC, kao i bitna dostignuća na istraživačkim poljima u oblasti SC koja se brzo razvijaju, i da se odredi mesto i efikasnost HSCT danas, u eri novih terapijskih pristupa i agenasa.

Ključne reči:
alograft; kriokonzervacija; hematološke bolesti; imunoterapija; multipli mijelom; ćelije, matične; transplantacija, autologna.

Introduction

Stem cells (SCs) are defined as cells with a unique ability for self-renewal, high proliferative capacity, and the potential to differentiate into mature blood or somatic cells, such as osteocytes, chondrocytes, hepatocytes, myocytes, cardiomyocytes, and even endothelial cells. The increasing clinical use of various cell-mediated therapeutic methods over the past decades has resulted in a growing demand for both hematopoietic SCs (HSCs) and the need to adapt and improve operating procedures to minimize cellular injury during collection, purification, and cryopreservation. A critical aspect of cell harvesting is obtaining improved SC yield, purity, and viability. The objective of fundamental and clinical cryoinvestigations is to decrease cellular damage during freeze/thaw procedures (cryoinjury). Although SC clinical use has become routine, a large number of questions related to optimal cell harvesting protocols, *ex vivo* processing, and cryopreservation remain unresolved [1–3].

Since the initial treatments with HSC transplants (HSCT), considerable changes and improvements have been made in the kind of medications used for peritransplant treatment of patients. In addition, new approaches and agents/drugs have recently been introduced for the therapy of various hematological diseases and disorders. For instance, the use of autologous HSCT (auto-HSCT) has long been a standard method for transplant-eligible patients. However, its status in treatment should be redefined due to the introduction of agents such as proteasome inhibitors, immunomodulatory drugs, monoclonal or bispecific antibodies (Abs), and chimeric antigen receptor T (CAR-T) cellular therapies [2–6].

The purpose of this paper is to recapitulate data in the field of conceptual aspects of SCs harvesting and extracorporeal "graft-engineering" systems adapted to specific cell categories. Moreover, some practical aspects of the place and justification of HSCTs in the era of new drugs and other biologically active agents will be briefly reviewed.

Stem cells – biology and *ex vivo* manipulations

The biology of various divisions of SCs is a fascinating and constantly expanding field of biomedicine that examines and describes both the fundamental properties of these cells, as well as the possibility and effectiveness of their therapeutic use in cell transplant and regenerative medicine [1–5]. There are different types and sources of SCs: embryonic SCs (ESCs – concerning their therapeutic use, ongoing regulations/directions are required), adult SCs (ASCs) or tissue-specific SCs (e.g., HSCs, and mesenchymal SCs – MSCs, etc.), as well as induced pluripotent SCs (iPSCs) – produced through "HSCs reprogramming" of somatic cells back into a pluri(multi)potent stage/phase. MSCs are important due to immunomodulation competence and the ability to differentiate into numerous cell types of mesodermal origin (tissue repair) [2, 6–14].

Cytopoiesis is a continuous biological process of producing a large number of "daughter cells" from the compartment of a single or solitary "parent" SC. The original explanation and description of SCs – that they are exclusive, high-class cells at an early developing stage, characterized by an almost limitless self-renewal ability (long-term possibility to create identical copies of themselves), high proliferative capacity, and extensive potential for differentiation into specialized and ever more mature cells of different lineages in the body – remains unsurpassed [2–10].

Thus, SCs are the "key" cells in the body functioning as special "antecedent" cells or precursors that precede (hemo)biological events or cellular evolution, producing a large quantity (proliferation) of mature (differentiation) cells within tissues, while simultaneously retaining the ability to reproduce themselves (self-renewal). This event is precisely regulated by intrinsic genetic/molecular pathways, which can be influenced by external signals from the extracellular matrix (ECM), as well as by the microenvironment provided by stromal cells [6–14].

Owing to the phenomenon of self-renewal, SCs maintain the constancy of their own population under steady-state conditions, but also in conditions when that physiological balance is disturbed – up to a certain limit. Through differentiation, primitive SCs create a "wide-ranging" series of less primitive cells: firstly, different pluri(multi)potent SCs with a somewhat decreased self-renewal ability, and then cells determined for more or just one cell lineage, which have a very moderate or no potential for self-renewal. To further clarify SCs, an "up-to-date" characteristic has been added–cell plas-

ticity (more precisely, intersystemic plasticity)–which is particularly important for their clinical application in regenerative medicine [2, 7–16].

Concisely, the most primitive SCs give rise to repopulation of the recipient's bone marrow (BM)–engraftment with the following complete and long-term reconstitution of hematopoiesis. They are also capable of "colonizing" targeted/damaged tissues ("homing")–by following "transdifferentiation" into the cell lineages of host organ, including collateral vessel formation ("neovascularization") [2, 17–22].

The use of HSCT is a highly specialized and often life-saving curative procedure in which a patient receives auto-HSCs or allogeneic HSCs (allo-HSCs) following high-dose chemotherapy (HDCT), and less frequently chemoradiotherapy, in order to replace damaged BM with healthy cells. In an autologous setting (auto-HSCT), a patient receives their own HSCs following HDCT. Allo-HSCT is a therapeutic method of replacing the patient's hematopoietic tissue with donor "blood-forming" cells/tissue, i.e., HSCs. It represents the concept of applying chemotherapy/radiotherapy with immunosuppressive treatment, after which donor HSCs are applied to the patient with high-risk hemato-malignancies [2–6].

In practice, HSCT represents a curative method of treating malignant disorders, such as Hodgkin's lymphoma (HL) and non-HL (NHL), multiple myeloma (MM), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), myelodysplastic syndrome, and myeloproliferative neoplasms (MPN) [6, 23–34]. Transplants are also indicated for the treatment of some non-malignant diseases and immune-mediated disorders, such as severe aplastic anemia and multiple sclerosis (MS) [2, 35–41].

Stem cell collection

In practice, SCs can be collected by multiple aspirations from BM, by harvesting mononuclear cells (MNCs) from peripheral blood (PB) after a mobilizing regimen, or by isolation from umbilical cord blood. Typically, the use of BM- or PB-derived grafts is a standard method in adult patients, whereas umbilical cord blood transplants have shown promising results, particularly in the pediatric setting [41–45].

Historically, BM was the primary source of SCs for transplantation in both experimental and clinical settings, obtained through multiple aspirations from the posterior and anterior iliac crests and, rarely, from the sternum. Nowadays, SCs are predominantly harvested from PB, accounting for ≥ 80% of HSCTs, after a mobilization regimen, using chemotherapy and/or granulocyte colony-stimulating factor (G-CSF), seldom in combination with plerixafor (mozobil) [2, 6, 24, 26, 42]. The use of G-CSF plus plerixafor increases the ratio of patients who respond to the cell mobilization ("good-mobilizers" – approximately 95% of cell harvestings) and enables the collection of enough cells for auto-HSCT (including patients with "mobilization-failure" or "poor-responders") [24].

Determining the best possible time for SC-harvesting from PB is the most critical event. For allogeneic donors, the first apheresis is regularly on the fifth day of G-CSF application. However, deciding on the optimal timing for autologous collection from patients who are primed by chemotherapy plus G-CSF is more complex and challenging. The circulating $CD34^+$ cells count evidently correlates with optimized harvesting time and SC quantity [target $CD34^+$ yield ≥ 2–5×10^6 cells/kg of patient's body mass (bm) in the harvest]. Nowadays, it is accepted that optimal timing to begin cell collection is when the number of $CD34^+$ cells is ≥ 20–40/μL of the patient's PB [3–6, 24, 41].

Our results confirmed the high-level efficacy of the large volume leukapheresis (LVL). For the approximately 90% of patients using one LVL, the mean $CD34^+$ yield was 8.4×10^6 cells/kg bm (allo-HSCT) and 5.5×10^6 cells/kg bm (auto-HSCT), respectively [2–4, 44]. Finally, in the group of patients requiring plerixafor, the use of the G-CSF plus plerixafor protocol reduced the rate of "poor-responders" and provided an adequate cell dose (mean $CD34^+$ cell yield was 7.6×10^6 cells/kg bm – to be able to perform a tandem auto-HSCT as well) with a superior therapeutic potential and safety profile of treatment [24].

Stem cell cryopreservation

The saving/banking of living cells, such as SCs, in a frozen state (cryopreservation) is required when cells appear to be biologically, chemically, or thermally unstable after liquid-state storage. Its key aim is to obtain better-quality cell count and viability recovery after thawing. Although SC-cryopreservation is nowadays a standard technique, recent cryoinvestigations recommend that freezing strategies be revised to minimize cryoinjuries and maximize cell recovery. Cryoinjuries result from the extensive cellular dehydration or "solution effect" and/or massive intracellular ice crystallization or "mechanical damage" [2, 4, 46–50].

The use of programmed or controlled-rate freezing, which ensures a precisely defined cooling rate, is a time-consuming process that requires high-level technical knowledge. The choice of an optimal freezing rate – specific for each cell type and cryobiosystem – should be determined. In practice, the cooling rate should be sufficiently rapid to prevent "solution effect", yet slow enough to allow possible water efflux from the cells and following "mechanical damage" [2, 6, 50]. Uncontrolled-rate technique ("dump-freezing") is less costly because it does not require a complex programmed freezing device. However, it has been confirmed that controlled-rate freezing systems are superior, as they provide better quantitative, morphological, ultrastructural, and functional cell recovery during cryopreservation and following thawing [45–50]. In addition, satisfactory numerical and functional recovery of cryopreserved SCs is achieved only when an appropriate cryoprotectant – most commonly dimethyl sulfoxide (DMSO) for SC cryopreservation—is added to the cryobiosystem at an optimal final concentration. Cryoprotectants express protective effects and consequences by decreasing cellular thermal damage, i.e., by reducing cell dehydration and intracellular ice crystallization [2, 50].

As previously demonstrated in our cryoinvestigations using a controlled-rate freezing system, the recovery of

committed hematopoietic progenitors – colony-forming unit (CFU)–spleen (CFU-Sd12) and CFU – granulocyte-macrophage (CFU-GM) – was improved in the presence of 5% DMSO [46]. On the other hand, we confirmed that the recovery of very primitive pluripotent SCs with marrow repopulating ability was better when 10% DMSO was used. Thus, our cryoinvestigation suggested various cryobiological characteristics and requirements of marrow repopulating ability cells compared with committed progenitors [46]. Finally, our early clinical studies showed that therapeutic use of controlled-rate cryopreserved SCs (10% DMSO) in the therapy of hemato-oncological patients resulted in high cell recovery and rapid post-transplant hematopoietic reconstitution, with neutrophil recovery occurring on average by day 11 and platelet recovery by day 13 [2, 24, 44].

The most frequent indications for auto-HSCT in hematology

As previously pointed out, auto-HSCT is a common procedure in hematology, primarily used to treat certain blood cancers. The main purpose of auto-HSCT is to "rescue" the patient's BM after damage caused by HDCT. Although HDCT is highly effective in eradicating malignant cells, it also destroys the healthy, "blood-forming" SCs, i.e., HSCs, in the BM [2–6].

In hematology, the most common indications for auto-HSCT are MM, HL, and NHL. In the treatment of patients with MM, auto-HSCT is often a standard part of the initial treatment for eligible patients, aiming to consolidate the initial therapeutic response. Conditioning regimen with standard high-dose melphalan (HD-Mel) is used to achieve a deep and long-lasting remission [51]. Based on the most recent data from the European Society for Blood and Marrow Transplantation (EBMT), MM is the most frequent indication for auto-HSCT. According to the 2023 EBMT report on HSCT and cellular therapies, plasma cell disorders, which primarily consist of MM, accounted for 58.2% of all auto-HSCT performed in Europe in 2023. The other main indications for autologous transplants in 2023 were lymphomas (32.2%) and solid tumors (6.6%). Among lymphomas are HL and NHL [52]. Additionally, auto-HSCc is a standard treatment for HL patients who have relapsed or whose disease is refractory to initial chemotherapy (relapsed/refractory – r/r HL), which accounts for approximately 40% of initial patients with this diagnosis [53]. Finally, auto-HSCT is frequently used as salvage therapy for patients with r/r NHL, or can also be used as consolidative treatment for high-risk patients, such as those with mantle cell lymphoma (MCL) or anaplastic large cell (ALC) lymphoma [54].

The role of auto-HSCT in MM, HL, and NHL in the era of new drugs and cellular therapies

Multiple myeloma

The field of MM treatment is constantly evolving, with the introduction of new drugs significantly impacting the role of auto-HSCT. While auto-HSCT has long been a standard of care for transplant-eligible patients (up to 65–70 years), its place in the treatment paradigm is being redefined by novel agents such as proteasome inhibitors, immunomodulatory drugs, monoclonal Abs, bispecific Abs, and CAR-T cellular therapies [55].

Despite all novel approaches in MM, an auto-HSCT remains a standard of care for many patients, since it is highly effective, particularly for younger and fit patients with newly diagnosed MM [56]. It is well known that it can provide deep and durable responses, which translate to longer progression-free survival. Some studies have even shown an overall survival (OS) benefit [57].

In the course of first-line treatment, auto-HSCT is often performed after a period of induction therapy. Initial treatment, which includes triple or, in high-risk patients, quadruplet combinations of new drugs, optimizes initial therapeutic response before the transplant procedure and changes the paradigm "when" and "how" to perform an auto-HSCT in MM patients.

There are a few pillars in the patient journey from diagnosis to possible optimal treatment response, and they are described in the passages that follow.

Improved induction with novel agents in combined regimens, including proteasome inhibitors (e.g., bortezomib, carfilzomib), immunomodulatory drugs (e.g., lenalidomide – LEN), and monoclonal Ab (e.g., daratumumab), is used before auto-HSCT. These drugs achieve a deeper initial response before the transplant.

The role of HD-Mel (200 mg/m^2) with possible addition of a new drug in the conditioning regimen before application of autologous HSCs in a single or tandem setting in high-risk patient [58].

New drugs are also used after auto-HSCT for maintenance therapy to prolong remission. This includes drugs like the immunomodulatory drug LEN, and ongoing research is exploring other options to maintain the depth of the post-transplant response [59].

The debate on upfront vs. delayed auto-HSCT: the excellent results achieved with novel drug combinations have led to a discussion regarding whether auto-HSCT should be performed right after induction therapy (upfront) or delayed until disease relapse. While some trials have shown a benefit to upfront auto-HSCT in terms of progression-free survival, the OS benefit is not always clear. This makes the decision highly personalized as part of an evidence-based approach in the patient's specific case [60].

The use of new immunotherapies like CAR-T-cell therapy and bispecific Abs is emerging as a highly effective option, particularly for patients with r/r MM [61]. These therapies are currently being explored for earlier use in the treatment pathway and may further impact the role of auto-HSCT in the future [62].

Hodgkin's lymphoma

In the r/r HL, new drugs such as brentuximab vedotin (BV) and checkpoint inhibitors play a great role as novel

treatment options [63]. Auto-HSCT exerts its principal benefit as consolidation therapy, particularly in achieving a second remission in chemosensitive r/r HL patients. The main purpose of auto-HSCT in these cases is to allow the administration of HDCT. The consolidation with BV after auto-HSCT in HL patients with high risk of relapse is the main achievement of this combined approach [64]. After treatment failure with BV, other options in r/r HL are different checkpoint inhibitors like nivolumab, pembrolizumab, and ipilimumab [65]. After relapse with all previously explained approaches in r/r HL, allo-HSCT could be the only reasonable treatment option. This type of transplant provides the additional benefit of creating a "new" immune system that may be able to recognize and fight any remaining lymphoma cells. However, it also carries a higher risk of complications, such as graft-versus-host disease (GvHD). The decision to use a different type of HSCT depends on various factors, including remission status, comorbidities, and the patient's preferences. While newer therapies are emerging, SC transplantation continues to play a vital role in the management of r/r HL.

Non-Hodgkin's lymphoma

Auto-HSCT plays a crucial role in the treatment of many subtypes of NHL, but its use and effectiveness vary substantially depending on the specific type of lymphoma. The biological nature of NHL is extremely heterogeneous and includes numerous pathohistological entities, 85% of which are of B-cell origin. Unlike in HL, where the treatment is more standardized, the diverse nature of NHL requires a tailored approach.

The main purpose of auto-HSCT in NHL is to enable the use of HDCT [54]. The most common representatives of aggressive B-cell lymphomas, which are candidates for auto-HSCT, are diffuse large B-cell lymphoma (DLBCL), MCL, indolent follicular lymphoma (FL), and, concerning T-cell types, peripheral T-cell lymphoma. Here is a summary of the role of auto-HSCT in these NHL subtypes.

Diffuse large B-cell lymphoma

First-line treatment: for most patients, DLBCL is successfully treated with standard immunochemotherapy (e.g., rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone – R-CHOP), achieving remission rates of approximately 60–70%. Auto-HSCT is not standard of care as consolidation therapy in first remission [66].

r/r DLBCL is a platform where auto-HSCT is considered a standard and potentially curative treatment option. However, before undergoing auto-HSCT, the disease status of r/r DLBCL must be chemosensitive after receiving salvage therapy. In chemotherapy-refractory patients, some other treatment options, such as monoclonal Abs-drug conjugates (e.g., polatuzumab vedotin), bispecific Abs (e.g., glofitamab), or CAR-T cells, provide an adequate therapeutic response [67].

Mantle cell lymphoma

Since MCL is a highly aggressive disease, consolidation of first remission with auto-HSCT has represented the standard of care for almost two decades [68]. The goal is to extend the duration of the first remission and improve long-term survival. This approach is a standard of care and an important part of treatment for many younger patients with MCL [69]. In chemo-refractory patients, the Bruton tyrosine kinase inhibitor ibrutinib or the immunomodulatory drug LEN can be treatment options, sometimes as a "bridge" to allo-HSCT. For patients with r/r MCL, allo-HSCT may also be considered. This approach is more intense and bears a higher risk, but it can be effective due to the graft-versus-lymphoma effect, where the donor's immune cells attack the remaining tumor cells.

Follicular lymphoma

Auto-HSCT is not typically part of the initial treatment for FL, which is often managed with less intensive therapies. For patients with r/r FL who no longer respond to other treatments, auto-HSCT can be used to achieve a long-lasting remission. Allo-HSCT may also be an option for a small, selected group of patients, particularly those with a high-risk FL or those who have failed auto-HSCT [70].

Key factors that historically predicted a good outcome with auto-HSCT in r/r FL include chemosensitivity to salvage therapy. Namely, patients who achieve a complete or partial response after a salvage chemotherapy regimen before transplant generally have better outcomes [71]. Patients who relapse more than 2 years after their initial therapy tend to have better outcomes with auto-HSCT compared to those with early relapse (within 24 months, also known as progression of disease within 24 months – POD24). However, some studies have shown that auto-HSCT can still be a valuable option even for patients with POD24, leading to improved survival [72]. Studies have found that patients who were sensitive to rituximab-based immune-chemotherapy prior to auto-HSCT had significantly better outcomes. The introduction of new drugs and cellular therapies has dramatically altered the treatment landscape for r/r FL. These new options have provided effective alternatives to intensive chemotherapy and auto-HSCT, particularly for patients who are not suitable candidates for a transplant or those who have failed prior therapies or were completely refractory. The use of targeted therapies, such as Bruton tyrosine kinase inhibitors, phosphoinositide 3-kinase inhibitors, and enhancer of zeste homolog 2 inhibitors, has become a cornerstone of treatment for r/r FL. These agents offer durable responses with a more favorable toxicity profile compared to traditional chemotherapy. The novel cellular approach, like CAR-T-cell therapy, has emerged as a powerful option for patients with multiply relapsed FL. It has shown impressive response rates, even in patients who have failed multiple prior lines of therapy, including rituximab and other targeted drugs. Additionally, a new class of immunotherapy called bispecific Abs is also

showing promise in r/r FL, offering another alternative to traditional treatments [73].

Peripheral T-cell lymphomas

Peripheral T-cell lymphomas are generally more aggressive and have a poorer prognosis than B-cell lymphomas. In many cases, auto-HSCT may be used as consolidation of first remission to prevent relapse. For patients with relapsed peripheral T-cell lymphoma, an allo-HSCT is often considered due to the role of donor T cells for the potential curative graft-versus-lymphoma effect [74].

The most frequent indications for allo-HSCT in hematology

Allo-HSCT works by replacing a patient's unhealthy or damaged BM with healthy donor HSCs, which then produce a new, well-functioning immune system. This new immune system can recognize and destroy remaining cancer cells, a process known as the graft-versus-tumor or graft-versus-leukemia (GvL) effect. The most frequent indications for allo-HSCT among hematologic disorders are malignant disorders, such as leukemias, followed by other hematologic malignancies and non-malignant disorders like BM failure syndromes. As *per* EBMT's last activity survey, AML is the most common indication for allo-HSCT and accounts for more than one-third of allo-HSCT [75].

Malignant disorders as indications for allo-HSCT

As mentioned, common indications for clinical application of allo-HSCT among hematological disorders include various malignant diseases, as presented below: in patients with AML who have intermediate- or high-risk disease in their first remission, or in those with relapsed disease in second or subsequent complete remission, allo-HSCT is considered the only curative option [76].

ALL with high-risk characteristics defined by well-established criteria is an indication for allo-HSCT, both in children and adults. For patients with relapsed disease in second or subsequent complete remission, allo-HSCT is also a reasonable treatment option [77]. In patients with *BCR-ABL+* ALL, treatment with second- or third-generation tyrosine kinase inhibitors may be preferred in certain circumstances.

Myelodysplastic syndrome with intermediate-2 to high-risk characteristics has a significant chance of transforming into AML and is a typical indication for allo-HSCT as a primary treatment option [78].

Myelofibrosis and other Philadelphia-negative MPN with high-risk scores and a high risk of disease progression are also standard indications for allo-HSCT as the only potentially curative treatment [79].

Chronic myeloid leukemia became potentially curable in the era of tyrosine kinase inhibitors, first to third generation. These drugs have largely replaced allo-HSCT for chronic myeloid leukemia, but when patients are intolerant or resistant to these drugs, it is still a reasonable treatment option [80].

The r/r lymphomas, especially when a prior auto-HSCT has failed, can be reasonable candidates for allo-HSCT, taking into consideration all risk factors connected to patients and disease status [81].

Non-malignant disorders as indications for allo-HSCT

Severe aplastic anemia as a BM failure syndrome is a significant indication for allo-HSCT, particularly for younger patients with a fully matched sibling donor. Congenital BM failure syndromes like Fanconi anemia and severe congenital neutropenia are also treated with allo-HSCT. Primary immunodeficiency syndromes, as severe inherited immune system disorders, can also be cured with allo-HSCT [82].

Congenital anemias like thalassemia major and sickle cell anemia can be treated with allo-HSCT as a curative treatment, especially in countries where these diseases are common genetic disorders [82].

Taking into account all the above, we must point out that allo-HSCT represents the most powerful form of adoptive immunotherapy for cancer, particularly for hematologic malignancies. Its curative potential is not solely linked to the HDCT +/- radiotherapy as part of the conditioning regimen, which is used to eliminate malignant cells, but is also present due to the GvL effect. Namely, allo-HSCT functions as an adoptive immunotherapy, and its mechanisms are based on the mentioned GvL effect.

The settings and application of adoptive immunotherapy

Adoptive immunotherapy is a form of treatment that uses the cells of our immune system (collected by apheresis, *ex vivo* modified, and then reinfused) to eliminate some tumor/cancer cells. In a broader sense, the term adoptive immunotherapy also includes the application of donor immunocompetent cells in order to achieve an anti-tumor effect (e.g., GvL effect).

The main aspects of adoptive immunotherapy

Adoptive immunotherapy is a form of treatment that uses donor immune cells, such as T cells, to fight against the patient's malignant cells [83]. In allo-HSCT, the "graft" of donor SCs is not just a source of new blood cells; it is a source of an entirely new immune system for the patient. This new immune system, derived from the donor, has a potent anti-tumor effect. The main principle is that the donor's immune cells recognize the patient's malignant cells as "foreign". This occurs because malignant cells, although originating from the patient, may express unique antigens or exhibit altered patterns of antigen presentation. Donor T cells and other immune cells, such as natural killer (NK) cells, can mount an immune response against the patient's malignant cells, leading to their destruction.

A few crucial points of the model of adoptive immunotherapy

The transfer of immunocompetent cells since the donor graft contains T cells, NK cells, and other immune cells that are already "programmed" to fight infections and recognize foreign threats, presents the mainstay of adoptive immunotherapy. These immune cells, infused into the patient, are able to attack the malignant clone directly [84].

The creation of a new, healthy immune system, as donor HSCs engraft in the patient's BM niche and begin to produce all types of blood cells, including a new, healthy population of cells and other immune cells, is the goal of adoptive immunotherapy. This new immune system can provide long-term surveillance against the disease relapse. Reconstitution of some donor immune cells is crucial for protecting a patient from disease relapse [85].

Controlling alloreactivity is crucial after allo-HSCT with strong monitoring and management of immunosuppressive therapy. Despite auto-HSCT, which provides autologous support with HSCs after a high-dose conditioning regimen, allo-HSC can produce a graft-versus-tumor effect. This effect represents the immune response of the donor's cells against the patient's allo-antigens, which can be a double-edged sword, leading to both a beneficial GvL effect and a dangerous side effect known as GvHD. Therefore, the GvL effect is the primary mechanism by which allo-HSCT provides its long-term curative benefit [86]. In the context of alloreactivity, GvHD is also one of the earliest and most powerful effects that can be present in a mild-to-moderate form that protects patients from leukemia relapse. Unfortunately, severe GvHD is a life-threatening complication that targets the patient's skin, lung, liver, upper and lower gastrointestinal tract, and all other healthy tissues [86].

In the setting of early leukemia relapse after allo-HSCT or a decrease in full donor chimerism, the additional application of donor lymphocytes, so-called donor lymphocyte infusion, can provide additional GvL effect. Namely, the infusion of more donor lymphocytes can induce a remission, even without additional chemotherapy. This shows that the donor immune cells, not just the conditioning regimen, are capable of eradicating the leukemia [87]. In order to deplete alpha-beta T-cell receptor (TCR) T lymphocytes while preserving gamma-delta TCR T lymphocytes, *ex vivo* graft manipulation or T-cell depletion has shown that removal of T cells from the donor graft before transplantation decreases the incidence of GvHD. However, this also leads to a significant increase in the risk of leukemia relapse, further proving that the donor T cells are crucial for the GvL effect [87–89].

Moreover, the type of conditioning regimen can either potentiate myeloablation in the myeloablative setting, so-called myeloablative conditioning, or enhance immunogenicity in a reduced-intensity setting [reduced-intensity conditioning (RIC) regimens]. The development of RIC regimens – also known as "mini-transplants" – provides a powerful GvL effect. These regimens use lower doses of chemotherapy that are sufficient to allow donor cell engraftment, but not to fully eradicate the malignant cells. The primary anti-tumor effect is then left to the donor's immune cells. This approach has made allo-HSCT an option for older and less fit patients [78].

As previously mentioned, the GvL effect can be potentiated by cellular mediators such as T cells (cytotoxic T-lymphocytes), NK cells, which can kill leukemia cells without prior sensitization, and are particularly important in haploidentical transplants, and by B cells, dendritic cells, and other immune cells that also contribute to the GvL effect by supporting and modulating the T-cell response [86, 87].

Our data also show that immune reconstitution after allo-HSCT is pivotal in achieving favorable long-term outcomes by influencing the rates of infection, GvHD, and relapse. Namely, we evaluated the clinical impact of immune reconstitution on NK cells on day +90 after allo-HSCT, as well as $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NKT cells by performing a landmark analysis in event-free patients. Our results showed that high NK cell counts on day +90 (>178 cells/μL) were associated with improved OS ($p = 0.039$) and lower rates of non-relapse mortality [1-year cumulative incidence of 5.7% vs. 31.4%, hazard ratio 0.16, 95% confidence interval: 0.04–0.69, $p = 0.014$] after T-cell-depleted allo-HSCT [85].

Conclusion

Auto-HSCT continues to be a standard treatment to consolidate first-line response for many patients with MM. However, its role is increasingly being integrated with and influenced by a growing number of novel drugs. These new agents are not only making auto-HSCT more effective but are also providing additional treatment options, leading to more personalized and long-lasting outcomes for multiple myeloma patients. Like in Hodgkin's lymphoma, the role of auto-HSCT in non-Hodgkin's lymphoma is being influenced by the emergence of new, highly effective therapies. Targeted drugs, CAR T-cell therapy, and bispecific antibodies are changing the treatment sequence and raising questions about whether transplantation can be delayed or even avoided for some patients. Auto-HSCT is generally reserved for lymphoma patients with relapsed or refractory disease who are chemosensitive following salvage therapy, and for consolidation of first remission in high-risk patients to improve the chances of long-term survival.

Allo-HSCT is a highly effective form of adoptive immunotherapy because it provides a new, healthy immune system capable of recognizing and destroying a patient's malignant cells. The most frequent indications among malignant disorders are acute leukemias, and in non-malignant settings, severe aplastic anemia and severe immunodeficiency. The transfer of immunocompetent cells from the donor graft presents the backbone of adoptive immunotherapy. A strong graft-versus-leukemia effect, mediated by donor immune cells, is a fundamental mechanism of this curative treatment, demonstrating the power of the immune system to combat aggressive hematologic malignant disorders.

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Received on August 22, 2025
Revised on November 8, 2025
Accepted on November 26, 2025
Online First January 2026

ORIGINAL ARTICLES

UDC: 616.831-006-08
DOI: https://doi.org/10.2298/VSP250525083L

Multiparametric structural imaging biomarkers of early white matter microstructural changes during and after glioblastoma chemoradiotherapy: diffusion tensor imaging and diffusion kurtosis imaging

Multiparametrijski strukturni slikovni biomarkeri ranih promena mikrostrukture bele mase tokom i nakon hemioradioterapije glioblastoma: difuziono tenzorsko snimanje i difuziono snimanje kurtoze

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Abstract

Background/Aim. Recognizing early irradiation-induced changes in the white matter is of great importance. The aim of this study was to investigate the potential use of diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI) parameters as biomarkers of early brain tissue microstructural changes during chemoradiotherapy (CRT) treatment of newly diagnosed glioblastoma. **Methods.** A total of 42 glioblastoma patients who underwent CRT after surgical resection/biopsy were scanned three times using magnetic resonance imaging (MRI): before treatment, after 16 fractions, and after 33 fractions. Regions of interest (ROI) with total irradiation doses of 59.4 Gy (ROI 1), 39.6 Gy (ROI 2), and 19.8 Gy (ROI 3) were identified using co-registered axial dose distribution plans/dose-volume histograms and MRI scans. For each ROI, the following DTI parameters were calculated, measured, and analyzed: fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD). The corresponding DKI parameters included: radial kurtosis (RK), axial kurtosis (AK), and mean kurtosis (MK). **Results.** A significant decrease in FA value was observed in ROI 1 (total delivered dose 59.4 Gy) after both 16 and 33 delivered fractions, while the other DTI parameters and MK showed a significant increase. In ROI 1, a decreasing trend in RK and AK was identified, which was statistically confirmed after both 16 and 33 delivered fractions. In ROI 2 (total delivered dose of 39.6 Gy), FA values were significantly reduced after both 16 and 33 fractions, whereas RD, AD, MD, and MK were increased after 16 fractions, followed by a decrease after 33 fractions. The RK value in ROI 2 showed a significant decrease after both 16 and 33 fractions, and no changes were observed in AK values. In ROI 3 (total delivered dose of 19.8 Gy), no significant changes in any of the measured DTI or DKI parameters were noticed. **Conclusion.** DTI and DKI metric parameters may serve as biomarkers of early changes during and after CRT, providing information that may offer a better understanding of the complex dynamics of early white matter microstructural changes in response to glioblastoma CRT.

Keywords:
biomarkers; brain injuries; chemoradiotherapy; diffusion magnetic resonance imaging; diffusion tensor imaging; glioblastoma.

Apstrakt

Uvod/Cilj. Prepoznavanje ranih promena izazvanih zračenjem u beloj masi je od velike važnosti. Cilj rada bio je da se ispita mogućnost primene parametara difuzionog tenzorskog snimanja (*diffusion tensor imaging* - DTI) i difuzionog snimanja kurtoze (*diffusion kurtosis imaging* - DKI) kao biomarkera ranih promena mikrostrukture moždanog tkiva, tokom lečenja novodijagnostikovanog glioblastoma hemioradioterapijom (HRT). **Metode.** Ukupno 42 bolesnika obolela od glioblastoma, koji su posle hirurške resekcije/biopsije podvrgnuti HRT, snimljena su tri puta magnetnom rezonancom (MR): pre terapije, posle 16 frakcija i posle 33 frakcije. Regioni od interesa (ROI), sa ukupnim

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dozama zračenja od 59,4 Gy (ROI 1), 39,6 Gy (ROI 2) i 19,8 Gy (ROI 3), identifikovani su korišćenjem koregistrovanih aksijalnih planova raspodele doze/histograma zapremine doze i snimaka MR. Za svaki ROI izračunati su, izmereni i analizirani sledeći DTI parametri: frakciona anizotropija (FA), radijalna difuzivnost (RD), aksijalna difuzivnost (AD) i srednja difuzivnost (SD). Odgovarajući DKI parametri uključivali su: radijalnu kurtozu (RK), aksijalnu kurtozu (AK) i srednju kurtozu (SK). **Rezultati.** Značajno smanjenje vrednosti FA utvrđeno je u ROI 1 (ukupna isporučena doza 59,4 Gy) posle isporučenih 16 i 33 frakcija, dok su ostali parametri DTI i SK pokazali značajno povećanje. U ROI 1 utvrđen je opadajući trend RK i AK, koji je potvrđen statistički i posle 16 i posle 33 isporučene frakcije. U ROI 2 (ukupna isporučena doza 39,6 Gy), vrednost FA bila je značajno smanjena i posle 16 i posle 33 frakcije, dok su RD, AD, SD i SK bili povećani posle 16 frakcija, a potom smanjeni posle 33 frakcije. Vrednost RK u ROI 2 pokazala je značajno smanjenje nakon 16 i 33 frakcije, a u vrednostima AK nisu ustanovljene promene. U ROI 3 (ukupna isporučena doza 19,8 Gy) nisu primećene značajne promene ni u jednom od izmerenih DTI ili DKI parametara. **Zaključak.** Metrički parametri DTI i DKI mogu poslužiti kao biomarkeri ranih promena tokom i posle HRT, obezbeđujući informacije koje pružaju bolje razumevanje složene dinamike ranih promena mikrostrukture bele mase, kao odgovor na HRT glioblastoma.

Ključne reči:
biomarkeri; mozak, povrede; radiohemioterapija; magnetna rezonanca, difuziona; snimanje, difuziono, tenzorsko; glioblastom.

Introduction

Treatment of newly diagnosed glioblastoma (GB), as one of the biologically most aggressive brain tumors in adult patients without chemoradiotherapy (CRT), is nowadays impossible to imagine [1–4]. It is believed that transient demyelination, axon impairment, and neuroinflammation are important markers of white matter injury during CRT in GB patients [5, 6]. Since these processes are vital for normal neurological functioning, treatment-induced alterations eventually lead to perivascular, diffuse demyelination and axon degeneration in later stages [6-8].

Recognizing early irradiation-induced changes in normal-appearing white matter is therefore of great importance. Although several other imaging modalities have recently been proposed, structural imaging based on diffusion-weighted magnetic resonance imaging (MRI) remains an important tool for assessing brain microstructural alterations during and after CRT treatment [9, 10]. Imaging of the brain's functional architecture became possible with the introduction of diffusion tensor imaging (DTI), a technique derived from diffusion-weighted imaging (DWI) that basically provides information on the Brownian motion of water molecules within brain tissue. The DTI technique provides insight into white matter tracts and brain tissue microstructural changes [11, 12] that could be detected even before the appearance of morphological changes, usually evaluated by conventional MRI techniques. Measurable DTI parameters such as fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD) are widely accepted for white matter microstructure assessment [13].

Differences in FA values, which provide information on white matter density and integrity, are observed not only between normal-appearing white matter and tumorous tissue but also between normal-appearing white matter and impaired white matter tissue during and after radiotherapy (RTh) treatment [14, 15]. RD represents diffusion magnitude perpendicular to the white matter fibers, and is associated with myelination changes, while AD represents diffusion magnitude parallel to the white matter fibers, and, as suggested by histologically correlated data, could be linked to the axonal injury [13, 16, 17]. MD is usually considered an inverse measure of membrane density, and is related to cellularity, edema, and necrosis [13, 17–19].

Besides DTI metrics, quantified with a diffusion tensor using a Gaussian distribution function, a whole new set of parameters that takes into account the non-Gaussian behavior of water molecular diffusion by mathematical calculation of the kurtosis of the diffusion displacement probability distribution, diffusion kurtosis imaging (DKI) has been translated from experimental to clinical settings [20, 21]. The degree of non-Gaussian water diffusion in kurtosis imaging is quantified using radial kurtosis (RK), axial kurtosis (AK), and mean kurtosis (MK). Some authors suggested that kurtosis metrics may complement standard diffusion metrics, and that DKI may be more sensitive to some aspects of brain tissue microstructure alterations [20, 22–24].

Several studies have shown that DKI values contribute to better discrimination between high-grade and low-grade gliomas than the conventional diffusion parameters [25–27]. DKI also appears to be a promising method for the early detection of healthy brain tissue damage during and/or after RTh treatment, potentially providing more information about mechanisms of white matter injury subsequent to brain irradiation [19]. Since both concomitant and adjuvant CRT with temozolomide have become an unavoidable part of the treatment of GB patients [1, 28, 29], and improve survival after resection or biopsy, the impact of irradiation on the healthy brain tissue, including the surrounding normal-appearing white matter, is inevitable [18, 30]. In recent years, numerous studies have been investigating chronic brain tissue and white matter injuries after the RTh treatment [30–34], among them several have used the advanced DWI MRI techniques in an attempt to identify early CRT-induced brain tissue injury [35–38].

The exact underlying pathophysiological mechanisms that result in the white matter irradiation changes are still not fully understood [35, 38–40]. Moreover, the impact of RTh doses and daily fractions on the changes that may be detectable by DTI or DKI parameters is still not discerned nor precisely determined [41, 42].

The aim of this prospective observational study was to examine the changes of DTI and DKI parameters within the white matter at three time points—before, during, and after the application of different daily and total radiation doses, as well as to evaluate whether the DTI and DKI parameters can be used as biomarkers of early white matter radiation damage during and immediately after CRT treatment in patients with GB.

Methods

Patients and treatment

The study included 42 patients with newly diagnosed GB who underwent concurrent CRT after neurosurgical resection/biopsy (25 males, 17 females; age range 31–72 years; mean age 57.28 ± 9.87). All patients were treated with fractionated focal irradiation, delivered in 33 fractions at 1.8 Gy *per* fraction, resulting in a total dose of 59.4 Gy to the gross tumor volume (GTV) with a 2–3 cm margin around the clinical target volume (CTV). Conformal RTh was delivered with linear accelerators with a nominal energy of 6 megavolts, and quality assurance was performed by means of individual case reviews. Concomitant chemotherapy, consisting of temozolomide at a dose of 75 mg/m^2/day, was applied from the beginning until the last day of RTh treatment (optimally for 42 days, but in real-life clinical settings, never longer than 49 days) [1, 43].

This study was approved by the Ethics Committee of the Oncology Institute of Vojvodina, Serbia (No. 4/18/1-972/2-8, from April 12, 2018). Written informed consent was obtained from all participants.

Delineation of target volumes

For delineation of the target volume, co-registration of axial dose distribution plans and dose volume histograms to the contrast enhanced T1-weighted (T1-w) and/or fluid-attenuated inversion recovery (FLAIR) MRI sequences were used in addition to the dedicated computerized tomography scans, that were used in the process of three-dimensional RTh planning (XiO 4.62 and Monaco 5.11, Elekta, Stockholm, Sweden).

The current European Society for Radiotherapy and Oncology (ESTRO)–Advisory Committee on Radiation Oncology Practice/European Organization for Research and Treatment of Cancer standards were used to delineate GTV, with CTV and planning target volume [44, 45]. Recently published ESTRO–European Society of Neuro-Oncology guidelines suggested a reduction of GTV to CTV margins of 0.5–1.5 cm, recommending the exclusion of subventricular zones, and margins reduced at anatomical barriers [46], while delineation of critical organs at risk remained unchanged [47].

Three regions of interest (ROI) with total irradiation doses of 59.4 Gy (ROI 1), 39.6 Gy (ROI 2), and 19.8 Gy (ROI 3) were identified using axial dose distribution plans co-registered with contrast-enhanced three-dimensional T1-w and/or FLAIR images (Figure 1A–C). Calculated daily doses were approximately 1.8 Gy for the areas with a total delivered dose of 59.4 Gy, 1.2 Gy for the areas with a delivered dose of 39.6 Gy, and 0.6 Gy for areas with a delivered dose of 19.8 Gy.

Magnetic resonance imaging data acquisition

Patients were scanned using a 3T MR scanner (Magnetom TIM Trio, Siemens, Erlangen, Germany) using a 48-channel head-phased array coil, at three different time points: immediately before the beginning of CRT treatment, after delivering 16 fractions (approximately half of the treatment), and after 33 fractions (immediately at the end of CRT treatment).

Imaging protocol included sagittal high-resolution magnetization-prepared rapid acquisition with gradient-echo

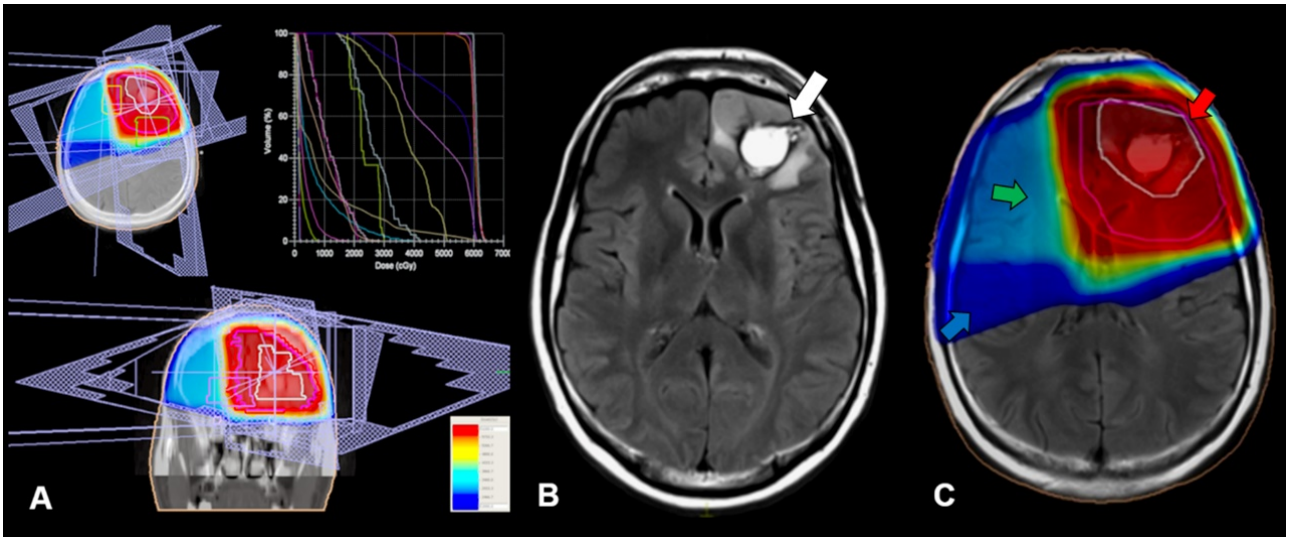


Fig. 1 – Axial dose distribution plan with dose volume histogram of a conformal radiation therapy plan for glioblastoma patient (A), superimposed to fluid-attenuated inversion recovery images, where postoperative glioblastoma is presented (white arrow) (B), and the irradiation dose distribution plan (C), with different isodose lines dividing the areas presented in different colors [red area 1.8 Gy daily/total delivered dose 59.4 Gy (red arrow); green area 1.2 Gy daily/total delivered dose 39.6 Gy (green arrow); blue area 0.6 Gy daily/total delivered dose 19.8 Gy (blue arrow)].

T1-weighted isotropic sequence before and after paramagnetic gadolinium-based contrast administration, and axial two-dimensional T2-weighted and FLAIR sequences (both 30 slices, 0.9 × 0.9 in-plane resolution, slice thickness 4 mm). For DWI acquisition, three b-values (0, 1,000, and 2,000 s/mm²) were applied along 30 diffusion-encoding directions, with 40 axial slices and an isotropic resolution of 3 mm, to calculate diffusion and kurtosis tensors. Based on these data, we measured and analyzed the following DTI parameters: FA, RD, AD, and MD. In addition, the corresponding DKI parameters—RK, AK, and MK—were also evaluated.

Only recently presented, kurtosis FA (KFA), as a potentially more accurate imaging biomarker than FA, has not been calculated or analyzed [48].

Image processing and region of data analysis

To generate FA, RD, AD, MD, RK, AK, and MK maps, the diffusional kurtosis estimator freely available software (version 2.5.1; Medical University of South Carolina, Charleston, South Carolina; https://www.nitrc.org/projects/dke/) [22] has been used.

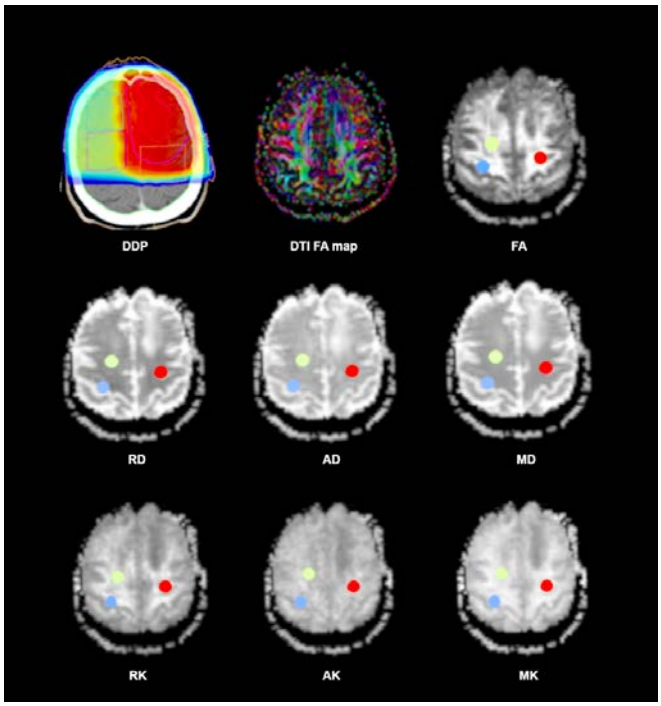
ROI were manually selected by two radiologists with more than 10 years of experience, who assessed the maps simultaneously in a joint session, reaching a consensus on where to place the ROI 1 (total irradiation dose of 59.4 Gy), ROI 2 (total irradiation dose of 39.6 Gy), and ROI 3 (total irradiation dose of 19.8 Gy). Each ROI was of the same size, and in each of them all observed DTI parameters (FA, RD, AD, MD) and all DKI parameters (RK, AK, MK) were separately measured on the same spots in all three observed areas in three different time points: immediately before CRT treatment, after 16 delivered fractions, and after 33 delivered fractions (immediately after the end of CRT treatment) (Figure 2).

Statistical analysis

Changes in DTI and DKI parameters at different time points were analyzed using a statistical data analysis software system (StatSoft, Inc., version 12.0.1133.15; www.statsoft.com). Student's *t*-test was executed to estimate pairwise differences between average values obtained from three different imaging time-points. Average cross-subject values of measured DTI and DKI parameters were used to identify the increasing or decreasing trend of the parameters in observing time points for each ROI.

Results

Statistical differences in observed DTI and DKI parameters over time, during and after CRT treatment, compared with the initial values measured before and during treatment, are presented in Table 1.



● **ROI 1** - 1.8 Gy daily fraction; overall delivered dose 59.4 Gy
● **ROI 2** - 1.2 Gy daily fraction; overall delivered dose 39.6 Gy
● **ROI 3** - 0.6 Gy daily fraction; overall delivered dose 19.8 Gy

Fig. 2 – Region of interest (ROI) identification according to co-registered radiotherapy dose distribution plan (DDP) in axial plane on different diffusion maps for measurement of diffusion tensor imaging (DTI) parameters: fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD), and diffusion kurtosis imaging (DKI) parameters: radial kurtosis (RK), axial kurtosis (AK), and mean kurtosis (MK) in areas with different delivered doses.

Table 1

Statistical differences of DTI and DKI parameters average values in predefined ROIs between observed time points before, during, and immediately after chemoradiotherapy

Parameter	ROI 1 (1.8 Gy/fraction)			ROI 2 (1.2 Gy/fraction)			ROI 3 (0.6 Gy/fraction)		
	16/0	33/0	33/16	16/0	33/0	33/16	16/0	33/0	33/16
DTI									
FA	0.348–0.396; < 0.05	0.304–0.396; < 0.01	0.304–0.348; < 0.05	0.324–0.368; < 0.05	0.318–0.368; < 0.05	0.318–0.324; > 0.05	0.361–0.364; > 0.05	0.359–0.364; > 0.05	0.359–0.361; > 0.05
RD	0.881–0.824; < 0.05	0.934–0.824; < 0.01	0.934–0.881; < 0.05	0.869–0.825; < 0.05	0.772–0.825; < 0.05	0.772–0.869; < 0.01	0.808–0.811; > 0.05	0.798–0.811; > 0.05	0.798–0.808; > 0.05
AD	1.489–1.376; < 0.05	1.501–1.376; < 0.05	1.501–1.489 > 0.05	1.511–1.399; < 0.05	1.288–1.399; < 0.05	1.288–1.511; < 0.01	1.387–1.377; > 0.05	1.391–1.377; > 0.05	1.391–1.387; > 0.05
MD	1.129–0.998; < 0.05	1.244–0.987; < 0.01	1.244–1.129; < 0.05	1.221–1.102; < 0.05	0.979–1.102; < 0.05	0.979–1.221; < 0.01	0.992–1.012; > 0.05	0.983–1.012; > 0.05	0.983–0.992; > 0.05
DKI									
RK	1.066–1.188; < 0.05	0.951–1.188; < 0.01	0.951–1.066; < 0.05	1.068–1.214; < 0.05	0.915–1.214; < 0.01	0.915–1.068; < 0.05	1.189–1.201; > 0.05	1.176–1.201; > 0.05	1.176–1.189; > 0.05
AK	0.675–0.799; < 0.05	0.661–0.799; < 0.05	0.601–0.675; > 0.05	0.761–0.783; > 0.05	0.774–0.783; > 0.05	0.774–0.761; > 0.05	0.776–0.791; > 0.05	0.762–0.791; > 0.05	0.762–0.776; > 0.05
MK	1.301–0.997; < 0.05	1.329–0.997; < 0.05	1.329–1.301; > 0.05	1.229–0.998; < 0.05	1.199–0.998; < 0.05	1.199–1.229; > 0.05	0.963–0.951; > 0.05	0.975–0.951; > 0.05	0.975–0.963; > 0.05

For abbreviations, see Figure 2.

All values are given as mean values. Statistical significance was set at $p < 0.05$.

Note. **16/0, 33/0, and 33/16 represent compared measurements at different time points – before, during, and after treatment: 16 vs. 0 fractions, 33 vs. 0 fractions, and 33 vs. 16 fractions.**

Within ROI 1, FA values were significantly lower in normal-appearing white matter, both after delivering 16 (1.8 Gy/fraction, total dose 28.8 Gy) and 33 fractions (1.8 Gy/fraction, total dose 59.4 Gy), compared with non-irradiated white matter before the irradiation. Average RD, AD, and MD values, as well as diffusion MK, showed a significant increase when comparing the same measurement time points within ROI 1. Regarding the DKI parameters RK and AK, a decrease in the values in the function of time was observed, and significant changes compared with pre-therapy values were noted both after 16 and 33 fractions. In ROI 1, only FA, RD, MD, and RK demonstrated a significant decrease between 16 and 33 fractions, whereas AD, AK, and MK did not show any significant changes between these time points.

In ROI 2, FA values were significantly lower after delivering 16 fractions (1.2 Gy/fraction, total dose 19.2 Gy) and 33 fractions (1.8 Gy/fraction, total dose 39.6 Gy) compared with pre-treatment values, with no significant differences between 16 and 33 fractions. Average RD, AD, and MD values increased significantly after delivering 16 fractions, but significantly decreased between 16 and 33 delivered fractions. Average RK values significantly decreased after 16 and 33 fractions within ROI 2, compared with the measured values before the CRT, while AK values remained without any significant changes. Average MK increased significantly after 16 fractions compared with pre-treatment values in ROI 2, then decreased significantly after 33 fractions. No significant changes in FA, AK, and MK values were observed between delivered 16 and 33 fractions in ROI 2.

Similar to the DTI parameters (FA, RD, AD, and MD), the DKI parameters (RK, AK, and MK) in ROI 3 did not exhibit any significant changes of the average values at the observed time points, neither after 16 delivered fractions (0.6 Gy/fraction, total dose 9.6 Gy) nor after 33 fractions (0.6 Gy/fraction, total dose 19.8 Gy), nor between the delivered 16 and 33 fractions.

Discussion

Since the initial introduction of molecular neuro-oncology into clinical practice [49], the impact of DTI metrics on the detection of brain tissue alteration of different origins, including irradiation-induced white matter injury, became the subject of research [7, 16, 17, 23, 35]. Fewer studies included non-Gaussian DKI metrics to detect early changes in brain parenchyma during CRT treatment [8, 14, 23, 50]. As the most prominent early indicators, the results of our study have demonstrated decrease of FA, and increase of MD and MK values in the normal appearing white matter, both during and immediately after CRT treatment in the brain areas with delivered dose of 28.8 Gy after 16 fractions, and total dose of 59.4 Gy after 33 fractions, and these results are coherent with the results of several other published studies [14, 19]. Both decreased FA and increased MD and MK values are considered as the consequences of demyelination, axonal loss, and/or transient cerebral edema. On the contrary, edema resolution, oligodendrocyte regeneration, and remyelination should result in increased FA and MD values [51]. As reported in the literature [19], in response to RTh, RD values typically increase, while AD values have been shown to either increase [10, 35, 42] or decrease [38, 41, 52]. In the brain areas with the delivered daily dose (DDD) of 1.8 Gy and a total dose of 59.4 Gy during and after CRT, both after delivering 16 and 33 fractions, an increase in RD values, correlating with demyelination, and AD values, which indicate axonal loss and reactive astrogliosis, was noticed [51, 53].

Unexpectedly, a decrease in RK and AK values was recorded at the same measurement time points in the same areas, even though they were expected to follow trends similar to those of RD and AD. Although the observed decreases in RK and AK values were unanticipated, they may potentially reflect transient/temporary microstructural alterations in white matter following irradiation, possibly related to processes such as edema resolution, axonal swelling, or reactive gliosis. Yet, the possibility of an unintentional computational error or another form of oversight cannot be entirely excluded. Nonetheless, the assumed explanation for the observed discrepancy between DTI- and DKI-derived parameters remains speculative and warrants validation through further investigation.

In a few published studies, we noticed a certain analogy in the discrepancy of the results perceived in the form of decreased RK and AK values in our study. Namely, opposite to the expected results of the majority of other studies, they have measured decreased MD, RD, and AD and slightly increased FA in patients after the end of CRT, though not as an early result, but in a follow-up period after 3 to 24 months [38, 41]. This, in turn, underscores the need for longer-term follow-up to clarify the sustained significance and interpretative value of both DTI and DKI metrics in GB CRT over time.

Although most conducted studies point out demyelination as the probable dominant consequence induced by RTh [19], Raschke et al. [38] concluded that neither edema nor demyelination could be a plausible reason for the decrease in diffusion. They speculated that the decreased diffusion could be the result of axonal swelling, possibly due to tissue oxygenation and vascular changes that occur after irradiation. Revealing reductions in RD and AD values after irradiation, Zhu et al. [41] proposed that RD decreases could be a sign of remyelination. In contrast, the AD decrease could be linked to astrogliosis, which may explain our results, but still does not provide a proper background for the opposite trends between RD and AD and between RK and AK in the area with a delivered total dose of 59.4 Gy, both after 16 and 33 fractions.

Still, recent investigations have revealed microstructural tissue changes not only in the hemisphere where GB is located, but also in the contralateral hemisphere, which differ even between right-sided and left-sided GB location [54]. Since this recently published feature was not considered in our study design, we strongly believe that further investigations to gain deeper insight into microstructural white matter alterations in the presence of high-grade glioma, or during and after irradiation, would be beneficial.

We found that AD, AK, and MK did not show a significant difference between 16 and 33 fractions in the area of DDD of 1.8 Gy and a total dose of 59.4 Gy. That does not

necessarily reflect a better sensitivity of MD compared to MK. The lack of statistical significance between these two time points may be explained by the higher susceptibility of MK to early irradiation-induced changes, leading to faster and more stable increases in MK values compared with MD.

In the treated brain areas with a DDD of 1.2 Gy, and a total dose of 39.6 Gy, where decrease of FA and an initial increase of RD, AD, and MD, but also MK, have been noted after 16 delivered fractions, with a later decrease of values after 33 delivered fractions. That leaves us in a belief that the initial increase and later decrease of RD, AD, MD, and MK suggest transient edema as a dominant mechanism of early white matter tissue injury, while the decrease of RK and AK both after delivering 16 and 33 fractions suggest that demyelination is included in the white matter alterations profile, rather than axonal impairment. Some authors suggest that the biological alterations are most likely occurring within the extracellular environment due to increased vascular permeability and/or reactive neuroinflammatory processes after irradiation [19, 42].

These findings are consistent with the statement that kurtosis measure is less sensitive to free fluid contamination, since the interpretability of DKI parameters depends on the accuracy of tensor estimation. Motion, noise, and/or imaging artifacts can cause significant errors, up to the level of physical/biological implausibility [15, 20–22]. That actually corresponds to our results, suggesting that DKI metrics were slightly less susceptible to radiation-induced edema detection than measured DTI parameters. Dose and time-dependent changes in irradiated white matter that have been found to correlate with several other studies' results [17, 19, 42], where measured MD, AD, RD, and MK increase and FA decrease appear earlier in areas receiving higher radiation doses. Hope et al. [35] found that MD, RD, and AD significantly increased in brain regions receiving more than 41 Gy in total dose, compared to those receiving less than 12 Gy in total, which corresponds with our results.

We found that daily doses of 0.6 Gy and total delivered dose of 19.8 Gy did not cause any significant changes in DTI or DKI metrics. This means that white matter alterations during and after CRT treatment could not be identified, neither after 16 delivered fractions, nor after 33 delivered fractions. Therefore, we may speculate that the threshold for detecting early white matter injury during or immediately after CRT treatment could actually lie somewhere above 20 Gy. Still, it should be emphasized that in several studies, changes in white matter structure for smaller delivered doses have been reported [41, 55], though not immediately, but later, in the period of 9 to 11 months after CRT [38, 42].

Although our study design includes imaging before, during, and immediately after CRT, it does not extend to post-treatment follow-up. This represents a clear limitation, as it prevents us from definitively characterizing the observed changes in DTI and DKI metrics as either transient and/or reversible effects, or as predictors of late or delayed radiation injury.

As late radiation injury was beyond the scope of the present investigation, we believe the current findings should be interpreted solely as indicative of early, and potentially transient effects of irradiation.

We would also like to emphasize that the present study did not employ repeated-measures analysis of variance or multivariate analyses, which might have provided a more robust statistical framework for assessing longitudinal changes and addressing multiple comparisons. As re-analysis is not feasible at this stage, this limitation is duly acknowledged and should be taken into consideration when interpreting the findings.

Accordingly, further comprehensive studies are warranted to fully elucidate the potential of DTI and DKI metrics as reliable biomarkers of white matter microstructural alterations during and after CRT in GB patients, in relation to time and radiation dose.

While several studies have investigated the association between neurocognitive impairment and/or clinical outcomes and both early and late radiation-induced brain injury [56–59], our study did not address this interconnection, which represents a limitation with respect to its translational relevance. Future investigations that integrate neurocognitive assessment with clinical outcomes during and after GB CRT would significantly enhance the clinical utility and applicability of DTI and DKI biomarkers in real-world neuro-oncological settings.

Recently published studies incorporating radiomics into the assessment of white matter alterations during CRT may contribute to a more profound comprehension of the sophisticated and interrelated pattern of radiation-induced brain injury [60, 61]. Furthermore, emerging and ongoing investigations focusing on cellular and molecular responses of brain tissue to specific RTh, including proton therapy, as well as the integration of artificial intelligence in the evaluation of irradiated white matter, are expected to offer novel insights into the pathophysiology of radiation-induced changes in the brain tissue [62–65].

Conclusion

Diffusion tensor imaging and diffusion kurtosis imaging metrics are susceptible to white matter alterations during and after chemoradiotherapy treatment. These metrics provide valuable insight into early irradiation-induced changes, even within normal-appearing white matter, and may prove useful as structural imaging biomarkers for identifying early, and potentially transient and/or reversible microstructural alterations related to irradiation. The application of diffusion tensor imaging and diffusion kurtosis imaging holds considerable potential to broaden our understanding of the complex dynamics underlying early microstructural alterations in white matter in response to glioblastoma chemoradiotherapy, potentially influencing future diagnostic and clinical strategies. Given that late radiation effects were not addressed within the scope of our present investigation, further longitudinal studies with extended follow-up periods would be essential to validate the potential of diffusion tensor imaging and diffusion kurtosis imaging metrics as predictors of late or delayed radiation-induced brain injury.

Acknowledgement

The authors acknowledge the financial support of the Provincial Secretariat for Science and Technology Development within the project "Multiparametric structural and metabolic imaging of the intratumorous bioarchitectonics in the function of the diagnosis and therapy improvement in patients with malignant lung and central nervous system tumours"; Grant No. 142-451-2433/2018.

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Received on May 25, 2025
Revised on September 19, 2025
Revised on October 29, 2025
Accepted on November 5, 2025
Online First December 2025

ORIGINAL ARTICLE

UDC: 616.61-008.6-08-06
DOI: https://doi.org/10.2298/VSP250819082A

Comparison of antimicrobial and thrombolytic central venous catheter lock solutions in preventing catheter-related complications in hemodialysis: a randomized controlled trial

Poređenje antimikrobnih i trombolitičkih rastvora za zatvaranje centralnih venskih katetera u prevenciji komplikacija povezanih sa kateterima kod bolesnika na hemodijalizi: randomizovano kontrolisano ispitivanje

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Abstract

Background/Aim. Central venous catheters (CVC) in hemodialysis (HD) patients are associated with serious complications, particularly catheter-related bloodstream infection (CRBSI) and thrombosis, leading to increased morbidity and mortality. The aim of this study was to evaluate the effectiveness of three different catheter lock solutions in preventing major catheter-related complications. **Methods.** This prospective, randomized, controlled, single-center study, conducted between June 2018 and June 2023, included 96 adult HD patients. Depending on the solutions they received, the patients were equally divided into three groups: gentamicin-citrate twice weekly plus taurolidine/urokinase after the third weekly session (TAURO); gentamicin-citrate three times weekly (GENTAM); unfractionated heparin 5,000 international units/mL three times weekly (HEPARIN). Lock solutions were administered for a minimum of three months post-CVC insertion and continued until catheter removal. Measured outcomes included CRBSI incidence, catheter thrombosis, and adverse events. **Results.** Over the course of 10,770 catheter-days, eight CRBSI episodes were recorded, with *Staphylococcus aureus* as the most common pathogen (50%). The incidence of CRBSI (*per* 1,000 catheter-days) was 0.27 in the TAURO group, 0.83 in the GENTAM group, and 1.15 in the HEPARIN group, without statistical significance ($p = 0.526$). Thrombosis incidence was similar across groups (1.09–1.15; $p = 0.990$). Cox proportional hazards analysis revealed no significant differences, although the TAURO group demonstrated a trend toward lower CRBSI risk compared to the HEPARIN group (hazard ratio = 0.236; 95% confidence interval 0.026–2.116). **Conclusion.** None of the evaluated lock regimens significantly reduced the risk of CRBSI or thrombosis. Nonetheless, the lowest CRBSI incidence was observed in the TAURO group, suggesting a potential benefit that warrants confirmation in larger, multicenter studies.

Keywords:
anticoagulants; bacteremia; catheter-related infections; catheterization, central venous; fibrinolytic agents; renal dialysis; thrombosis.

Apstrakt

Uvod/Cilj. Centralni venski kateteri (CVK) kod bolesnika na hemodijalizi (HD) povezani su sa ozbiljnim komplikacijama, posebno bakterijemijom povezanom sa kateterom (BPSK) i trombozom, što dovodi do povećanog morbiditeta i mortaliteta. Cilj rada bio je da se proceni efikasnost tri različita rastvora za zatvaranje katetera u sprečavanju većih komplikacija povezanih sa kateterom. **Metode.** Ova prospektivna, randomizovana, kontrolisana studija sprovedena u jednom centru, od juna 2018. do juna 2023. godine, obuhvatila je 96 odraslih bolesnika na HD. U zavisnosti od rastvora koji su primali, bolesnici su bili ravnomerno podeljeni u tri različite grupe: gentamicin–citrat dva puta nedeljno uz taurolidin/urokinazu nakon treće nedeljne dijalize (TAURO); gentamicin–citrat tri puta nedeljno (GENTAM); nefrakcionisani heparin 5 000 internacionalnih jedinica/mL tri puta nedeljno (HEPARIN). Rastvori za zatvaranje katetera su primenjivani najmanje tri meseca nakon plasiranja CVK i njihova primena je nastavljena do uklanjanja katetera. Praćeni ishodi obuhvatali su incidenciju BPSK, trombozu

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katetera i neželjene događaje. **Rezultati.** Tokom 10 770 „kateter-dana" registrovano je osam epizoda BPSK, a najčešći uzročnik bio je *Staphylococcus aureus* (50%). Incidencija BPSK (na 1 000 „kateter-dana") bila je 0,27 u TAURO grupi, 0,83 u GENTAM grupi i 1,15 u HEPARIN grupi, bez statističke značajnosti (p = 0,526). Incidencija tromboze katetera bila je slična u svim grupama (1,09–1,15; p = 0,990). Koksova analiza proporcionalnih rizika nije pokazala značajne razlike, iako je TAURO grupa pokazala trend ka manjem riziku od BPSK u poređenju sa HEPARIN grupom (*hazard ratio* = 0,236; 95% interval poverenja 0,026–2,116). **Zaključak.** Nijedan ispitivani režim rastvora za zatvaranje katetera nije značajno smanjio rizik od BPSK ili tromboze. Ipak, najniža incidencija BPSK zabeležena je u TAURO grupi, što ukazuje na potencijalnu korist koju treba potvrditi u većim, multicentričnim studijama.

Ključne reči:
antikoagulansi; bakterijemija; kateter, povezane infekcije; kateterizacija, centralna, venska; fibrinolitici; bubreg, dijaliza; tromboza.

Introduction

Vascular access is essential for effective hemodialysis (HD), yet it also represents its most fragile component. While arteriovenous fistulas (AVF) remain the gold standard due to their superior long-term outcomes, temporary central venous catheters (CVCs) for dialysis are still widely used in incident HD patients in Serbia, with a prevalence of 63.5% according to data from the Serbian National Vascular Surgery Registry, due to their ability to provide immediate vascular access [1]. However, frequent failures in primary AVF creation and the rise in marginal fistulas have resulted in many patients depending on prolonged CVC use. Long-term use of CVC leads to complications, the most common of which are CVC dysfunction and thrombosis, and a high rate of catheter-related bloodstream infections (CRBSI) [2, 3]. The risk of sepsis in patients dialyzed *via* a CVC is two to five times higher than in patients with AVF and arteriovenous grafts (AVG), and initial septic episodes can double the incidence of adverse cardiovascular events such as myocardial infarction and congestive heart failure [3, 4]. These complications often necessitate catheter replacement, further limiting available vascular access options.

Preventive strategies for catheter-related complications have traditionally focused on using unfractionated heparin as a lock solution. However, high concentrations [5,000–10,000 international units (IU)/mL] raise concerns about systemic anticoagulation and promote *Staphylococcus* (*S.*) *aureus* biofilm formation in a dose-dependent manner [5, 6]. As an alternative, trisodium citrate (TSC) offers both anticoagulant and antimicrobial properties without systemic effects. *In vitro* studies have shown that the use of TSC reduces the formation of biofilms inside dialysis catheters [5]. Different concentrations of TSC have been tested, with 4% TSC being safe and effective in preventing catheter thrombosis, but it did not lead to the expected reduction in the incidence of CRBSI [5, 7]. Several studies have explored the addition of antibiotics to anticoagulant lock solutions to reduce the incidence of CRBSI [8, 9]. Moran et al. [8] reported a significant decrease in CRBSI rates (0.28 episodes *per* 1,000 catheter-days) using a gentamicin and 4% TSC combination, compared to heparin (1,000 IU/mL). While this combination reduced CRBSI, it showed no benefit in preventing thrombotic complications. A meta-analysis by Lai et al. [9], including 17 randomized controlled trials, confirmed a significant reduction in CRBSI with citrate-based locks compared to heparin [relative risk: 0.48; 95% confidence interval (CI): 0.31–0.73; p = 0.001]. Subsequent studies have highlighted citrate-based lock solutions combined with antimicrobial agents as the most effective strategy for preventing catheter-related infections [5]. Other research has focused on reducing catheter thrombosis. Weekly use of thrombolytics, such as recombinant tissue plasminogen activator (1 mg) or urokinase (25,000 IU) dissolved in 4% TSC with taurolidine (taurolock-urokinase), combined with standard anticoagulants twice weekly, was associated with reduced thrombosis and less frequent catheter replacement [5, 10].

There were no data regarding the preventive use of urokinase (25,000 IU) dissolved in 4% TSC with taurolidine (taurolock-urokinase) combined with gentamicin-citrate antibiotic catheter locks.

The aim of this study was to compare the effects of a combination of prophylactic catheter lock solutions to gentamicin-citrate three times a week or heparin on the incidence of catheter-related complications (CRBSI and catheter-related thrombosis – CRT).

Methods

This was a randomized controlled trial with an open-label approach that included 96 adult patients with end-stage chronic kidney disease undergoing HD at the Clinic for Nephrology and Clinical Immunology, University Clinical Center of Vojvodina, Novi Sad, Serbia, from June 2018 to June 2023.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-20/243, from February 22, 2018). Written informed consent was obtained from all participants prior to enrollment.

Participants were excluded if they had confirmed malignancy, significant bleeding within the previous four weeks, clinical or laboratory signs of active infection, psychiatric illness, pregnancy, or were receiving concomitant therapy affecting thrombogenicity.

Temporary double-lumen, polyurethane, non-tunneled catheters (REF CV 12122 F, 16 cm; Arrow International, Pennsylvania, USA) were inserted by anesthesiologists or nephrologists using a modified Seldinger technique under strict aseptic conditions.

Patients were randomly assigned in a 1:1:1 ratio to receive interdialytic catheter locking with either gentamicin-citrate twice weekly plus taurolidine/urokinase after the third

weekly session (TAURO group), gentamicin-citrate three times weekly (GENTAM group), or unfractionated heparin 5,000 IU/mL three times weekly (HEPARIN group). Patients in all three groups were predominantly male, with no significant differences in age or the underlying cause of end-stage kidney disease.

Patients underwent thrice-weekly 4-hr bicarbonate HD using high-flux polysulfone capillary membranes (1.1–1.3 m²), with a blood flow rate of 250–300 mL/min, a dialysate flow of 500 mL/min, and standard bicarbonate dialysate.

Following each dialysis session, both lumens were locked with the assigned solutions appropriate to the randomized group. Lock solutions were prepared by dialysis nurses at the end of each session and instilled into the catheter lumen according to protocol. Gentamicin-citrate lock was prepared by mixing 0.2 mL of gentamicin (40 mg/mL; 2 mL) with 3 mL of 4% TSC solution (IntraLock®, Fresenius Medical Care, Bad Homburg, Germany) in a sterile syringe. This solution contains gentamicin 2.5 mg/mL and 4% TSC. TauroLock™-U25,000 (TauroPharm GmbH, Waldbüttelbrunn, Germany) is a commercially available product that contains taurolidine, 4% TSC, and 25,000 IU urokinase *per* vial. When used as a catheter lock solution, unfractionated heparin (5,000 IU/mL; Heparin Galenika 25,000 IU/5 mL, Galenika A.D., Belgrade, Serbia) was prepared from the original ampoule and instilled in a volume recommended by the catheter manufacturer using a 2 mL syringe. Lock volumes adhered to manufacturer recommendations, and catheter lock solutions were administered for at least 3 months after CVC insertion or until one of the predefined outcomes occurred.

During follow-up, catheter care was performed at each dialysis session following infection control protocols. Catheters were disinfected with chlorhexidine and 70% alcohol. Exit sites were treated aseptically with hydrogen peroxide, iodine, or Codan (colorless alcohol-based solution), and covered with 3M™ Tegaderm™ Transparent Film Dressing (Saint Paul, Minneapolis, USA) or sterile gauze [11]. If signs of local infection were observed, venous blood samples for hemocultures and catheter exit site swabs were collected. Swabs were sent for microbiological analysis using standard culture and biochemical identification techniques [12]. In the case of systemic infection signs, blood was sampled from both catheter lumens and a peripheral vein (10–20 mL *per* sample), and inoculated into commercial blood culture bottles (Himedia HiCombi HiSafe, 40 mL, HiMedia Laboratories, Thane, India). Cultures were processed using the BacT/Alert® 3D system (bioMérieux, Marcy-l'Étoile, France) for continuous monitoring. At the end of the study, after aseptic catheter removal, the distal 5 cm tip was placed in a sterile container and processed using standard microbiological techniques [12]. Catheter tip colonization was determined using a semi-quantitative technique [2].

The primary outcomes were the occurrence of CRBSI, exit-site infections, and CRT, within 90 days post-CVC insertion (or until AVF maturation if longer). Patients were censored at catheter removal or death. CRT and CRBSI were defined according to the 2019 Kidney Disease Outcomes Quality Initiative (KDOQI) vascular access guidelines [2]. Since non-tunneled CVCs were used, thrombosis was further defined as the inability to initiate dialysis after previous successful use or to maintain a blood flow rate ≥ 150 mL/min during three consecutive dialysis sessions. Patients with CRBSI continued follow-up unless the catheter was removed. No patient experienced more than one episode of CRBSI. The number of catheter-days was calculated from CVC insertion to removal. The incidence of CVC thrombosis and CRBSI was expressed as events *per* 1,000 catheter-days, using the formula: (number of events/total catheter-days) × 1,000.

Detailed patient laboratory data are presented in Table 1 of Azasevac et al. [13].

Statistical analysis

The data were evaluated using descriptive statistics (mean values and standard deviations). Group differences

Table 1

Demographic and clinical characteristics at baseline by study group

Parameters	Group			*p*-value
	TAURO	GENTAM	HEPARIN	
Gender				
male	14	14	14	1.0
female	18	18	18	
Age, years	58.6 ± 15.3	60.4 ± 12.2	62.1 ± 14.6	0.1
Cause of ESKD				
diabetes	10 (31.3)	11 (34.4)	11 (34.4)	0.9
hypertension	11 (34.4)	12 (37.5)	9 (28.1)	0.8
polycystic kidney disease	3 (9.4)	3 (9.4)	3 (9.4)	1.0
glomerulonephritis	5 (15.6)	2 (6.3)	2 (6.3)	0.4
chronic pyelonephritis	0 (0)	1 (3.1)	3 (9.4)	0.1
other	3 (9.4)	3 (9.4)	4 (12.5)	0.9
Clinical characteristics				
overweight and obesity	17 (53.1)	21 (65.6)	17 (53.1)	0.5
CKD vintage, year	5.0 ± 4.4	4.9 ± 4.7	4.5 ± 4.8	0.9
antiplatelet drugs	18 (46.0)	18 (46.0)	16 (50.0)	0.9

ESKD – end-stage kidney disease; CDK – chronic kidney disease.
All values are given as numbers (percentages) or mean ± standard deviation.
***Note*. TAURO group – gentamicin-citrate twice weekly plus taurolidine/urokinase after the third weekly session; GENTAM group – gentamicin-citrate three times weekly; HEPARIN group – unfractionated heparin 5,000 international units/mL three times weekly.**

were tested with the *t*-test or Mann-Whitney *U* test for two groups, and analysis of variance or Kruskal-Wallis test for three or more groups. Categorical data were compared using the Chi-square test. Survival was analyzed using the Kaplan-Meier method and the log-rank test. Cox proportional hazards regression was used to examine the association between predictor variables and the occurrence of key catheter-related complications, with hazard ratios (HR) and 95% CI reported. A p-value < 0.05 was considered statistically significant.

Results

Baseline demographic, clinical, and laboratory data

A total of 96 patients (32 *per* group) were enrolled, and no patient was lost to follow-up or discontinued the catheter-lock solution. The mean patient age was 60.35 ± 14.25 years; 56.3% were male, without significant differences between groups. Approximately one-third of the study population had diabetes mellitus, with comparable rates of diabetic kidney disease across the three treatment groups. Together with arterial hypertension, diabetic kidney disease was the leading underlying cause of chronic kidney disease. Analysis of anthropometric parameters revealed that more than half of the study participants were overweight or obese (BMI > 25 kg/m²). Baseline demographic and clinical characteristics of the study population, presented by study group, are summarized in Table 1.

Among the patients included in this study, the majority (86 patients) received a CVC for the initiation of renal replacement therapy with HD, making it their first vascular access. A smaller proportion began HD *via* a functional AVF or AVG, with CVC placement required only after thrombosis of the existing access. One patient was initially treated with peritoneal dialysis and underwent CVC insertion upon transition to HD. Regarding CVC positioning, the majority of catheters were inserted *via* the right internal jugular vein (90.6% vs. 84.4% vs. 84.4%), followed by the left internal jugular vein (6.3% vs. 9.4% vs. 12.5%). There was no statistically significant difference in the distribution of jugular vein catheter placements among the three groups (p = 0.693).

Regarding laboratory findings, parameters were mostly similar across the three groups (TAURO, GENTAM, HEPARIN), with no statistically significant differences observed in white blood cells, hemoglobin, platelets, creatinine, albumin, calcium, phosphorus, ferritin, transferrin, CRP, or fibrinogen levels (p > 0.05). However, parathyroid hormone (PTH) levels differed significantly between the groups, with mean values of 428 ± 304, 412 ± 334, and 263 ± 231 pg/mL, respectively (p = 0.03).

Across 10,770 catheter-days, median catheter dwell time was similar: 125.94 ± 87.48, 113.44 ± 35.68, and 108.59 ± 37.84 days, respectively (p = 0.831).

Catheter-related bloodstream infection

The CRBSI rate was lowest in the TAURO group (0.27 episodes/1,000 catheter-days), compared to the GENTAM group (0.83 episodes/1,000 catheter-days) and the control HEPARIN group (1.15 episodes/1,000 catheter-days), although the difference was not statistically significant (p = 0.526). All isolated pathogens causing CRBSI were gram-positive. In 87.5% of cases, a single organism was isolated, while polymicrobial growth was identified in 12.5%. The most common pathogen was *S. aureus* (50.0%). Table 2 presents the distribution of isolated CRBSI pathogens across study groups.

The average interval from catheter insertion to CRBSI onset was 45.47 ± 38.93 days. While the TAURO group exhibited the longest duration prior to infection (115.99 ± 58.61 days), compared to the HEPARIN (37.75 ± 35.37 days) and GENTAM (33 ± 24.27 days) groups, this difference did not reach statistical significance (p = 0.212) (Table 3). The total number of catheter-days until CRBSI onset was 935. There was no statistically significant difference in cumulative CVC

Table 2

Distribution of pathogens isolated from catheter-related bloodstream infections by study group

Pathogen	Group			Total
	TAURO	GENTAM	HEPARIN	
Staphylococcus species, coagulase-negative	1 (12.5)	1 (12.5)	1 (12.5)	3 (37.5)
Staphylococcus aureus	0 (0)	2 (25.0)	2 (25.0)	4 (50.0)
Polimicrobic flora	0 (0)	0 (0)	1 (12.5)	1 (12.5)
Total	1 (12.5)	3 (37.5)	4 (50.0)	8 (100)

All values are given as numbers (percentages).

Table 3

Number and time to onset of catheter-related complication following catheter insertion by study group

Catheter-related complication	Group			*p*-value
	TAURO	GENTAM	HEPARIN	
CRBSI	1 (3.12)	3 (9.37)	4 (12.5)	0.385
Time to CRBSI onset, days	115.99 ± 58.61	33 ± 24.27	37.75 ± 35.37	0.212
Exit-site infections	24 (75.0)	26 (81.2)	19 (59.4)	0.968
Time to exit-site infections onset, days	141.47 ± 111.747	122.19 ± 36.694	107.05 ± 32.667	0.344
Catheter-related thrombosis	4 (12.5)	4 (12.5)	4 (12.5)	1.000
Time to catheter-related thrombosis onset, days	67.75 ± 14.886	99.50 ± 49.568	132.00 ± 57.486	0.182

CRBSI – catheter-related bloodstream infection; n – number. For other abbreviations, see Table 1.
All values are given as numbers (percentages) or mean ± standard deviation.

survival free from CRBSI between the antibiotic lock groups (TAURO and GENTAM) and the control HEPARIN group (log-rank test, $p = 0.38$) (Figure 1). Cox proportional analysis regarding the occurrence of CRBSI showed the following results: HR (HEPARIN vs. TAURO) = 0.236 (95% CI: 0.026–2.116), $p = 0.197$; HR (HEPARIN vs. GENTA) = 0.737 (95% CI: 0.165–3.292), $p = 0.689$; HR (TAURO vs. GENTA) = 3.115 (95% CI: 0.324–29.95), $p = 0.325$.

Exit-site infections

A total of 89 exit-site infections were documented during the follow-up period, corresponding to an overall incidence of 8.26 episodes *per* 1,000 catheter-days. The cumulative number of catheter-days until the first recorded exit-site infection was 7,219. The number of exit-site infections and the time to their onset by group are presented in Table 3. The incidence of exit-site infections was 8.45 episodes/1,000 catheter-days in the TAURO group, 10.46 in the GENTAM group, and 5.75 in the HEPARIN group ($p = 0.078$). Regarding the frequency of exit-site infections *per* patient, 23 (23.9%) patients experienced one episode, 27 (28.1%) experienced two episodes, and 4 (4.2%) experienced three or more episodes. The remaining 42 (43.7%) patients did not develop any exit-site infections during the study period. There was no statistically significant difference in cumulative CVC survival free from exit-site infections between the antibiotic lock groups (TAURO and GENTAM) and the control HEPARIN group ($p = 0.21$) (Figure 2). Cox proportional analysis for exit-site infections occurrence showed no significant associations with lock type: $HR_{TAURO\ vs.\ HEPARIN} = 1.384$ (95% CI: 0.687–2.787) ($p = 0.362$), and $HR_{GENTAM\ vs.\ HEPARIN} = 1.836$ (95% CI: 0.925–3.641) ($p = 0.082$).

Catheter thrombosis

The overall incidence of CRT was 1.11 events *per* 1,000 catheter-days, with a total of 1,197 catheter-days until thrombosis occurred. The incidence of CVC thrombosis in

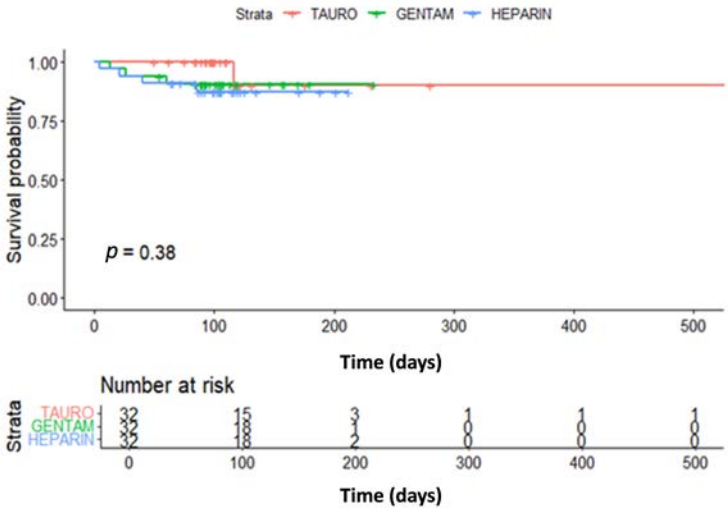

Fig. 1 – Kaplan-Meier analysis of cumulative catheter-related bloodstream infection-free catheter survival among the three catheter lock groups. For abbreviations, see Table 1.

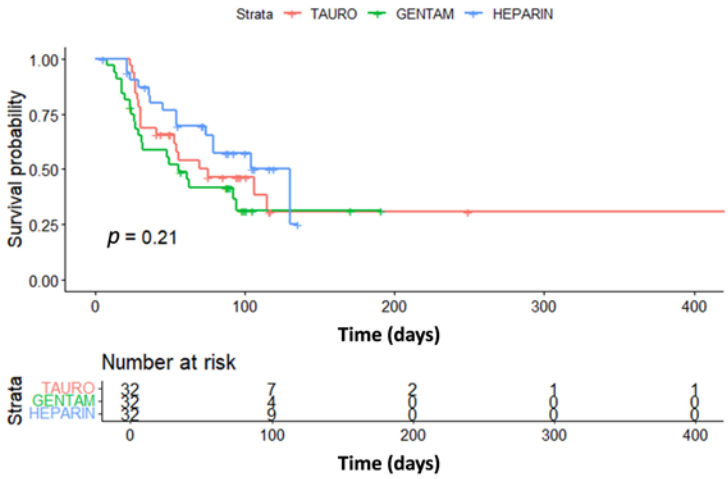

Fig. 2 – Kaplan-Meier analysis of cumulative exit-site infection-free central venous catheter survival among the three catheter lock solution groups. For abbreviations, see Table 1.

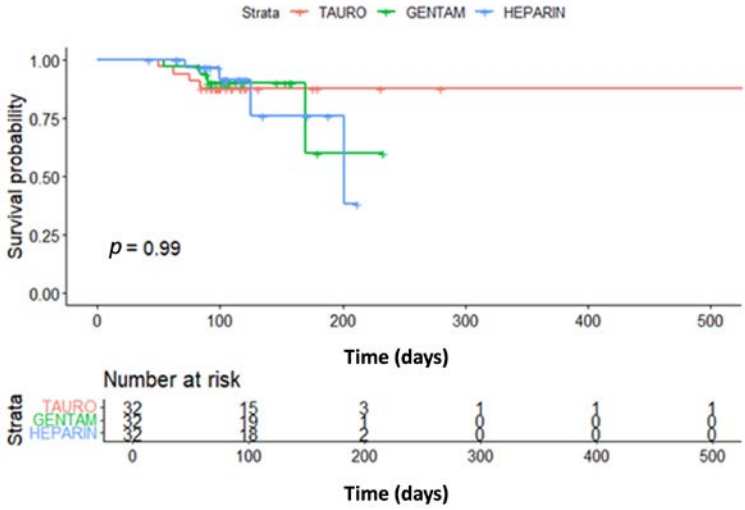

Fig. 3 – Kaplan-Meier analysis of cumulative thrombosis-free catheter survival among the three catheter lock solution groups.
For abbreviations, see Table 1.

the TAURO group was 1.09/1,000 catheter-days, 1.10/1,000 catheter-days in the GENTAM group, and 1.15/1,000 catheter-days in the control HEPARIN group ($p = 0.990$). The mean time to CRT onset was 99.75 ± 48.81 days, with the number of thrombosis and time to onset by groups presented in Table 3. Cumulative catheter survival without thrombosis was not significantly different between groups receiving anticoagulant and fibrinolytic locks (TAURO and GENTAM groups) compared with the HEPARIN group ($p = 0.990$) (Figure 3). The Cox proportional model regarding the development of CVC thrombosis in relation to the lock type showed the following result: $HR_{TAURO\ vs.\ HEPARIN} = 0.958$ (95% CI: 0.239–3.838) ($p = 0.952$); $HR_{GENTA\ vs.\ HEPARIN} = 1.002$ (95% CI: 0.249–4.032) ($p = 0.997$); $HR_{TAURO\ vs.\ GENTA} = 1.082$ (95% CI: 0.267–4.379) ($p = 0.912$).

Adverse effects

No differences were observed in the distribution of gentamicin-resistant strains after completion of the tested lock regimens and removal of the CVC ($p = 0.799$). No symptoms were recorded that would indicate toxicity from the administered drugs or solutions.

Discussion

Despite frequent complications, the use of temporary CVCs for dialysis remains remarkably high among incident HD patients in Serbia [1]. Depending on the dialysis center, the incidence of CRBSIs associated with CVCs ranges from 1.0 to 6.2 episodes *per* 1,000 catheter-days [7], while the incidence of CRT is reported to be 0.5 to 3.0 episodes *per* 1,000 catheter-days when heparin is used as the locking solution [2]. To prevent CRBSIs and CRT, the KDOQI guidelines for vascular access recommend the use of anticoagulant agents such as heparin or citrate. In centers with a high incidence of these complications, or in high-risk patients with a history of recurrent infections and/or thrombosis, the guidelines suggest combining these agents with thrombolytics and antibiotic locks to further reduce the risk of recurrence [2]. These combinations have yielded varying degrees of success in reducing the incidence of both CRBSIs [5, 8, 9] and CRT [5, 11]. To the best of our knowledge, this is the first study to use a combination of gentamicin-citrate and taurolock-urokinase to prevent catheter-related complications, aiming to reduce both infections and thrombosis in HD patients. Although the use of these catheter locks showed a non-significant trend toward reducing CRBSIs compared to other solutions, it did not prove superior in preventing catheter thrombosis.

As noted in previous studies, diabetes may contribute to CRBSI risk [3]. In our study, about one-third of patients had diabetes, with similar rates of diabetic kidney disease across groups. Mohazzab et al. [14] identified obesity as a potential risk factor for catheter dysfunction and thrombosis in tunneled dialysis catheters. Notably, in the present study, over half of the patients in each group were overweight, with a balanced distribution among the groups. Analysis of biochemical parameters revealed that the only significant difference between the groups was a higher PTH level in the TAURO group compared to the HEPARIN group. However, according to the literature, PTH is not considered a recognized risk factor for the development of CRBSI or CVC thrombosis [5, 14–17].

In our study, over 90% of CVCs were inserted *via* the right internal jugular vein, followed by the left internal jugular vein. Previous studies have shown that right internal jugular access is associated with longer catheter survival, but potentially higher risk of bacteremia due to prolonged use [18]. In contrast, femoral vein catheterization has been linked to an increased risk of CRBSIs [15, 19, 20], likely due to its anatomical proximity to the perineum, a moist area conducive to bacterial growth. Prolonged catheter duration further increases the risk of infection, regardless of the insertion site [18–20].

Catheters in the TAURO group had the longest median duration (125.9 days), compared with the GENTAM (113.4 days) and HEPARIN (108.6 days) groups, although the difference was not statistically significant. Compared to published data, the duration of catheter use in our study was notably longer. Prior studies have reported much shorter average lifespans for temporary CVCs, ranging from 6 to 58 days, depending on catheter type and insertion site [20–22]. For instance, Stolić et al. [22] reported a much shorter average duration of 17.4 ± 13.2 days, with most catheters placed in the femoral vein. Weldetensae et al. [21] found a mean duration of 57.9 ± 95.5 days for the first catheter, irrespective of insertion site. In contrast, Van Oevelen et al. [23] demonstrated significantly longer durations for tunneled and precurved non-tunneled jugular catheters, while Slovenian data showed that precurved non-tunneled catheters used as long-term access remained functional for over 9 months on average [24]. As expected, tunneled permanent catheters have the longest duration, typically ranging from 504 (366-3,802) days [25]. Our findings suggest that the catheter locks used in the TAURO group may contribute to enhanced catheter longevity.

The overall incidence of CRBSIs in our study was 0.74/1,000 catheter-days, with the lowest rate (*per* 1,000 catheter-days) observed in the TAURO group (0.27), followed by the GENTAM group (0.83), and the HEPARIN group (1.15), though without statistical significance. Notably, this represents a marked reduction compared to a previous study conducted at our center between 2012 and 2015, in which the CRBSI incidence associated with heparin locks (5,000 IU/mL) was 3.72/1,000 catheter-days [26]. This improvement likely reflects better adherence to vascular access guidelines and catheter care protocols, which may have contributed to the lower infection rates even in the control (HEPARIN) group. Additionally, another study from our region have reported CRBSI rates affecting 3.7% to 4.8% of patients during the 2003–2006 period [22]. International data show a wide range of CRBSI incidence with temporary catheters locked with heparin (3.55–7.74/1,000 catheter-days), and reduced infection rates when precurved catheters or gentamicin-heparin locks were used [19, 21, 23, 24]. Antibiotic-based catheter locks, particularly those containing gentamicin-citrate, have been shown to reduce CRBSI incidence, with reported effectiveness ranging from 31% to 100% [27]. Although randomized trials investigating taurolidine-urokinase combinations have not reached statistical significance, a meta-analysis including this agent reported a favorable trend in reducing infection rates [10, 28]. All CRBSI isolates in our study were gram-positive organisms, with *S. aureus* responsible for 50% of cases, consistent with findings reported in the literature [29]. Although gentamicin primarily targets gram-negative bacteria, none of these organisms were identified in our patients. Similarly, no gram-negative pathogens were isolated in the HEPARIN control group, consistent with previous data from our center, where gram-negative bacteria represented only 6.4% of all CRBSI isolates [26].

In contrast to a meta-analysis by Sheng et al. [30], which demonstrated that citrate-based lock solutions significantly reduced the incidence of catheter exit-site infections compared to heparin at 5,000 IU/mL, our study found the highest incidence in the GENTAM group and the lowest in the HEPARIN group ($p = 0.078$).

The incidence of CRT did not differ across groups, with an overall incidence of 1.11 events *per* 1,000 catheter-days. These rates are lower than previously reported thrombosis incidences associated with heparin locks, which reach up to 3.0 events/1,000 catheter days [2]. Previous study using 4% TSC as an anticoagulant reported catheter thrombosis rates of around 3.2 events *per* 1,000 catheter-days [24]. However, one meta-analysis concluded that citrate locks, regardless of concentration or combination with antimicrobials such as gentamicin, did not significantly reduce catheter malfunction [30]. In contrast, a separate meta-analysis found that urokinase combined with 4% TSC and taurolidine significantly reduced the incidence of catheter thrombosis compared to heparin locks [28].

This study has several limitations, including a relatively small sample size and a single-center design, which limit the generalizability of the findings. A notable strength of our study is the novel application of antimicrobial and thrombolytic lock combinations for the prevention of CRBSI and catheter thrombosis. Although the observed reductions in complication rates were not statistically significant, the results suggest potential benefit in high-risk populations and settings with high CRBSI incidence.

Conclusion

The findings of this study indicate that the use of gentamicin-citrate in combination with taurolock-urokinase is safe and feasible in routine clinical practice. While no statistically significant reductions in catheter-related complications were observed, the consistent trend toward lower catheter-related bloodstream infection rates and enhanced catheter longevity supports further investigation. Future multicenter studies with larger sample sizes are needed to validate these results and guide individualized catheter care strategies in hemodialysis patients.

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Received on August 19, 2025
Revised on October 17, 2025
Accepted on November 5, 2025
Online First December 2025

ORIGINAL ARTICLE

UDC: 616.314.5-007-02
DOI: https://doi.org/10.2298/VSP250317001G

Possible relationship between blood groups and impacted lower third molars categorized according to the Pell and Gregory and the Winter classifications

Moguća povezanost krvnih grupa i impaktiranih donjih trećih molara kategorisanih prema Pell i Gregory i Winter klasifikacijama

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Abstract

Background/Aim. Teeth that fail to reach their normal position within the jaws are considered to be impacted, and the etiology of impacted teeth is multifactorial. The aim of this study was to examine the possible relationship between blood groups and classifications of impacted lower third molars (LTMs) according to the Pell and Gregory and the Winter classifications. **Methods.** This retrospective, cross-sectional study included 534 patients (274 males and 260 females) with at least one impacted LTM. A total of 890 LTMs were assessed using panoramic radiographs. Teeth were classified based on the Pell and Gregory and the Winter classifications, and blood groups were documented for each patient. Statistical analysis was performed using Fisher-Freeman-Halton test and Fisher's exact test, with the Bonferroni correction *Z* test for multiple comparisons. **Results.** Significant relationships were identified between blood groups and the Pell and Gregory classification ($p = 0.008$), but not with the Winter classification. Notably, women with A Rh-negative (Rh-), AB Rh-, and B Rh- blood groups exhibited higher prevalence rates for the I/B, I/C, and III/C positions, respectively. Additionally, women with A Rh-positive (Rh+) and O Rh+ blood groups demonstrated higher prevalence rates for the II/A position, while men with A Rh+ showed higher rates for the II/C position. Gender-specific differences in impaction patterns were observed for A Rh+ and O Rh+ blood groups ($p = 0.006$, $p = 0.038$, respectively). **Conclusion.** Blood group antigens might influence LTM impaction patterns, particularly in relation to the Pell and Gregory classification.

Keywords:
blood group antigens; molar, third; radiography, panoramic; tooth, impacted.

Apstrakt

Uvod/Cilj. Zubi koji ne dostignu svoj normalan položaj u vilicama smatraju se impaktiranim, a etiologija impaktiranih zuba ima više uzroka. Cilj rada bio je da se ispita moguća povezanost između krvnih grupa i impaktiranih donjih trećih molara (*lower third molars* – LTM) kategorisanih prema Pell i Gregory i Winter klasifikacijama. **Metode.** Ova retrospektivna, studija preseka obuhvatila je 534 pacijenta (274 muškarca i 260 žena) sa najmanje jednim impaktiranim LTM. Ukupno je analizirano 890 impaktiranih LTM pomoću panoramske radiografije. Zubi su klasifikovani prema Pell i Gregory i Winter klasifikacijama, a krvne grupe su zabeležene za svakog pacijenta. Statistička analiza sprovedena je korišćenjem Fisher-Freeman-Halton-ovog testa i Fisher-ovog egzaktnog testa, uz Bonferroni-jevu korekciju *Z* testa za višestruka poređenja. **Rezultati.** Utvrđena je značajna povezanost između krvnih grupa i Pell i Gregory klasifikacije ($p = 0{,}008$), ali ne i povezanost sa Winter klasifikacijom. Posebno je uočeno da su žene sa krvnim grupama A Rh-negativnom (Rh-), AB Rh- i B Rh- pokazale višu prevalenciju za pozicije I/B, I/C i III/C, redom. Takođe, žene sa krvnim grupama A Rh-pozitivnom (Rh+) i O Rh+ imale su više stope učestalosti za poziciju II/A, dok su muškarci sa A Rh+ krvnom grupom imali višu stopu učestalosti za poziciju II/C. Polno specifične razlike u obrascima impakcije primećene su kod krvnih grupa A Rh+ i O Rh+ ($p = 0{,}006$, $p = 0{,}038$, redom). **Zaključak.** Antigeni krvnih grupa mogu uticati na obrasce impakcije LTM, posebno u odnosu na Pell i Gregory klasifikaciju.

Ključne reči:
krvne grupe, antigeni; molar, treći; ortopantomografija; zub, impakcija.

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Introduction

Tooth impaction is defined as the failure of a tooth to erupt into its normal functional position in the dental arch, and this condition occurs most frequently in the region of the lower third molars (LTMs) [1, 2]. The impaction of LTMs can be diagnosed during both routine dental examinations and the treatment of another tooth or teeth coincidentally [3, 4]. The impaction of LTMs can be associated with insufficient space, limited skeletal growth, reduced vertical condylar growth, increased crown dimension, delayed maturation of third molars, local factors, and certain systemic conditions (e.g., cleidocranial dysplasia, Down syndrome) [5, 6]. Furthermore, genetic factors may be linked with tooth impaction according to many previous studies [7].

Numerous classification systems have been proposed for impacted LTMs (ILTMs) to evaluate their angulation and extraction difficulty. Among these, the Winter and the Pell and Gregory classifications are the most preferred [8]. According to the Winter classification, the LTMs are categorized based on the angle formed between their longitudinal axis and that of the adjacent second molar as mesioangular, distoangular, horizontal, vertical, or inverted [9]. Concerning the relationship to the anterior margin of the mandibular ramus, the Pell and Gregory classification is performed to understand the level (depth) of the occlusal plane and the difficulty of impaction of the LTM [10].

A number of studies have demonstrated a correlation between blood groups (BGs) and the occurrence of various diseases [11, 12]. The ABO classification and Rhesus (Rh) system are the most commonly used around the world. The ABO classification comprises four blood groups (A, B, O, and AB) and is based on the presence or absence of A and B antigens on the surface of red blood cells. Specifically, blood group A is characterized by the presence of the A antigen, blood group B by the presence of the B antigen, blood group AB by the presence of both A and B antigens, and blood group O by the absence of both antigens [13]. In contrast, the Rh system is a classification system that categorizes groups according to the presence or absence of the RhD antigen on the red blood cell membrane. If a red blood cell presents the RhD antigen, it is designated as Rh-positive (Rh+), while if it does not, it is designated as Rh-negative (Rh-) [14].

Previous studies have reported associations between BGs and various medical and dental conditions, including cancer, cardiovascular disease, and dental caries [15–17]. However, a limited number of studies have examined the possible association between BGs and oral diseases. With respect to this matter, the evidence indicated that considering BGs separately, AB individuals have a greater risk of developing early childhood dental caries compared with individuals of other BGs [15, 18].

A great deal of controversy and inconsistency remains concerning the possible significance of BGs, specifically those of ABO system, as either diagnostic or prognostic factors in the context of oral and dental diseases, especially due to the diversity of methodological approaches employed in the various studies. Additionally, the varying geographical distributions and genetic profiles of BGs in different populations may contribute to the observed discrepancies [19]. The association between impacted teeth and ABO and Rh types within BGs is of great importance in predicting which populations are more susceptible to developing impacted third molars.

The aim of this study was to examine the possible relationship between ILTM classifications and BGs.

Methods

Study design

This retrospective cross-sectional study was conducted on all patients referred to the Department of Oral Radiology, Faculty of Dentistry, Alanya Alaaddin Keykubat University, Türkiye, between May 1 and December 31, 2023. The study was approved by the Ethics Committee of the Faculty of Dentistry, Alanya Alaaddin Keykubat University (No. 10354421-2023/14-15, from October 18, 2023). All individuals participating in the study were informed verbally about the study and provided written informed consent.

The study population consisted of all patients who underwent panoramic radiographs for dental examination or treatment. Out of 8,880 patients, those older than 20 years with at least one ILTM with complete root development, with no systemic disease, and no radiological lesions such as cysts or tumors in the posterior mandibular area were included in our study.

Among these individuals, patients lacking a lower second molar in the relevant region, those with crown destruction of the mandibular second molar due to caries or other reasons, those with a history of orthodontic treatment, and those with any trauma or pathology that would affect the dentition were excluded from the study. Ultimately, 534 patients (274 males and 260 females) who met the inclusion criteria were enrolled in the study, contributing a total of 890 ILTMs, of which 454 were in males and 436 in females.

Measures and procedures

All panoramic radiographs were obtained by a single operator using standardized parameters (66 kV, 8 mA, exposure time 15.8 s) on a panoramic X-ray unit (ProMax® 2D S3, Planmeca, Helsinki, Finland), with a magnification factor set to 1. A patient informed consent form, designed specifically for use within the context of our study, was utilized to conduct interviews with the participants. The participants were examined by a single dental practitioner (G.I.T.) to ensure standardization and reliability in the evaluation process. This approach minimized inter-observer variability, particularly given that subjective interpretations were required for the classifications of third molars. Additionally, the dental practitioner was calibrated through a test-retest process, achieving a substantial agreement ($\kappa = 0.879$), which ensured consistency in applying the classification criteria across all participants. The radiographs were analyzed at the same time of day and on the same

computer. During data collection, a 5-min rest period was taken after every 30 min of evaluating radiographs. This was done to prevent visual fatigue and to ensure the integrity of the process.

The collected data included the patient's gender, BG, classification of impacted third molars based on both the Pell and Gregory and the Winter classification systems, and the side (left or right) of the impacted tooth. BG (categorized as A, B, AB, or O) and Rh factor (positive or negative) were retrieved from medical documentation, the e-Nabız system (a digital health portal provided by the Turkish Ministry of Health), and driver's licenses. A tooth was defined as impacted if the occlusal surface of the LTM was positioned below the occlusal plane and partially embedded within the jawbone.

The ILTMs were evaluated using the Pell and Gregory classification system, which assesses the tooth's position based on two parameters: its vertical alignment relative to the adjacent second molar's occlusal plane, and the extent to which it is obstructed by the mandibular ramus.

Regarding the ramus relationship, Class I is identified when the space between the anterior border of the mandibular ramus and the distal surface of the second molar is wide enough to accommodate the full mesiodistal width of the third molar. Class II applies when the available space is smaller than the third molar's width, resulting in partial coverage of the tooth by the ramus. Class III indicates that the mandibular ramus entirely overlaps the area where the third molar would erupt, with no discernible gap between the ramus and the distal side of the second molar.

Regarding the vertical positioning relative to the second molar, position A denotes that the top surface (occlusal surface) of the third molar is at or above the level of the second molar's biting surface. Position B describes a scenario where the third molar lies between the occlusal surface and the cervical (neck) area of the second molar. Position C is used when the occlusal surface of the third molar is situated beneath the cervical margin of the second molar.

Additionally, the ILTMs were categorized according to the Winter classification, which is based on the angulation of the third molar in relation to the long axis of the second molar. This included vertical, mesioangular, horizontal, distoangular, and inverse positions. The buccoangular and linguoangular orientations were excluded from the analysis due to limitations in their accurate visualization on panoramic radiographs.

Statistical analysis

A sample size calculation was conducted using the G*Power software (latest version 3.1.9.7, Heinrich Heine University of Düsseldorf, Düsseldorf, Germany). The sample size was determined by utilizing the prevalence of impacted third molars by the Winter and the Pell and Gregory classification on radiographic assessment in relation to ABO BGs, which was published in a previous study [20]. All statistical analyses were performed using IBM SPSS Statistics version 23 (Chicago, USA) in a Windows environment. The Fisher-Freeman-Halton test and Fisher's exact test were employed to compare categorical data, with multiple comparisons analyzed by a Bonferroni-corrected *Z* test. The results of the analyses were presented in the form of a frequency table, with the corresponding percentage values. The significance level was set at $p < 0.05$.

Based on the results of the reference study [20], the minimum number of ILTMs required for sampling was 835, as determined by the power analysis, with a 95% confidence level (1-α), a 95% power test (1-β), and an effect size of w: 0.198.

Results

Demographic characteristics

A total of 534 patients (274 males and 260 females) with a mean age of 30.8 ± 8.7 years (range: 20–73 years) were included in the study. Among the 890 ILTMs, 454 belonged to males and 436 to females. The teeth were nearly evenly distributed between the right (444 teeth) and left (446 teeth) sides. The distribution of BGs was as follows: A Rh+ 39.1%, O Rh+ 28.3%, B Rh+ 13.3%, AB Rh+ 5.8%, O Rh- 5.4%, A Rh- 5.1%, B Rh- 1.7%, and AB Rh- 1.3% (Table 1).

Table 1

Demographic characteristics of the study population (n = 534)

Characteristics	Value
Gender	
male	274 (51.3)
female	260 (48.7)
Age, years	30.8 ± 8.7 (20–73)
Blood group	
A Rh+	39.1
O Rh+	28.3
B Rh+	13.3
AB Rh+	5.8
O Rh-	5.4
A Rh-	5.1
B Rh-	1.7
AB Rh-	1.3

All values are given as numbers (percentages), except for age, which is shown as mean ± standard deviation (range).

Classification of ımpacted teeth

In accordance with the Pell and Gregory classification, the most prevalent position was II/B (36.85%), followed by II/A (33.14%), and the least prevalent was III/A (1.01%). Concerning the Winter classification, the most common angulation was mesioangular (28.36%), followed by vertical (27.57%), and the least common was inverted (1.56%) (Table 2).

Relationships between classifications and blood groups

No statistically significant relationship was observed between BGs and classes in the Winter classification, regardless of gender or side ($p = 0.476$) (Table 3).

In contrast, a statistically significant relationship was found between BGs and ILTMs in the Pell and Gregory classification, regardless of gender or side ($p = 0.008$) (Table 4).

Table 2

Distribution of impacted lower third molars by the Pell and Gregory and the Winter classifications

Classification/Position	Prevalence (%)
Pell and Gregory	
II/B	36.85
II/A	33.14
I/A	8.31
II/C	6.85
III/B	5.39
I/B	3.25
III/C	2.92
I/C	2.24
III/A	1.01
Winter	
mesioangular	28.36
vertical	27.57
distoangular	23.87
horizontal	18.60
inverted	1.56

Table 3

Comparison of the Winter classification classes by blood groups for right and left sides, and overall distribution without gender distinction

Side/Class	Blood group								Test statistic	*p-value
	A Rh+	A Rh-	B Rh+	B Rh-	O Rh+	O Rh-	AB Rh+	AB Rh-		
Right										
mesioangular	49 (28.3)	8 (34.8)	16 (26.7)	2 (28.6)	30 (22.7)	8 (32.0)	7 (30.4)	0 (0)		
distoangular	40 (23.1)	4 (17.4)	18 (30.0)	1 (14.3)	34 (26.2)	5 (20.0)	3 (13.0)	2 (66.7)		
vertical	41 (23.7)	5 (21.7)	17 (28.3)	2 (28.6)	36 (27.3)	9 (36.0)	6 (26.1)	0 (0)	18.315	0.940
horizontal	40 (23.1)	6 (26.1)	9 (15.0)	2 (28.6)	27 (20.5)	3 (12.0)	7 (30.4)	1 (33.3)		
inverted	3 (1.7)	0 (0)	0 (0)	0 (0)	3 (2.3)	0 (0)	0 (0)	0 (0)		
total	173 (100)	23 (100)	60 (100)	7 (100)	130 (100)	25 (100)	23 (100)	3 (100)		
Left										
mesioangular	58 (35.4)	4 (18.2)	16 (27.1)	1 (12.5)	40 (30.1)	6 (24.0)	7 (24.1)	1 (16.7)		
distoangular	32 (19.5)	3 (13.6)	13 (22.0)	0 (0)	34 (25.6)	6 (24.0)	13 (44.8)	3 (50.0)		
vertical	45 (27.4)	10 (45.5)	21 (35.6)	3 (37.5)	37 (27.8)	9 (36.0)	4 (13.8)	1 (16.7)	30.026	0.268
horizontal	26 (15.9)	5 (22.7)	8 (13.6)	4 (50.0)	20 (15.0)	3 (12.0)	4 (13.8)	1 (16.7)		
inverted	3 (1.8)	0 (0)	1 (1.7)	0 (0)	2 (1.5)	1 (4.0)	1 (3.4)	0 (0)		
total	164 (100)	22 (100)	59 (100)	8 (100)	133 (100)	25 (100)	29 (100)	6 (100)		
Total										
mesioangular	107 (31.8)	12 (26.7)	32 (26.9)	3 (20.0)	70 (26.4)	14 (28.0)	14 (26.9)	1 (11.1)		
distoangular	72 (21.4)	7 (15.6)	31 (26.1)	1 (6.7)	68 (26.4)	11 (22.0)	16 (30.8)	5 (55.6)		
vertical	86 (25.5)	15 (33.3)	38 (31.9)	5 (33.3)	73 (27.5)	18 (36.0)	10 (19.2)	1 (11.1)	26.504	0.476
horizontal	66 (19.6)	11 (24.4)	17 (14.3)	6 (40.0)	47 (17.7)	6 (12.0)	11 (21.2)	2 (22.2)		
inverted	6 (1.8)	0 (0)	1 (0.8)	0 (0)	5 (1.9)	1 (2.0)	1 (1.9)	0 (0)		
total	337 (100)	45 (100)	119 (100)	15 (100)	263 (100)	50 (100)	52 (100)	9 (100)		

All values are given as numbers (percentages).

***Note*: * Fisher-Freeman-Halton test was used.**

Table 4

Sample distribution by the Pell and Gregory classification across blood groups, regardless of gender or side

Scale/Class	Blood group								Test statistic	*p-value
	A Rh+	A Rh-	B Rh+	B Rh-	O Rh+	O Rh-	AB Rh+	AB Rh-		
Right										
I/A	15 (8.7)	2 (8.7)	6 (10.0)	0 (0)	10 (7.7)	7 (28.0)	1 (4.3)	0 (0)		
I/B	3 (1.7)	2 (8.7)	3 (5.0)	0 (0)	2 (1.5)	1 (4.0)	2 (8.7)	0 (0)		
I/C	2 (1.2)	0 (0)	2 (3.3)	0 (0)	1 (0.8)	0 (0)	0 (0)	0 (0)		
II/A	63 (36.4)	4 (17.4)	20 (33.3)	4 (57.1)	51 (39.2)	9 (36.0)	7 (30.4)	2 (66.7)		
II/B	54 (31.2)	10 (43.5)	25 (41.7)	2 (28.6)	46 (35.4)	7 (28.0)	9 (39.1)	1 (33.3)	62.671	0.134
II/C	17 (9.8)	0 (0)	0 (0)	0 (0)	9 (6.9)	0 (0)	1 (4.3)	0 (0)		
III/A	2 (1.2)	0 (0)	0 (0)	0 (0)	2 (1.5)	0 (0)	0 (0)	0 (0)		
III/B	12 (6.9)	3 (13.0)	3 (5.0)	0 (0)	8 (6.2)	1 (4.0)	0 (0)	0 (0)		
III/C	5 (2.9)	2 (8.7)	1 (1.7)	1 (14.3)	1 (0.8)	0 (0)	3 (13.0)	0 (0)		
total	173 (100)	23 (100)	60 (100)	7 (100)	130 (100)	25 (100)	23 (100)	3 (100)		
Left										
I/A	15 (9.1)	0 (0)	2 (3.4)	0 (0)	10 (7.5)	3 (12)	3 (10.3)	0 (0)		
I/B	3 (1.8)	2 (9.1)	3 (5.1)	1 (12.5)	5 (3.8)	2 (8.0)	0 (0)	0 (0)		
I/C	3 (1.8)	0 (0)	3 (5.1)	0 (0)	8 (6)	0 (0)	0 (0)	1 (16.7)		
II/A	56 (34.1)	9 (40.9)	13 (22.0)	4 (50.0)	35 (26.3)	7 (28.0)	10 (34.5)	1 (16.7)		
II/B	57 (34.8)	6 (27.3)	28 (47.5)	2 (25.0)	56 (42.1)	11 (44.0)	12 (41.4)	2 (33.3)	59.421	0.137
II/C	14 (8.5)	2 (9.1)	6 (10.2)	0 (0)	9 (6.8)	2 (8.0)	1 (3.4)	0 (0)		
III/A	2 (1.2)	2 (9.1)	0 (0)	0 (0)	1 (0.8)	0 (0)	0 (0)	0 (0)		
III/B	8 (4.9)	0 (0)	3 (5.1)	0 (0)	8 (6.0)	0 (0)	0 (0)	2 (33.3)		
III/C	6 (3.7)	1 (4.5)	1 (1.7)	1 (12.5)	1 (0.8)	0 (0)	3 (10.3)	0 (0)		
total	164 (100)	22 (100)	59 (100)	8 (100)	133 (100)	25 (100)	29 (100)	6 (100)		
Total										
I/A	30 (8.9)	2 (4.4)	8 (6.7)	0 (0)	20 (7.6)	10 (20.0)	4 (7.7)	0 (0)		
I/B	6 (1.8)	4 (8.9)	6 (5.0)	1 (6.7)	7 (2.7)	3 (6.0)	2 (3.8)	0 (0)		
I/C	5 (1.5)	0 (0)	5 (4.2)	0 (0)	9 (3.4)	0 (0)	0 (0)	1 (11.1)		
II/A	119 (35.3)	13 (28.9)	33 (27.7)	8 (53.3)	86 (32.7)	16 (32.0)	17 (32.7)	3 (33.3)		
II/B	111 (32.9)	16 (35.6)	53 (44.5)	4 (26.7)	102 (38.8)	18 (36.0)	21 (40.4)	3 (33.3)		
II/C	31 (9.2)	2 (4.4)	6 (5.0)	0 (0)	18 (6.8)	2 (4.0)	2 (3.8)	0 (0)	75.721	**0.008**
III/A	4 (1.2)	2 (4.4)	0 (0)	0 (0)	3 (1.1)	0 (0)	0 (0)	0 (0)		
III/B	20 (5.9)^{ab}	3 (6.7)^{ab}	6 (5.0)^{ab}	0 (0)^{ab}	16 (6.1)^{ab}	1 (2.0)^{ab}	0 (0)^{b}	2 (22.2)^{a}		
III/C	11 (3.3)^{abc}	3 (6.7)^{abc}	2 (1.7)^{abc}	2 (13.3)^{c}	2 (0.8)^{b}	0 (0)^{abc}	6 (11.5)^{ac}	0 (0)^{abc}		
total	337 (100)	45 (100)	119 (100)	15 (100)	263 (100)	50 (100)	52 (100)	9 (100)		

All values are given as numbers (percentages). Bold values indicate statistical significance, $p < 0.05$.
***Note*: * Fisher-Freeman-Halton test was used; $^{a\text{-}c}$ there was no difference between groups with the same letter.**

Gender-specific analyses

Separate analyses were conducted to investigate the relationships between BGs and classifications of ILTMs in males and females, across both the Pell and Gregory and the Winter classification systems.

For females, a statistically significant relationship was found between BGs and classifications in the Pell and Gregory system, regardless of the side ($p = 0.041$) (Table 5).

No statistically significant relationships were found in the Winter classification for females ($p > 0.05$).

For males, no statistically significant relationships were identified between BGs and classifications in either the Pell and Gregory or the Winter classification systems, regardless of the side ($p > 0.05$).

Table 5

Female sample distribution by the Pell and Gregory classification across blood groups, regardless of side

Scale/Class	Blood group								Test statistic	*p-value
	A Rh+	A Rh-	B Rh+	B Rh-	O Rh+	O Rh-	AB Rh+	AB Rh-		
Right										
I/A	10 (10.5)	1 (12.5)	2 (6.3)	0 (0)	3 (4.7)	3 (25)	0 (0)	0 (0)		
I/B	1 (1.1)	1 (12.5)	2 (6.3)	0 (0)	1 (1.6)	1 (8.3)	1 (9.1)	0 (0)		
I/C	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
II/A	38 (40.0)	2 (25.0)	9 (28.1)	1 (33.3)	33 (51.6)	4 (33.3)	4 (36.4)	2 (100)		
II/B	33 (34.7)	3 (37.5)	17 (53.1)	1 (33.3)	19 (29.7)	4 (33.3)	5 (45.5)	0 (0)	63.334	0.390
II/C	4 (4.2)	0 (0)	0 (0)	0 (0)	4 (6.3)	0 (0)	0 (0)	0 (0)		
III/A	1 (1.1)	0 (0)	0 (0)	0 (0)	2 (3.1)	0 (0)	0 (0)	0 (0)		
III/B	4 (4.2)	1 (12.5)	1 (3.1)	0 (0)	2 (3.1)	0 (0)	0 (0)	0 (0)		
III/C	3 (3.2)	0 (0)	1 (3.1)	1 (33.3)	0 (0)	0 (0)	1 (9.1)	0 (0)		
total	95 (100)	8 (100)	32 (100)	3 (100)	64 (100)	12 (100)	11 (100)	2 (100)		

Table 5 (continued)

Scale/Class	Blood group								Test statistic	*p-value
	A Rh+	A Rh-	B Rh+	B Rh-	O Rh+	O Rh-	AB Rh+	AB Rh-		
Left										
I/A	8 (10.0)	0 (0)	2 (6.5)	0 (0)	3 (5.1)	1 (10.0)	2 (13.3)	0 (0)		
I/B	1 (1.3)	1 (14.3)	2 (6.5)	0 (0)	1 (1.7)	1 (10.0)	0 (0)	0 (0)		
I/C	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.4)	0 (0)	0 (0)	1 (25.0)		
II/A	35 (43.8)	4 (57.1)	5 (16.1)	2 (66.7)	17 (28.8)	4 (40.0)	5 (33.3)	1 (25.0)		
II/B	27 (33.8)	2 (28.6)	18 (58.1)	0 (0)	31 (52.5)	4 (40.0)	6 (40.0)	1 (25.0)	67.538	0.130
II/C	4 (5.0)	0 (0)	2 (6.5)	0 (0)	3 (5.1)	0 (0)	1 (6.7)	0 (0)		
III/A	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
III/B	3 (3.8)	0 (0)	2 (6.5)	0 (0)	2 (3.4)	0 (0)	0 (0)	1 (25.0)		
III/C	1 (1.3)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	1 (6.7)	0 (0)		
total	80 (100)	7 (100)	31 (100)	3 (100)	59 (100)	10 (100)	15 (100)	4 (100)		
Total										
I/A	18 (10.3)	1 (6.7)	4 (6.3)	0 (0)	6 (4.9)	4 (18.2)	2 (7.7)	0 (0)		
I/B	2 (1.1)^{a}	2 (13.3)^{b}	4 (6.3)^{ab}	0 (0)^{ab}	2 (1.6)^{ab}	2 (9.1)^{ab}	1 (3.8)^{ab}	0 (0)^{ab}		
I/C	1 (0.6)^{a}	0 (0)^{ab}	0 (0)^{a}	0 (0)^{ab}	2 (1.6)^{ab}	0 (0)^{ab}	0 (0)^{ab}	1 (16.7)^{b}		
II/A	73 (41.7)	6 (40.0)	14 (22.2)	3 (50.0)	50 (40.7)	8 (36.4)	9 (34.6)	3 (50.0)		
II/B	60 (34.3)	5 (33.3)	35 (55.6)	1 (16.7)	50 (40.7)	8 (36.4)	11 (42.3)	1 (16.7)	68.123	**0.041**
II/C	8 (4.6)	0 (0)	2 (3.2)	0 (0)	7 (5.7)	0 (0)	1 (3.8)	0 (0)		
III/A	2 (1.1)	0 (0)	0 (0)	0 (0)	2 (1.6)	0 (0)	0 (0)	0 (0)		
III/B	7 (4.0)	1 (6.7)	3 (4.8)	0 (0)	4 (3.3)	0 (0)	0 (0)	1 (16.7)		
III/C	4 (2.3)^{a}	0 (0)^{ab}	1 (1.6)^{a}	2 (33.3)^{b}	0 (0)^{a}	0 (0)^{ab}	2 (7.7)^{ab}	0 (0)^{ab}		
total	175 (100)	15 (100)	63 (100)	6 (100)	123 (100)	22 (100)	26 (100)	6 (100)		

All values are given as numbers (percentages). Bold values indicate statistical significance, $p < 0.05$.

***Note*: * Fisher-Freeman-Halton test was used; [a, b] there was no difference between groups with the same letter.**

Investigation of blood groups and ımpaction relationships

Each BG was investigated to compare impacted tooth subgroups according to gender, both with and without side discrimination. Among all BGs, A Rh+ and O Rh+ were the only ones that showed a statistically significant relationship between gender and impacted tooth subgroups. Importantly, this relationship was observed exclusively in the Pell and Gregory classification.

For the A Rh+ BG, a statistically significant relationship between gender and impacted tooth subgroups was identified without side discrimination (p = 0.006). These results are detailed in Table 6, where differences between males and females in specific subgroups are highlighted. For instance, significant differences were noted for Class II/A (males: 28.4%, females: 41.7%) and Class II/C (males: 14.2%, females: 4.6%), indicating notable gender-based variations within these subgroups.

For the O Rh+ BG, a similar statistically significant relationship was found (p = 0.038) (Table 7). These findings underscore the unique relevance of these two BGs in relation to ILTMs, as no other BG demonstrated significant associations.

Table 6

Comparison of impacted tooth subgroups by gender in the A Rh+ blood group based on the Pell and Gregory classification, considering sides and overall distribution

Scale/Class	Gender		Test statistic	*p-value
	male	female		
Right				
I/A	5 (6.4)	10 (10.5)		
I/B	2 (2.6)	1 (1.1)		
I/C	1 (1.3)	1 (1.1)		
II/A	25 (32.1)	38 (40.0)		
II/B	21 (26.9)	33 (34.7)	12.529	0.086
II/C	13 (16.7)	4 (4.2)		
III/A	1 (1.3)	1 (1.1)		
III/B	8 (10.3)	4 (4.2)		
III/C	2 (2.6)	3 (3.2)		
total	78 (100)	95 (100)		

Table 6 (continued)

Scale/Class	Gender		Test statistic	*p-value
	male	female		
Left				
I/A	7 (8.3)	8 (10.0)		
I/B	2 (2.4)	1 (1.3)		
I/C	3 (3.6)	0 (0)		
II/A	21 (25.0)	35 (43.8)		
II/B	30 (35.7)	27 (33.8)	12.408	0.097
II/C	10 (11.9)	4 (5.0)		
III/A	1 (1.2)	1 (1.3)		
III/B	5 (6.0)	3 (3.8)		
III/C	5 (6.0)	1 (1.3)		
total	84 (100)	80 (100)		
Total				
I/A	12 (7.4)	18 (10.3)		
I/B	4 (2.5)	2 (1.1)		
I/C	4 (2.5)	1 (0.6)		
II/A	46 (28.4)[a]	73 (41.7)[b]		
II/B	51 (31.5)	60 (34.3)	19.880	**0.006**
II/C	23 (14.2)[a]	8 (4.6)[b]		
III/A	2 (1.2)	2 (1.1)		
III/B	13 (8.0)	7 (4.0)		
III/C	7 (4.3)	4 (2.3)		
total	162 (100)	175 (100)		

All values are given as numbers (percentages).
Bold values indicate statistical significance, $p < 0.05$.
***Note*: * Fisher-Freeman-Halton test was used; [a, b] there was no difference between groups with the same letter.**

Table 7

Comparison of impacted tooth subgroups by gender in the O Rh+ blood group based on the Pell and Gregory classification, considering sides and overall distribution

Scale/Class	Gender		Test statistic	*p-value
	male	female		
Right				
I/A	7 (10.6)	3 (4.7)		
I/B	1 (1.5)	1 (1.6)		
I/C	1 (1.5)	0 (0)		
II/A	18 (27.3)	33 (51.6)		
II/B	27 (40.9)	19 (29.7)	13.068	0.051
II/C	5 (7.6)	4 (6.3)		
III/A	0 (0)	2 (3.1)		
III/B	6 (9.1)	2 (3.1)		
III/C	1 (1.5)	0 (0)		
total	66 (100)	64 (100)		
Left				
I/A	7 (9.5)	3 (5.1)		
I/B	4 (5.4)	1 (1.7)		
I/C	6 (8.1)	2 (3.4)		
II/A	18 (24.3)	17 (28.8)		
II/B	25 (33.8)	31 (52.5)	8.911	0.306
II/C	6 (8.1)	3 (5.1)		
III/A	1 (1.4)	0 (0)		
III/B	6 (8.1)	2 (3.4)		
III/C	1 (1.4)	0 (0)		
total	74 (100)	59 (100)		

Table 7 (continued)

Scale/Class	Gender		Test statistic	*p-value
	male	female		
Total				
I/A	14 (10)	6 (4.9)		
I/B	5 (3.6)	2 (1.6)		
I/C	7 (5.0)	2 (1.6)		
II/A	36 (25.7)[a]	50 (40.7)[b]		
II/B	52 (37.1)	50 (40.7)	15.133	**0.038**
II/C	11 (7.9)	7 (5.7)		
III/A	1 (0.7)	2 (1.6)		
III/B	12 (8.6)	4 (3.3)		
III/C	2 (1.4)	0 (0)		
total	140 (100)	123 (100)		

All values are given as numbers (percentages).
Bold values indicate statistical significance, $p < 0.05$.
***Note*: *Fisher-Freeman-Halton test was used; [a, b] there was no difference between groups with the same letter.**

Discussion

Teeth that fail to reach their normal position within the jaws at the expected time of eruption, for a variety of reasons, are considered to be impacted [21]. The etiology of impacted teeth is multifactorial, with numerous contributing factors. These include physical disintegration of the lamina of the teeth, insufficient space for the eruption of the teeth, a natural defect of the tooth lamina, and failure of the induction of the mesenchyme [22].

There is a paucity of data regarding potential differences in impaction incidence between genders, populations, age groups, and over time [2]. Although some researchers have reported a higher occurrence in females compared to males [23–25], a substantial body of literature indicates no statistically significant association between gender and the presence of impacted third molars [26–29]. These findings are consistent with the results of the present study. Similarly, the current results revealed no significant difference in the frequency of impaction between the right and left sides of the mandible, which aligns with observations reported in several other studies [23, 30, 31].

The LTMs have the highest incidence of impaction. In order to evaluate these teeth radiographically, it is necessary to determine the angulation of the molars according to the Winter classification, the level of depth in the bone, and the relationship of the teeth to the ramus of the mandible. Furthermore, the relationship of the second molars to the aforementioned structures should be determined according to the Pell and Gregory classification. Similar to the present study, mesioangular and inverted positions were observed to be the most and least prevalent, respectively, according to the Winter classification, as reported by Santos et al. [32]. In accordance with the Pell and Gregory classification, some authors have indicated that the C level is the most prevalent at the depth level [33], while others have suggested that the A level is the most common [34]. Previous research [35] reported that the most commonly observed level was B, consistent with the findings of this study. When considering the ramus of the mandible, although some authors have found that Position I is the most common position, followed by Position II [35], many studies have reported that Position II is the most common position [32, 36, 37]. This is similar to the present study. In contrast to prior study [38], which identified II/A as the most prevalent category followed by II/B within the Pell and Gregory classification, the present study yielded different results. In our study, the II/B position (36.85%) was the most prevalent, followed by II/A (33.14%).

The most common cause of impacted third molars is insufficient space for eruption, which has a genetic component. This raises the possibility of a potential link between BG and the occurrence of impacted third molars [39]. The association has been investigated previously. However, unlike the findings presented in the current research, Ahmadi et al. [40] did not demonstrate a statistically significant correlation between BGs and the presence of impacted teeth. The sample size and geographical distribution may have been responsible. In previous research [3], the prevalence of impacted teeth in both jaws was analyzed, and the existing impacted teeth were classified according to the Winter classification. In this study, given that the prevalence of impacted teeth was the highest, only impacted teeth in the lower jaw were evaluated, and all impacted teeth were grouped according to both the Pell and Gregory and the Winter classifications.

The significant association observed between BGs and impacted teeth according to the Pell and Gregory classification suggests a potential genetic or biological link. Among the different BGs, individuals with AB Rh- showed a higher prevalence of the III/B position, while those with B Rh- and AB Rh+ were more likely to present with the III/C position. These findings provide new insights into the possible role of genetic factors.

Gender-specific analyses revealed further distinctions. Women with A Rh- BG were more likely to present with the I/B position, while those with AB Rh- and B Rh- BGs were more likely to present with I/C and III/C positions, respectively. Additionally, women with A Rh+

and O Rh+ BGs exhibited higher prevalence rates for the II/A position, whereas men with A Rh+ showed higher rates for the II/C position. These findings could have significant clinical implications for identifying individuals at greater risk of specific impaction patterns based on their gender and BG.

This study's limitations warrant careful consideration. First, all evaluations were conducted by a single operator, which, while ensuring consistency, limits the inter-rater reliability of the findings. Future studies should involve multiple independent observers to enhance objectivity. Second, the exclusion of certain classification angles, such as bucoangular and distoangular, due to the limitations of panoramic radiography, may have excluded relevant data. Third, the study design did not include a *post hoc* power analysis, which could have further strengthened the statistical robustness of the findings. Moreover, clinical implications could be influenced by these limitations. For instance, the lack of inter-observer variability may impact the reproducibility of these results in broader clinical settings. Fourth, the sample size and geographic focus may limit the generalizability of the results. Future research should aim to include larger, more diverse populations to validate these findings and explore advanced imaging techniques to capture a more comprehensive classification of impacted teeth. Lastly, employing multi-center trials and advanced statistical techniques may help better elucidate the relationship between genetic factors, such as BG, and impact patterns.

Conclusion

This study provides novel insights into the relationship between blood groups and impacted third molars, particularly with respect to the Pell and Gregory classification. The findings highlight the possible clinical significance of incorporating genetic and demographic factors into dental practice. Future research should investigate the underlying biological mechanisms that drive these associations and assess their implications in larger, more diverse cohorts.

Conflict of interest

The authors declare no conflict of interest.

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Received on March 17, 2025
Revised on November 24, 2025
Accepted on December 10, 2025
Online First January 2026

ORIGINAL ARTICLE

UDC: 616.31-74
DOI: https://doi.org/10.2298/VSP250822079Y

Fracture resistance of five intra-orifice barriers in endodontically treated mandibular premolars: an *in vitro* study

Otpornost na prelom pet različitih barijera koje se postavljaju na ulazu u kanal endodontski lečenih premolara u donjoj vilici: *in vitro* studija

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Abstract

Background/Aim. Endodontically treated teeth (ETT) are more prone to fractures than vital teeth, and insertion of an intra-orifice barrier (IOB) can increase their fracture resistance (FR). The aim of this study was to compare and evaluate the FR of ETT using smart dentin replacement (SDR), everX Flow (EXF), resin-modified glass-ionomer cements (RMGIC), calcium-enriched mixture (CEM), and universal flowable composite (UFC) as IOBs. **Methods.** After performing root canal treatment on 70 human mandibular premolars with a single root canal, the coronal 3 mm of root fillings were removed with heated instruments, except for the control specimens. Based on the IOB above the root canal obturation, the filled specimens were divided into six groups: RMGIC (n = 13), UFC (n = 13), SDR (n = 13), CEM (n = 13), EXF (n = 13), and a control group (CG; n = 5). A spherical steel insert with a diameter of 2 mm was used in the strength test with a universal testing machine. Data were analyzed using the Shapiro-Wilk test, analysis of variance, and least significant difference tests. The value of $p < 0.05$ is considered statistically significant. **Results.** The EXF group showed the highest mean FR of 759.9 ± 177.9 Newtons. The groups RMGIC, UFC, and EXF demonstrated a statistically significant difference compared to CG. CEM had a lower FR value than all groups except the SDR and CG. However, the FR of SDR was lower than that of the UFC and EXF groups. There were no significant differences between EXF, UFC, and RMGIC groups. **Conclusion.** Except for SDR and CEM, all other groups showed an increase in FR compared to CG. The results indicate that using EXF, UFC, or RMGIC as IOB can significantly enhance the FR of ETT compared to untreated controls. Clinically, selecting these IOBs may help prevent root fractures and improve the long-term prognosis of ETT.

Keywords:
bicuspid; dental cements; endodontics; in vitro; materials testing; root canal preparation.

Apstrakt

Uvod/Cilj. Endodontski lečeni zubi (*endodontically treated teeth* – ETT) skloniji su prelomima od vitalnih zuba, a umetanje barijera koje se postavljaju na ulazu u kanal (*intra-orifice barriers* – IOB) može povećati njihovu otpornost na prelom (*fracture resistance* – FR). Cilj rada bio je da se uporedi i proceni FR ETT korišćenjem „pametne“ zamene dentina (*smart dentin replacement* – SDR), *everX Flow* (EXF), smolom modifikovanog staklenojonomernog cementa (*resin-modified glass-ionomer cements* – RMGIC), kalcijum silikatnog cementa (*calcium-enriched mixture* – CEM) i univerzalnog tečnog kompozita (*universal flowable composite* – UFC) kao IOB. **Metode.** Nakon lečenja korena kanala 70 humanih jednokanalnih mandibularnih premolara, zagrejanim instrumentima su uklonjena koronarna 3 mm korenskih punjenja, osim kod kontrolnih uzoraka. Na osnovu IOB iznad opturacije korenskog kanala, uzorci pripremljeni za punjenje podeljeni su u šest grupa : RMGIC (n = 13), UFC (n = 13), SDR (n = 13), CEM (n = 13), EXF (n = 13) i kontrolna grupa (KG; n = 5). U testu čvrstoće univerzalnom mašinom za ispitivanje korišćen je sferični čelični umetak prečnika 2 mm. Za analizu podataka korišćeni su Shapiro-Wilk test, analiza varijanse i test najmanje značajne razlike. Vrednost $p < 0,05$ smatrana je statistički značajnom. **Rezultati.** Grupa EXF pokazala je najvišu srednju FR od 759,9 ± 177,9 Njutna. Grupe RMGIC, UFC i EXF pokazale su statistički značajnu razliku u odnosu na KG. CEM je imao nižu vrednost FR od svih grupa osim SDR i KG. Međutim, FR SDR bio je niži nego kod UFC i EXF grupa. Nije bilo značajnih razlika između grupa EXF, UFC i RMGIC. **Zaključak.** Osim SDR i CEM, sve ostale grupe pokazale su povećanje FR u poređenju sa KG. Rezultati ukazuju da korišćenje EXF, UFC ili RMGIC kao IOB može značajno poboljšati FR ETT u poređenju sa netretiranim kontrolama. Klinički, izbor ovih IOB može pomoći u sprečavanju preloma korena zuba i poboljšanju dugoročne prognoze ETT.

Ključne reči:
premolari; zub, cement; endodoncija; in vitro; materijali, testiranje; zub, korenski kanal, prıprema.

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Introduction

Preservation of the remaining tooth structure is one of the main objectives of endodontic treatment [1]. Canal preparation with rotary instruments, which have a greater taper, heightens the risk of fracture in the coronal third of teeth. Consequently, it is essential to emphasize the reinforcement of these vulnerable regions [2]. After dental caries and periodontal disease, vertical root fractures (VRFs), described as fractures in teeth that run along the longitudinal axis of the root, are one of the common causes of tooth extraction [3]. Endodontically treated teeth (ETT) are more prone to fractures than vital teeth, which may vary from a typical cusp fracture to a catastrophic root fracture that requires extraction [4]. This can be prevented by strengthening the remaining radicular tooth structure, particularly in teeth exposed to high occlusal stresses. For this reinforcement, it is recommended to use restorative materials with compressive strength and modulus of elasticity similar to dentin, which helps reduce stress concentration at the dentin-restoration interface [5].

It is recommended to remove the 3 mm gutta-percha at the orifice of the root canal and replace it with a restorative material [6]. Insertion of an intra-orifice barrier (IOB) increases the strength of the ETT [7]. To replicate the stress-absorbing qualities of dentin, short fiber-reinforced composites have been developed to be utilized as a bulk basis for the restoration of high-stress teeth. The composite everX Flow (EXF) is recommended as an ideal IOB, especially in large cavities, as it allows better stress distribution [8]. One of the most popular materials for ETT restoration is smart dentin replacement (SDR) Plus Bulk Fill Flowable, a low-viscosity flowable composite that minimizes air bubble formation and enables it to reach deep places [9]. Another common option is resin-modified glass-ionomer cements (RMGICs), which chemically bond with dentin [10]. The RMGICs perform a similar acid-base reaction, but with the inclusion of resins. They allow for an effective setting and greater initial strength than glass-ionomer cements [11]. Calcium-enriched mixture (CEM), a water-based cement, is made up of calcium compounds. CEM is less toxic and more biocompatible, and is used for vital pulp treatment [12].

Despite the variety of materials available, limited data exist comparing their effects on fracture resistance (FR) when used as IOBs.

The aim of this study was to compare and evaluate the FR of ETT using SDR, EXF, RMGIC, CEM, and universal flowable composite (UFC) as IOBs. The null hypothesis was that the IOBs do not affect the FR of ETT and that there are no differences in FR offered by the five IOBs.

Methods

This *in vitro* study was conducted within four weeks, from January to February 2024, at the Department of Endodontics, Faculty of Dentistry, Gaziantep University, Gaziantep, Türkiye. This study was approved by the Ethics Committee of Gaziantep University (No. 2023/354, from November 01, 2023).

Sample size calculation

This sample size was computed using the mean and standard deviation (SD) values reported in two previous studies [13, 14]. Therefore, using G*Power 3.1 software (Universität Düsseldorf, Germany) and Cohen's *d* method with $\alpha = 0.05$, power of 95%, and computed effect size $f = 2.05$, a sample size of 13 was determined for each group. Five samples were chosen for the control group (CG). Thus, a total sample of 70 was determined.

Inclusion and exclusion criteria

Seventy human mandibular premolars with single root canals extracted for orthodontic procedures were chosen because they were of comparable size and had straight roots. The samples were first inspected under a stereomicroscope to ensure there were no cracks, and teeth with short and curved roots were eliminated. To rule out teeth with resorptive abnormalities and verify the existence of a single canal, intraoral periapical radiographs were also obtained. An ultrasonic scaler handpiece (Woodpecker HW-5L, Guangxi, China) with an ultrasonic scaler tip (G1) was subsequently utilized to clean all samples in order to remove calculus and debris. To avoid dehydration, the samples were then kept in distilled water for two weeks before use.

Specimen preparation

After measuring 14 mm from the root apex with a digital vernier caliper and marking it with a fine-point marker, the samples were decoronated along the marking using a low-speed handpiece with a diamond disc under water cooling. A size 10 K-type file (Dentsply Maillefer, Tulsa, USA) was inserted into the canal until it was observable through the apical foramen. The working length was determined 1 mm below this length.

Root canal preparation

Crown-down endodontic treatment of the specimens was carried out utilizing a nickel-titanium rotary instrument set (ProTaper Next, Dentsply Tulsa Dental, Tulsa, USA). A number 15 K-type file (Dentsply Maillefer, Tulsa, USA) was used to establish a glide path in the canals. In each sample, files X1 (#17.04), X2 (#25.06), and X3 (#30.04) were used in that order. During the preparation process, 2 mL of 5.25% sodium hypochlorite (NaOCl) (Wizard, Rehber Kimya San. ve Tic., Istanbul, Türkiye) was used for irrigation. After each file was replaced, 2 mL of 17% ethylenediaminetetraacetic acid (EDTA) was added. NaviTip sideport 31-gauge side perforated flushing needles (Ultradent, South Jordan, Utah, USA) were used for canal irrigation. After the root canals were prepared, the smear layer was removed by irrigating them with 3 mL of 5% NaOCl solution, followed by 3 mL of

15% EDTA for 1 min, and finally with 3 mL of 5% NaOCl solution. To ensure the effectiveness of the treatments, the root canals were lastly cleaned with 10 mL of distilled water and dried with sterile paper points (Aceonedent, Geonggi-Do, Korea).

The canals were subsequently filled with AH Plus Jet (Dentsply DeTrey, Konstanz, Germany) canal sealer and gutta-percha with 0.2% taper, according to the instructions of the manufacturer, using the lateral condensation technique. Then, the coronal 3 mm of root fillings was carefully removed using heated tools, with the exception of control specimens. By using a William's periodontal probe, this depth was verified. Microbrushes soaked with 70% ethanol were used to remove any remaining gutta-percha or sealer. Lastly, based on the IOB located above the root canal obturation, the filled specimens were split into the groups by manual allocation.

Placement of intra-orifice barriers

In the RMGIC group (n = 13), Equia Forte HT Fil (GC, Tokyo, Japan) was used. This is a high-viscosity glass ionomer restorative material consisting of fluoroaluminosilicate glass and an aqueous polyacrylic acid solution. The glass component contains SiO_2, Al_2O_3, CaF_2, AlF_3, and $AlPO_4$, which undergo an acid–base reaction to form a strong ionic bond with the tooth structure. The RMGIC capsule was mixed in the amalgamator for the duration specified by the manufacturer. Then, RMGIC was inserted into the prepared canal openings and polymerized for 20 s using a light-curing device (Elipar DeepCure, 3M ESPE).

In the UFC group (n = 13), G-ænial Universal Injectable (GC, Tokyo, Japan) was used. This is a highly filled, light-cured injectable composite containing urethane dimethacrylate, bisphenol A-ethoxylate dimethacrylate, and strontium glass fillers [69 weight percent (wt%)]. The root canal orifices were etched with 37% phosphoric acid for 20 s. The surface was then cleaned with water, and extra water was removed using an air syringe. The sample was then rinsed with water and dried with air. The bonding agent (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) was applied to the area with a microbrush and polymerized with a light-curing device for 10 s. The pre-prepared canal orifices were filled with flowable composite in 1.5 mm increments, using a 470 nm visible light curing device placed 2 mm away for 20 s, in two passes.

In the SDR Plus group (n = 13), SDR Plus (Dentsply, Sirona, Germany) material was used, which contains modified urethane dimethacrylate resin matrix and filler (68 wt%). Root canal orifices were etched for 20 s using 37% phosphoric acid prior to restoration. After that, water was used to rinse the surface, and an air syringe was employed to remove any extra water. The enamel and dentin were then coated with Adper Single Bond 2, which was light polymerized for 10 s. Then, the SDR was installed and exposed to light polymerization for 20 s.

In the CEM group (n = 13), the material used was CEM (Bioniquedent Co., Tehran, Iran), and it contains calcium oxide, calcium phosphate, calcium carbonate, and calcium sulfate. The powder and liquid portions were added according to the manufacturer's instructions. The powder was progressively combined with the liquid for 15–30 s to wet all powder particles and achieve a dense consistency with a plastic spatula. Then, a ball-shaped mass of CEM cement was taken from the mixture and gently pushed into the canal orifices with a hand instrument. A wet cotton pellet was put on the IOB and allowed to set for 1 hr.

The material EXF (GC Dental, Tokyo, Japan), used in the EXF group (n = 13), contains E-glass fibers (1–2 mm), bisphenol A-glycidyl methacrylate, triethylene glycol dimethacrylate, and barium glass filler. Before restoration, the surfaces were etched with 37% phosphoric acid, rinsed with water, and dried using an air syringe. The bonding agent (Single Bond 2) was coated and lightly polymerized for 10 s. Finally, EXF was applied and lightly polymerized for 20 s.

In the CG (n = 5), there was no gutta-percha removal or IOB application.

For full setting, the specimens were then kept for 48 hrs at 37 °C and 100% humidity. The roots' apical 5 mm were submerged in molten wax. This resulted in a periodontal ligament gap of around 0.2–0.3 mm. Then, all specimens were placed in plastic cylinder molds that were 20 mm in diameter and 20 mm in height and embedded in acrylic resin (Imicryl, Konya, Türkiye).

Fracture testing

To prevent bias, all of the aforementioned steps were completed by a single operator (IY). The operator was not blinded to the group allocation. This represents a potential source of operator bias and is acknowledged as a limitation of the study. A spherical steel insert with a diameter of 2 mm (perpendicular to the tooth's long axis) and a constant crosshead speed of 1 mm/min was used in the strength test, which was carried out using a universal testing machine (AGS-X, Shimadzu Corporation, Tokyo, Japan). Until the root broke, the loading segment with the tip was positioned in the middle of each specimen's groove opening. The moment at which a sharp decline of more than 25% of the force utilized became evident was considered a fracture [15]. Newtons (N) were used to record the force at fracture.

Statistical analysis

The numerical variables' conformance to the normal distribution was verified using the Shapiro-Wilk test. The analysis of variance (ANOVA) and least significant difference tests were used for analyzing normally distributed data across six groups. For the recorded forces, we derived the mean and SD. The analyses were carried out using SPSS 22.0 Windows package application (SPSS Inc., Chicago, IL, USA). The value of $p < 0.05$ was accepted as significant.

Results

There was a significant relationship between groups (Table 1 and Figure 1).

The highest mean fracture strength value (759.9 ± 177.9) among the tested groups was observed in the teeth with EXF, followed by UFC (741.2 ± 163.4). The lowest average FR value (519.6 ± 60.2) was observed in CG, and the closest value to CG (539.2 ± 107.6) was in the CEM group.

In comparing normally distributed variables across six groups, ANOVA and least significant difference tests revealed a significant difference between the FR values obtained from the groups (p = 0.001). When the FR of the groups was evaluated using the pairwise comparison test, all groups except SDR and CEM showed a statistically significant difference compared to CG. The UFC group showed significantly higher FR than all groups except EXF and RMGIC, while the CEM group showed a lower FR value than all groups except SDR and CG. The EXF group showed significantly higher FR against all groups except the UFC and RMGIC groups. However, SDR showed lower FR against UFC and EXF groups. There was no significant difference between EXF, UFC, and RMGIC.

Table 1

Fracture resistance of experimental groups and statistical comparisons

Group	Mean ± SD	Median (Min–Max)	Homogeneous subset*
EXF (n = 13)	759.9 ± 177.9	709 (527–1,098)	c
UFC (n = 13)	741.2 ± 163.4	772 (516–1,036)	c
RMGIC (n = 13)	659.8 ± 107.5	699 (461–856)	bc
SDR (n = 13)	617.5 ± 87.8	596 (501–839)	ab
CEM (n = 13)	539.2 ± 107.6	558 (399–695)	a
CG (n = 5)	519.6 ± 60.2	520 (435–605)	a

SD – standard deviation; EXF – everX Flow; UFC – universal flowable composite; RMGIC – resin-modified glass-ionomer cements; SDR – smart dentin replacement; CEM – calcium-enriched mixture; CG – control group; n – number; Min – minimum; Max – maximum.

***Note*. *Lowercase letters indicate statistical groups; groups sharing the same letter are not significantly different, while statistically significant differences (p < 0.05, analysis of variance and least significant difference test) occur between all groups with different letters, including comparisons involving multiple groups simultaneously.**

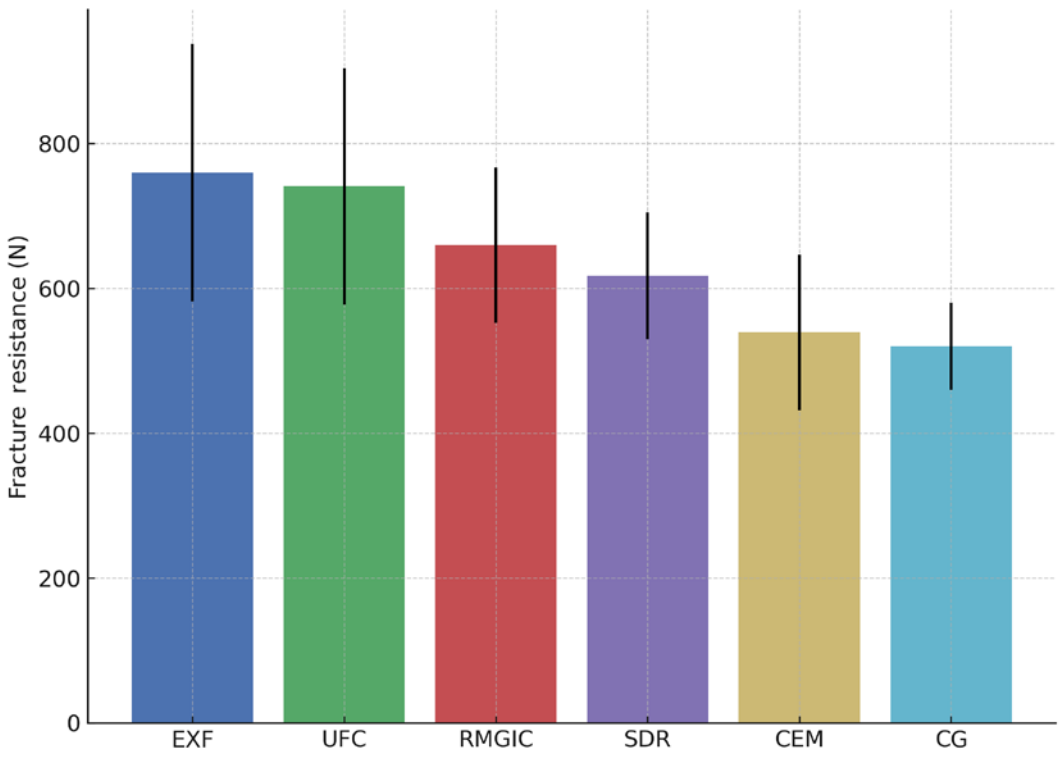

Fig. 1 – Fracture resistance results.
For other abbreviations, see Table 1.
***Note*. The bar graph illustrates the mean fracture resistance for each experimental group, with standard deviations indicated as error bars.**

Discussion

Due to extensive dentin removal, particularly in the cervical area, the larger preparation that results from the use of rotary instruments can lead to VRF [3]. Furthermore, dehydration and irrigant exposure weaken dentin and make it more susceptible to VRF [16]. Additionally, while evaluating the VRF risk of ETT, additional characteristics, including the size and curvature of the external root, as well as the form and morphology of the root canal, should be taken into account [17]. Strengthening the radicular part and the coronal structure should be the main priority. IOB has been suggested to lower the risk of fracture and support ETT [13].

Using five distinct IOBs, our study estimated the FR of mandibular premolars that had undergone endodontic treatment. Fracture strength data of the test groups showed that the type of IOB used had a substantial impact on the roots' FR. According to the results, CG had the lowest FR, whereas EXF and UFC had the greatest FR. The lowest FR levels were observed in CG, which was consistent with previous research [5, 14, 18]. Our findings also showed significant differences in FR between these groups. IOB placement of EXF, UFC, and RMGIC significantly increased the FR of ETT. Therefore, the null hypothesis of the present study, that there would be no significant difference between the tested groups, was rejected. The higher FR values observed in our research may be mostly attributable to the tested IOBs' strong adhesive qualities to dentin. This explains why RMGIC had a strong FR relative to CG and CEM, but less than the UFC, and no discernible difference in FR compared to the UFC and EXF groups in the current study. Additionally, the EXF group showed greater FR, in line with other studies [19, 20]. The EXF group has been shown to be useful in strengthening ETT as IOB [21]. The presence of short fibers integrated into the matrix, which greatly enhanced the material's resistance to crack propagation and reduced the stress intensity at the crack tip and its unstable propagation, may have contributed to the increased FR of EXF [19]. However, this contrasts with the findings of Gupta et al. [18], which indicated that the FR of RMGIC was greater than that of fiber-reinforced composite. This discrepancy might be attributed to the fact that in the study mentioned earlier, the fiber was employed as a separate layer (Ribbond, Seattle, Washington, USA) on the cavity floor alongside the composite filling.

Previous research has demonstrated that UFCs are more successful than RMGIC at improving FR as an IOB, as was the case in our study [5, 13, 14]. RMGIC has an elastic modulus similar to that of dentin and a strong flexural strength. As a result, the material may bear the stress before the load is transferred to the root. Additionally, the dentin-RMGIC contact is more resistant due to its chemical connection to the dentin surface [18]. Compared to traditional composites, UFCs have been reported to provide increased flowability, better adaptability to the interior cavity wall, and more elasticity [22]. However, UFC's lower stiffness and greater polymerization shrinkage are significant disadvantages compared to composite resins [23]. This may help explain why the FR of RMGIC in the current study was not significantly different from that of UFC, showing a good FR relative to CG but still lower than UFC.

UFC showed more FR than CEM in the present research. This could be because the resin's low viscosity made it easier for UFC to adhere to the intra-orifice dentin. Compared to mineral trioxide aggregate (MTA), CEM has a smaller film thickness, a higher flow rate, and a faster setting time [24]. Although CEM increased the FR of immature anterior teeth at 6 months [25], there were no significant differences between the tested groups in our study, except the CEM group. The elastic modulus of CEM is comparable to that of dentin. However, the impact of FR as IOB is minimal. MTA, which has mechanical properties consistent with CEM, provided the lowest FR in multiple studies comparing the FR of RMGIC, fiber-reinforced composite, and MTA as IOB [14, 26]. Regardless of the type of adhesive employed, the study by Savadi Oskoee et al. [27] revealed that RMGIC's shear bond strength was much higher than CEM's. Since the adhesive system allows them to penetrate and interlock into surface pores and imperfections, the bonding process of CEM is most likely micromechanical. Additionally, the combination of powder and liquid alters the mechanical characteristics of the CEM, which in turn influences the FR values [28]. Furthermore, as shown below, rather than having a favorable modulus of elasticity, CEM's inability to strengthen roots is most likely due to its weakening under stresses and its absence of bonding to dentin.

Although tooth strength is determined by the amount of tooth structure remaining, the FR could be increased by inserting a further 3 mm barrier into the root canal. Stress transmission along the length of the tooth depends on the pericervical dentin, which is situated close to the alveolar crest and extends around 4 mm coronally and apically from the crestal bone. The tooth may be prone to fracture if this pericervical dentin is lost [5]. Gao et al. [29] highlighted this point by stating that the cervical portion of the root experiences the greatest stress due to occlusal pressures in ETT, and that this stress increases as the instrument's taper becomes larger. To restore the missing pericervical dentin in the experimental groups, 3 mm of barrier material was used in place of gutta-percha. This enhanced FR when the restorative materials flexed under occlusal loading, dispersing the stresses equally over the dentin-restoration contact [5, 14]. Up to 4 mm of SDR can be polymerized at once, which is 1 mm deeper than the depth required by the IOB. Additionally, it has a modified methacrylate resin that lowers the pressures generated by shrinkage *via* slowing down the rate of polymerization [30].

According to Atalay et al. [31], ETT restored with fiber-reinforced composite (everX posterior) or bulk-fill/fluid bulk-fill, like SDR in our study, does not have a different FR than those restored with conventional nanohybrid resin composite similar to UFC. Similarly, no significant difference was found between UFC and EXF in our study. The results of our research were corroborated by Ozsevik et al. [20], who found that teeth with root canal therapy and those

reconstructed with fiber-reinforced composite material (everX posterior, GC, Tokyo, Japan) had fracture strength values that were quite similar to those of the intact tooth group. Even though their findings were comparable to those of our study, the aforementioned study differs in certain ways from the current investigation. First, rather than evaluating IOBs, their goal was to assess the FR of composite fillings. Additionally, they tested fillings in molars rather than uncrowned, single-rooted premolars, and they used everX posterior instead of EXF as the short fiber-reinforced flowable resin.

Within the limitations of this *in vitro* study, EXF and UFC demonstrated the highest FR and may therefore be recommended as IOB materials for ETT under high occlusal stress. EXF, containing short fibers that mimic dentin's elasticity, provides effective stress distribution and is particularly suitable for posterior teeth with wide canal openings. On the other hand, UFC offers easier handling, excellent adaptation, and lower cost, making it a practical choice for routine cases. Although RMGIC showed slightly lower resistance, its chemical adhesion and fluoride release can be advantageous in cases requiring enhanced sealing. CEM and SDR, with lower reinforcement potential, may not be ideal in teeth subject to high masticatory forces. Further long-term clinical studies are needed to evaluate the performance of these materials under cyclic loading and thermomechanical stress.

This *in vitro* study inherently has several limitations. Firstly, there was a possibility of sampling and representation bias because the study included only healthy mandibular premolars. Secondly, despite extensive efforts to standardize the form and size of the premolars, undetectable canal defects may still have been present and could have influenced the force readings. Thirdly, in the current investigation, the force was applied in a single direction and at a single spot to evaluate FR, which does not accurately replicate intraoral environments. A fourth limitation is that the operator was not blinded to group allocation, creating the potential for operator bias. As a fifth limitation, this study applied a single, static vertical load to the fracture, which does not fully reflect the complex occlusal stresses occurring within the mouth. Cyclic loading and thermomechanical aging could have provided a more accurate simulation of clinical conditions. Therefore, the results should be interpreted with caution, and further *in vitro* studies that include dynamic and thermal fatigue are recommended. Lastly, the inability to apply the study's findings in clinical settings is another shortcoming. In addition, it is necessary to explore the possibilities of using a potential IOB candidate for further studies. Future research should include cyclic loading and other demanding simulations to replicate clinical conditions better.

Conclusion

Among the tested intra-orifice barrier materials, everX Flow exhibited the highest fracture resistance, while universal flowable composite and resin-modified glass-ionomer cements showed comparable performance. Except for smart dentin replacement and calcium-enriched mixture, all materials significantly increased fracture resistance compared with the control group. These results indicate that everX Flow, universal flowable composite, and resin-modified glass-ionomer cements may provide superior reinforcement in endodontically treated teeth, making them preferable choices in clinical practice. Further *in vitro* and clinical studies are needed to confirm their long-term performance under functional conditions.

Conflict of interest

The authors declare no conflict of interest.

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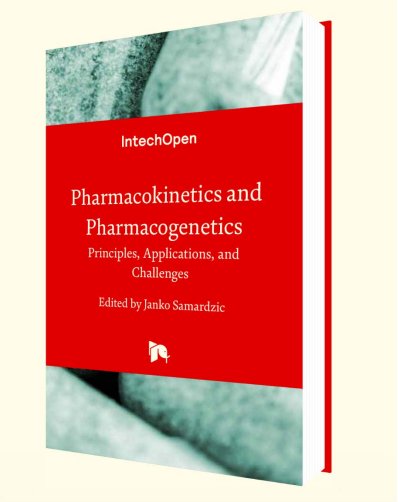
Received on August 22, 2025
Revised on October 5, 2025
Revised on November 4, 2025
Accepted on November 26, 2025
Online First December 2025

BOOK REVIEW/PRIKAZ KNJIGE

Pharmacokinetics and pharmacogenetics – principles, applications, and challenges

Farmakokinetika i farmakogenetika – principi, primene i izazovi



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Publisher/Izdavač: IntechOpen, London, UK
Year of publication/Godina izdanja: 2025
ISBN: 978-1-83635-359-1
ISSN: 3033-3318; 102 pages

The book *Pharmacokinetics and Pharmacogenetics – Principles, Applications, and Challenges*, edited by Prof. Janko Samardžić, brings together contributions from distinguished scientists whose expertise encompasses the molecular, preclinical, and clinical aspects of drug research. It is intended for academic researchers, pharmacologists, geneticists, clinicians, and professionals engaged in drug development and precision medicine. The volume will be particularly valuable to readers seeking an integrated understanding of pharmacokinetics and pharmacogenetics, as well as to those interested in translational applications, including central nervous system drug delivery, ocular pharmacokinetics, analytical method development, and population-level pharmacogenomics.

The central aim of the book is to illustrate how pharmacokinetics and pharmacogenetics intersect to advance precision pharmacotherapy. The six chapters, each reviewed by experts in the field, present up-to-date research and review data in pharmacokinetics and pharmacogenetics – two of the most dynamic and rapidly evolving disciplines within contemporary pharmaceutical and biomedical sciences. Each contributing author provides insights grounded in rigorous scientific investigation, ensuring that the content is both methodologically sound and directly relevant to current challenges in therapeutic development. The scientific content demonstrates a strong methodological foundation, drawing on molecular, preclinical, and clinical research, as well as advanced analytical and computational approaches. Several chapters address ongoing scientific and clinical challenges, including optimization of central nervous system drug delivery, overcoming ocular barriers, and managing interindividual variability in antipsychotic response driven by genetic polymorphisms. The integration of artificial intelligence, machine learning, and multi-omics approaches further underscores the book's alignment with emerging technological and clinical trends shaping modern precision therapeutics.

The opening chapter, "Integrating Pharmacokinetics and Pharmacogenetics in the Era of Personalized Medicine," outlines the fundamental principles of pharmacokinetics and pharmacogenetics, emphasizing how their integration supports more precise dosing strategies, optimization of clinical trial design, and the development of safer and more effective therapeutics. The chapter also underscores the expanding role of artificial intelligence, machine learning, and pharmacogenetic databases in predicting individual drug responses. Building on this conceptual foundation, Chapter 2, "Pharmacokinetic Considerations for Drugs Targeting the Central Nervous System (CNS)," addresses one of the most complex and clinically relevant domains of pharmacokinetics. It examines the intricate interplay between drug physico-

chemical properties, the blood–brain barrier, and CNS pharmacodynamics. The chapter provides a structured overview of how factors such as lipid solubility, protein binding, active transport mechanisms, and pathological alterations in the blood–brain barrier permeability influence CNS drug disposition. The chapter also discusses emerging strategies to overcome these barriers, including nanocarrier-based delivery systems. Chapter 3, "Ocular Pharmacokinetic Studies: Challenges and Best Practices," focuses on another organ system in which distinct anatomical and physiological characteristics significantly affect drug disposition. The chapter highlights the limitations of extrapolating systemic pharmacokinetic data to ocular exposure and offers a comprehensive discussion of experimental and computational methodologies. It outlines best practices in study design, sample collection, and pharmacokinetic modeling, emphasizing the translational importance of understanding intraocular drug kinetics. Chapter 4, "Development and Validation of an HPLC–PDA Method for Quantitative Bioanalysis of Curcuminoids and Its Application to Preclinical Pharmacokinetics," addresses the analytical dimension of pharmacokinetic research. It describes the development and validation of a robust high-performance liquid chromatography–photodiode array (HPLC–PDA) method for the simultaneous quantification of curcuminoids, bioactive compounds with considerable therapeutic potential but limited bioavailability. Chapter 5, "Comprehensive Pharmacogenetic Allele Landscape from Whole Exome Sequencing: Single-Center Cohort Analysis in the Population of North Macedonia," extends the focus from molecular analysis to population-level pharmacogenetics. Utilizing whole exome sequencing data, this chapter presents a comprehensive pharmacogenetic landscape of the population, identifying both common and novel variants with potential clinical relevance. The final chapter, "The State of the Art: Pharmacogenomics, Multi-Omics, and Translational Barriers in Antipsychotic Therapy," rounds the discussion off by applying pharmacogenetics concepts to one of the most challenging areas of clinical pharmacology – neuropsychopharmacology. It synthesizes current knowledge on key metabolic enzymes, receptor polymorphisms, and emerging multi-omics data that collectively influence therapeutic response and clinical outcomes.

Together, the chapters in this volume demonstrate that the integration of pharmacokinetics and pharmacogenetics is not merely a theoretical concept but a necessary progression toward precision pharmacotherapy. The multidisciplinary perspective further promotes collaboration across pharmacology, genetics, and clinical sciences. Certain limitations should also be noted, such as the specialized nature of some chapters focusing on ocular pharmacokinetic methods or HPLC–PDA analytical techniques, which may reduce accessibility to readers without a strong technical background. However, given the book's target audience, primarily researchers and specialists, this limitation is understandable and does not diminish the overall value. In summary, *Pharmacokinetics and Pharmacogenetics – Principles, Applications, and Challenges* offers a cohesive, informative, and methodologically rigorous examination of two foundational disciplines driving the advancement of personalized medicine.

The book is available both in print and as a freely accessible online edition on the following link: https://www.intechopen.com/books/1004701

Prof. Silva Dobrić, PhD

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

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

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A Case Report must not have more than five authors.

QUESTIONNAIRES

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

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

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

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ACKNOWLEDGEMENTS

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Volume with a Supplement

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

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

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

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

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

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

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

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Printed Book

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

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

Chapter

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

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

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

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

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

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

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

Other published articles

News article

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

Holy Bible

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

Dictionaries and similar references

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

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ISSN Online 2406-0720 OPEN ACCESS

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

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

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

ЕТИЧКА САГЛАСНОСТ

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

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

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

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

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

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

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

ПЛАГИЈАРИЗАМ

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

КОРИШЋЕЊЕ AI

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

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

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

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

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

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

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

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

ТИПОВИ РУКОПИСА

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

ОРИГИНАЛНИ ЧЛАНАК

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

ПРЕТХОДНО САОПШТЕЊЕ

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

КРАТКО САОПШТЕЊЕ

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

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МЕТА-АНАЛИЗА

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

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

АКТУЕЛНА ТЕМА

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

У ФОКУСУ

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

КАЗУИСТИКА

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

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

УВОДНИК

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

ПИСМО УРЕДНИКУ

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

ИСТРАЖИВАЧКО ПИСМО

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

ИСТОРИЈА МЕДИЦИНЕ/СТОМАТОЛОГИЈЕ/ФАРМАЦИЈЕ

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

КЛИНИЧКО ИСТРАЖИВАЊЕ

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

ПРИКАЗ КЊИГЕ

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

ИЗВЕШТАЈ СА НАУЧНОГ ИЛИ СТРУЧНОГ СКУПА

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

ОБИМ РУКОПИСА

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

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

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

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

ПРИПРЕМА РАДА

НАСЛОВНА СТРАНА

На првој страници рукописа треба навести следеће:

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

АПСТРАКТ

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

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

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

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

КЉУЧНЕ РЕЧИ

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

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

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

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

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

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

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

ПРИЛОЗИ

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

Табеле

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

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

Слике (илустрације)

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

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

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

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

СКРАЋЕНИЦЕ

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

ДЕЦИМАЛНИ БРОЈЕВИ

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

ЈЕДИНИЦЕ МЕРА

Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – m, килограм (грам) – kg (g), литар – L) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), притисак крви у милиметрима живиног стуба (mm Hg). Резултате клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (SI).

ЗАХВАЛНИЦА

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

СТАТИСТИЧКА АНАЛИЗА

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

ЛИТЕРАТУРА

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

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

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

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

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

Примери цитирања:

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