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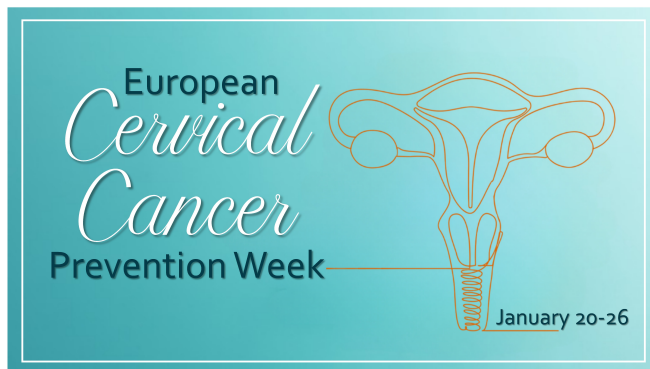
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According to data from the European Center for Disease Prevention and Control, cervical cancer is the second most common cancer, after breast cancer, that affects women aged 15 to 44. Every year, about 33,000 women in the European Union get cervical cancer, and about 15,000 cases end in death. The European Cervical Cancer Prevention Week is observed from January 20 to 26 to inform about the cervical cancer risk factors, symptoms, and prevention methods. It is crucial to raise awareness about the need for frequent screenings and the availability of a vaccine that is effective in protecting against certain high-risk types of human papillomavirus that can cause cervical cancer.

Prema podacima Evropskog centra za sprečavanje i kontrolu bolesti, rak grlića materice je drugi najčešći rak, posle raka dojke, koji pogađa žene starosti od 15 do 44 godine. Svake godine u Evropskoj uniji oko 33 000 žena oboli od raka grlića materice a oko 15 000 slučajeva završi se smrtnim ishodom. Evropska nedelja prevencije raka grlića materice obeležava se od 20. do 26. januara u cilju informisanja o faktorima rizika od raka grlića materice, simptomima i načinima prevencije. Od ključne je važnosti podizanje svesti o neophodnosti čestih pregleda i dostupnosti vakcine koja je efikasna u zaštiti od određenih visokorizičnih tipova humanog papiloma virusa koji mogu uzrokovati rak grlića materice.



Vojnosanitetski pregled – New year, new challenges

Vojnosanitetski pregled – Nova godina, novi izazovi

Dragana Vučević

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Introduction

After saying farewell to 2024, the Editorial Office of *Vojnosanitetski pregled* (VSP), one of the oldest professional medical journals in the region, will remember this past year for two significant events. First, the great jubilee—VSP celebrated 80 years of existence in September 2024 ([click here](#)). The second important event was the constitution of the new international and domestic Editorial Board (EB) ([click here](#)).

Moreover, the last year will be remembered for two additional significant jubilees for the Medical Services of the Serbian Army – 180 years of existence of the Military Medical Academy ([click here](#)) and 15 years of constitution of the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade ([click here](#)).

The first half of 2024 marked three years since the election of the previous domestic VSP EB. Considering the time-limited mandate of the EB members (except for academicians and those of very specific scientific fields), the termination of the mandate of EB members selected in 2021 was the opportunity to rejuvenate the EB by selecting new members.

The following are the new EB members from the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade: Assoc. Prof. Vesna Begović-Kuprešanin, MD, PhD (Infectious Diseases); Assist. Prof. Mihailo Bezmarević, MD, PhD (General Surgery); Prof. Snežana Cerović, MD, PhD (Pathology); Prof. Viktorija Dragojević Simić, MD, PhD (Clinical Pharmacology); Col. Assist. Prof. Branko Košević, MD, PhD (Urology); Assoc. Prof. Boško Milev, MD, PhD (General Surgery); Col. Prof. Milan Petronijević, MD, PhD (Rheumatology); Assist. Prof. Dejan Pilčević, MD, PhD (Nephrology); Assist. Prof. Nemanja Rančić, MD, PhD (Pharmacology/Radiology/Medical Statistics); Assist. Prof. Aleksandra Vukomanović, MD, PhD (Physical Medicine and Rehabilitation) (in alphabetical order). The following are the

new EB members from the Faculty of Medicine, University of Belgrade order: Assist. Prof. Suzana Bojić, MD, PhD (Anesthesiology); Assist. Prof. Igor Končar, MD, PhD (Vascular Surgery); Prof. Olivera Kontić-Vučinić, MD, PhD (Gynecology and Obstetrics); Assoc. Prof. Dragana Miljić, MD, PhD (Endocrinology, Diabetes, and Metabolism); Assoc. Prof. Dejan Orlić, MD, PhD (Cardiology) (in alphabetical order). The new member from the Faculty of Medical Sciences, University of Kragujevac, is Assist. Prof. Raša Mladenović, DDM, PhD (Dentistry). The new member from the Faculty of Medicine, University of Novi Sad, is Prof. Marija Jevtić, MD, PhD (Public Health).

The EB kept some of its previous members, namely the following ones: full members of the Serbian Academy of Science and Arts – Prof. (ret.) Bela Balint, MD, PhD, FSASA (Transfusion Medicine/Hemobiology), Brigadier General (ret.) Prof. Miodrag Čolić, MD, PhD, FSASA (Immunology), Prof. (ret.) Miodrag Ostojić, MD, PhD, FSASA (Cardiology), Prof. (ret.) Đorđe Radak, MD, PhD, FSASA (Vascular Surgery) (in alphabetical order); members from the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade – Prof. Dragana Daković, DDM, PhD (Dentistry/Oral Medicine and Periodontology), Prof. (ret.) Silva Dobrić, BPharm, PhD (Pharmacy), Col. Prof. Boban Đorđević, MD, PhD (Plastic and Reconstructive Surgery), Assoc. Prof. Željko Mijušković, MD, PhD (Dermatology), Lieut. Col. Assoc. Prof. Aleksandar Perić, MD, PhD (Otorhinolaryngology), Prof. Dušica Stamenković, MD, PhD (Anesthesiology), Assoc. Prof. Zvezdana Stojanović, MD, PhD (Neuropsychiatry), and Col. Prof. Miroslav Vukosavljević, MD, PhD (Ophthalmology) (in alphabetical order); one member from the Faculty of Medical Sciences, University of Kragujevac, Prof. Vladimir Jakovljević, MD, PhD (Medical Physiology).

Additionally, the international EB of VSP welcomed new members – distinguished subject matter experts with great professional reputations in the international scientific community. It was a privilege that the following experts accepted to become international EB members of VSP: Assoc. Prof. (ret.) Mario Abinun, MD, PhD, from the Faculty of Medical Sciences, Newcastle University, UK (Pediatric Immunology); Prof. Dejan Bokonjić, MD, PhD, from the Faculty of Medicine Foča, University of East Sarajevo, Bosnia and Herzegovina (Pediatric Pulmonology); Prof. Marla Dubinsky, MD, from the Icahn School of Medicine, Mount Sinai New York, USA (Gastroenterology); Prof. David A. Geller, MD, from the University of Pittsburgh, USA (Hepatobiliary and Pancreatic Surgery); Prof. Predrag Gligorović, MD, MHA, from the Wake Forest University School of Medicine, USA (Psychiatry/Behavioral Medicine); Prof. Zoran Ivanović, MD, PhD, from the French Blood Institute *Nouvelle Aquitaine*, Bordeaux, France (Stem and Progenitor Cell Biology/Cell Therapy); Prof. Nebojša Nick Knežević, MD, PhD, from the College of Medicine, University of Illinois, Chicago, USA (Anesthesiology/Pain medicine); Assist. Prof. Boštjan Lanišnik, MD, PhD, from the University Medical Center Maribor, Slovenia (Rhinology/Otolaryngology); Prof. (ret.) Desa Lilić, MD, PhD, from the Faculty of Medical Sciences, Newcastle University, UK (Clinical Immunology); Prof. Janko Ž. Nikolich, MD, PhD, from the University of Arizona College of Medicine-Tucson, USA (Immunology/Gerontology); Prof. Mirjana D. Pavlović, MD, PhD, from the Florida Atlantic University, Florida, USA (Bioengineering); Prof. Vesna Petronić-Rosić, MD, MSc, from the University of Illinois Chicago, USA (Dermatology); Assoc. Prof. Chaitanya P. Puranik, MDS, PhD, from the School of Dental Medicine University of Colorado, USA (Pediatric Dentistry/Dental Materials); Prof. Corey A. Siegel, MD, MSc, from the Dartmouth Hitchcock Medical Center, New Hampshire, USA (Gastroenterology); Assoc. Prof. Lina Zuccatosta, MD, from the Interventional Pulmonology Unit Antonio Cardarelli Hospital Naples, Italy (Respiratory Diseases/Interventional Pulmonology) (in alphabetical order).

We would like to thank all former EB members of VSP whose mandate has been terminated. Special thanks go to the following former members, in alphabetical order: Col. Assoc. Prof. Srđan Lazić, MD, PhD; Col. Prof. (ret.) Dragan Mikić, MD, PhD; Prof. Branka Nikolić, MD, PhD; Col. Prof. Slobodan Obradović, MD, PhD; Assoc. Prof. Biserka Vukomanović Đurđević, MD, PhD. These people spared no time in the last three years and invested great effort to elevate the journal's reputation. We truly believe that our successful collaboration will continue in the future.

Once again, we would like to thank the new members of the EB for their willingness to accept this responsible duty and make the maximum contribution to the further improvement of the journal. We wish all the members of the EB a lot of success in their editorial work, and we hope that in the next three years, we will achieve, if not all, most of the set goals.

According to the previous practice, the first issue of the journal's new volume will present an overview of the work of

its Editorial Office and EB in the past year, emphasizing the main characteristics of the received and published articles.

Last year, a total of 292 manuscripts were received. The number of received manuscripts was higher for 66 manuscripts compared to the previous year when that number was 226 and got closer to the number of manuscripts received in 2020 and 2021, 280 and 278, respectively. The submitted articles covered various topics related to clinical and experimental medicine, dentistry, and pharmacy. As usual, the largest number was from the category Original Articles (207 or 70.9%) and Case Reports (46 or 15.8%) (Table 1).

Table 1
Categories and the number of manuscripts submitted to the *Vojnosanitetski Pregled* in 2024

Category	Manuscripts
	n (%)
Original Articles	207 (70.9)
Case Reports	46 (15.8)
Current Topics	10 (3.4)
General Review	8 (2.7)
History of Medicine	6 (2.1)
Letter to the Editor	5 (1.7)
Editorial	4 (1.4)
Meta-analysis	4 (1.4)
Short Communications	1 (0.3)
Book Review	1 (0.3)
Total	292 (100)

The analysis of the institutions where the authors of submitted papers work showed that 88.8% of authors came from so-called civilian health and academic institutions, of which about 46.5% were from abroad, and 11.2% were from the University of Defence in Belgrade.

We are especially proud of foreign authors for submitting their manuscripts from 16 countries, not only from our region but also from all around the world: Bosnia and Herzegovina, Bulgaria, China, Croatia, India, Iran, Italia, Montenegro, North Macedonia, Qatar, Russia, Saudi Arabia, Slovakia, Slovenia, Turkey, and the United States of America (in alphabetical order). Thank you for your trust. A particularly pleasing fact is that the percentage of papers submitted by (co)authors from abroad increased in 2024 (47.7%) compared to 2023 (32.1%), indicating an increased interest in our journal in the international scientific community. It is expected that the number of papers published by foreign authors will increase in the next period, thus further affecting the international recognition of the VSP.

We declined 49.1% of manuscripts in the pre-review phase, and 50.8% entered the review stage.

Among reviewed manuscripts, until December 30, 2024, we accepted 48.6% for publication (after correction) and rejected 20.5%. The remaining 30.8% are still in the process of peer review. The total number of articles that were in the process of receiving DOI numbers in 2024 (including the ones submitted for consideration before 2024) was 92. They will be published in printed form in future issues.

In 2024, we published 102 articles, including 2 book reviews (Table 2). The number of published manuscripts was lower than in the previous years. Part of the reason for that was the decision of the EB to reduce the number of published papers *per* volume since the influx of papers during 2022 and 2023 was smaller, 264 and 222, respectively, compared to previous years. Simultaneously, the percentage of rejected papers in the pre-review stage in 2024 increased by 5% compared to 2023. As in previous years, the majority of the published articles were classified as Original Articles (68 or 66.7%) and Case Reports (15 or 14.7%). After analyzing the authors' affiliations from the published articles, it could be seen that the largest number of authors were from the so-called civilian institutions, domestic and foreign (81.4%), of which about 22.4% were from abroad, followed by authors from the University of Defence in Belgrade (18.6%).

Once more, we would like to thank most sincerely all the authors who chose VSP to present their results, especially the foreign authors. We hope that with your assistance and support, we will be able to increase the VSP's impact in the scientific and academic community.

According to the Center for Evaluation in Education and Science database, the average time from submission to first decision in 2024 was 47 days, and from submission to online ahead-of-print publication was 175 days which was approximate to the value of the same parameters of the editorial review process in 2023, 51 and 209 days, respectively.

According to the EBSCO database, the number of downloads of the VSP articles published in 2024 was 4,903. The number of downloads was 1,264 times higher compared to the previous year. In total, 10 articles published in 2024 were downloaded more than 100 times (Table 3), and 77 articles were downloaded more than 20 times. The highest

Table 2

**Categories and the number of articles published
in the *Vojnosanitetski Pregled* in 2024**

Category	Articles
	n (%)
Original Articles	68 (66.7)
Case Reports	15 (14.7)
Editorial	4 (3.9)
History of Medicine / History of Dentistry	4 (3.9)
Letter to the Editor	3 (2.9)
General Review	2 (2)
Current Topic	2 (2)
Book Review	2 (2)
Short Communications	1 (1)
Preliminary Report	1 (1)
Total	102 (100)

Table 3

The articles downloaded more than 100 times arranged according to their number of downloads (N)

Article title (DOI)	Corresponding author	N
Association between eating habits and low physical activity in adolescents (https://doi.org/10.2298/VSP230131058D)	Dragan Djurdjević	396
Assessment of depression in patients with open-angle glaucoma (https://doi.org/10.2298/VSP230831008C)	Aleksandra Cvetković	197
Job satisfaction of healthcare professionals in palliative care departments and factors affecting job satisfaction during the COVID-19 pandemic (https://doi.org/10.2298/VSP230831060S)	Olivera Milovanović	161
Hyperoxia therapy for prevention of postoperative nausea and vomiting after breast cancer surgery (https://doi.org/10.2298/VSP230522059M)	Nora Mihalek	131
Does hospital medical staff maintain hospital disaster resilience? (https://doi.org/10.2298/VSP230727067G)	Mariya Georgieva Georgieva	123
Sex differences in the prognostic value of computed tomography pulmonary angiography parameters for intrahospital acute pulmonary embolism-related death (https://doi.org/10.2298/VSP240109023B)	Slobodan Obradović	121
Sociodemographic factors related to internet addiction among adolescents in Serbia (https://doi.org/10.2298/VSP240307037P)	Teodora Safiye	117
Hospital professional staff awareness of hospital plans for the provision of teams for medical support in case of disasters (https://doi.org/10.2298/VSP231229015G)	Mariya Georgieva Georgieva	112
Translation, transcultural adaptation, and validation of the Serbian version of the University of Washington Quality of Life (UW-QoL) Questionnaire – a pilot study (https://doi.org/10.2298/VSP231020002S)	Dragana Stanišić	109
Thymic hyperplasia as a rare etiology of pure red cell aplasia (https://doi.org/10.2298/VSP230803045A)	Isidora Arsenović	102

number of downloads of an article was 396, and the title of that article is *Association between eating habits and low physical activity in adolescents* (DOI: <https://doi.org/10.2298/VSP230131058D>). In total, 2,489 universities, colleges, libraries, and other institutions worldwide accessed the papers published in VSP in 2024 more than 69,970 times. In the first two places were Pukyong National University (South Korea) with 9,631 downloads and Stanford University (USA) with 6,894 downloads. That encourages us to continue our editorial policy in 2025. We hope that, in the future, we will be able to continue publishing educational and important papers for all our readers.

The VSP impact factor for 2023, published in July 2024, did not change compared to the previous year (2022), and it was 0.2. Therefore, one of our key goals should be to restrict the selection of papers and publish only those that will attract the scientific community's attention and have the potential to be cited for the quality and topic they cover. To accomplish this goal, we require great involvement of all members of the EB of VSP. In addition, it is necessary to select reviewers who are not only subject matter experts but are also willing to invest their time and knowledge in enhancing the journal's quality.

During 2024, we were very fortunate to receive the support of 229 reviewers (Table 4). We are using this opportunity to express our gratitude to all of them. Our special thanks go to Professors Gordana Dedić, Ljubica

Đukanović, Slobodan Janković, Smiljana Kostić, Milan Lepić, Saša Milenković, Nataša Petronijević, and Marina Svetel (in alphabetical order) who reviewed more than two manuscripts during 2024.

The equally big challenge we have been facing for many years is how to motivate domestic authors to publish the best results of their scientific work in VSP and not in "stronger" scientific journals. To overcome this challenge, we expect support from the VSP Publishing Advisory Board, whose members are always willing to listen to the key issues in the Editorial Office activities and offer support in critical times. Their understanding and willingness to help were and will remain outstanding support for the journal.

Furthermore, this is the opportunity to thank all the associates of the VSP Editorial Office, especially those who contributed maximally to publishing all 12 journal issue numbers planned for 2024 on time.

Looking ahead, we encourage all our authors and readers to suggest the changes they consider relevant to help us strengthen our position among the most valued journals from the biomedical science field.

As we begin this new year, let us all have a renewed sense of purpose for our work. We hope 2025 brings us highly cited publications and, of course, excellent health and good relationships with our family and friends. We wish you all a happy and prosperous New Year and warmly welcome submissions of excellent new articles in 2024.

Table 4

Reviewers of the *Vojnosanitetski pregled* in 2024

Adžić Tatjana	Ćupurdija Vojislav	Gebauer-Bukurov Ksenija	Končar Igor
Ajduk Marko		Georgievski Brkić Biljana	Kostić Dejan
Amidžić Jelena	Dacić Sanja	Glišić Miodrag	Kostić Milena
Andrić Filipović Snežana	Damjanov Dimitrije	Grubović Rastvorčeva Rada	Kostić Smiljana
Antić Ana	Darkwah Oppong Marvin	Gullo Giuseppe	Kovačević Aleksandra
Arsenović-Ranin Nevena	Davidović Lazar		Kravljaja Milica
Atanasković Lavinika	Dedić Gordana	Ilić Jugoslav	Krivošejev Vladimir
	Delić Dragan	Ilić Živojinović Jelena	Krstovski Nada
Babić Aleksandra	Denda Dalibor	Ivetić Dražen	
Bajkin Branislav	Dimić Janjić Sanja		Laušević Mirjana
Bančević Vladimir	Dinčić Evica	Jačević Vesna	Lazarević Gordana
Basta Nikolić Marijana	Doknić Mirjana	Janković Slobodan	Lepić Milan
Baščarević Zoran	Dragičević Danijela	Janković Srdja	Lepić Toplica
Begović Kuprešanin Vesna	Dragović Tamara	Jelić Svetlana	Lučić Miloš A.
Bezmarević Mihailo	Dukanac Vesna	Jocić Miodrag	Lučić Silvija
Bila Jelena		Jovanović Dejan	Lukić Ljiljana
Biočanin Vladimir	Džopalić Tanja	Jovanović Dragan	
Božić Bojana		Jovanović Dragana	Manojlović Nebojša
Božić Marija	Djokić Dragoljub	Jovanović Vesna	Marčetić Mirjana
Božić Milena	Djordjević Boban	Jović Marko	Marić Dragana
Brajušković Goran	Djordjević Dragan	Jović Rajko	Marić Gorica
Brašanac Dimitrije	Djordjević Miroslav	Jović Zoran	Marić Nadja
Brdareski Zorica	Djukanović Ljubica	Jovičić Bojan	Marić Nebojša
Brkić Zlata	Djurić Milanko	Jovičić Jelena	Marjanović Ivan
Burić Nikola			Marković Evgenija
	El Rahal Amir	Karan Radmila	Marković Vladana
Cerovac Nataša	Elez Marija	Kesić Ljiljana	Matić Milan
Cikota Bojana		Kezić Aleksandra	Matijašević Jovan
	Filipović Branko	Kiković Saša	Matijević Stevo
Čvorović Ljiljana	Folić Miljan	Knežević Vladimir	Mihailović-Stanojević
		Kocić Gordana	Nevena
Ćeranić Miljan	Gajić Branislava	Kojović Vladimir	Mijatović Jovanović Vesna

Table 4 (continued)

Mijušković Željko	Odalović Marina	Ristanović Elizabeta	Tadić Ivana
Milenković Branislava		Ristić Dragana	Tarabar Dino
Milenković Marina	Padjen Višnja	Ristić Petar	Tepšić Ostojić Vesna
Milenković Saša	Pajić Matić Ivana	Roganović Jelena	Testarelli Luca
Miličić Vesna	Pasin Ōzge	Roš Tatjana	Tiplica George-Sorin
Milić-Lemić Aleksandra	Pavlović Milorad	Rotim Krešimir	Todorović Balint Milena
Miljević Čedo	Pavlović Nebojša		Todorović Ljubomir
Milojević Milan	Pejčić Ana	Safiye Teodora	Todorović Veljko
Miloradović Vladimir	Pekmezović Tatjana	Samardžić Predojević	Todorović Zoran
Milosavljević Marko	Perić Aneta	Jelica	Trifunović Zoran
Milošević Ivana	Pešić Anita	Savić Slobodan	Trivunić Dajko Sandra
Milošević Vuk	Pešut Dragica	Sekulić Igor	
Milovanović Andjela	Petronijević Milan	Semnic Marija	Unić Dragana
Milovanović Dragan	Petronijević Miloš	Sinobad Vladimir	Urošević Ivana
Milovanović Srdjan	Petronijević Nataša	Sladojević Miloš	
Mirković Ljiljana	Petrović Sladjana	Smiljanić Kosara	Vanmarcke Cedric
Mišović Miroslav	Pilčević Dejan	Srzentić Snežana	Vasović Dolika
Mitevska Irena	Polovina Snežana	Stanetić Mirko	Veličkov Aleksandra
Mitić Branka	Popadić Svetlana	Stankov Karmen	Vlahović Zoran
Mitković Milan	Popović Dušan	Stanković Nebojša	Vojinović Radiša
Mladenović Raša	Popović Jelena	Stanojević Ivan	Vorgučin Ivana
Mojsilović Slavko	Popović Milica	Stepić Nenad	Vučković Ljiljana
Munitlak Staša		Stević Marija	Vujašković Mirjana
	Radjen Slavica	Stojičić Milan	Vujčić Isidora
Nejković Lazar	Radević Svetlana	Stojković Branislava	Vukmirović Saša
Nešković Vojislava	Radoičić Dragan	Stojković Mirjana	Vukomanović Aleksandra
Nikolić Jakoba Nataša	Radunović Aleksandar	Svetel Marina	Vuković Natalija
Nikolić Jelena	Rahelić Dario		
Nožić Darko	Rančić Nemanja	Šarac Sanja	Živanović Željko
	Rasulić Lukas	Šuljagić Vesna	Živković Slavoljub
Obradović Rada	Ratković Dragana	Šurbatović Maja	
Obradović Slobodan	Resan Mirko	Šušnjević Sonja	Zubelić Aleksa



Bibliometric analysis of the 200 most cited papers in the journal *Vojnosanitetski pregled*

Bibliometrijska analiza 200 najcitiranijih radova časopisa *Vojnosanitetski pregled*

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Equal contribution and shared first authorship

Abstract

Background/Aim. *Vojnosanitetski pregled* (VSP) is the official scientific and professional journal of the University of Defence in Belgrade, Serbia. VSP is a peer-reviewed journal that publishes a wide range of scientific and professional articles. The aim of the study was to perform a bibliometric analysis of the 200 most cited articles published in VSP, and to assess the impact, significance, and scientific contribution of the journal. **Methods.** Using the Web of Science (WoS) Core Collection (WoSCC) database, 2,664 articles published from 2008 to December 31, 2022, were analyzed, focusing on citation counts, author productivity, and collaboration networks. **Results.** The analysis revealed that the majority of the most cited articles came from Serbian institutions, with the University of Belgrade being the most prolific contributor. The top five most cited authors as well as the most cited article were identified, and a trend of increasing significance of research on bioactive compounds was noticed. The study also observed a shift in key word usage over time, reflecting changes in research trends within the journal. The collaboration network analysis showed a strong clustering among the leading authors, indicating a collaborative culture that contributes to the journal's influence. **Conclusion.** Emphasizing the importance of targeted collaborations makes it evident that maintaining and expanding the journal's impact on the scientific community is the key to improving medical research. Despite limitations such as reliance on a single database, the findings provide valuable insights into the journal's role in advancing medical research and highlight the importance of targeted collaboration.

Key words:

bibliometrics; interdisciplinary communication; journal impact factor; research.

Apstrakt

Uvod/Cilj. *Vojnosanitetski pregled* (VSP) je naučni i stručni časopis Univerziteta odbrane u Beogradu, Srbija. VSP je časopis sa recenziranim radovima, koji objavljuje širok spektar naučnih i stručnih članaka. Cilj studije bio je da se izvrši bibliometrijska analiza 200 najcitiranijih članaka objavljenih u VSP-u, i da se procene uticaj, značaj i naučni doprinos časopisa. **Metode.** Korišćenjem baze podataka *Web of Science* (WoS) *Core Collection* (WoSCC), analizirano je 2 664 članaka objavljenih od 2008. do 31. decembra 2022. godine, sa fokusom na broj citata, produktivnost autora i mreže saradnje. **Rezultati.** Analiza je pokazala da je većina najcitiranijih članaka potekla iz srpskih institucija, pri čemu je Univerzitet u Beogradu imao najplodniji doprinos. Identifikovano je prvih pet najcitiranijih autora kao i najcitiraniji članak, i zapažen je trend porasta značaja istraživanja bioaktivnih jedinjenja. Uočena je i promena u korišćenju ključnih reči tokom vremena, koja odražava promene u istraživačkim trendovima u okviru časopisa. Analiza mreže saradnje pokazala je snažno grupisanje vodećih autora, što ukazuje na kulturu saradnje koja doprinosi uticaju časopisa. **Zaključak.** Isticanjem važnosti ciljanih saradnji evidentno je da je održavanje i proširivanje uticaja časopisa na naučnu zajednicu ključno za unapređenje medicinskih istraživanja. Uprkos ograničenjima, kao što je oslanjanje na samo jednu bazu podataka, nalazi pružaju dragocene uvide u ulogu časopisa u unapređenju medicinskog istraživanja i naglašavaju važnost ciljanih saradnji.

Ključne reči:

bibliometrija; komunikacija, interdisciplinarna; časopis, impakt faktor; istraživanja.

Introduction

Vojnosanitetski pregled (VSP) is the official scientific and professional journal of the University of Defence in Belgrade, Serbia¹. It is a peer-reviewed journal that publishes original research, review articles, and case reports on a wide range of topics related to military medicine, including surgery, internal medicine, neurology, otorhinolaryngology, dermatology, radiology, and anesthesiology². The journal also publishes editorials, book reviews, and reports on scientific meetings and conferences. VSP is published in English, but the abstracts are written both in Serbian and English. The journal is available online through the website <https://www.vsp.mod.gov.rs>. It is indexed in several databases, including PubMed (1950-2017), Web of Science (WoS) Core Collection (WoSCC), and Scopus³. Several factors can influence the impact and influence of a scientific journal, such as the quality of the research published, the journal's reputation, the editorial board, the number of citations received by its articles, and the journal's visibility and accessibility. Other influential factors include the journal's impact factor (citation frequency of the journal's articles) and the submission and acceptance rates, which indicate the journal's competitiveness and selectivity^{4,5}. Ultimately, the influence of a scientific journal depends on the recognition and respect it garners from the scientific community, influenced by various factors⁶. Bibliometric analysis is a study of publication characteristics, such as citation counts or impact factors, that can be used to evaluate the quality and significance of the work. Bibliometric analyses are often employed to assess the impact of research or identify trends in a particular field^{7,8}. They are useful for identifying influential scholars, institutions, or research topics and comparing the relative importance of different publications. Common metrics used in bibliometric analysis include citation counts, impact factor, and the H-index, which measures the productivity and impact of a researcher's work^{9,10}. However, it is important to acknowledge that bibliometric analyses have limitations and should be used with caution, as they do not always accurately reflect the quality or importance of a publication. Conducting a bibliometric analysis of VSP can serve several purposes. For instance, such an analysis can provide insight into the journal's impact and influence, its authors' productivity and citation patterns, and broader trends within the field. This information is valuable for researchers, institutions, and funding agencies because it can help them evaluate the quality and significance of the research published in the journal and identify emerging trends and patterns in military medicine and related fields.

This study presents a bibliometric analysis of the 200 most cited articles in VSP. The primary aim was to evaluate the impact and influence of the journal, as well as to understand the scholarly contributions and citation patterns.

Methods

The WoS database analysis was performed from January 1, 2008 to December 31, 2022, for manuscripts published in VSP. The search of the WoSCC using the search term

"vojnosanitetski pregled" returned 2,664 articles. The year 2008 was selected to align with the impact factor (IF) calculation process, which considers citation data from two years prior. This choice allows us to capture the earliest relevant citation activity that contributes to the journal's IF, offering a comprehensive view of its scholarly impact from the beginning of the IF assessment period. Starting in 2008, we ensure a more accurate and representative analysis of the journal's influence over time. Manuscripts were ranked according to citation count, and the 200 most cited articles, along with their publishing data assorted into digital files, were analyzed. The top 200 most cited articles were selected as they represent the most impactful and frequently referenced studies within the journal, providing a focused view of the core contributions that have shaped its academic reputation. Analyzing this subset allows for a meaningful evaluation of the journal's influence on the scientific community by highlighting the research that has gained the greatest recognition and citation over time. This text file was imported into Bibliometrix¹¹ software, where a detailed analysis was performed. The results were presented in tables and figures. The WoS database was chosen for its high recognition and established credibility in assessing journal impact and citation metrics. The WoS is considered a more selective database, indexing only journals that meet stringent quality standards, which makes it particularly suitable for evaluating the global influence of a journal. Additionally, using the WoS provides consistent and comparable citation data, aligning with the aim of the study to provide a robust bibliometric analysis based on the most authoritative source. Within this analysis, a directed graph visualizes elements as nodes, with the connections between them represented by links. Nodes that share the same color are grouped into clusters or communities. The size of each node and its labels reflect its significance, with larger nodes indicating a greater number of connections to others. The length of the links demonstrates the strength of the relationship between nodes, and shorter links denote stronger connections.

Results

A total of 200 VSP publications with the highest citation count were mostly written by authors from Serbia (178 articles, 2,233 citations), followed by authors from Montenegro (6 articles, 62 citations), North Macedonia (4 articles, 50 citations), Bosnia and Herzegovina (4 articles, 46 citations), and Romania (2 articles, 35 citations) (Figure 1).

Authors from the University of Belgrade contributed to 161 articles, the University of Defence in Belgrade to 105 articles, the University of Novi Sad, Serbia to 46 articles, and the University of Kragujevac, Serbia and the University of Niš, Serbia to 43 articles each (Table 1).

The top five authors were Jovanović Milan (13 publications, 174 citations, H-index 10), Lazić Zoran (8 publications, 103 citations, H-index 8), Janković Slobodan (6 publications, 96 citations, H-index 8), Stojanović Miodrag (6 publications, 75 citations, H-index 6), and Pekmezović Tatjana (5 publications, 65 citations, H-index 5) (Table 2). The authorship timeline is given in Figure 2.

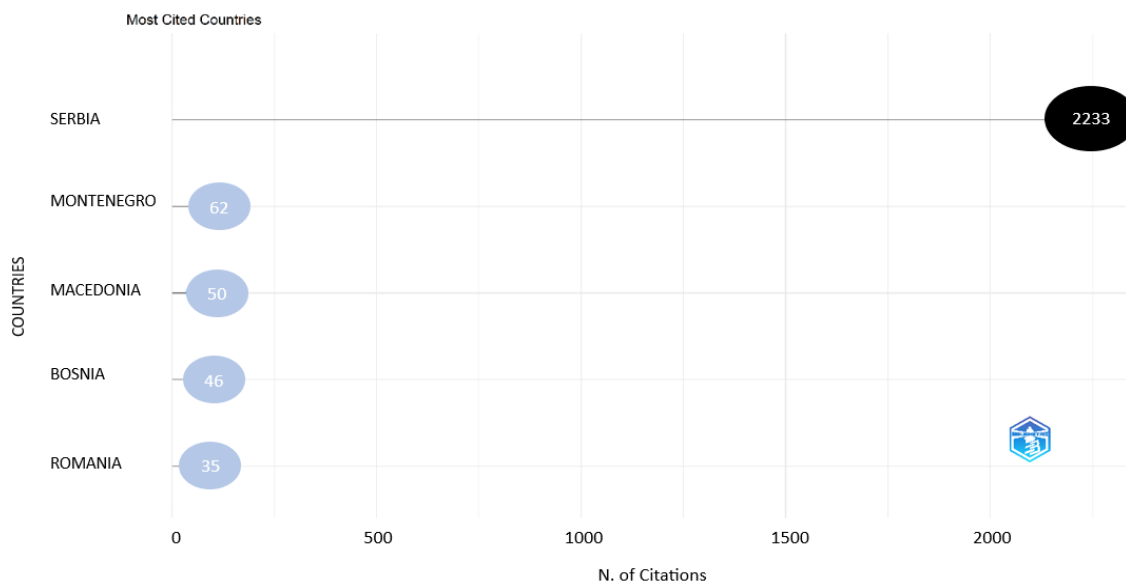


Fig. 1 – Most cited countries.

Table 1

Institutional contribution

Institution	Publication count
University of Belgrade, Belgrade, Serbia	161
University of Defence, Belgrade, Serbia	105
University of Novi Sad, Novi Sad, Serbia	46
University of Niš, Niš, Serbia	43
University of Kragujevac, Kragujevac, Serbia	43

Table 2

Top 5 authors

Author	Publication count	Citation count	H-index
Jovanović Milan	13	174	10
Lazić Zoran	8	103	8
Janković Slobodan	6	96	8
Stojanović Miodrag	6	75	6
Pekmezović Tatjana	5	65	5

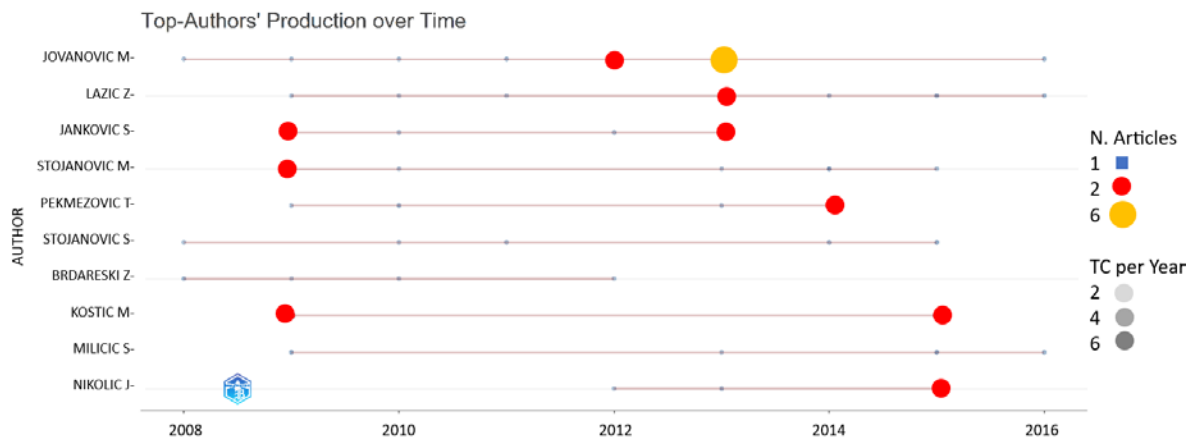


Fig. 2 – Top 10 author production timeline.

The document with the highest citation count was authored by Kuntić V, Brborić J, Holclajtner-Antunović I, and Uskoković-Marković S, titled “Evaluating the bioactive effects of flavonoid hesperidin – a new literature data survey,” published in VSP in January 2014. It was cited 38

times in the WoSCC. The authors with the most co-authorship connections were Jovanović Milan and Lazić Zoran; the latter author’s network inter-acted with three other networks (Figure 3). This figure illustrates the collaboration networks among the most prolific authors, highlighting the

strength and extent of their collaborative efforts. The size of the nodes indicates the number of articles published by each author, while the thickness of the links represents the strength of co-authorship connections. The five most common key words were “treatment outcome”

(27 occurrences), “risk factors” (25 occurrences), “serbia” (19 occurrences), “diagnosis” (18 occurrences), and “quality of life” (17 occurrences). The timeline of word dynamics, key word evolution, and thematic mapping is presented in Figures 4, 5, and 6.

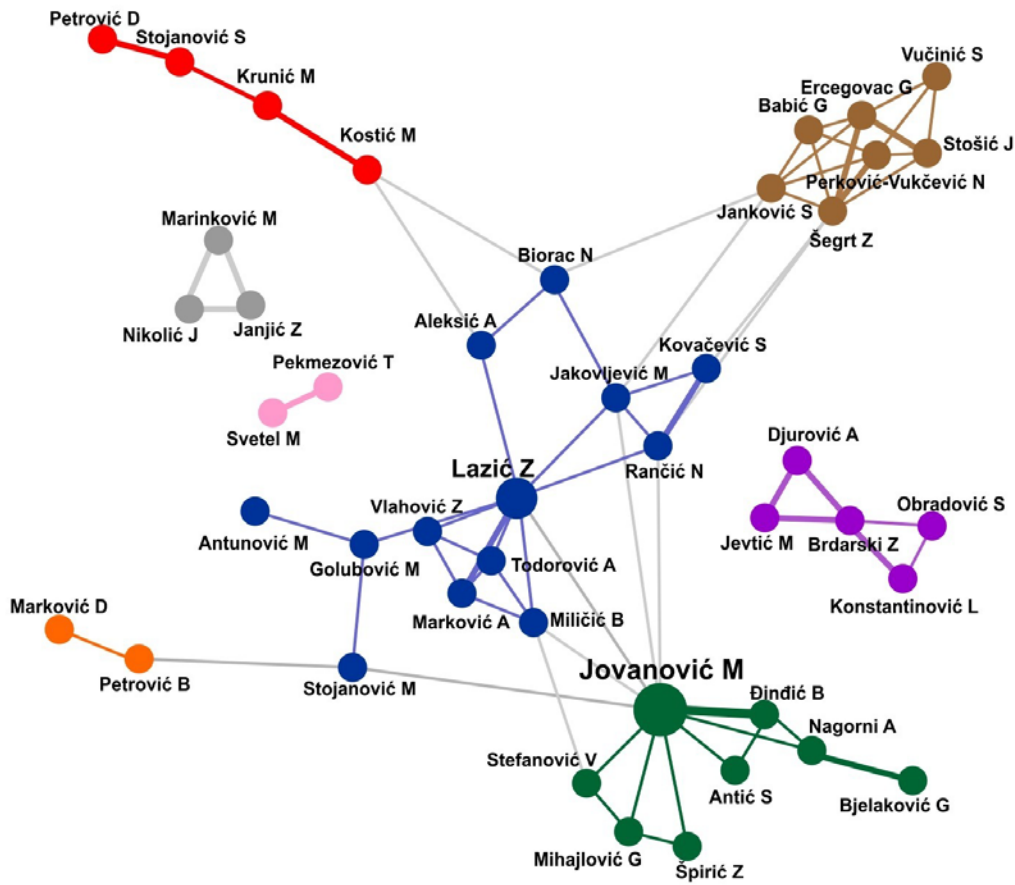


Fig. 3 – Collaboration network.

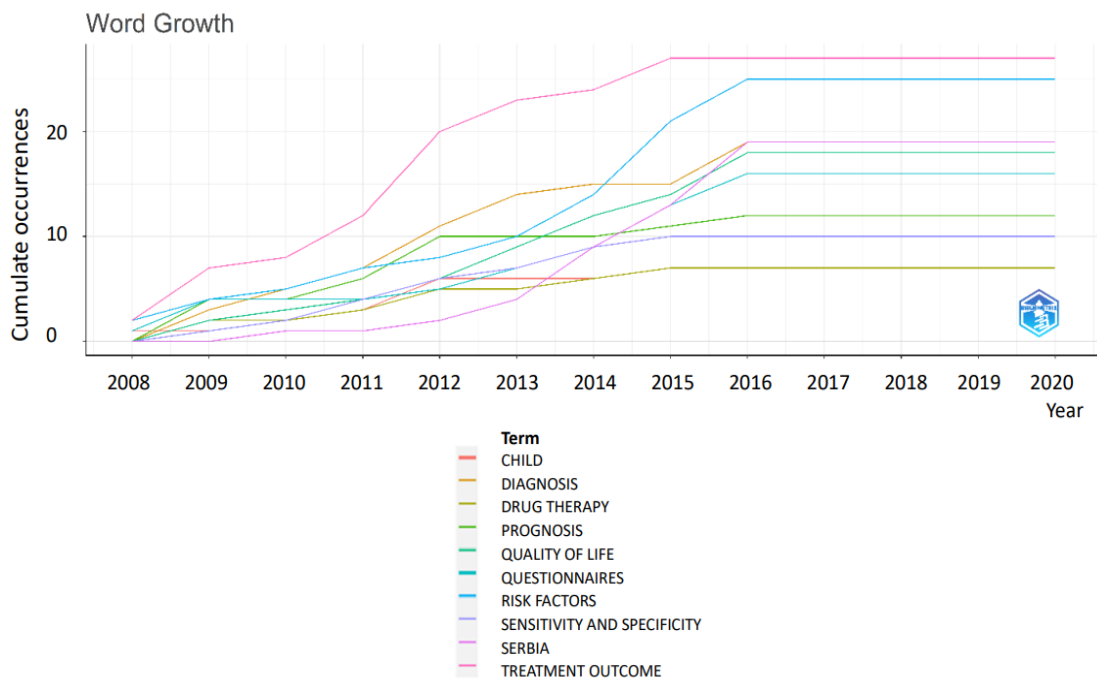


Fig. 4 – Key word dynamic.

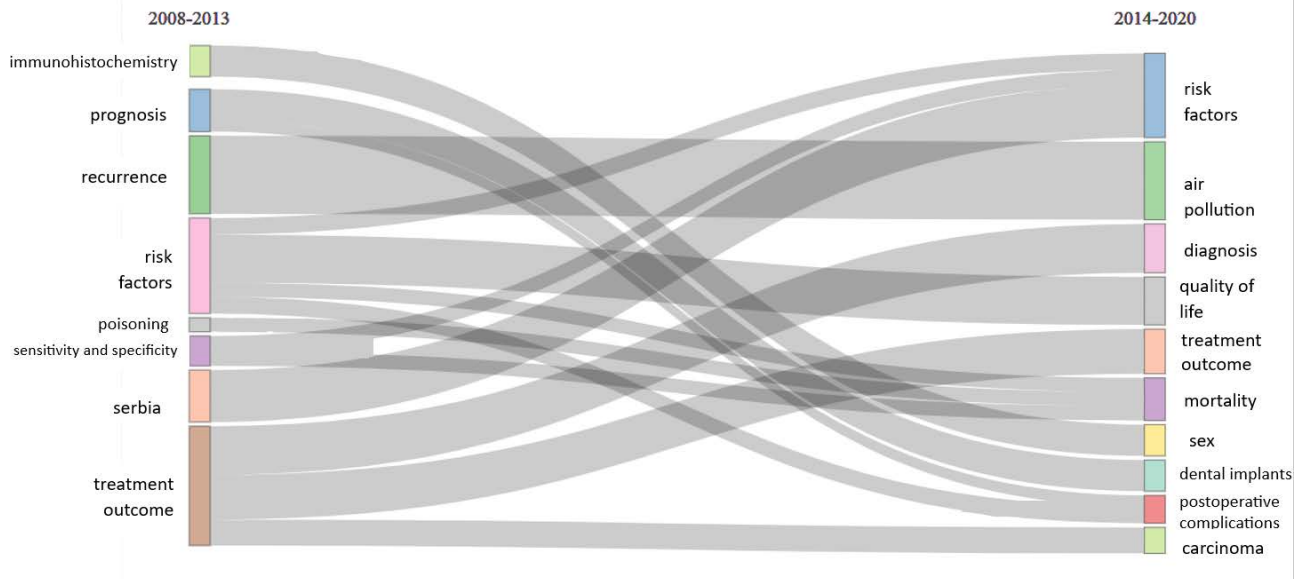


Fig. 5 – Key word evolution.

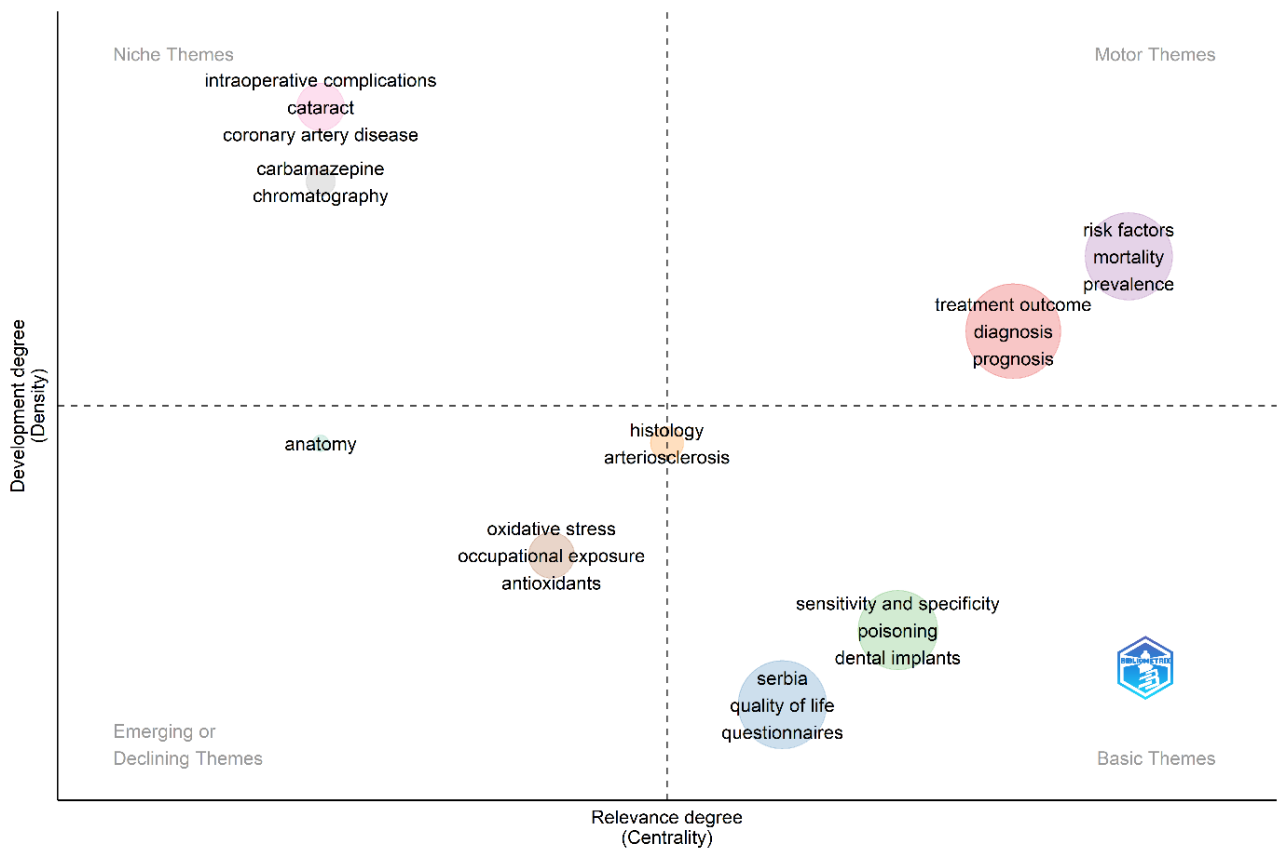


Fig. 6 – Key word thematic map.

Discussion

The analysis identified Jovanović Milan as the most cited author, with the highest number of publications and the highest H-index. The most cited individual article was authored by Kuntić V et al. ¹², highlighting the influence of research on bioactive compounds in the journal’s citation landscape. The key word analysis revealed a shift from

general key words (e.g., “risk factors,” “outcome”) used between 2008 and 2013 to more specific key words (e.g., “quality of life,” “dental implants”) from 2014 to 2020. This shift likely reflects the evolving focus of the journal in response to global and national trends in medical research. For instance, the increased use of “air pollution” as a key word after 2014 aligns with Serbia’s national efforts to address air quality issues and public awareness

campaigns¹³. The collaboration network analysis demonstrated the interconnectedness of the leading authors, with significant clustering around a few key individuals, such as Jovanović Milan and Lazić Zoran. This suggests a strong collaborative culture within the journal, which may contribute to its influence in the field.

While this bibliometric analysis provides valuable insights into VSP, the method has inherent limitations. The limitation of the study was that the analysis was restricted to articles indexed only in the WoSCC, potentially excluding relevant papers not captured by this database¹⁴. Despite this limitation, using a reputable and widely recognized database like the WoSCC ensures that the results represent the impact of the journal in the broader scientific community.

Conclusion

This bibliometric analysis offers a detailed overview of the most cited articles in *Vojnosanitetski pregled*, highlighting key contributors, evolving research trends, and the impact of the journal on the medical field. Further studies could expand on this work by incorporating additional databases and exploring the relationship between bibliometric indicators and the journal's influence over time. Future research could explore the evolving role of collaborative networks in enhancing the visibility and impact of research published in the journal, particularly in emerging fields of study. The findings underscore the importance of collaboration and targeted research in advancing both clinical practice and academic knowledge.

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Deficits in naming and auditory comprehension of terms in individuals with vascular dementia

Deficiti imenovanja i auditivnog razumevanja pojmova kod osoba sa vaskularnom demencijom

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Abstract

Background/Aim. Although deficits in naming and understanding named terms are characteristic of dementia, they have seldom been investigated in individuals with vascular dementia (VaD). The aim of the study was to determine deficits in naming objects and actions in individuals with VaD and the ability to understand the meanings of words used to name objects and actions. **Methods.** The study included 30 participants with VaD, who represented the clinical group, while the control group consisted of 30 neurologically healthy participants. Participants with VaD were first assessed using the Mini-Mental State Examination to determine the severity of dementia. The Northwestern Naming Battery was used to assess naming and auditory comprehension of terms. Descriptive and inferential statistical methods were used for data analysis. Group comparisons were conducted using the Chi-squared (χ^2) test of independence, while for 2×2 contingency frequency tables, continuity correction according to Yates was

applied, and the *phi* (ϕ) coefficient was calculated as an indicator of effect size. The relationships between continuous variables were expressed using Spearman's rank correlation coefficient *rho* (ρ). **Results.** Participants with VaD demonstrated significantly lower performance on the subtest of naming and auditory comprehension of named objects and actions compared to neurologically healthy participants. The severity of dementia significantly influenced the performance on the administered test. Specifically, participants with moderate dementia exhibited significantly lower scores on all subtests of naming and comprehension of named objects compared to participants with milder dementia. **Conclusion.** Individuals with VaD exhibit pronounced deficits in naming and understanding named terms. The ability to name and understand named terms significantly declines with the progression of dementia.

Key words:

auditory perceptual disorders; cerebrovascular disorders; cognition; dementia, vascular; neuropsychological tests.

Apstrakt

Uvod/Cilj. Mada su deficiti imenovanja i razumevanja imenovanih pojmova karakteristični za demenciju, oni su veoma malo istraživani kod osoba sa vaskularnom demencijom (VaD). Cilj rada bio je da se utvrdi deficit imenovanja predmeta i radnji kod osoba sa VaD, kao i sposobnost razumevanja značenja reči kojima se imenuju predmeti i radnje. **Metode.** U studiju je bilo uključeno 30 ispitanika sa VaD koji su predstavljali kliničku grupu dok je kontrolnu grupu činilo 30 neurološki zdravih ispitanika. Ispitanici sa VaD su najpre testirani korišćenjem *Mini-Mental State Examination* upitnika radi utvrđivanja težine demencije. Za procenu imenovanja i auditivnog razumevanja pojmova primenjena je Severozapadna baterija testova za imenovanje. U statističkoj obradi

podataka korišćene su metode deskriptivne i inferencijalne statistike. Za poređenje grupa primenjen je Hi-kvadrat (χ^2) test nezavisnosti, dok je za bivarijantne frekvencijske nacрте tipа 2×2 uračunata korekcija neprekidnosti prema Jejtсу, a koeficijent *phi* (ϕ) izračunat je kao pokazatelj veličine uticaja. Odnosi između neprekidnih varijabli izraženi su koeficijentom Spirmanove korelacije ranga *rho* (ρ). **Rezultati.** Ispitanici sa VaD imali su značajno niža postignuća na subtestu imenovanja i auditivnog razumevanja imenovanih objekata i radnji u poređenju sa neurološki zdravim ispitanicima. Težina demencije značajano je uticala na rezultate na primenjenom testu. Posebno, ispitanici sa umerenim stepenom demencije imali su značajno niža postignuća na svim subtestovima imenovanja i razumevanja imenovanih pojmova u poređenju sa ispitanicima sa lakšim stepenom demencije.

Zaključak. Osobe sa VaD imaju izražene deficite u imenovanju i razumevanju imenovanih pojmova. Sposobnost imenovanja kao i razumevanja imenovanih pojmova značajno slabi sa napredovanjem demencije.

Ključne reči: slušna percepcija, poremećaji; cerebrovaskularni poremećaji; saznanje; demencija, vaskularna; testovi, neurofiziološki.

Introduction

Vascular dementia (VaD) is a cognitive function disorder caused by vascular brain lesions¹. Cognitive deficits are observed in the domain of complex attention, executive functions, and language. Although language disorders in VaD clinically manifest clearly, they receive little attention in scientific literature. Regarding linguistic abilities, naming has been the focus of most research²⁻⁴, and disturbances in this aspect of language are most frequently described in the literature^{5,6}.

Naming objects or abstract entities is a multimodal process of cortical networks involving visual processing and recognition, semantic processing, abstract representation, and verbal word production^{7,8}. Various cortical areas are involved in the naming process, including the temporal, temporoparietal, temporooccipital, and frontal regions of the left hemisphere⁸⁻¹¹. Considering that the naming process involves multiple brain regions^{11,12} and that VaD represents a heterogeneous group of disorders, including multi-infarct dementia and dementia due to strategically placed infarcts, it is expected that patients with this dementia exhibit a deficit in finding lexical units⁵.

Since most studies on dementia state naming deficits as a significant symptom of language impairment, assessing nominative function may be particularly important for diagnosing cognitive disorders of vascular etiology^{13,14}. Previous studies have shown that individuals with VaD exhibit deficits in confrontation naming (CN) and word finding during spontaneous speech^{5,6,15-17}.

A review of the literature indicates that individuals with aphasia and progressive language disorders exhibit differences in the ability to name specific categories of terms, including differences in naming objects and actions¹¹. For instance, in some studies, patients with Broca's and Wernicke's aphasia were more successful in naming objects than actions¹⁸. However, some studies show that patients with aphasia are more successful in naming and understanding actions compared to naming and understanding objects¹⁹. Differences in the ability to name these two categories of terms have also been observed in patients with primary progressive aphasia. For instance, it has been found that patients with the semantic variant of primary progressive aphasia have more pronounced deficits in naming objects than actions, while patients with non-fluent primary progressive aphasia exhibit greater deficits in naming actions than objects^{20,21}.

Interesting data also come from studies that examined differences in naming ability and auditory comprehension (AC) of different semantic categories. For example, Silveri et al.²² found that verbs in patients with semantic dementia

were more preserved than nouns. The authors also note that patients with semantic dementia are more successful in naming artificial objects compared to natural items. Similar differences were observed in the domain of understanding named terms, where patients achieved higher performance in understanding words naming artificial objects compared to natural objects.

Given that previous studies indicate the presence of naming disorders in individuals with VaD, the aim of this study was to determine differences in the ability to find words naming objects (nouns) and words naming actions (verbs). We also aimed to determine the abilities of AC of words naming objects and actions.

Methods

Sample

The study was conducted from 2022 to 2023. The sample consisted of 30 participants with VaD who represented the clinical group and 30 neurologically healthy adults without data on language development disorders in the control group. All the patients signed an informed consent to participate in the research. The study was approved by the Ethics Committee of the Faculty of Special Education and Rehabilitation (No. 8911). Respondents of both sexes, aged 67 to 94, with at least eight years of formal education, were included in the sample.

The inclusion criteria for the clinical group were the following: patients with VaD, which was established based on the International Classification of Diseases, Tenth Revision (ICD-10)²³ and the National Institute of Neurological Disorders and Stroke – *Association Internationale pour la Recherche et l'Enseignement en Neurosciences* (NINDS-AIREN) for the diagnosis of VaD²⁴; patients who are able to be tested; patients whose mother tongue is Serbian.

The exclusion criteria were a severe degree of dementia (i.e., the subject was not testable), the presence of another form of dementia, or a psychiatric illness.

Instruments and procedures

In the study, the Mini-Mental State Examination (MMSE)²⁵ and the Northwestern Naming Battery (NNB)²⁶ were administered. The MMSE was used to assess the degree of cognitive impairment in persons with VaD and to exclude cognitive deficits in participants in the control group. Based on the overall MMSE score, participants with VaD were divided into two groups. The first group consisted of 20

participants with mild dementia (scores from 20 to 24). The second group consisted of 10 participants with moderate dementia (scores from 11 to 19)^{10, 11}.

The NNB was used to assess the ability to name objects and actions, as well as to understand words naming objects and actions. This provides insight into the ability to produce and comprehend nouns and verbs. The NNB consists of seven subtests: 1) Auditory discrimination; 2) Auditory lexical decision; 3) Confrontation naming; 4) Auditory comprehension; 5) Semantic associations; 6) Non-word repetition; 7) Word repetition. In this study, CN and AC (CNAC) subtests were used. These subtests assessed the ability to produce and understand nouns in the following categories: artificial objects (tools and clothing) and natural objects (animals and fruits/vegetables). Additionally, the ability to name and understand body parts and colors was tested.

CN is assessed by showing the participant one picture/drawing at a time. The correct response is marked in the designated template. In this part of the test, the participant can achieve a maximum of 90 points, where each correct answer receives 1 point. The distribution of the maximum number of points according to semantic categories is as follows: animals (8 points), fruits and vegetables (8 points), tools (8 points), clothing (8 points), body parts (8 points), colors (8 points), and "other objects": syringe, chimney, pen, nail, crown, wreath, binoculars, pliers, left-handed, and rocket (10 points). Additionally, this subtest includes the assessment of the ability to name actions. Actions named with one argument (14 points), two arguments (14 points), and three arguments (4 points) are included.

In the AC subtest, participants are asked to point to an object or action in a drawing that the examiner has named. In this subtest, participants can achieve a maximum of 50 points. The AC subtest also includes the following semantic categories: animals (5 points), fruits and vegetables (5 points), tools (8 points), clothing (5 points), other objects (5 points), body parts (5 points), and colors (5 points). Additionally, participants are asked to point to actions named with a verb with one argument (5 points), a verb with two arguments (5 points), and a verb with three arguments (5 points).

Statistical analysis

Both descriptive and inferential statistical methods were utilized in the study. Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) for Windows, version 23.0, 2015. Descriptive statistical measures included absolute frequency, percentage, median, mean, range (minimum-maximum), interquartile range, standard deviation, and standard error. Inferential statistical techniques employed for group comparisons included the Chi-squared (χ^2) test of independence. For 2x2 contingency frequency tables, Yates continuity correction was applied, and the *phi* (ϕ) coefficient was calculated as an indicator of effect size, classified as small effect (0.10), moderate effect (0.30), or large effect (0.50). The relationships between continuous variables were expressed using Spearman's

rank correlation coefficient *rho* (ρ). The strength of the relationship was determined according to guidelines: small or low (0.10–0.29), moderate (0.30–0.49), and large or high (above 0.50).

Results

VaD group and control group each included 9 (15%) male participants and 21 (35%) female participants. By applying the χ^2 test of independence (with Yates continuity correction), there was no statistically significant difference in the sample distribution according to participants' gender ($\chi^2 = 0.000$, $df = 1$, $p = 1.000$, $\phi = 0.00$).

The mean age of participants with VaD was 79.83 years, and in the control group, it was 76.83 years. There was no statistically significant difference in age between the clinical and control groups ($p = 0.097$).

The mean years of education for participants with VaD were 13.20 [standard deviation (SD) = 2.50] years, and for participants in the control group, it was 13.07 (SD = 3.00) years. There was no statistically significant difference in years of education between the tested groups of participants ($p = 0.554$).

Regarding cognitive status, the total score on MMSE averaged 19.87 (SD = 3.66) in the VaD group, ranging from 11 to 23. On the other hand, the control group achieved a mean score of 29.00 (SD = 0.87) on the same variable, ranging from 28 to 30. The groups differed significantly in MMSE performance ($p < 0.001$).

The average score for the participants with mild dementia was 22.20 (SD = 0.83), while participants with moderate dementia scored 15.20 (SD = 2.30) on average. The two groups differed significantly in MMSE ($p < 0.001$).

Table 1 shows the achievements of participants with VaD and the control group on the CN subtest.

Based on the analysis of the obtained results, it was determined that participants in the control group achieved significantly higher scores on tasks naming objects and actions compared to participants with VaD. By applying the Mann-Whitney *U*-test, it was shown that participants with VaD performed significantly worse than the participants in the control group in the following categories: animals ($U = 165.00$, $z = -5.12$, $p < 0.001$), fruits and vegetables ($U = 75.00$, $z = -6.21$, $p < 0.001$), total natural items ($U = 60.00$, $z = -6.21$, $p < 0.001$), tools ($U = 285.00$, $z = -3.62$, $p < 0.001$), clothing ($U = 240.00$, $z = -4.21$, $p < 0.001$), total artificial items ($U = 180.00$, $z = -4.94$, $p < 0.001$), other objects ($U = 30.00$, $z = -6.75$, $p < 0.001$), total objects ($U = 15.00$, $z = -6.93$, $p < 0.001$), body parts ($U = 255.00$, $z = -4.01$, $p < 0.001$), total nouns ($U = 15.00$, $z = -6.93$, $p < 0.001$), total colors ($U = 255.00$, $z = -4.00$, $p < 0.001$), verbs with one argument ($U = 105.00$, $z = -5.84$, $p < 0.001$), verbs with two arguments ($U = 15.00$, $z = -6.94$, $p < 0.001$), verbs with three arguments ($U = 105.00$, $z = -5.86$, $p < 0.001$), total verbs ($U = 0.00$, $z = -7.12$, $p < 0.001$), total CN ($U = 45.00$, $z = -6.56$, $p < 0.001$).

Table 1
Achievements of the VaD group (n = 30) and control group (n = 30)
participants on the Confrontation Naming subtest

Parameters	M ± SD	SE	Mdn	IQR	Min–Max	<i>r</i>
Animals						
VaD	6.63 ± 1.56	0.29	7.00	2.00	2–8	0.66
control	8.00 ± 0.00	0.00	8.00	-	8–8	
Fruits and vegetables						
VaD	6.00 ± 1.36	0.25	6.00	2.00	4–8	0.80
control	8.00 ± 0.00	0.00	8.00	-	8–8	
Total natural items						
VaD	12.63 ± 2.47	0.45	13.00	3.25	6–16	0.82
control	16.00 ± 0.00	0.00	16.00	-	16–16	
Tools						
VaD	7.33 ± 1.03	0.19	8.00	1.00	5–8	0.47
control	8.00 ± 0.00	0.00	8.00	-	8–8	
Clothing						
VaD	7.30 ± 0.99	0.18	8.00	1.00	4–8	0.54
control	8.00 ± 0.00	0.00	8.00	-	8–8	
Total artificial items						
VaD	14.63 ± 1.73	0.32	15.00	2.00	9–16	0.64
control	16.00 ± 0.00	0.00	16.00	-	16–16	
Other objects						
VaD	5.83 ± 2.78	0.51	5.50	4.25	1–10	0.87
control	10.00 ± 0.00	0.00	10.00	-	10–10	
Total objects						
VaD	33.10 ± 5.89	1.07	33.50	8.50	20–42	0.89
control	42.00 ± 0.00	0.00	42.00	-	42–42	
Body parts						
VaD	7.03 ± 1.22	0.22	8.00	2.00	4–8	0.52
control	8.00 ± 0.00	0.00	8.00	-	8–8	
Total nouns						
VaD	40.13 ± 6.78	1.24	41.00	8.50	24–50	0.89
control	50.00 ± 0.00	0.00	50.00	-	50–50	
Total colors						
VaD	7.07 ± 1.34	0.24	8.00	2.00	3–8	0.52
control	8.00 ± 0.00	0.00	8.00	-	8–8	
Verbs with one argument						
VaD	10.43 ± 2.69	0.49	10.00	3.50	5–14	0.75
control	14.00 ± 0.00	0.00	14.00	-	14–14	
Verbs with two arguments						
VaD	8.57 ± 2.79	0.51	7.50	5.25	5–14	0.90
control	14.00 ± 0.00	0.00	14.00	-	14–14	
Verbs with three arguments						
VaD	2.57 ± 1.04	0.19	2.50	1.25	1–4	0.76
control	14.00 ± 0.00	0.00	14.00	-	14–14	
Total verbs						
VaD	21.73 ± 5.91	1.08	21.00	11.25	12–30	0.92
control	32.00 ± 0.00	0.00	32.00	-	32–32	
Total CN						
VaD	68.93 ± 12.33	2.25	68.50	18.25	39–87	0.92
control	90.00 ± 0.00	0.00	90.00	-	90–90	

VaD – vascular dementia; CN – confrontation naming; M – mean; SD – standard deviation; SE – standard error; Mdn – median; IQR – interquartile range; Min – minimum; Max – maximum; *r* – strength of association.

Table 2 shows the results of the participants' achievements on the AC subtest.

Results of the AC subtest also show that participants with VaD performed significantly worse in understanding named objects and actions compared to the participants from the control group in tasks: animals ($U = 270.00$, $z = -3.82$, $p < 0.001$), fruits and vegetables ($U = 180.00$, $z = -4.78$, $p < 0.001$), total natural items ($U = 150.00$, $z = -5.15$, $p < 0.001$), tools ($U = 285.00$, $z = -3.40$, $p < 0.001$), clothing

($U = 300.00$, $z = -3.42$, $p < 0.001$), total artificial items ($U = 255.00$, $z = -3.80$, $p < 0.001$), other objects ($U = 225.00$, $z = -4.39$, $p < 0.001$), total objects ($U = 105.00$, $z = -5.70$, $p < 0.001$), body parts ($U = 285.00$, $z = -3.63$, $p < 0.001$), total nouns ($U = 105.00$, $z = -5.70$, $p < 0.001$), total colors ($U = 225.00$, $z = -4.39$, $p < 0.001$), verbs with one argument ($U = 150.00$, $z = -5.30$, $p < 0.001$), verbs with two arguments ($U = 75.00$, $z = -6.23$, $p < 0.001$), verbs with three arguments ($U = 135.00$, $z = -5.48$, $p < 0.001$), total verbs ($U = 75.00$,

$z = -6.20, p < 0.001$), total AC ($U = 45.00, z = -6.56, p < 0.001$).

Tables 3 and 4 show the results of the comparison of achievements on CNAC subtests among participants with different degrees of VaD severity.

Participants with mild dementia were statistically significantly better compared to those with moderate dementia in naming the following categories: animals ($U = 35.00, z = -2.98, p = 0.003$), total natural items ($U = 45.40, z = -2.43, p = 0.015$), tools ($U = 61.00, z = -1.99, p = 0.046$), clothing ($U = 58.50, z = -2.03, p = 0.043$), total artificial items ($U = 52.00, z = -2.22, p = 0.027$), other

objects ($U = 39.50, z = -2.68, p = 0.007$), total objects ($U = 34.59, z = -2.89, p = 0.004$), body parts ($U = 21.00, z = -3.91, p < 0.001$), total nouns ($U = 25.50, z = -3.29, p = 0.001$), verbs with one argument ($U = 42.00, z = -2.59, p = 0.010$), verbs with two arguments ($U = 47.00, z = -2.37, p = 0.018$), total verbs ($U = 42.00, z = -2.56, p = 0.010$), total CN ($U = 28.00, z = -3.18, p = 0.001$) (Table 3).

Participants with mild dementia achieved statistically significantly better results compared to those with moderate dementia on the following categories of the AC subtest: animals ($U = 66.50, z = -1.98, p = 0.048$), total objects ($U = 55.50, z = -1.98, p = 0.048$), verbs with three arguments

Table 2

Participants' achievements on the Auditory Comprehension subtest

Parameters	M ± SD	SE	Mdn	IQR	Min–Max	<i>r</i>
Animals						
VaD	4.40 ± 0.86	0.16	5.00	1.00	2–5	0.49
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Fruits and vegetables						
VaD	3.97 ± 1.07	0.19	4.00	2.00	2–6	0.62
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Total natural items						
VaD	8.37 ± 1.73	0.32	9.00	2.26	5–11	0.66
control	10.00 ± 0.00	0.00	10.00	-	10–10	
Tools						
VaD	4.53 ± 0.73	0.13	5.00	1.00	3–6	0.44
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Clothing						
VaD	4.57 ± 0.68	0.12	5.00	1.00	3–5	0.44
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Total artificial items						
VaD	9.10 ± 1.24	0.23	10.00	2.00	7–11	0.49
control	10.00 ± 0.00	0.00	10.00	-	10–10	
Other objects						
VaD	4.27 ± 0.94	0.17	4.50	1.00	1–5	0.57
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Total objects						
VaD	21.73 ± 3.10	0.57	23.00	5.00	15–26	0.74
control	25.00 ± 0.00	0.00	25.00	-	25–25	
Body parts						
VaD	4.50 ± 0.86	0.16	5.00	1.00	1–5	0.47
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Total nouns						
VaD	26.23 ± 3.52	0.64	27.00	6.00	16–31	0.74
control	30.00 ± 0.00	0.00	30.00	-	30–30	
Total colors						
VaD	4.27 ± 0.94	0.17	4.50	1.00	1–5	0.57
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Verbs with one argument						
VaD	3.73 ± 1.20	0.22	4.00	2.00	1–5	0.68
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Verbs with two arguments						
VaD	3.27 ± 1.20	0.22	3.00	1.00	0–5	0.80
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Verbs with three arguments						
VaD	3.30 ± 1.56	0.28	4.00	3.00	0–5	0.71
control	5.00 ± 0.00	0.00	5.00	-	5–5	
Total verbs						
VaD	10.30 ± 3.24	0.59	10.00	4.25	4–15	0.80
control	15.00 ± 0.00	0.00	15.00	-	15–15	
Total AC						
VaD	40.80 ± 6.09	1.11	40.50	7.00	28–50	0.85
control	50.00 ± 0.00	0.00	50.00	-	50–50	

AC – auditory comprehension. For other abbreviations, see Table 1.

($U = 40.50$, $z = -2.68$, $p = 0.007$), total verbs ($U = 35.00$, $z = -2.89$, $p = 0.004$), and total AC ($U = 37.00$, $z = -2.79$, $p = 0.005$) (Table 4).

The Spearman correlation was used to examine the relationship between achievements on the MMSE and the NNB. A statistically significant positive and strong correlation was observed between the total CN score and the overall score on the MMSE, indicating that higher

scores on the total CN were associated with higher overall scores on the MMSE ($\rho = 0.626$, $p < 0.01$), and *vice versa*. Similarly, a statistically significant positive and strong correlation was found between the total AC score and the overall score on the MMSE, with higher scores on the total AC associated with higher overall scores on the MMSE ($\rho = 0.683$, $p < 0.01$), and *vice versa*.

Table 3

Achievements on the Confrontation Naming subtest to determine severity of dementia

Parameters	M ± SD	Mdn	IQR	Mean rank	p	r
Animals						
mild	7.25 ± 1.02	7.50	1.00	18.75	0.003	0.54
moderate	5.40 ± 1.78	5.50	3.00	9.00		
Fruits and vegetables						
mild	6.20 ± 1.36	6.00	2.00	16.78	0.250	0.21
moderate	5.60 ± 1.35	6.00	2.25	12.95		
Total natural items						
mild	13.45 ± 1.90	14.00	3.50	18.23	0.015	0.44
moderate	11.00 ± 2.75	10.50	3.75	10.05		
Tools						
mild	7.60 ± 0.82	8.00	0.75	17.45	0.046	0.36
moderate	6.80 ± 1.23	7.00	2.25	11.60		
Clothing						
mild	7.55 ± 0.76	8.00	1.00	17.58	0.043	0.37
moderate	6.80 ± 1.23	7.00	2.00	11.35		
Total artificial items						
mild	15.15 ± 1.23	15.50	1.00	17.90	0.027	0.40
moderate	13.60 ± 2.17	14.00	3.25	10.70		
Other objects						
mild	6.80 ± 2.55	7.50	4.00	18.53	0.007	0.49
moderate	3.90 ± 2.23	4.00	3.50	9.45		
Total objects						
mild	35.40 ± 4.64	35.50	6.75	18.78	0.004	0.53
moderate	28.50 ± 5.56	30.50	10.00	8.95		
Body parts						
mild	7.65 ± 0.75	8.00	0.00	19.45	< 0.001	0.71
moderate	5.80 ± 1.03	6.00	1.00	7.60		
Total nouns						
mild	43.05 ± 4.91	42.00	6.75	19.23	0.001	0.60
moderate	34.30 ± 6.34	36.00	10.25	8.05		
Total colors						
mild	7.30 ± 1.17	8.00	1.75	17.18	0.102	0.30
moderate	6.60 ± 1.58	7.00	2.25	12.15		
Verbs with one argument						
mild	11.35 ± 2.21	11.50	4.00	18.40	0.010	0.47
moderate	8.60 ± 2.72	9.00	3.50	9.70		
Verbs with two arguments						
mild	9.40 ± 2.70	9.00	5.00	18.15	0.018	0.43
moderate	6.90 ± 2.23	7.00	2.50	10.20		
Verbs with three arguments						
mild	2.75 ± 1.02	3.00	2.00	16.95	0.185	0.24
moderate	2.20 ± 1.03	2.00	2.00	12.60		
Total verbs						
mild	23.75 ± 5.24	24.00	9.75	18.40	0.010	0.47
moderate	17.70 ± 5.23	16.50	8.25	9.70		
Total CN						
mild	74.10 ± 9.56	72.50	15.75	19.10	0.001	0.58
moderate	58.60 ± 10.91	56.00	14.75	8.30		

CN – confrontation naming. For other abbreviations, see Table 1.

Table 4
Participants' achievements on the Auditory Comprehension subtest to determine severity of dementia

Parameters	M ± SD	Mdn	IQR	Mean rank	<i>p</i>	<i>r</i>
Animals						
mild	4.65 ± 0.59	5.00	1.00	17.48	0.048	0.36
moderate	3.90 ± 1.10	4.00	2.00	11.55		
Fruits and vegetables						
mild	4.15 ± 1.04	4.00	2.00	16.90	0.200	0.23
moderate	3.60 ± 1.07	4.00	1.50	12.70		
Total natural items						
mild	8.80 ± 1.40	9.00	2.00	17.28	0.110	0.29
moderate	7.50 ± 2.07	8.00	4.20	11.95		
Tools						
mild	4.70 ± 0.66	5.00	1.00	17.25	0.082	0.32
moderate	4.20 ± 0.79	4.00	1.25	12.00		
Clothing						
mild	4.65 ± 0.67	5.00	0.75	16.63	0.234	0.22
moderate	4.40 ± 0.70	4.50	1.00	13.25		
Total artificial items						
mild	9.35 ± 1.17	10.00	1.00	17.00	0.155	0.26
moderate	8.60 ± 1.35	8.50	3.00	12.50		
Other objects						
mild	4.45 ± 0.76	5.00	1.00	16.95	0.163	0.25
moderate	3.90 ± 1.20	4.00	1.25	12.60		
Total objects						
mild	22.60 ± 2.48	23.50	3.75	17.73	0.048	0.36
moderate	20.00 ± 3.59	19.50	6.25	11.05		
Body Parts						
mild	4.60 ± 0.60	5.00	1.00	15.85	0.717	0.07
moderate	4.30 ± 1.25	5.00	1.00	14.80		
Total nouns						
mild	27.20 ± 2.71	27.50	4.75	17.60	0.063	0.34
moderate	24.30 ± 4.27	24.50	5.75	11.30		
Total colors						
mild	4.40 ± 0.75	5.00	1.00	16.33	0.427	0.14
moderate	4.00 ± 1.24	4.00	1.25	13.85		
Verbs with one argument						
mild	4.00 ± 0.97	4.00	1.75	17.08	0.150	0.26
moderate	3.20 ± 1.48	3.00	3.00	12.35		
Verbs with two arguments						
mild	3.60 ± 0.88	3.00	1.00	17.58	0.057	0.35
moderate	2.60 ± 1.51	2.50	2.25	11.35		
Verbs with three arguments						
mild	3.90 ± 1.07	4.00	2.00	18.48	0.007	0.49
moderate	2.10 ± 1.73	1.50	2.50	9.55		
Total verbs						
mild	11.50 ± 2.26	11.00	3.75	18.75	0.004	0.53
moderate	7.90 ± 3.67	7.50	5.25	9.00		
Total AC						
mild	43.10 ± 4.02	41.50	6.75	18.65	0.005	0.51
moderate	36.20 ± 7.07	38.50	10.50	9.20		

AC – auditory comprehension. For other abbreviations, see Table 1.

Discussion

This study aimed to determine deficits in naming objects and actions and understanding the meanings of words used to name those objects and actions, as well as to explore how these abilities relate to the severity of dementia.

Our findings indicate that participants with VaD achieved significantly lower scores compared to neurologically healthy participants, which is consistent with

the results of other authors^{20, 25}. When assessing the ability to name natural items, we found that participants with VaD performed better in naming animals compared to naming fruits and vegetables, while individuals without neurological disorders accurately named all items in the listed categories. In assessing the ability to name artificial items, it was shown that individuals with VaD were equally (un)successful in naming tools and clothing. Further analysis of the obtained results showed that individuals with VaD were more

successful in naming artificial items compared to naming natural items, consistent with previous research indicating that dementia patients are more successful in naming artificial items compared to natural items²². Additionally, studies show that some individuals with semantic dementia have a preserved ability to find verbs compared to nouns in CN tasks²². However, our findings suggest that participants with VaD have a better ability to find nouns than verbs, as they were more successful in naming objects than actions. Regarding the action naming results, it is interesting to note that participants with VaD were more successful in finding verbs with one argument compared to verbs with two or three arguments. On the other hand, participants in the control group successfully named all actions regardless of the structure of the verb arguments. This finding suggests that individuals with VaD experience difficulties in finding verbs with complex argument structures, similar to individuals with aphasia^{12,26}.

Regarding AC of the meanings of words used to name objects and actions, the results of our study show that participants with VaD were poorer in some categories compared to neurologically healthy participants. Specifically, participants with VaD were better at AC of words naming objects (nouns) compared to words naming actions (verbs). Additionally, a dissociation was found regarding the participants' ability to understand verbs with a different number of arguments, where participants with VaD had slightly higher achievements in understanding verbs with three arguments compared to verbs with two arguments. These findings indicate the importance of further investigating the AC of verbs in individuals with VaD.

The degree of cognitive deficit or dementia significantly influences naming ability. Patients with milder VaD had higher achievements in CNAC of words

naming objects and actions compared to participants with moderate dementia. The impact of dementia severity on naming ability and AC of terms has been demonstrated in other studies as well. For instance, some authors find that individuals with mild dementia are more successful in CN tasks compared to individuals with more severe cognitive impairment²⁷. The influence of dementia severity on the ability to find words in naming tasks is also evident in the results of previous research^{28,29}. Finally, the results of our study indicate a statistically significant correlation between the severity of dementia and the ability of CNAC of named terms in individuals with VaD. Individuals with milder dementia have better abilities to name objects and actions, as well as to understand the meanings of named terms.

Conclusion

Based on the analysis and discussion of the obtained results, it can be concluded that individuals with vascular dementia have significantly pronounced deficits in visual confrontation naming. This finding indicates that vascular dementia leads to impairments in the ability to find nouns and verbs. It was also concluded that individuals with vascular dementia had pronounced deficits in auditory comprehension of certain semantic categories. The severity of cognitive impairment, i.e., dementia, correlates with performance on naming and understanding named term tasks. Patients with more severe dementia performed poorly in confrontation naming and auditory comprehension of words used to name objects and actions compared to patients identified with milder dementia. This finding suggests that as dementia progresses, naming and auditory comprehension abilities significantly worsen.

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Bacterial flora of chronic venous leg ulcers: shifts over a two-decade period

Bakterijska flora hroničnih venskih ulceracija nogu: promene tokom dvadesetogodišnjeg perioda

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Abstract

Background/Aim. Venous leg ulcers (VLUs) are often colonized by various types of bacteria, which can lead to infection that requires empirical antibiotic therapy. Bacterial types and their antibiotic susceptibility vary widely over time and by geographic location. The aim of the study was to determine the change trends of causative bacteria and antibiotic susceptibility in patients with VLUs over the past two decades. **Methods.** The study was conducted at a tertiary care dermatovenereology clinic. Data on the microbiological analysis of ulcer swabs from patients with VLUs were collected over two consecutive months at three time points: 2001, 2014, and 2020. **Results.** The study included 250 patients divided into three groups. The first group (2001) had 111 participants, the second group (2014) had 64, and the third group (2020) had 75 participants. Out of the 250 swabs sent for microbiological testing, only 4 (1.6%) samples yielded negative results. In 2001, the bacteria identified were 31.83% Gram-positive (G+) and 68.13% Gram-negative (G-). In 2014, the ratio between G+ and G- bacteria was 27.18%

vs. 72.82%. In 2020, the predominance of G- bacteria was even more pronounced, with 89.86% compared to 10.14% G+ ($p < 0.001$). In 2001, *Pseudomonas (P.) aeruginosa (P. aeruginosa)* (26.54%) was the predominant G- bacterium, and *Staphylococcus (S.) aureus* (24.78%) was the most common G+ bacterium. In 2014, *P. aeruginosa* (30.10%) and *S. aureus* (23.30%) remained prevalent. By 2020, *P. aeruginosa* (35.14%) increased further, while *S. aureus* (7.43%) decreased significantly ($p < 0.001$). Antibiotic sensitivity varied over the years, with older antibiotics showing decreased efficacy and newer classes demonstrating increased sensitivity. **Conclusion.** Throughout the study period, there was a notable shift toward G- bacterial dominance, particularly *P. aeruginosa*. Antibiotic resistance patterns also evolved. This study highlights the importance of local surveillance of bacterial flora of VLUs and antibiotic resistance profiles.

Key words:

anti-bacterial agents; bacterial infections; drug resistance, bacterial; gram-negative bacteria; gram-positive bacteria; varicose ulcer; microbiology.

Apstrakt

Uvod/Cilj. Venske ulceracije nogu (VUN) su često kolonizovane različitim vrstama bakterija, što može dovesti do infekcije koja zahteva empirijsku terapiju antibioticima. Vrste bakterija i njihova osetljivost na antibiotike znatno variraju tokom vremena i u zavisnosti od geografske lokacije. Cilj istraživanja bio je da se utvrde trendovi promene uzročnih bakterija i osetljivosti na antibiotike kod bolesnika sa VUN u poslednje dve decenije. **Metode.** Istraživanje je sprovedeno u tercijarnoj dermatovenerološkoj klinici. Podaci o mikrobiološkoj analizi briseva uzetih od bolesnika sa VUN prikupljeni su tokom dva uzastopna meseca, u tri vremenska perioda: 2001, 2014. i 2020. godine. **Rezultati.** Istraživanjem je

obuhvaćeno 250 bolesnika, podeljenih u tri grupe. Prva grupa (2001) imala je 111 učesnika, druga grupa (2014) imala je 64, a treća grupa (2020) 75 učesnika. Od 250 briseva poslatih na mikrobiološku analizu, rezultat je bio negativan kod samo 4 (1,6%) uzorka. U 2001. godini identifikovano je 31,83% Gram-pozitivnih (G+) i 68,13% Gram-negativnih (G-) bakterija. Tokom 2014. godine, odnos G+ i G- bakterija bio je 27,18% vs. 72,82%. U 2020. godini, dominacija G- bakterija postala je još izraženija, sa 89,86% u poređenju sa 10,14% G+ ($p < 0,001$). U 2001. godini, *Pseudomonas (P.) aeruginosa* (26,54%) bila je pretežna G- bakterija, dok je *Staphylococcus (S.) aureus* (24,78%) bila najčešća G+ bakterija. U 2014. godini, *P. aeruginosa* (30,10%) i *S. aureus* (23,30%) i dalje su preovlađivali. Do 2020. godine, učestalost javljanja *P.*

aeruginosa (35,14%) je dodatno povišena, dok je učestalost *S. aureus* (7,43%) značajno smanjena ($p < 0,001$). Osetljivost na antibiotike varirala je tokom godina, pri čemu su stariji antibiotici pokazivali smanjenu efikasnost, dok su novije klase pokazale povećanu osetljivost. **Zaključak.** Tokom perioda istraživanja, došlo je do značajnog prelaska ka dominaciji G- bakterijske flore, posebno *P. aeruginosa*. Obrasci rezistencije na antibiotike su

se takođe menjali. Ova studija naglašava značaj lokalnog praćenja bakterijske flore kod VUN i profila otpornosti na antibiotike.

Ključne reči:
antibiotici; infekcija, bakterijska; lekovi, rezistencija bakterija; gram-negativne bakterije; gram-pozitivne bakterije; venska ulceracija; mikrobiologija.

Introduction

Venous leg ulcers (VLUs) represent the most common type of chronic wounds. It is estimated that 70–90% of all chronic leg wounds are caused by chronic venous insufficiency (CVI)^{1,2}. VLUs are often colonized by various types of bacteria without showing signs of infection. However, in certain cases, signs of infection may develop. If left unrecognized and untreated, this can lead to prolonged healing times, enlargement of the ulcer surface, and an increased risk of complications.

Recognizing signs of infection and promptly initiating treatment is one of the most challenging tasks for doctors treating such patients. This assessment is highly subjective and relies primarily on the physician's experience. It is recommended that VLUs with clinical evidence of infection be treated with systemic antibiotics³. When antibiotic therapy is necessary, it is typically initiated empirically, based on assumptions about the type of bacteria and their antibiotic sensitivity, until the results of the microbiological analysis of the swab taken from the ulcer are obtained.

Significant variations exist in the most frequently isolated bacteria depending on geographical distribution. Moreover, the commonly isolated types of bacteria and their antibiotic sensitivity can change over time.

The aim of the study was to investigate whether a change has occurred in the types of most commonly isolated bacteria and their antibiotic sensitivity over a twenty-year period.

Methods

The study was conducted at the Phlebology Department of the Clinic of Dermatovenereology Diseases, University Clinical Center of Vojvodina, Serbia, the only subspecialist department of its kind in the region, serving approximately 2 million inhabitants and specializing in the treatment of VLUs. The study was approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-08/332, from September 26, 2024). We anonymously collected microbiological data from ulcer swabs of patients with single or multiple VLUs. Data were gathered over two consecutive months at three time points: 2001, 2014, and 2020. All patients treated at the Phlebology Department during these periods were included, provided they met the inclusion criteria. Only outpatients with CVI confirmed by duplex scan, ankle-brachial index (ABI) values between 0.9 and 1.3, and those over 18 years were eligible. Microbiological

samples were collected from patients who had not received systemic antibiotics, topical antibiotics, or topical antiseptics for at least four weeks prior to swab collection. All patients received standard local therapy for VLUs (regular wound cleaning with saline solutions and maintaining a moist wound environment by applying hydrocolloids, hydrogels, alginates, and foam dressings).

The study included an initial ulcer swab for each patient. Prior to sampling, each ulcer was thoroughly cleaned with a saline solution, and any necrotic material or crust was removed. The sample was taken using the Levine technique. The swab was gently pressed into the wound bed over an area of about 1 cm². It was rotated while applying enough pressure to express fluid from the tissue. Afterward, each swab was sent for standard microbiological testing. The time between sampling and incubation was no more than 2 hrs. The swabs were tested for microbial sensitivity and resistance using a standard antibiotic set.

Statistical analysis

A database was compiled with data on patient age, gender, the number and species of bacteria isolated from ulcer swabs, and their antimicrobial sensitivity. Statistical analyses were performed using SPSS for Windows, version 26 (IBM SPSS, Chicago, Illinois). Descriptive statistics, including arithmetic means and minimum and maximum values, were used to summarize quantitative variables. Qualitative variables were expressed as frequencies and percentages. The Chi-square test was applied to analyze attributive variables, allowing for comparisons across the different study groups. All statistical tests were two-tailed, with a significance level set at $p < 0.05$.

Results

The study included 250 patients divided into three groups according to the time periods. The first group (2001) comprised 111 participants, 70 females and 41 males, aged 46 to 91 years (the average age was 66.9 years). The second group (2014) included 64 respondents, 47 females and 17 males, aged 51 to 87 years (the average age was 65). The third group (2020) consisted of 75 patients, 51 females and 24 males, aged 47 to 82 years (the average age was 65.3 years).

Out of the 250 swabs sent for microbiological testing, the result was negative in only 4 (1.6%) samples. In the analyzed samples, a single bacterium was isolated in most

cases, 100 (40%) out of 250, while four bacteria were found in only 18 (7.2%) samples. Detailed data on the number of isolated bacteria in individual samples are presented in Table 1.

In 2001, a total of 223 bacteria were isolated from 111 samples (mean = 2.01), identifying 33 different bacterial species. Of these, 31.83% were Gram-positive (G+) bacteria, while 68.13% were Gram-negative (G-). In 2014, the average number of bacteria *per* sample decreased to 1.61, and the number of bacterial species dropped to 23. The ratio of G+ to G- bacteria was 28 (27.18%) to 75 (72.82%). By 2020, the average number of bacteria *per* sample increased slightly to 1.97, but the number of bacterial species fell further to just 18. The predominance of G- bacteria became even more pronounced, with 133 (89.86%) compared to 15 (10.14%) G+. The difference in

the frequency of isolation of G- compared to G+ bacteria through three study periods was highly significant, at $p < 0.001$ (Table 2).

In 2001, the most common G- bacterium was *Pseudomonas (P.) aeruginosa* (26.54% of the total number of bacteria), while the most common G+ bacterium was *Staphylococcus (S.) aureus* (24.78%). In 2014, the situation was somewhat different. The most common G- and G+ bacteria were still *P. aeruginosa* and *S. aureus*, but with different frequencies: 30.10% and 23.30%, respectively. In 2020, we observed a further widening in the frequency difference between the most common G- and G+ bacteria, with *P. aeruginosa* accounting for 35.14% of cases, while *S. aureus* was present in only 7.43%, and this difference in frequency was also highly significant, with $p < 0.001$ (Table 3).

Table 1

Number of isolated bacteria in individual samples of chronic venous leg ulcers across ¹three time points

Number of isolated bacteria	2001 (n = 111)	2014 (n = 64)	2020 (n = 75)
0	2 (1.80)	1 (1.56)	1 (1.33)
1	37 (33.33)	36 (56.25)	27 (36)
2	38 (34.24)	17 (26.56)	27 (36)
3	26 (23.42)	7 (10.94)	13 (17.33)
4	8 (7.21)	3 (4.69)	7 (9.34)
Total number (mean)	223 (2.01)	103 (1.61)	148 (1.97)

Results are given as numbers (percentages) except for the total number.

¹Note: the term three time points is referred to entire individual years, i.e., 2001, 2014, and 2020. n – number of samples (i.e., number of respondents) during that year.

Table 2

Gram-positive and Gram-negative bacteria isolated in study samples of chronic venous leg ulcers across ¹three time points

Year	Total number of isolated bacteria in all samples	Average number of bacteria <i>per</i> sample	Number of bacterial species	Gram-positive bacteria	Gram-negative bacteria	<i>p</i> -value
2001	223	2.01	33	71 (31.83)	152 (68.13)	
2014	103	1.61	23	28 (27.18)	75 (72.82)	< 0.0001
2020	148	1.97	18	15 (10.14)	133 (89.86)	

Results are shown as numbers (percentages).

¹Note: see explanation in Table 1.

Table 3

Most common isolated bacterial species of chronic venous leg ulcer samples across ¹three time points

2001		2014		2020	
bacterial species	%	bacterial species	%	bacterial species	%
<i>Pseudomonas aeruginosa</i>	26.54	<i>Pseudomonas aeruginosa</i>	30.10	<i>Pseudomonas aeruginosa</i>	35.14
<i>Staphylococcus aureus</i>	24.78	<i>Staphylococcus aureus</i>	23.30	<i>Enterobacter</i> species	12.84
<i>Escherichia coli</i>	4.87	<i>Enterobacter</i> species	8.73	<i>Acinetobacter</i> species	8.78
<i>Enterobacter</i> species	3.98	<i>Proteus mirabilis</i>	5.82	<i>Staphylococcus aureus</i>	7.43
<i>Proteus mirabilis</i>	3.98	<i>Acinteobacter</i> species	5.82	<i>Serratia</i> species	6.76

¹Note: see explanation in Table 1.

Table 4

**Three most effective antibiotics against isolated
Gram-positive (G+) and Gram-negative (G-) bacteria across ¹three time points**

	2001	2014	2020
Antibiotics against G+			
chloramphenicol (65.46)		trimethoprim + sulphamethoxazole (83.33)	cephalosporins (90.32)
gentamicin (50.91)		cephalosporins (80.28)	gentamicin (77.78)
trimethoprim + sulphamethoxazole (47.28)		tetracycline (73.91)	quinolones (76.20)
Antibiotics against G-			
ciprofloxacin (80.70)		piperacillin-tazobactam (84.48)	carbapenems (91.70)
amikacin (74.56)		carbapenems (82.03)	piperacillin + tazobactam (81.67)
ofloxacin (68.42)		cephalosporins (75.37)	cephalosporins (75.60)

Results are shown as percentages of bacteria sensitive to the specific antibiotic.

¹Note: see explanations in Table 1.

In 2001, G+ bacteria exhibited the highest sensitivity to chloramphenicol and gentamicin, while G- bacteria were most susceptible to antibiotics from the quinolone group. According to the results of the antibiogram in 2014, G+ was most sensitive to trimethoprim combined with sulphamethoxazole (83.33%), cephalosporins (80.28%), and tetracycline (73.91), and G- was most sensitive to piperacillin-tazobactam (84.48%), carbapenems (82.03), and cephalosporins (75.37%). In 2020, G+ bacteria exhibited the highest sensitivity to cephalosporins, gentamicin, and quinolones, while G- bacteria were most susceptible to the carbapenem group, piperacillin with tazobactam, and newer-generation cephalosporins (Table 4).

Discussion

A chronic lower leg ulcer is a type of wound that fails to progress through the usual stages of healing within the expected timeframe, typically over six weeks⁴. These wounds are associated with underlying health conditions such as venous insufficiency, arterial disease, diabetes mellitus, or prolonged pressure and require specialized medical treatment to promote healing and prevent complications. VLU is a typical example of chronic wounds. The prevalence of lower leg wounds is around 1%, and CVI is by far the most common cause⁵.

Bacteria inhabit the wound bed from the onset of wound formation, with their population increasing over time. Although virtually all VLUs are colonized by bacteria, some may develop into overt infections. Chronic open wounds create an ideal environment for bacteria, which allows them to establish, survive, and evolve^{6,7}.

The infection status of chronic wounds can be stratified into four stages⁸⁻¹⁰. The first stage is contamination – non-replicating bacteria are present in the ulcer and do not cause any damage or host response. The second stage is colonization – bacteria begin to replicate and grow on the surface of a wound, but the host's immune response is very weak. Colonization does not lead to deterioration of the ulcer's status, and healing is not compromised. The third stage is critical colonization – the number of bacteria increases significantly, which interferes with the ulcer's healing process and may cause subtle local signs of infection.

However, there is still no strong activation of the patient's immune system. Finally, the fourth stage is infection – the number of bacteria is rapidly increasing. The local clinical findings of the ulcer are deteriorating. Healing is compromised. Signs of infection are prominent, and the patient complains of intense pain. There is a marked activation of the patient's immune system.

In the first two phases, the presence of microorganisms usually does not affect the healing of ulcerations. In the third and fourth phase, therapeutic intervention is necessary. The difference between colonization and infection is typically subtle and not easily determined. It primarily relies on clinical assessment rather than the microbiological status of the ulcer⁹.

Various studies have recorded a large number of different bacteria isolated from VLUs. Regardless of the geographic location where the studies were conducted, the predominant G- microorganisms found in leg ulcer infections were *P. aeruginosa* and *Escherichia coli*, whereas *S. aureus* was the leading G+ microorganism^{6,9,11,12}. However, since bacterial colonization of VLUs tends to be polymicrobial, many other species of bacteria can be isolated from this type of chronic wound. Nevertheless, it is important to note that even extensive colonization of VLUs by various bacteria does not always result in clinically evident infections. This can likely be explained by the fact that many of the bacteria isolated from these ulcers are part of the skin's saprophytic microflora. When these microorganisms colonize the wound, they activate the body's innate immunity mechanisms, effectively preventing their overgrowth and the onset of infection¹¹.

It is known that there are geographic and temporal differences in the species of bacteria most commonly isolated from VLUs and their antibiotic susceptibility. Geographic differences are influenced by factors such as local healthcare practices, hygiene conditions, climate, and access to medical care. Temporal differences can be caused by changes in medical protocols, the emergence of antibiotic resistance, and the development of new treatment methods. As a result, the types of bacteria most frequently isolated from these wounds can vary considerably between different regions and over various time periods¹³.

In our study, we observed significant changes in the frequency of occurrence of certain bacteria and their susceptibility to antibiotics over time. We noted that over 20 years, there was a markedly higher prevalence of G-bacteria in isolates from VLU. *P. aeruginosa*, the most prevalent in our samples from the beginning, accounted for more than one-third of all isolated bacteria after 20 years. The most common G+ bacterium, *S. aureus*, appeared significantly less frequently in later periods. Sensitivity to antibiotics has also changed significantly. We noted resistance to older types of antibiotics and considerable sensitivity to newer classes.

There is a concerning rapid increase in bacterial resistance to antibiotics, outpacing the development of new antibiotics entering the market^{14, 15}. This trend poses a significant threat to public health, as it limits the effectiveness of current treatments. It is crucial to keep this in mind when selecting and prescribing antibiotics.

Although there are numerous guidelines for treating infections in VLUs and chronic wounds in general, there is still no consensus on the methods for diagnosing and implementing therapy. Most wound care practitioners generally evaluate and manage bacterial infections in chronic wounds by observing clinical signs and patient-reported symptoms rather than depending exclusively on objective measures¹⁶. After an infection is diagnosed, two types of antimicrobial treatments are available for infected VLUs: systemic antibiotics and topical preparations¹⁷.

Most guidelines recommend systemic antibiotics as the first choice of therapy for infections, but only in cases with clear signs of the infection. No evidence suggests that the

routine or preventive use of systemic antibiotics enhances healing rates for non-infected VLUs^{7, 17}. Since this therapy usually needs to begin immediately after determining the infection of the ulcer and before the ulcer swab results are available, the treatment often starts empirically with later reevaluation and possible adjustment. Therefore, it is crucial to have up-to-date local data on the predominant bacterial flora and their antibiotic resistance.

The topical application of antibiotics in the treatment of VLUs is generally not recommended³. It leads to a temporary reduction in bacterial counts and inflammation. However, bacteria can survive in very high antibiotic concentrations, leading to the development of multi-resistant strains of microorganisms. Additionally, local antibiotic therapy often results in contact sensitization^{18, 19}. Therefore, in the local treatment of an infection, we typically employ adequate debridement and the application of antiseptics and appropriate dressings.

Conclusion

Over the study period, there was a notable shift towards Gram-negative bacterial dominance, particularly *Pseudomonas aeruginosa*. Antibiotic resistance patterns also evolved, with older antibiotics showing decreased efficacy and newer classes demonstrating increased sensitivity.

This study highlights the importance of local surveillance to guide effective treatment strategies in managing venous leg ulcers, emphasizing the need for updated knowledge on bacterial flora and antibiotic resistance profiles.

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Association between C-reactive protein-albumin-lymphocyte (CALLY) index and cerebral edema in acute ischemic stroke patients

Povezanost između indeksa C-reaktivni protein-albumin-limfociti (CALLY) i cerebralnog edema kod bolesnika sa akutnim ishemijskim moždanim udarom

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Abstract

Background/Aim. There is an association between brain edema and inflammation that may occur in a stroke. The aim of the study was to determine the relationship between the C-reactive protein-albumin-lymphocyte (CALLY) index and cerebral edema developed as a consequence in stroke patients. **Methods.** The retrospective study included patients aged 18 years and above who presented to the emergency department from January 2021 to December 2023 and were admitted to the neurology/intensive care unit with a diagnosis of stroke. The following hematological parameters were included in the research: the pan-immune-inflammation value (PIV), systemic inflammatory response index (SIRI), systemic immune-inflammation index (SII), and the CALLY index. These parameters were calculated from blood samples of patients taken during admission, and neurosurgeons assessed the development of cerebral edema and the need for decompression. The parameters were compared between two groups of

patients: the surgical group (SG), with patients in need of decompression, and the nonsurgical group (NSG), with patients who do not have such a need. **Results.** The study included 274 patients in total, of which 189 (68.90%) were in NSG and 85 (31.10%) in SG. It was found that the CALLY index was significantly higher in patients in NSG than in patients in SG (16.47 vs. 0.79; $p < 0.001$). SIRI and SII levels were significantly higher in SG patients compared to NSG ($p < 0.001$ and $p = 0.001$, respectively). PIV level was also significantly higher in SG than in NSG ($p < 0.001$). At a cut-off value of 4.06, the CALLY index had a sensitivity of 69.40% and a specificity of 83.00%. **Conclusion.** The CALLY index is an easily accessible and calculable marker, which can be used to predict the need for decompression that may develop in stroke patients.

Key words: brain edema; c-reactive protein; hematologic tests; stroke.

Apstrakt

Uvod/Cilj. Postoji povezanost između edema mozga i zapaljenja koje se može pojaviti u moždanom udaru. Cilj rada bio je da se utvrdi povezanost između indeksa C-reaktivni protein-albumin-limfociti (*C-reactive protein-albumin-lymphocyte* – CALLY) i posleđičnim edemom mozga kod bolesnika sa moždanim udarom. **Metode.** Retrospektivnom studijom obuhvaćeni su bolesnici stari 18 ili više godina, koji su se javili u hitnu pomoć od januara 2021. do decembra 2023. godine i bili primljeni na neurologiju/oddeljenje intenzivne nege sa dijagnozom moždanog udara. U istraživanje su bili uključeni sledeći hematološki parametri: zbirni imunsko-zapaljenski količnik (*pan-immune-inflammation value* – PIV), indeks sistemskog zapaljenskog odgovora

(*systemic inflammatory response index* – SIRI), sistemski imunsko-zapaljenski indeks (*systemic immune-inflammation index* – SII) i indeks CALLY. Ovi parametri izračunati su iz uzoraka krvi bolesnika uzetih prilikom prijema, a neurohirurzi su procenili razvoj cerebralnog edema i potrebu za dekompresijom. Parametri su upoređivani između dve grupe bolesnika: onih kod kojih je postojala potreba za dekompresijom, hirurška grupa (*surgical group* – SG) i onih bez takve potrebe, nehirurška grupa (*nonsurgical group* – NSG). **Rezultati.** Istraživanjem je obuhvaćeno ukupno 274 bolesnika, od kojih je 189 (68,90%) bilo u NSG, a 85 (31,10%) u SG. Utvrđeno je da je indeks CALLY bio značajno viši kod bolesnika NSG u odnosu na SG (16,47 vs. 0,79; $p < 0,001$). Nivoi SIRI i SII bili su značajno viši kod bolesnika SG u odnosu na NSG ($p < 0,001$ i $p = 0,001$,

redom). Nivo PIV bio je takođe značajno viši kod bolesnika SG u odnosu na NSG ($p < 0,001$). Na *cut-off* vrednosti 4,06, utvrđena je senzitivnost 69,40% i specifičnost 83,00% CALLY indeksa **Zaključak.** Indeks CALLY je lako dostupan marker koji je jednostavno izračunati, kojim se može predvideti

potreba za dekompresijom koja se može razviti kod bolesnika sa moždanim udarom.

Ključne reči:**mozak, edem; c-reaktivni protein; hematološki testovi; moždani udar.**

Introduction

A cerebrovascular accident is a disorder of blood vessels in the brain. The main cause of this disorder is difficulty in getting oxygen and nutrients as a result of the interruption of blood flow to the brain¹. Symptoms of a cerebrovascular accident can lead to admission to the emergency department with different symptoms, such as numbness or weakness in the face and body, imbalance or lack of coordination, loss of vision, or blurred vision, depending on the location of the affected area in the brain and the damage^{2,3}. Treatment of a cerebrovascular event may require immediate intervention and is usually carried out in an emergency department. The aim of the treatment is to minimize damage to brain tissue, restore blood flow, and prevent complications. There are a number of ways to do this, including drugs, endovascular interventions, or surgery^{4,5}.

Cerebral edema (CE) is a condition caused by the build-up of excess fluid in the brain, which can put pressure on brain tissue and disrupt normal brain function⁶. CE can occur for many reasons, including brain trauma, infection, tumor, or blockage in the brain vessels. Symptoms of CE can include headache, dizziness, nausea, vomiting, visual problems, behavioral changes, and even loss of consciousness^{7,8}. Treatment may vary depending on the cause of the edema and may include medication, surgery, or other treatments. CE can be a serious condition, and it is important to seek medical attention immediately⁹. This pathological increase in intracerebral hemispheric volume can lead to a mass effect with an increase in compartment pressure, accelerating subfalcine and transtentorial herniation¹⁰. This type of CE is responsible for the majority of neurological impairment and carries a mortality of approximately 80% without surgical decompression. If there is a sudden deterioration in mental status, a malignant edema clinic should be prioritized¹¹.

Since cerebrovascular events damage brain tissue, an inflammatory response can occur. Inflammation increases the activity of immune cells in the brain to eliminate damaged cells in brain tissue^{7,11}. The effects of inflammation resulting from a cerebrovascular event can include further damage to brain tissue, the formation of edema, and deterioration in brain function^{11,12}. In addition, inflammation following a cerebrovascular event may contribute to long-term damage to brain blood vessels and an increased risk of recurrent events. Studies have shown an association between CE and the inflammation that can occur after a stroke^{12,13}. Recently, the relationship between malignancy, gastrointestinal disease, and pneumonia and the C-

reactive protein (CRP)-albumin-lymphocyte (CALLY) index, calculated from albumin, lymphocyte, and CRP parameters, has been reported¹⁴⁻¹⁶. Very few studies in the literature investigate the relationship between CE, which can develop in stroke patients, and the CALLY index. Therefore, the aim of this study was to determine the relationship between the CALLY index and CE developed in stroke patients.

Methods

Patients

This retrospective study included a total of 274 patients aged 18 and above who presented to the emergency department of a tertiary teaching and research hospital from January 2021 to December 2023 and were admitted to the neurology department/intensive care unit with a diagnosis of stroke. Stroke was diagnosed using physical examination, clinical and imaging techniques, and internationally accepted guidelines.

The study was approved by the Ethics Committee of the Antalya Training and Research Hospital (from March 21, 2024).

Exclusion criteria included the following patients: those transferred to another hospital for neurological follow-up and treatment, those with chronic malnutrition, autoimmune, hematological, and renal diseases that could affect the CALLY index, those with additional pathology that could cause CE, then, patients with malignancy, patients using steroids and the like, and patients who did not accept medical treatment. They were defined as patients who did not accept medical treatment.

Laboratory and clinical data

Demographic data and laboratory parameters were collected from patients diagnosed with stroke who met the inclusion criteria for the study. The parameters were calculated from the blood drawn from the patients on admission, and neurosurgeons determined the development of CE and the need for decompression in patients under 70 years of age with a large middle cerebral artery infarct in the non-dominant hemisphere and in other patients within the first 48 hrs. Indication for surgery was based on criteria such as the shift of midline structures on tomography, and relevant parameters were compared between patients with and without the need for decompression.

According to the need for decompression, all patients were divided into two groups: surgical group (SG) with

85 patients and nonsurgical group (NSG) with 189 patients.

The pan-immune-inflammation value (PIV), one of the hematological parameters, was calculated using the following formula: neutrophil count ($10^9/L$) \times platelet count ($10^9/L$) \times monocyte count ($10^9/L$)/lymphocyte count ($10^9/L$). The systemic inflammatory response index (SIRI), an inflammatory marker that has been increasingly used in recent years, is calculated using the formula: neutrophil count ($10^9/L$) \times monocyte count ($10^9/L$)/lymphocyte count ($10^9/L$). The systemic immune-inflammation index (SII) was calculated using the formula: neutrophil count ($10^9/L$) \times platelet count ($10^9/L$) /lymphocyte count ($10^9/L$). The CALLY index was calculated using the formula: albumin value (g/dL) \times lymphocyte count (μL)/CRP value (mg/dL) $\times 10^4$. Parameters were compared between patients in these two groups.

Statistical analysis

For statistical analysis of the data in our study, the SPSS 25.0 was used. In the analysis of demographic and laboratory data of patients who underwent decompression, frequency for categorical data is expressed with numbers (percentages). For the analysis of continuous variables, the mean \pm standard deviation (SD) was used, while for categorical data, Pearson Chi-square and Fisher's exact test were used. The Student *t*-test and Mann-Whitney *U* test showed the effects of hematological parameters on the need for decompression. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal values of these markers for the need for decompression.

The value of $p < 0.05$ was considered statistically significant.

Results

A total of 274 patients who met the inclusion criteria were included in our study, of which 189 (68.90%) were in NSG and 85 (31.10%) in SG. When both groups were compared in terms of gender, the percentage of females was higher in SG than in NSG (44.70 vs. 31.20; $p = 0.022$). Hemoglobin and albumin levels were significantly higher in NSG patients compared to SG patients. Mean neutrophil count, monocyte count, and CRP levels were significantly higher in SG patients compared to NSG patients. A comparison of demographics and laboratory values between groups is shown in Table 1.

The CALLY index, one of the parameters obtained from the combination of blood parameters, was significantly higher in NSG patients than in SG (16.47 vs. 0.79; $p < 0.001$). SIRI and SII levels were significantly higher in patients in SG than in NSG ($p < 0.001$ and $p = 0.001$, respectively). PIV level was also significantly higher in SG patients than in NSG ($p < 0.001$). The values obtained from the combination of hemogram parameters are compared in Table 1. These values were calculated by drawing ROC curves to determine the need for decompression in stroke patients (Figure 1).

The effectiveness of the CALLY index, SIRI, SII, and PIV parameters in predicting the need for decompression was statistically significant. At a cut-off value of 4.06, the CALLY index had a sensitivity of 69.40% and a specificity of 83.00% (Table 2).

Table 1

Demographic and laboratory findings for examined patients with acute ischemic stroke

Parameters	Non-Surgery Group (n = 189)	Surgery Group (n = 85)	<i>p</i> -value
Female	59 (31.20)	38 (44.70)	0.022
Age, years	64.05 \pm 13.91	60.25 \pm 18.01	0.092
White blood cells	8.00 \pm 2.57	12.30 \pm 6.52	< 0.001
Hemoglobin	12.94 \pm 1.65	11.38 \pm 2.55	< 0.001
Platelets	238.91 \pm 80.17	240.21 \pm 99.34	0.167
Neutrophils	4.89 \pm 2.21	9.94 \pm 6.49	< 0.001
Lymphocytes	2.17 \pm 0.77	1.44 \pm 0.92	0.498
Monocytes	0.68 \pm 0.22	0.77 \pm 0.47	< 0.001
CRP	4.0 (6.93)	41.60 (111.68)	< 0.001
Albumin	36.78 \pm 6.45	30.12 \pm 7.43	0.006
CALLY	16.47 (28.59)	0.79 (9.39)	< 0.001
SII	499.35 (327.40)	1,645.75 (2,120.34)	0.001
SIRI	1.39 (1.17)	4.73 (8.89)	< 0.001
PIV	320.96 (315.21)	955.44 (2,029.67)	< 0.001

CRP – C-reactive protein; CALLY – CRP-albumin-lymphocyte; SII – systemic immune-inflammation; SIRI – systemic response inflammatory index; PIV – pan-immune-inflammation value.

All values are given as mean \pm standard deviation or median (interquartile range), except for Female parameter, which is expressed as number (percentage).

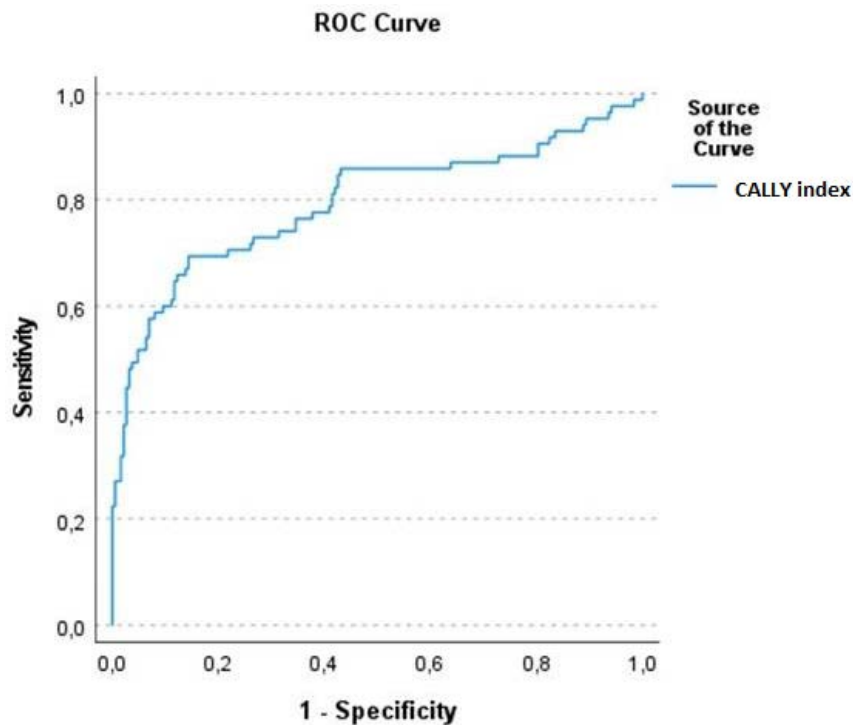


Fig. 1 – Receiver operating characteristic (ROC) curve analysis – C-reactive protein albumin-lymphocyte (CALLY) index of acute ischemic stroke patients predict cerebral edema.

Table 2

Receiver operating characteristic curves for hematologic parameters

Parameters	AUC	<i>p</i> -value	Cut-off	Sensitivity (%)	Specificity (%)
CALLY	0.795	0.001	4.06	69.40	83.00
SII	0.824	0.001	956.38	67.10	89.90
SIRI	0.775	0.001	2.96	63.50	88.40
PIV	0.765	0.001	709.68	57.60	89.40

AUC – area under the curve.

For other abbreviations, see Table 1.

Discussion

Although CE occurs approximately two to five days after stroke, the biological process that causes CE begins within hours of stroke onset¹⁷. A combination of cytotoxic, ionic, and vasogenic edema contributes to increased brain volume and can lead to increased pressure in this region¹⁸. Studies have shown that CE is associated with stroke size, National Institutes of Health Stroke Scale (NIHSS) score, and admission glucose levels with poor prognosis^{14–18}. Studies have shown the prognostic efficacy of lymphocyte count as an indicator of immune competence, especially in patients with malignancies^{12–16}. Acute phase reactants such as CRP and albumin are also used effectively in many diseases, especially in malignancy patients¹⁹. In our study, we investigated the relationship between the CALLY index and CE, which can develop in stroke patients.

The CALLY index was first shown to be prognostic in patients with malignancies and has been used effectively in the diagnosis and prognosis of many bowel diseases. In a study of oral cancer, preoperative CALLY was reported to be

a simple and inexpensive prognostic marker²⁰. Similarly, in another study on colorectal malignancies, the CALLY index appeared as an independently associated prognostic marker¹⁵. In a study of hepatocellular carcinoma patients with hepatitis C infection, the prognostic efficacy of the CALLY index was demonstrated at the optimal value of 5¹³. A study by Yang et al.¹⁵ demonstrated the long-term prognostic efficacy after surgery in 1,260 colorectal cancer patients and the importance of early assessment of the immuno-inflammatory response. In a study of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected patients, the CALLY index was reported to predict in-hospital mortality with a sensitivity of 76.15% and a specificity of 62.50%²¹. Another study demonstrated the utility of a high CALLY index, with an optimal cut-off value of 3.0, as a new prognostic biomarker and reported its effectiveness in patients suitable for postoperative adjuvant treatment with poor prognosis¹⁵. Recently, while conducting a study concerning the CALLY index, we found in the literature that the CALLY index can be considered an easily applicable indicator for the mortality of glioblastoma patients²². In a study by Gürbüz and To-

zođlu²³, it was found that new inflammatory, immunonutritive, and cardiovascular biomarkers SII, SIRI, atherogenic index of plasma (AIP), and CALLY index could be promising clinical tools for evaluating the severity, potential complications, and treatment response of alcohol use disorder. In a study by Fukushima et al.²⁴, the preoperative CALLY index < 2 was independently associated with a poor prognosis for patients after gastrectomy for gastric cancer. In our study, we found that the CALLY index could indicate the need for decompression with high sensitivity and specificity (69.40% and 83.00%), with the optimal value for the CALLY index being 4.06.

Limitations of the study

Our study has some limitations. First of all, this is a retrospective, single-center study. It is known that the parameters and the inflammatory process used for the CALLY index represent a dynamic situation, and the inability to follow the process and the changes in these parameters during the follow-up of these patients is an important limitation. In addition, the inability to compare inflammatory parameters such

as CRP, interleukins (e.g., tumor necrosis factor), or white blood cells is another limitation. Although parameters such as the inflammation-related CALLY index are associated with many diseases, it may not be correct to make a surgical decision and associate mortality entirely with this parameter, representing yet another limitation. Finally, the extent to which the parameters are useful for the clinical follow-up of the disease is also a limitation, including the fact that the disease onset time cannot be clearly determined. A prospective multicenter study is needed so that the data in our study can be analyzed.

Conclusion

Based on the results of our study, the C-reactive protein-albumin-lymphocyte index is an easily accessible and calculable marker that can predict the need for decompression in stroke patients.

Conflict of interest

The authors declare no conflict of interest.

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The effect of three different acrylic intraocular lenses on the glistening formation

Uticaj tri različita akrilna intraokularna sočiva na formiranje *glistening*-a

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Abstract

Background/Aim. One of the postoperative complications of phacoemulsification is the formation of fluid-filled microvacuoles inside the implanted intraocular lens (IOL). This condition is known as ‘glistening’. The aim of this study was to determine the incidence of glistening formation after the implantation of three different acrylic IOLs during the two-year follow-up period. **Methods.** Cataract surgery was performed in 93 patients (93 eyes) with developed senile cataracts. According to the implanted IOL, patients were equally divided into three groups: group with single-piece hydrophilic (SPHphil) acrylic IOL, group with single-piece hydrophobic (SPHphob) acrylic IOL, and group with three-piece hydrophobic (TPHphob) acrylic IOL. The presence of glistening was measured five times: 1, 6, 12, 18, and 24 months after phacoemulsification. **Results.** Statistically significantly lower glistening incidence was recorded in the SPHphil group compared to the SPHphob and TPHphob group, six months after phacoemulsification ($p < 0.05$). That difference was even higher one year after the cataract surgery ($p < 0.01$) and remained at that level until the end of the study. During the whole follow-up period, no statistically significant difference was recorded among SPHphob and TPHphob groups ($p > 0.05$). **Conclusion.** The presence of glistening was recorded in all groups. Our results strongly suggest that the progression of glistening was the most pronounced in the first postoperative year. However, a very low glistening incidence associated with SPHphil IOL could be particularly beneficial in patients expected to develop increased postoperative inflammation.

Key words:

cataract; lenses, intraocular; ophthalmologic surgical procedures; phacoemulsification.

Apstrakt

Uvod/Cilj. Jedna od postoperativnih komplikacija fakoemulzifikacije je formiranje mikrovakuola ispunjenih tečnošću unutar implantiranog intraokularnog sočiva (IS). Ovo stanje poznato je kao *glistening*. Cilj rada bio je da se utvrdi incidencija stvaranja *glistening*-a posle implantacije tri različita akrilna IS tokom dve godine praćenja. **Metode.** Operacija katarakte izvršena je kod 93 bolesnika (93 oka) sa razvijenom formom senilne katarakte. Prema implantiranom IS, bolesnici su podeljeni u tri jednake grupe: grupu sa jednodelnim hidrofilnim (JDHfil) akrilnim IS, grupu sa jednodelnim hidrofobnim (JDHfob) akrilnim IS i grupu sa trodelnim hidrofobnim (TDHfob) akrilnim IS. Prisustvo *glistening*-a mereno je pet puta: 1, 6, 12, 18 i 24 meseca nakon fakoemulzifikacije. **Rezultati.** Statistički značajno niža incidencija *glistening*-a zabeležena je u grupi JDHfil, u poređenju sa bolesnicima grupe JDHfob i TDHfob, šest meseci posle fakoemulzifikacije ($p < 0,05$). Ta razlika bila je još veća godinu dana posle operacije katarakte ($p < 0,01$) i ostala je na tom nivou do kraja studije. Tokom čitavog perioda praćenja nije zabeležena statistički značajna razlika među grupama JDHfob i TDHfob ($p > 0,05$). **Zaključak.** Prisustvo *glistening*-a zabeleženo je u svim grupama. Naši rezultati snažno sugerišu da je progresija *glistening*-a bila najizraženija u prvoj postoperativnoj godini. Međutim, veoma niska incidencija *glistening*-a povezana JDHfil IS može biti posebno korisna kod bolesnika kod kojih se očekuje razvoj povećane postoperativne inflamacije.

Ključne reči:

katarakta; sočiva, intraokularna; hirurgija, oftalmološka, procedure; fakoemulzifikacija.

Introduction

Cataract surgery (CS) is the most commonly performed surgery worldwide^{1,2}. Phacoemulsification has represented a standard technique for CS in the last few decades^{3,4}. It is known that this technique decreased the intraoperative and postoperative complications rate compared to previously used intracapsular and extracapsular cataract extraction⁵. Moreover, phacoemulsification provides far better recovery of the postoperative visual function^{6,7}. However, this procedure also has some limitations and possible complications. One of the complications is the formation of fluid-filled microvacuoles inside the implanted intraocular lens (IOL). This condition is known as 'glistening'⁸. Glistening occurs in the postoperative period (POP) and can cause light scattering as well as decreased visual acuity (VA) and glare by changing the refractive index between IOL and aqueous humor. These symptoms can cause dissatisfaction and even fear in patients in the POP, especially if the patients know that the CS was performed without complications⁹. The high frequency of uneventful phacoemulsification, along with increased life expectancy of patients, the increased number of lens surgeries performed on younger patients, and the existence of numerous ocular comorbidities are factors that support the development of glistening in the POP¹⁰. Knowing that the only way to treat developed glistening is the IOL exchange gives this condition even more importance.

Glistening was mentioned for the first time in 1984 after the implantation of polymethyl methacrylate (PMMA) IOL¹⁰. So far, many studies have confirmed the presence of glistening in all materials used in the production of IOLs, including PMMA, silicone, hydrogel, and hydrophobic and hydrophilic acrylate^{9,11,12}.

According to their chemical structure, IOLs are polymers. During polymerization, small interspaces occur where the monomers are not ideally bound. Because of this, different parts characterized by higher and lower density are created inside the IOL. The water diffuses within the IOL and tends to accumulate precisely in the parts with lower density, such as in the cavities in which the monomers are not ideally bound to each other. These hollow spaces filled with fluid are clinically manifested as glistening¹³ (Figure 1). The degree of water absorption depends on the IOL material and temperature¹⁴. When the IOL is surrounded by warm water, glistening does not form. Due to the drop in temperature, there is a supersaturation of the water inside the polymer and the glistening is formed¹⁵. In addition to the influence of the IOL material, more frequent glistening development was found in patients with glaucoma, uveitis, and IOLs of higher diopter strength¹⁶. Moreover, patients with a longer POP, as well as those who underwent combined cataract and glaucoma surgery, i.e., phacotrabeculectomy, had a higher incidence of glistening formation¹⁷.

Glistening can be diagnosed during a detailed patient examination with a biomicroscope. Due to the change in the IOL optical property, a small percentage of light is reflected towards the biomicroscope, which enables the visualization

of glistening¹⁸. The diameter of the clinically visible vacuole averages from 1 to 20 μm . Miyata et al.¹⁹ graded glistening in the following way: grade 0 – no glistening; grade I – 50 vacuoles/ mm^2 ; grade II – 51–100 vacuoles/ mm^2 ; grade III – 100–200 vacuoles/ mm^2 .

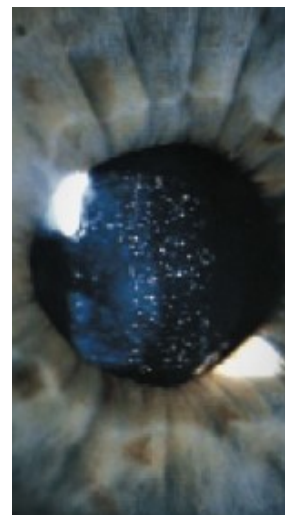


Fig. 1 – Biomicroscope image of implanted acrylic intraocular lens with developed glistening.

It is well-known that phacoemulsification leads to the occurrence of postoperative inflammation due to blood-aqueous barrier breakdown². Long-term usage of antiglaucoma eye drops and the presence of uveitis or diabetes mellitus also contribute to intraocular inflammation, and thus the development of glistening. Current approaches in glistening reduction refer to the choice of an adequate surgical technique and IOL type, as well as the treatment of ocular comorbidities, in order to decrease postoperative inflammation^{20–22}.

The aim of this study was to determine the incidence of glistening formation after the implantation of three different acrylic IOLs during the two-year follow-up period.

Methods

The study was conducted at the Clinic for Ophthalmology of the University Clinical Center Kragujevac. It was designed as a prospective, randomized clinical study that lasted two years. The study was carried out according to the Declaration of Helsinki and approved by the Ethics Committee of the University Clinical Center Kragujevac (No. 01/17/1829, from May 25, 2017). Initially, all the patients gave their written consent to participate in the study. The research included 93 patients with senile cataracts. All the patients were operated on by one experienced surgeon using the same surgical technique and followed for 24 months after the phacoemulsification. A complete ophthalmological examination was performed one day before the surgery, on the first postoperative day, and 1, 6, 12, 18, and 24 months after the phacoemulsification. It included the measurement of VA and intraocular pressure, biomicroscope examination, keratometry, ophthalmoscopy,

ocular ultrasonography, and ultrasound biometry. The development of glistening was measured five times: 1, 6, 12, 18, and 24 months after CS. In this study, we applied the glistening gradation that Miyata et al.¹⁹ performed using high-resolution images made at the biomicroscope.

The study included participants of both sexes, older than 65 years, with a confirmed diagnosis of senile cataract. Patients with presenile, traumatic, complicated, iatrogenic, congenital, and all other types of cataracts were excluded from the study. Those with a history of previous intraocular surgery and eye injuries or patients with corneal diseases, glaucoma, strabismus, uveitis, and retinal diseases could not participate in the study. Patients with complications during and after phacomemulsification, as well as those patients suffering from systemic autoimmune diseases, were excluded. Therefore, only patients with developed senile cataracts, without any underlying ocular condition, scheduled to be operated on by one surgeon using the same surgical technique were included in the study. Depending on the type of IOL which was going to be implanted during CS, the 93 patients (i.e., 93 eyes) were equally randomized into three groups: first group – single-piece hydrophilic acrylic IOL (SPHphil) (Eyecryl plus 600, Biotech visioncare, Luzern, Switzerland); second group – single-piece hydrophobic acrylic IOL (SPHphob) (AcrySof SA60AT, Alcon-Couvreur NV, Puurs, Belgium); third group – three-piece hydrophobic acrylic IOL (TPHphob) (AcrySof MA60AC, Alcon-Couvreur NV, Puurs, Belgium).

These IOLs were chosen because the Clinic for Ophthalmology, where the study was conducted, is part of the University Clinical Center, which belongs to the Public Health System and possesses exactly these three types of IOLs implanted in all patients who underwent CS.

All the surgeries were performed under local anesthesia, using topical tetracaine. Adequate mydriasis was achieved preoperatively using topical phenylephrine and tropicamide (2.5% phenylephrine, 0.5% tropicamide, Pharmacy “Zaječar”, Zaječar, Serbia). The Phaco machine used in all surgeries was Stellaris Elite™ (Bausch & Lomb). The skin of the eyelids and periorbites was cleansed with a 10% solution of povidone-iodide, and the conjunctival fornix was washed with a 5% solution of povidone-iodide. After placing blepharostat and self-adhesive sterile compress, CS was started by creating two lateral paracentesis 1.5 mm wide at 2 and 10 o'clock. If necessary, trypan blue (0.06% ophthalmic solution 1 mL, Sidapharm, Thessaloniki, Greece) was used for better visualization of the anterior lens capsule. A cohesive viscoelastic (Bio-Hyalur plus, Biotech visioncare) was injected into the anterior chamber, and a “clear cornea” incision 2.75 mm wide was made at 12 o'clock. Continuous capsulorhexis, hydrodissection, and nucleus rotation followed. The lens phacofragmentation technique was “divide and conquer”, after which the lens fragments were aspirated. Aspiration of the remaining epicortex and polishing of the posterior lens capsule were performed using bimanual irrigation and aspiration. The anterior chamber and capsular bag were filled with cohesive hyaluronate and IOL was implanted in the capsular bag. Viscoelastic was aspirated from the anterior

chamber of the eye and the capsular bag. A diluted solution of cefuroxime (Nilacef®, Hemofarm AD, Vršac, Serbia; 1 mg/0.1 mL of balanced saline) was injected into the anterior chamber. Corneal incision wounds were hydrated. Postoperatively, topical dexamethasone-tobramycin (Tobradex®, Alcon-Couvreur NV, Puurs, Belgium) was administered six times a day for one week, then four times a day for another three weeks, and nepafenac (Nevanac®, Alcon-Couvreur NV, Puurs, Belgium) was administered four times a day for two weeks.

In the follow-up period, the influence of the patient's sex and age was also analyzed due to their possible effect on glistening formation. Therefore, each group was further divided into patients aged 65 to 75 and patients over 76, as well as male and female patients. The presence of clinical signs of glistening in the form of glare was also analyzed in the study.

Statistical analysis

SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA) was used in statistical analysis. The significance at different time intervals during the research was tested with the Student's *t*-test or by the Wilcoxon equivalence test in cases where the distribution was not normal. The incidence of glistening grades according to the IOL type was done using the Chi-Square test (χ^2) test and ANOVA ($p < 0.05$ value was accepted as statistically significant).

Results

The study included 93 patients (i.e., 93 eyes) equally divided into three groups ($n = 31$ each) according to the type of implanted IOL. In the first postoperative month, one patient from the third group died, so the total number of the followed participants was 92. Of that number, 48 (52.2%) patients were male and 44 (47.8%) were female. No statistically significant differences between the sexes were noticed in all groups ($\chi^2 = 0.17$, $df = 1$, $p > 0.05$).

Demographic characteristics of patients are shown in Table 1. The mean age of the patients was 73.5 ± 5.95 years (range 65–87). No statistically significant difference was observed in the mean age of the patients among the groups ($F = 0.26$, $df = 2$, $p > 0.05$).

During the study glistening was noticed in 43 patients (46.7%). Of that number, 20 (21.7%) patients were male and 23 (25%) female. No statistically significant differences between the sexes were recorded in glistening formation ($p > 0.05$).

One month after the surgery, 94.6% of patients had glistening grade 0, while only 5.4% had glistening grade I. During the first postoperative year, a statistically significant difference was noticed in every following visit compared to the previous one ($p < 0.05$). From that moment until the end of the research, no statistically significant glistening progression was observed ($p > 0.05$) (Table 2).

Two years after phacoemulsification, glistening was not recorded in 53.3% of patients (grade 0), while 18.5% of

Table 1

Demographic characteristics of patients with senile cataracts according to the type of implanted acrylic intraocular lens

Parameter	Groups			<i>p</i> ^a
	SPHphil (n = 31)	SPHphob (n = 31)	TPHphob (n = 30)	
Sex				
male	14	17	17	> 0.05
female	17	14	13	
Age, years				
mean ± SD	72.94 ± 6.12	73.42 ± 5.39	74.03 ± 6.44	> 0.05
min–max	65–86	65–85	65–87	

SPHphil – single-piece hydrophilic; SPHphob – single-piece hydrophobic; TPHphob – three-piece hydrophobic; SD – standard deviation; min – minimum; max – maximum.

^aANOVA.

Table 2

Progression of glistening in patients with senile cataracts during the follow-up period

Time after the cataract surgery	Glistening grade				<i>p</i> ^a
	0	I	II	III	
1 month	87 (94.6)	5 (5.4)	0 (0)	0 (0)	
6 months	60 (65.2)	18 (19.6)	10 (10.9)	4 (4.3)	< 0.05*
12 months	49 (53.3)	18 (19.6)	15 (16.3)	10 (10.9)	
18 months	49 (53.3)	17 (18.5)	16 (17.4)	10 (10.9)	
24 months	49 (53.3)	17 (18.5)	16 (17.4)	10 (10.9)	> 0.05

Values are given as numbers (percentages) of patients.

*statistically significant during the first 12 months; ^a Chi-Square test.

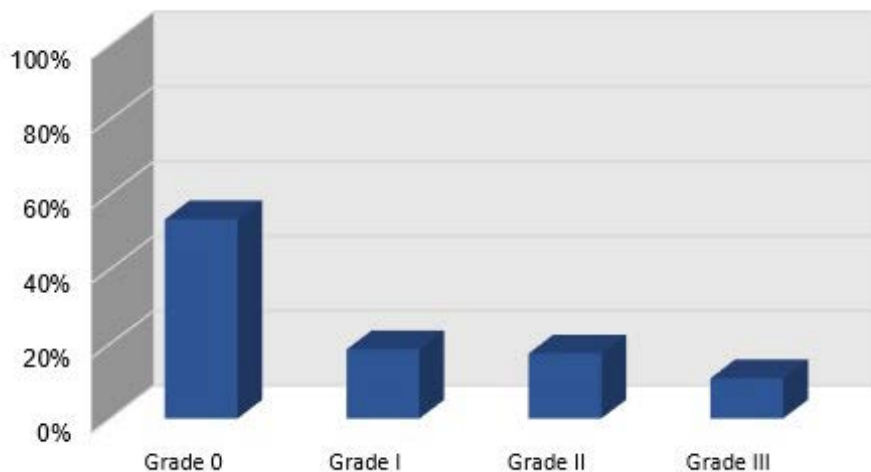


Fig. 2 – Incidence of glistening grades at the end of the research ¹.

Note: ¹ – two years after phacoemulsification.

patients had grade I, 17.4% had grade II, and 10.9% had grade III glistening (Figure 2).

Glistening expressed in grades according to the IOL type during the follow-up period is presented in Table 3.

The analysis of glistening depending on the IOL type one month after the CS showed no statistically significant difference among the groups ($p > 0.05$) (Table 4).

Six months after phacoemulsification the lowest frequency of glistening grades was recorded in the SPHphil group. Intergroup analysis revealed the existence of a significant difference in glistening between the SPHphil and SPHphob groups ($\chi^2 = 45.45$, $df = 28$, $p < 0.05$), as well as be-

tween SPHphil and TPHphob groups ($\chi^2 = 33.56$, $df = 29$, $p < 0.05$). The difference among the SPHphob and TPHphob groups was not significant ($\chi^2 = 2.47$, $df = 6$, $p > 0.05$).

One year after the CS, the difference was statistically significant between SPHphil and SPHphob groups ($\chi^2 = 55.74$, $df = 12$, $p < 0.01$), as well as between SPHphil and TPHphob groups ($\chi^2 = 47.21$, $df = 28$, $p < 0.01$). The difference between the groups with implanted hydrophobic IOLs was not statistically significant ($\chi^2 = 4.54$, $df = 6$, $p > 0.05$).

In the last two measurements, 18 and 24 months after CS, the results have not changed significantly compared with the results recorded on the 12th postoperative month. The

Table 3

Time after the CS/IOL type	Glistening grade			
	0	I	II	III
1 month				
SPHphil	30 (96.8)	1 (3.2)	0 (0)	0 (0)
SPHphob	29 (93.5)	2 (6.5)	0 (0)	0 (0)
TPHphob	28 (93.3)	2 (6.7)	0 (0)	0 (0)
6 months				
SPHphil	22 (71.0)	6 (19.4)	3 (9.6)	0 (0)
SPHphob	19 (61.3)	6 (19.4)	4 (12.8)	2 (6.5)
TPHphob	19 (63.3)	6 (20)	3 (10.0)	2 (6.7)
12 months				
SPHphil	20 (64.5)	6 (19.4)	3 (9.6)	2 (6.5)
SPHphob	14 (45.2)	5 (16.1)	7 (22.6)	5 (16.1)
TPHphob	15 (50.0)	7 (23.3)	5 (16.7)	3 (10.0)
18 months				
SPHphil	20 (64.5)	5 (16.1)	4 (12.9)	2 (6.5)
SPHphob	14 (45.2)	5 (16.1)	7 (22.6)	5 (16.1)
TPHphob	15 (50.0)	7 (23.3)	5 (16.7)	3 (10.0)
24 months				
SPHphil	20 (64.5)	5 (16.1)	4 (12.9)	2 (6.5)
SPHphob	14 (45.2)	5 (16.1)	7 (22.6)	5 (16.1)
TPHphob	15 (50.0)	7 (23.3)	5 (16.7)	3 (10.0)

CS – cataract surgery. For other abbreviations, see Table 1.

Values are given as numbers (percentages) of patients.

Table 4

Statistical significance of glistening depending on the IOL type and the time of measurement			
Time after the CS	SPHphil vs. SPHphob	SPHphil vs. TPHphob	SPHphob vs. TPHphob
1 month	$p > 0.05$	$p > 0.05$	$p > 0.05$
6 months	$p < 0.05^*$	$p < 0.05^*$	$p > 0.05$
12 months	$p < 0.01^{**}$	$p < 0.01^{**}$	$p < 0.05$
18 months	$p < 0.01^{**}$	$p < 0.01^{**}$	$p > 0.05$
24 months	$p < 0.01^{**}$	$p < 0.01^{**}$	$p > 0.05$

For abbreviations, see Tables 1 and 2. Chi-Square test.

*statistically significant; **highly statistically significant.

Table 5

Glistening depending on sex in each group during the follow-up period					
IOL type/Glistening grade	Months				
	1	6	12	18	24
SPHphil					
0	14/16	9/13	8/12	8/12	8/12
I	0/1	3/3	3/3	3/2	3/2
II	0/0	2/1	2/1	2/2	2/2
III	0/0	0/0	1/1	1/1	1/1
SPHphob					
0	16/13	10/9	7/7	7/7	7/7
I	1/1	4/2	3/2	3/2	3/2
II	0/0	2/2	4/3	4/3	4/3
III	0/0	1/1	3/2	3/2	3/2
TPHphob					
0	16/12	11/8	9/6	9/6	9/6
I	1/1	4/2	4/3	4/3	4/3
II	0/0	1/2	3/2	3/2	3/2
III	0/0	1/1	1/1	1/2	1/2

For abbreviations, see Table 1. Values are given as numbers of patients (males/females). Chi-Square test.

No statistically significant influence of patients' sex was recorded in glistening development in all groups ($p > 0.05$).

difference between the SPHphil group and the other two groups remained highly statistically significant ($p < 0.01$), while no significant difference was measured between the hydrophobic groups ($p > 0.05$) (Table 4).

The glistening formation was also analyzed depending on the patient's sex (Table 5) and age (Table 6). During all measurements, no statistically significant influence of the patient's sex and age was recorded in glistening development in all groups.

Table 6

Glistening depending on patients' age in each group during the follow-up period

IOL type/Glistening grade	1 month		6 months		12 months		18 months		24 months	
	65–75	76–86	65–75	76–86	65–75	76–86	65–75	76–86	65–75	76–86
SPHphil										
0	17	13	12	10	11	9	11	9	11	9
I	1	0	2	4	2	4	2	3	2	3
II	0	0	2	1	2	1	2	2	2	2
III	0	0	0	0	1	1	1	1	1	1
SPHphob										
0	15	14	11	8	8	6	8	6	8	6
I	1	1	3	3	2	3	2	3	2	3
II	0	0	1	3	5	2	5	2	5	2
III	0	0	1	1	3	2	3	2	3	2
TPHphob										
0	13	15	8	11	7	8	7	8	7	8
I	2	0	3	3	4	3	4	3	4	3
II	0	0	2	1	2	3	2	3	2	3
III	0	0	1	1	1	2	1	2	1	2

For abbreviations, see Table 1. Values are given as numbers of patients. Chi-Square test.

No statistically significant influence of patients' age was recorded in glistening development in all groups ($p > 0.05$).

Note: Patients were classified into two age groups: 65–75 and 76–86 years of age.

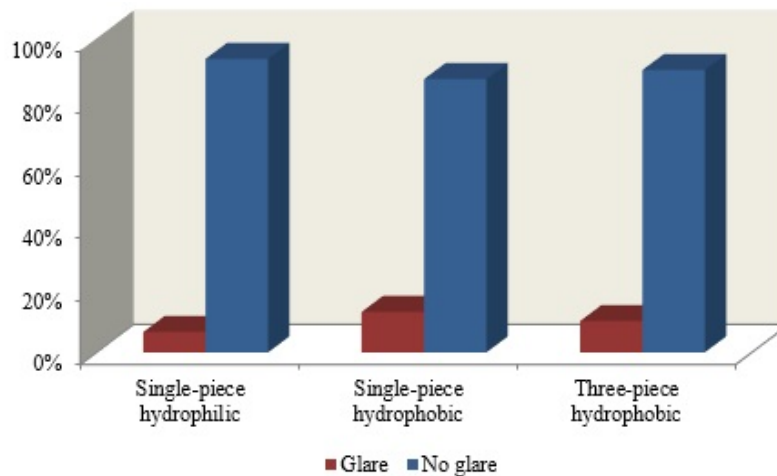


Fig. 3 – The incidence of glare according to the intraocular lens type.

Clinical signs of glistening in the form of glare were present in 9 (9.8%) patients. Four patients with glare were in the SPHphob group, three in TPHphob, and two in the SPHphil group. No significant difference in glare was observed among the groups ($\chi^2 = 0.73$, $df = 2$, $p > 0.05$) (Figure 3). All patients who had glare belonged to glistening grades II and III.

Discussion

Glistening is the formation of fluid-filled microvacuoles inside IOL. It is believed that the water content in the structure of the IOL is the most important predictive factor in glistening development. Miyata et al.¹⁹ first examined the mechanism of glistening formation. IOL was immersed in a bottle of physiological saline *in vitro* at 50 °C. The polymers from which IOLs are made absorb water when they are in an aqueous medium. The amount of liquid that IOL would absorb depended on the characteristics of the IOL material and

temperature. After spending 2 hrs at 50 °C, the IOL was immersed in a bottle of saline at 35 °C. Due to temperature difference, water oversaturation within the IOL followed, forming the microvacuoles, i.e., glistening. This process simulated an accelerated pace of the glistening formation *in vivo* in the eye. Although intraocular conditions are characterized by very small temperature fluctuations, glistening can develop even over a prolonged period of time. Kato et al.²³ demonstrated that a temperature change of only 3 °C causes glistening formation. Due to the difference in the refractive index between IOL and fluid-filled microvacuoles, light scattering occurs, which can be clinically manifested more often by the appearance of glare than by decreased contrast sensitivity or VA^{15, 24}. Glare can be the reason for patients' dissatisfaction after CS. Nowadays, phacoemulsification is performed more often in younger patients. Hence, glare can affect the patient's ability to work and lead to numerous problems in everyday life, such as reading, writing, walking, driving a car, taking prescribed therapy, and many other activities. This

way, glistening can affect the quality of vision and complete quality of life in the POP^{24, 25}.

In our study, the incidence of glistening was measured depending on the type of implanted IOL, as well as the occurrence of glare. During the two-year period of the study, the presence of glistening was noticed in 43 (46.7%) patients. Researchers are not unanimous about the incidence of glistening, and its occurrence in previous studies varies widely²⁶⁻³¹. Examining the incidence of acrylic hydrophobic IOLs, Colin et al.³² discovered glistening in 86.5% of patients. However, in a study published in 2018, Ton Van and Tran³³ did not prove the presence of glistening during the three years of follow-up after the implantation of the en-Vista® MX60 IOL (Bausch & Lomb) on a sample of 245 eyes. It has been proven that IOL material has a great influence on glistening development. Although acrylic IOLs have primacy in CS today due to their optical and immunological characteristics, Rønbeck et al.³⁴ found a statistically significantly higher incidence of glistening in these IOLs compared to PMMA and silicone.

Our results indicate that glistening formation started in the first postoperative month. In the following months, the progression of glistening was observed, with a significant difference until the 12th postoperative month, after which the progression was almost interrupted. During the research, a difference was noticed between IOLs made of hydrophilic and hydrophobic acrylate. Eyecryl plus 600 IOL contains 26% of the fluid. This percentage is significantly higher compared to hydrophobic acrylate IOLs (the percentage of liquid does not exceed 1%). Therefore, it is believed there was a higher water absorption and consequent glistening development in these IOLs. From the sixth postoperative month to the end of the study, a statistically significantly lower incidence of glistening was observed in the SPHphil group compared to the hydrophobic groups. On the other hand, the difference recorded between hydrophobic IOL groups was not significant. That difference between IOLs made of the same material can be explained by the different structures of monomers used in the production of these IOLs. During the polymerization of these monomers, cavities are formed. These cavities, in which glistening will be formed later, are different in morphology and quantity, depending on the IOL type. Many studies suggest that not only IOL material but also manufacturing technique, IOL packaging, IOL diopter, duration of the follow-up period, ocular disease, and patient age have a huge impact on glistening formation. Omar et al.³⁵ reported an *in vitro* study comparing glistening development in AcrySof acrylic hydrophobic IOLs based on AcryPack and Wagon Wheel packing systems. Glistening was recorded in both IOL types. However, IOLs packaged in Wagon Wheel did not form glistening when they were kept under constant temperature, while IOLs based on AcryPack displayed significantly more microvacuoles. IOL diopter can also have an influence on glistening formation.

Some studies reported that less glistening was developed in lower IOL diopters^{12, 26, 27}. It can be explained by the fact that IOL thickness is directly correlated with the IOL diopter. Therefore, fluid has more space to accumulate

in the thicker IOLs presented in higher IOL diopters. Researchers are quite unanimous in saying that glistening increases with time and that its incidence depends on the study duration^{23, 36}. Analyzing our results, after the intensive formation of glistening in all groups during the first postoperative year, a quite small degree of glistening progression was noticed during the last 12 months. These results are in contrast with the study by Wilkins and Olson³⁷, which recorded the progression of glistening continuously until the end of the third postoperative year. Interruption of progression is thought to occur when all cavities in the IOL polymer become filled with glistening. In our research, that period was 12 months. From that moment until the end of the study, glistening progression was seen in only one patient with hydrophilic IOL. Glistening formation is associated with different ocular comorbidities such as glaucoma, uveitis, or retinal diseases. In these conditions, the degree of intraocular inflammation and blood-aqueous barrier breakdown is increased, which in the POP contributes to the occurrence of glistening. Schweitzer et al.³⁸ and Colin and Orignac³⁹ separately showed the impact of glaucoma on the increased incidence of glistening. In glaucoma patients, in addition to the disease itself, various materials present in antiglaucoma medications can affect the permeability of the blood-aqueous barrier.

Glistening formation has decreased a lot in recent years with the use of modern materials and technologies in the production of IOLs. Nowadays, a large number of studies in ophthalmology are based on the implantation of IOLs, known as glistening-free IOLs. These IOLs are characterized by outstanding optical characteristics, with the absence of glistening formation^{40, 41}. They are made of new monomers that bond significantly better during the polymerization process, limiting the formation of spaces where liquid could accumulate in the POP.

We examined the influence of sex and age on glistening development. Statistical analysis did not determine the influence of sex on the development of glistening in any group. It is in accordance with numerous previous studies where no gender dominance has been proven in the development of glistening^{17, 18, 29}. Furthermore, our results did not show the influence of patients' age on the development of glistening. This can be explained by the fact that only patients with senile cataracts, over 65 years of age, without ocular comorbidities participated in our study. For that reason, a relatively similar inflammatory response to CS could be expected in all patients, and the development of glistening depended on the type of IOLs.

Our study had certain limitations, such as the number of patients, the follow-up period of two years, the usage of only three types of acrylate in the implanted intraocular lens, the absence of ocular complications, and cataract surgery performed only in people older than 65 years. However, our results can represent an excellent starting point for future research examining the development of glistening in younger patients with ocular comorbidities who will be implanted with an intraocular lens made of more modern materials.

Conclusion

In conclusion, we found the existence of glistening in all types of tested acrylic intraocular lenses. Our study has shown a huge impact of hydrophobic material on the glistening formation. On the other hand, no effect of the intraocular lens design was recorded on the glistening development. Our results also pointed out that the progression of glistening was the most intensive during the first postoperative year. Knowing that there is still no effective treatment for developed glistening, we believe that

the best treatment is prevention, and the main role in that prevention is the selection of an adequate intraocular lens.

Acknowledgement

The study is part of a doctoral dissertation titled "The influence of material and design of intraocular lens on the posterior capsule opacification development in patients who underwent cataract surgery by the phacoemulsification method" defended at the Faculty of Medical Sciences, University of Kragujevac, Serbia.

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Sarcoidosis-like reaction induced by immune checkpoint inhibitors in patients with advanced melanoma: a report of two cases and a brief review of the literature

Reakcija slična sarkoidozi izazvana inhibitorima kontrolnih tačaka kod bolesnika sa uznapredovalim melanomom: prikaz dva slučaja i kratak pregled literature

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Abstract

Introduction. Immunotherapy is associated with a wide range of adverse events. A drug-induced sarcoidosis-like reaction is a systemic granulomatous reaction that is no different from sarcoidosis and occurs in a certain temporal relationship with the initiation of the drug. **Case report.** The first presented patient was a 61-year-old male with stage IIIC BRAF-positive melanoma treated with adjuvant nivolumab therapy. After four cycles of therapy, enlarged mediastinal lymph nodes were confirmed using computed tomography. Bronchoscopy with bronchoalveolar lavage and transbronchial biopsy showed chronic granulomatous inflammation. After 12 cycles, grouped brownish-red papules and plaques covered with whitish scales were observed in the skin of both knees, and a histopathology finding indicated a sarcoidosis-like reaction. He was treated with oral prednisone, 60 mg daily in decreasing doses, and after 16 months, the enlarged mediastinal lymph nodes and skin lesions disappeared completely. The second presented patient was a 45-year-old male with stage

IIIC BRAF-positive melanoma treated with adjuvant pembrolizumab therapy. After four cycles, enlarged mediastinal lymph nodes were observed. Bronchoscopy with bronchoalveolar lavage revealed granulomatous inflammation, and transbronchial biopsy confirmed sarcoidosis. Therapy with oral prednisone 40 mg daily in decreasing doses was performed in the next three months, and immunotherapy was continued. The enlarged mediastinal lymph nodes resolved after completion of adjuvant therapy. **Conclusion.** In most cases, a diagnosis of a sarcoidosis-like reaction requires a biopsy of the suspected lesions. It is not usually necessary to stop immunotherapy, but sometimes standard corticosteroid therapy is indicated. An interdisciplinary approach is important to distinguish true disease progression from adverse drug reaction.

Key words:
biopsy; diagnosis, differential; disease progression; drug-related side effects and adverse reactions; melanoma; sarcoidosis.

Apstrakt

Uvod. Imunoterapija je povezana sa širokim spektrom neželjenih događaja. Reakcija slična sarkoidozi, izazvana lekom, je sistemska granulomatозна reakcija koja se ne razlikuje od sarkoidoze i vremenski je povezana sa uzimanjem leka kojim je izazvana. **Prikaz bolesnika.** Prvi prikazani bolesnik bio je muškarac star 61 godinu sa BRAF pozitivnim melanomom u IIIC stadijumu, lečen adjuvantnom terapijom nivolumabom. Posle četiri ciklusa terapije, primenom kompjuterizovane tomografije utvrđeni su uvećani medijastinalni limfni čvorovi. Bronhoskopijom

sa bronhoalveolarnom lavazom i transbronhijalnom biopsijom viđena je hronična granulomatозна inflamacija. Posle 12 ciklusa, na koži oba kolena uočene su braonkastrocrvene papule i plakovi prekriveni beličastim ljuspama, a histopatološkim nalazom utvrđena je reakcija slična sarkoidozi. Lečen je prednizonom oralno, 60 mg dnevno u opadajućim dozama i posle 16 meseci je došlo do potpunog nestanka uvećanih medijastinalnih limfnih čvorova i promena na koži. Drugi prikazani bolesnik bio je muškarac star 45 godina sa BRAF pozitivnim melanomom u IIIC stadijumu, lečen adjuvantnom terapijom pembrolizumabom. Posle četiri ciklusa, uočeno je uvećanje

medijastinalnih limfnih čvorova. Bronhoskopijom sa bronhoalveolarnom lavezom otkriveno je granulomatozno zapaljenje, a transbronhijalnom biopsijom potvrđena je sarkoidoza. Terapija prednizonom oralno 40 mg dnevno u opadajućim dozama primenjavana je naredna tri meseca, a imunoterapija je nastavljena. Uvećani medijastinalni limfni čvorovi su se povukli posle završetka adjuvantne terapije. **Zaključak.** U većini slučajeva, za postavljanje dijagnoze reakcije slične sarkoidozi potrebna je biopsija sumnjivih

lezija. Obično nije potrebno prekinuti imunoterapiju, ali je ponekad indikovana standardna terapija kortikosteroidima. Interdisciplinarni pristup je važan kako bi se razlikovala prava progresija bolesti od neželjene reakcije na lek.

Ključne reči:

biopsija; dijagnoza, diferencijalna; bolest, progresija; lekovi, neželjeni efekti i neželjene reakcije; melanom; sarkoidoza.

Introduction

Therapy with immune checkpoint inhibitors (ICIs) is associated with a wide spectrum of adverse events (AEs) related to their mechanism of action ¹. A drug-induced sarcoidosis-like reaction (DISR) is a systemic granulomatous reaction that is indistinguishable from sarcoidosis and occurs in a temporal relationship with the initiation of an offending drug ². Here, we report two cases of DSIR induced by adjuvant therapy of melanoma with ICIs.

Case report

Case 1

A 61-year-old man underwent melanoma excision from the skin of his right lower leg in October 2018. The histopathological (HP) findings were consistent with nodular melanoma: Breslow thickness 3.6 mm, Clark level IV with

ulceration, and primary tumor (pT) defined as pT3b. In November 2018, a re-excision and sentinel lymph node biopsy (SLNB) was performed, and no metastasis was observed. In June 2019 and January and November 2020, excisions of satellite and in-transit metastases from the right lower leg were performed. Due to the presence of lymphadenopathy, evacuation of the right inguinal fossa was performed in December 2020, when metastasis in one lymph node had been confirmed on HP analysis. During that period, until January 2021, the patient did not receive any systemic therapy. BRAF testing from the primary tumor was performed, and a V600E mutation in the *BRAF* gene was detected. The patient started therapy with nivolumab in January 2021 in a standard adjuvant regime. After four cycles of therapy, follow-up thoracic computed tomography (CT) imaging showed mediastinal lymph nodes enlarged up to 28 mm in diameter (Figures 1a and 1b). From the diagnosis of primary melanoma in October 2018 until the observed enlarged mediastinal lymph nodes, the patient

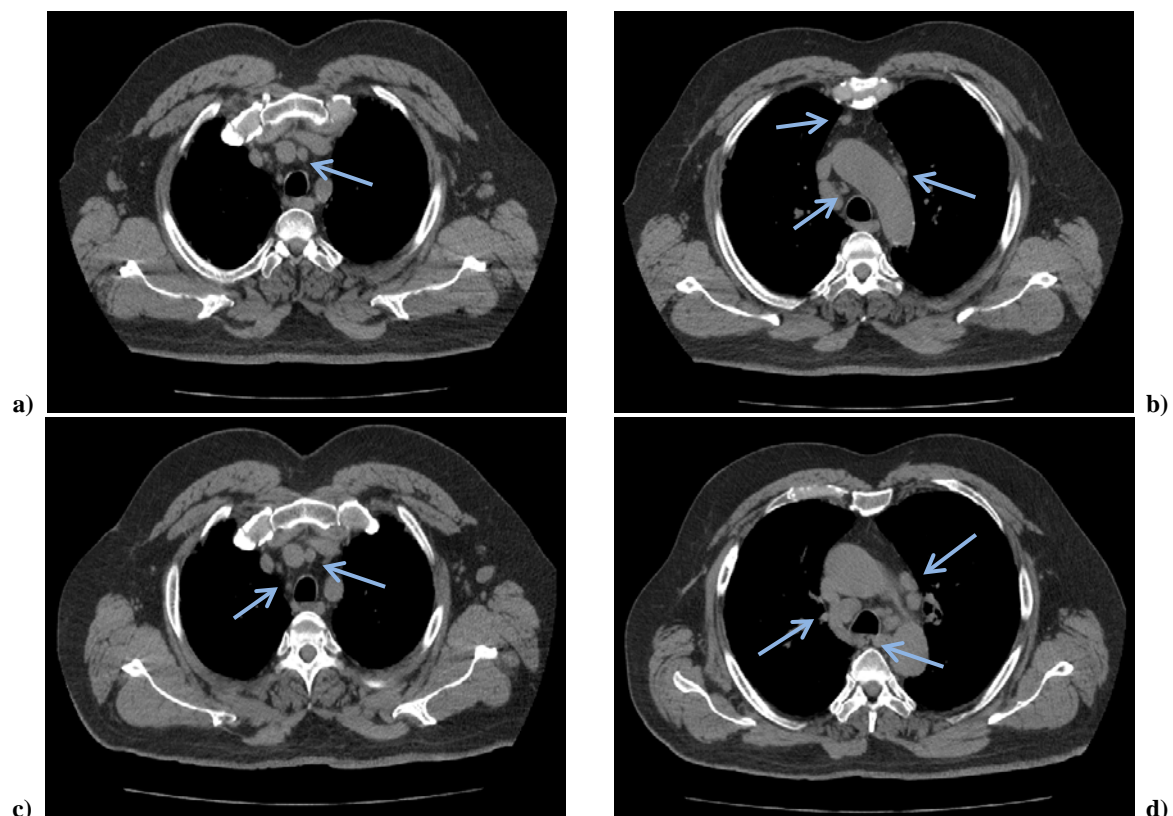


Fig. 1 – Mediastinal lymphadenopathy (blue arrows) after 4 cycles of nivolumab therapy up to 28 mm (a,b) and after 12 cycles of nivolumab therapy up to 17 mm (c, d).

underwent full body CT images every six months, which were completely normal. After 12 cycles of therapy, thoracic CT imaging showed persistence of the enlarged mediastinal lymph nodes (Figures 1c and 1d). Furthermore, grouped brownish-red papules and plaques covered with whitish scales were observed on the skin of the lower legs (Figure 2a) and dermatoscopy revealed a pattern of granulomatous inflammation – the presence of yellowish-orange globular fields, permeated with linear blood vessels (Figure 2b). HP analysis of the skin sample revealed DISR of the skin (Figure 3a). Bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsy were taken, and HP analysis was consistent with chronic granulomatous inflammation (Figure 3b). Histochemical analyses showed no visible Periodic acid-Schiff (PAS)-sensitive fungal or acid-resistant mycobacterial infectious agents (Ziehl-Nielsen). The 24-hour urine calcium was slightly elevated, the angiotensin-converting enzyme (ACE) level was normal, and the QuantiFERON® TB test was negative. Therapy with oral prednisone 60 mg daily in decreasing doses in the next three months was started, and nivolumab therapy was discontinued after 12 cycles due to the observed progression of the disease in the form of in-transit metastases. After three months, skin lesions on the lower extremities were in

regression, leaving hyperpigmented macules, and after 16 months, there was a confirmed complete remission of the enlarged mediastinal lymph nodes.

Case 2

A 45-year-old man has been diagnosed with nodular melanoma (Breslow thickness 5.2 mm, Clark level IV with ulceration) in February 2016, followed by re-excision and SLNB of the left inguinal region, without metastasis in the lymph nodes. Excision of in-transit metastases was performed in February, May, and October 2019. BRAF testing was performed, and mutation in the *BRAF* V600 gene was detected. From December 2019 to December 2020, adjuvant therapy with dabrafenib and trametinib was carried out. In July 2021, new in-transit metastases were excised, and in October 2021, adjuvant therapy with pembrolizumab was started. After four cycles of adjuvant therapy with pembrolizumab, follow-up thoracic CT imaging showed enlarged mediastinal lymph nodes (Figures 4a and 4b). From the diagnosis of melanoma, during the application of adjuvant target therapy until the detection of mediastinal lymphadenopathy, CT imaging of the whole body was performed every six months, with



Fig. 2 – Grouped brownish-red papules and plaques covered with whitish scales in the skin of the knee, after 12 cycles of nivolumab therapy (a); Dermatoscopy of the changes on the knee reveals a pattern of granulomatous inflammation – the presence of yellowish-orange globular fields, permeated with linear blood vessels (b).

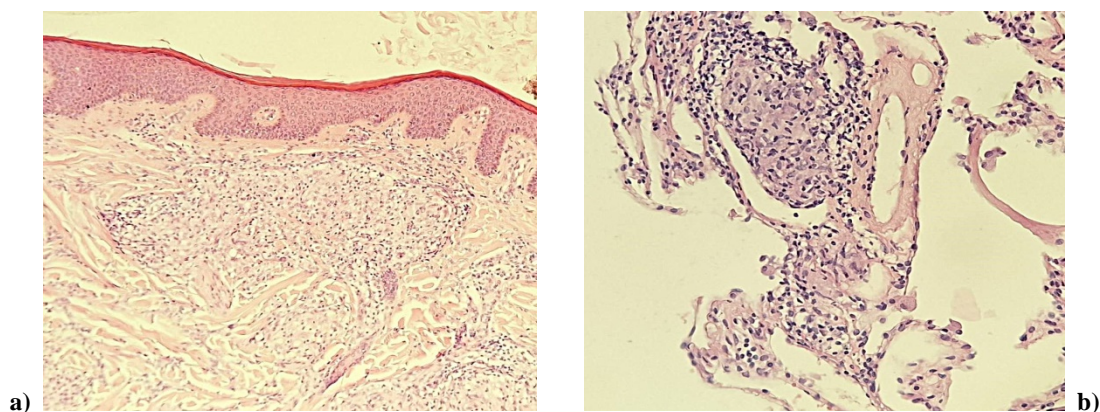
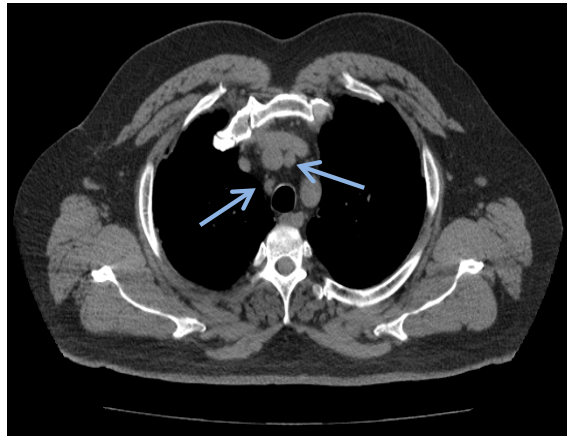


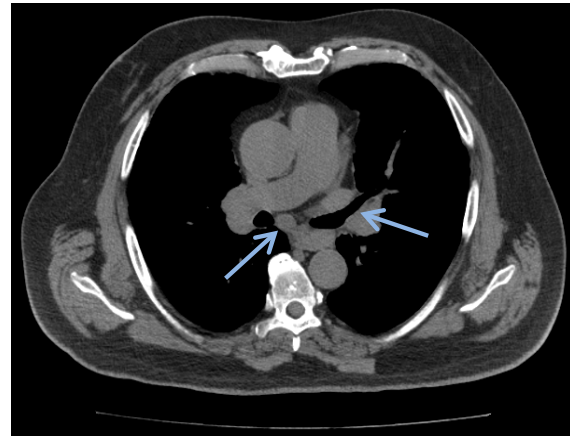
Fig. 3 – Histopathological (HP) analysis of the skin sample (a) reveals the sarcoidosis-like reaction of the skin and HP analysis of the transbronchial biopsy (b) point out to the pattern of chronic granulomatous inflammation (hematoxylin and eosin, ×10).

completely normal findings. Bronchoscopy with BAL revealed granulomatous inflammation and transbronchial biopsy confirmed sarcoidosis on HP analysis (Figures 5a and 5b). The 24-hour urine calcium was normal, the level of ACE was slightly elevated, and the Quantiferon TB test was negative. Therapy with oral prednisone 40 mg daily in

decreasing doses in the next three months was performed, and pembrolizumab therapy was continued until the planned completion after nine cycles. After adjuvant therapy with pembrolizumab was completed, remission of enlarged mediastinal lymph nodes occurred (Figures 6a and 6b).

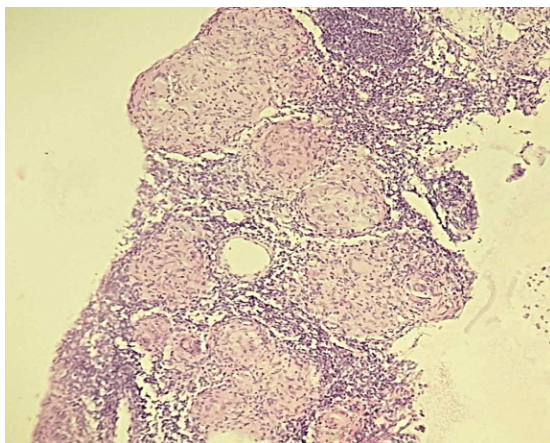


a)

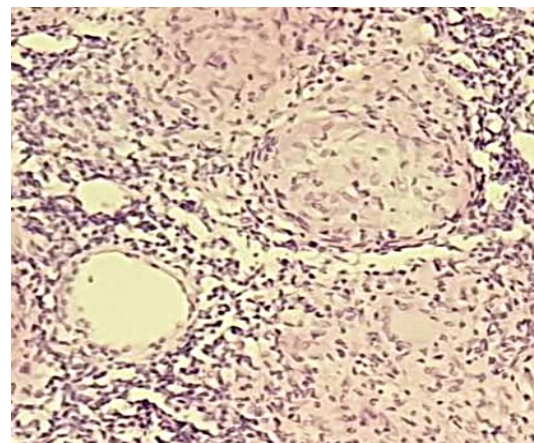


b)

Fig. 4 – Mediastinal lymphadenopathy (blue arrows) after 4 cycles of pembrolizumab therapy up to 22 mm (a, b).

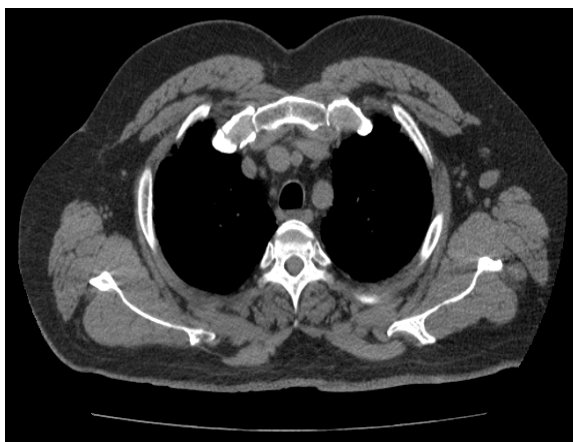


a)

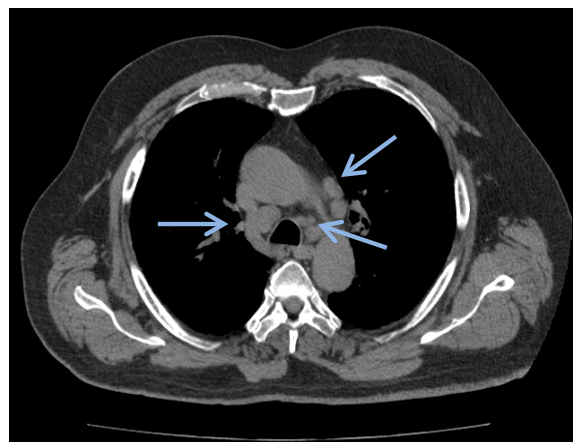


b)

Fig. 5 – Histopathological analysis of the transbronchial biopsy point out to the pattern of chronic granulomatous inflammation [hematoxylin and eosin, $\times 10$ (a); $\times 20$ (b)].



a)



b)

Fig. 6 – Mediastinal lymphadenopathy (blue arrows) after 9 cycles, that is the completion of adjuvant therapy with pembrolizumab up to 10 mm (a, b).

Discussion

Current treatment options for melanoma, such as immunotherapy, have significantly changed the prognosis of patients with advanced melanoma. With the increase in the number of patients receiving immunotherapy, new AEs of the therapy are discovered, and new knowledge is gained in diagnosing and treating already well-described AEs. DISR is a systemic granulomatous tissue reaction that is indistinguishable from sarcoidosis and occurs in a temporal relationship with the initiation of an offending drug, such as ICIs, tumor necrosis factor (TNF)- α antagonists, interferons (IFNs), antiretroviral therapy, and others². Histopathologically, DISR granulomas most often completely resemble sarcoid granulomas with the presence of non-caseous epithelioid granulomas of giant cells surrounded by lymphocytes, as well as with the occasional presence of birefringent foreign bodies, asteroid bodies, and Schaumann bodies³⁻⁶. Anti-programmed death-1 (PD-1) antibodies promote CD4⁺ T-lymphocyte activation [T helper (Th) type 1 – Th1 and Th17 cells] and cytokine secretion, including IFN- α , TNF- α , IL-17, and IL-2, which may be the cause of DISR⁷. In a retrospective study of patients treated with ICIs at the University of California Medical Center, DISR occurred in approximately 3.7% of patients treated with anti-cytotoxic T lymphocyte antigen-4 (anti-CTLA-4) or anti-PD-1 antibody and 6.3% of patients treated with a combination of both¹. In the same study, the median interval from initiation of immunotherapy to the development of DISR was 5.5 months. The median interval from radiologic detection of DISR to evidence of resolution was 5.8 months¹. In contrast, in a review of the literature by Melin et al.⁸, the median time to onset of symptoms associated with granulomatosis after the introduction of immunotherapy was three months. Although it is a rare cutaneous immune-mediated AE, sarcoidosis has been shown to occur more frequently in melanoma patients treated with immunotherapy than in those treated for other cancers⁹⁻¹¹. Previous studies have shown that the most commonly affected organs are lymph nodes, lungs, skin, and eyes^{8, 12-14}. Previous research has shown that the diagnosis of DSIR is established by excluding alternative causes of granulomatous inflammation as well as idiopathic sarcoidosis, with certain specific etiologies that require special attention². It is always necessary to rule out an underlying malignancy (disease progression) first because the neoplastic lymph node involvement detected by CT/positron emission tomography studies can mimic sarcoidosis¹⁵. Therefore, in many cases it is important to confirm HP diagnosis in any new or growing enlargement of lymph nodes, most often hilar and mediastinal, in patients in whom we suspect DSIR. Certainly, in the case of skin changes, it is always necessary to perform a dermatoscopic examination and a biopsy of the lesion to eliminate the suspicion of cutaneous/subcutaneous metastases. As both interstitial lung disease and interstitial pneumonitis represent one of the possible side effects of immuno-oncology therapy, it is sometimes difficult to

distinguish them from pulmonary sarcoidosis. What can help us in the differential diagnosis of these two AEs is that pulmonary sarcoidosis caused by immunotherapy is usually asymptomatic or with milder symptoms, while pneumonitis is usually accompanied by respiratory symptoms¹⁶. Excluding pulmonary infections can also be challenging based on radiological imaging alone, in which case, clinical correlation and laboratory findings are crucial¹⁷. In most cases of ICI treatment, DISR has been reported to improve after discontinuation of ICI⁷. In a study by Cornejo et al.¹⁸, 57% of patients received systemic corticosteroids, 49% had withholding or discontinuation of immunotherapy, whereas 17.9–41% of individuals used systemic corticosteroids in other trials^{16, 19}. Looking at the data from the available literature and comparing it with idiopathic sarcoidosis, DSIR, in most cases, has milder clinical manifestations, better prognosis, and less need for systemic corticosteroids or other immunosuppressive therapy^{12, 20, 21}. It has not been established yet why this is so, but it is considered that it is most likely because of an earlier diagnosis of DSIR compared to idiopathic sarcoidosis due to the regular radiological controls and clinical examination of these patients. The Society for Immunotherapy of Cancer Toxicity Management Working Group has issued useful consensus guidelines for the management of ICI-related DSIR. The guidelines are as follows: in grade 1 of pulmonary sarcoidosis, consider the use of corticosteroids, continue immunotherapy, and closely monitor the patient; in grade 2, or when progressive radiographic changes with persistent and/or disturbing pulmonary symptoms occur, along with the worsening of the lung function, simultaneous involvement of critical extrapulmonary organ systems, or hypercalcemia associated with sarcoidosis, it is necessary to start systemic corticosteroid therapy (prednisone 1 mg/kg or IV equivalent of methylprednisolone with taper steroids over 2–4 months, depending on response)²². The main goal in these patients is certainly to avoid discontinuation of therapy, especially in patients with a good response, but the occurrence of grade 2 or higher immune-related AEs most often leads to discontinuation of ICIs²³. The results of previous studies suggest that ICI can be safely continued in DSIR grade 1^{7, 12}. Results obtained from a literature review by Melin et al.⁸ suggest that the occurrence of a granulomatous reaction during ICI treatment may be associated with clinical benefit. The observed response rates (75% and 69%, respectively) are higher than those typically reported with ICIs (about 43% for anti-PD-1 and 58% with the combination of ipilimumab plus nivolumab)⁸. We conducted a literature review of ICI-induced sarcoidosis in patients treated for melanoma. In total, we found 88 patients described. The majority of patients are men (52%), and the most common localization is the thoracic region, skin, and lymph nodes. All other relevant data obtained from the literature review are presented in Table 1^{3, 8, 9, 11, 19, 24-67}. Certainly, further research and a larger number of patients are needed to prove this data.

Table 1

Patient characteristics from literature review (n = 88) ²⁴⁻⁶⁷	
Parameter	Value
Gender	
female	42 (48)
male	46 (52)
Age (years), mean (range)	56 (22–83)
Type of ICIs	
anti-CTLA-4 monotherapy	23 (26)
anti-PD-1 monotherapy	34 (39)
anti-CTLA-4 + anti-PD-1 combined	31 (35)
Time since ICI initiation (months), median (range)	3 (1–43)
Sites and organs involved with granulomatous reaction	
thoracic	69 (78)
dermatological	36 (41)
lymph node	8 (9)
bone	5 (6)
hepatic	4 (5)
ophthalmologic	2 (2)
renal	1 (1)
Therapeutic management for DSIR	
systemic corticosteroids	34 (39)
discontinuation of immunotherapy	40 (45)
Response to treatment of DSIR (n = 83)	
stability	17 (20)
partial or complete regression	66 (80)

ICIs – immune checkpoint inhibitors; CTLA-4 – cytotoxic T-lymphocyte associated antigen-4; PD-1 – programmed death-1; DSIR – drug-induced sarcoidosis-like reaction.

Values are given as numbers (percentages) except for age and time since ICI initiation.

Conclusion

ICI-induced sarcoidosis has been reported in different cancers and using different ICIs. Further larger multicenter studies are needed to reveal the true incidence of DISR in melanoma patients treated with ICIs. Certainly, all previous research and case reports support the fact that the most common localizations of DISR are the lymph nodes, lungs, and skin. The differential diagnosis of DISR vs. melanoma progression requires a biopsy of the suspicious lesion in the majority of cases, but an interdisciplinary approach is always needed to distinguish the true progression of the

disease from an adverse reaction, as well as to decide to continue ICIs. In most cases, it is not necessary to discontinue ICIs. Usually, the changes resolve or regress spontaneously after discontinuation of therapy, which should be considered in the adjuvant setting. Sometimes it is necessary to start standard therapy with systemic corticosteroids, which, in most cases, have shown a good therapeutic response.

Conflict of interest

The authors declare no conflict of interest.

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Tuberculous lymphadenitis – still on the scene: a case report

Tuberkulozni limfadenitis – još uvek na sceni

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Abstract

Introduction. Tuberculosis (TB) continues to be a global health problem, with various manifestations affecting different organs and organ systems. Tuberculous lymphadenitis (TBL), also known as scrofula or lymph node (LN) TB, is a manifestation of *Mycobacterium tuberculosis* infection primarily affecting the LNs. Our region has a low annual incidence rate of TB, which is why the expected prevalence of TBL is also low. **Case report.** We present two cases of TBL diagnosed three months apart. Both presented patients had the disease localized in the LNs of the neck. These were young people without chronic diseases and previously in good health condition. The diagnosis was confirmed by surgical biopsy of the LNs in the first patient and fine needle aspiration biopsy in the second patient. Both patients were treated with standard protocols for TBL, with favorable outcomes and without significant complications and side effects. **Conclusion.** Presented cases highlight the importance of considering TBL in the differential diagnosis of neck masses, even in regions with a low prevalence of TB. Timely diagnosis and treatment are essential for preventing complications and ensuring a successful outcome.

Key words:

biopsy; biopsy, fine-needle; diagnosis, differential; drug therapy; lymphadenitis; neck; tuberculosis; treatment outcome.

Apstrakt

Uvod. Tuberkuloza (TB) i dalje predstavlja globalni zdravstveni problem, sa različitim manifestacijama koje utiču na različite organe i organske sisteme. Tuberkulozni limfadenitis (TBL), poznat i kao škrofula ili TB limfnih čvorova (LČ), manifestacija je infekcije *Mycobacterium tuberculosis*, koja prvenstveno zahvata LČ. Naš region ima nisku godišnju stopu incidencije TB, zbog čega je niska i očekivana prevalencija TBL. **Prikaz bolesnika.** Prikazana su dva slučaja TBL-a, koja smo dijagnostikovali u razmaku od tri meseca. Oba prikazana bolesnika imala su bolest lokalizovanu u LČ vrata. Radilo se o mladim osobama bez hroničnih bolesti, koje su prethodno bile u dobrom zdravstvenom stanju. Dijagnoza je kod prvog bolesnika potvrđena hirurškom biopsijom LČ, a kod drugog bolesnika aspiracionom biopsijom „finom iglom“. Oba bolesnika lečena su standardnim protokolima za TBL, sa povoljnim ishodom i bez značajnih komplikacija i neželjenih efekata terapije. **Zaključak.** Prikazani slučajevi potvrđuju važnost razmatranja TBL u diferencijalnoj dijagnozi izraslina na vratu, čak i u regionima sa niskom prevalencijom TB. Pravovremena dijagnoza i lečenje su ključni za sprečavanje komplikacija i postizanje uspešnog ishoda lečenja.

Ključne reči:

biopsija; biopsija tankom iglom; dijagnoza, diferencijalna; lečenje lekovima; limfadenitis; vrat; tuberkuloza; lečenje, ishod.

Introduction

Tuberculosis (TB) remains a global health concern, with various manifestations affecting different organs. According to the latest published statistical data, in 2022, an estimated 10 million people fell ill with TB worldwide. There were approximately 1.5 million TB-related deaths in 2022.

TB is more prevalent in certain regions, with over 95% of TB deaths occurring in low- and middle-income countries. The regions most affected include sub-Saharan Africa and Southeast Asia. These are also the regions with the highest prevalence of human immunodeficiency virus (HIV) infection, which is considered the most important risk factor for TB^{1,2}.

Serbia is classified as a country with a low TB incidence rate of around seven to ten people *per* 100,000 inhabitants, which has been slightly increasing in the last five years ³.

Pulmonary TB is the most common form. However, other organs may also be affected. The most frequently affected sites were lymph nodes (LNs) (48.5%), and the next three ranked were genitourinary TB (20.5%), pleural TB (12.0%), and osteoarticular TB (10.1%) ⁴.

Tuberculous lymphadenitis (TBL), also known as scrofula or LN TB, is a manifestation of *Mycobacterium tuberculosis* (*M. tuberculosis*) infection primarily affecting the LNs ⁵.

Historically, TBL has been recognized as a significant health issue. It has been documented in ancient Egyptian mummies and was prevalent during the Middle Ages and the Renaissance. In the past, the disease was commonly associated with poor living conditions and malnutrition. However, with advancements in healthcare and the availability of anti-tubercular medications, the incidence of TBL has decreased in many parts of the world ⁶.

TBL occurs when *M. tuberculosis* infects the LNs through hematogenous spread or direct extension from a nearby primary focus, usually in the lungs. The bacilli then replicate within LNs, leading to granuloma formation and subsequent caseous necrosis. The immune response often encapsulates the affected nodes, resulting in the characteristic appearance of firm, matted lymphadenopathy ⁷.

The clinical presentation of TBL can vary widely, making it a diagnostic challenge. Patients may present with painless, slowly enlarging LNs, commonly involving the cervical region. The LNs are usually firm, non-tender, and may become matted together. Constitutional symptoms such as fever,

night sweats, and weight loss can accompany TBL, mimicking other infectious or neoplastic etiologies such as lymphoma ⁸.

Accurate diagnosis is imperative for appropriate management. TBL diagnosis often involves a combination of clinical, radiological, and laboratory methods. Fine needle aspiration (FNA) biopsy is a commonly employed diagnostic tool, providing a minimally invasive means to obtain tissue for microscopic examination and culture. Polymerase chain reaction assays can enhance sensitivity by detecting *M. tuberculosis* deoxyribonucleic acid. Radiological imaging, such as contrast-enhanced computed tomography (CT) scans, helps visualize the extent of LN involvement and identify associated complications ⁹⁻¹¹.

The cornerstone of TBL management is a multidrug anti-tubercular therapy (ATT) regimen. First-line drugs, including isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z), are typically administered for an extended period. Surgical intervention may be considered for refractory cases, complications (e.g., abscess formation), or diagnostic uncertainty. Ongoing research explores the potential role of novel anti-tubercular agents and immunomodulatory therapies to improve treatment outcomes ¹².

Case report

Case I

A 19-year-old male construction worker presented to the outpatient department with complaints of a gradually increasing painless swelling in the left side of his neck for the past four months. He denied any history of trauma, fever, or weight

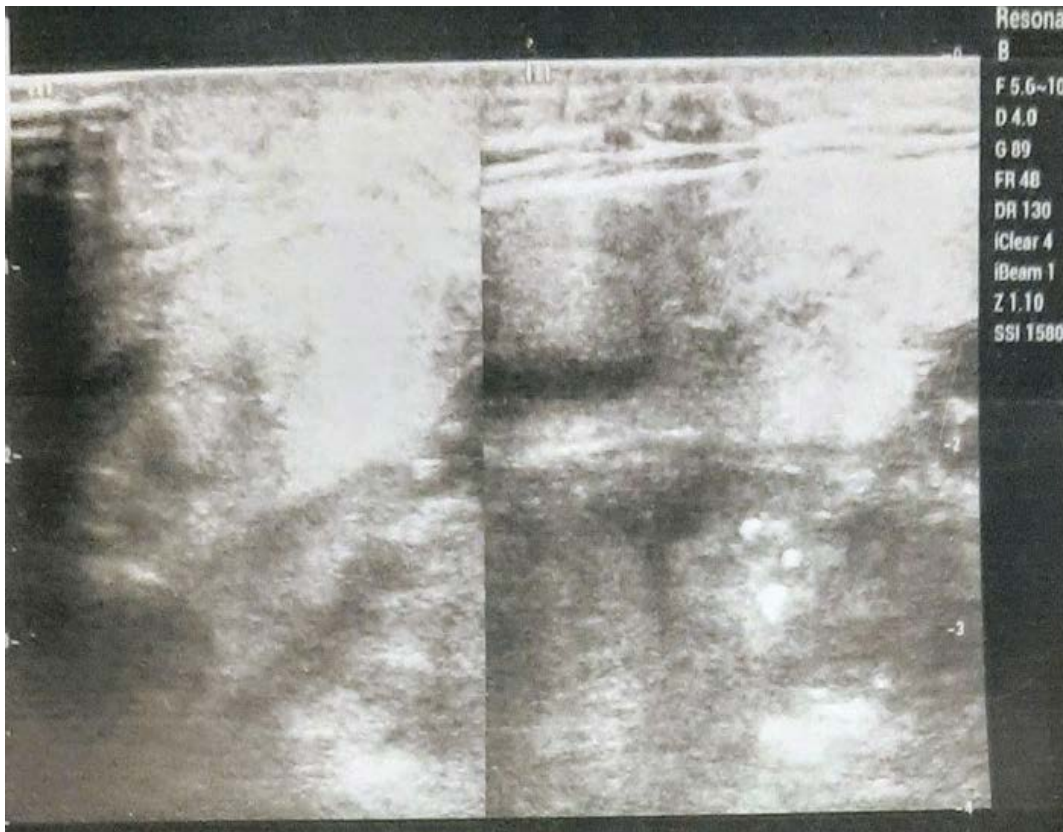


Fig. 1 – Ultrasound finding of enlarged and necrotic lymph nodes in the left submandibular region.

loss. On further inquiry, he reported occasional night sweats and a feeling of weakness. He had no significant medical history and was not on any regular medications. Upon physical examination, a firm, non-tender, and mobile LN mass was palpable in the left submandibular region. The overlying skin was normal, and there was no evidence of sinus formation or discharge. General physical examination was unremarkable, with no signs of respiratory distress or abnormal lung sounds. The systemic examination did not reveal any other abnormal findings. The complete blood count was within normal limits. Laboratory tests for viruses (HIV, hepatitis A and B, cytomegalovirus, and Epstein-Barr virus) were negative. Chest X-ray revealed no significant abnormalities in the lungs. Ultrasound examination of the neck showed enlarged and necrotic LNs in the left submandibular region, the largest of which was 4 cm in diameter (Figure 1). Chest CT scan revealed numerous enlarged and necrotic LNs in the mediastinum, the largest of which was 5.5×6 cm in the area of the right hilum. No pathological changes were observed in the lungs (Figure 2).

Given the presence of pronounced symptoms and a strong suspicion of lymphoproliferative disease, we decided to perform a surgical biopsy of the LNs. The procedure was done without immediate or delayed complications (Figure 3).

Histopathological (HP) findings showed caseous necrosis and acid-fast bacilli observed under the microscope (Figures 4 and 5).

After confirming the diagnosis, we started the treatment. The patient was started on a standard ATT regimen comprising an initial phase of two months of H, R, Z, and E, followed by a continuation phase of four months of H and R, 2HRZE/4HR. He was educated on the importance of medication adherence and the need for regular follow-up. The patient showed significant improvement after two months of treatment, with a reduction in the size of LNs. Repeated chest X-ray remained normal, and the patient reported a resolution of night sweats and weakness. ATT was continued for a total duration of six months.

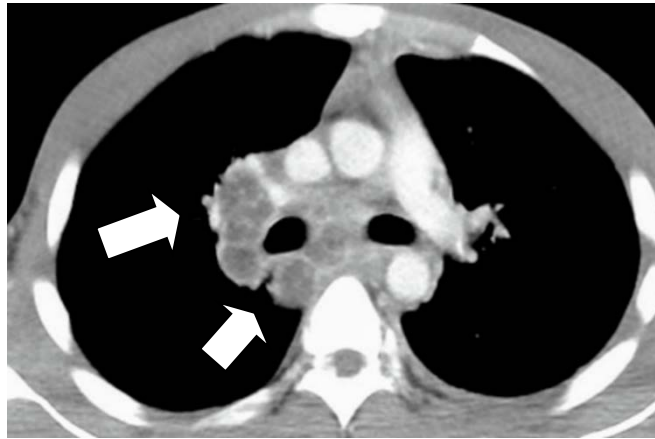


Fig. 2 – Chest computed tomography scan – numerous enlarged and necrotic lymph nodes in the mediastinum.



Fig. 3 – Submandibular mass after surgical biopsy.

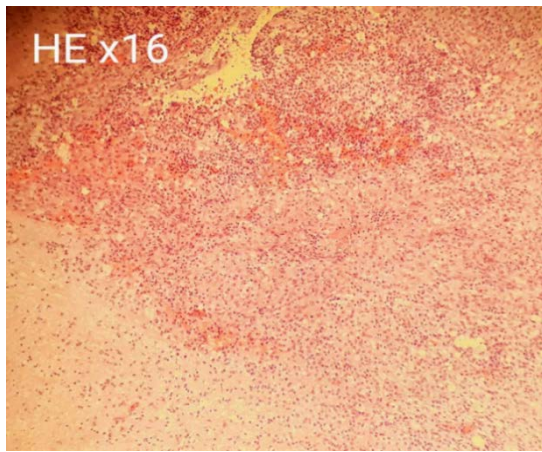


Fig. 4 – Lymph node histopathological examination: mononuclear cell infiltrate, epithelioid granuloma, and caseous necrosis [hematoxylin-eosin (HE) staining, $\times 16$].

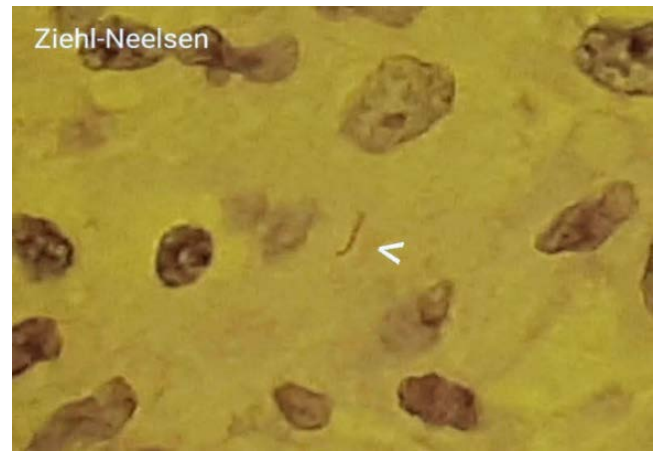


Fig. 5 – Microscopic lymph node examination revealed acid-fast bacilli (Ziehl-Neelsen stain, $\times 100$).

Case II

A 21-year-old male student of the Military Academy presented with a two-month history of painless neck swelling and low-grade fever. The patient denied any significant weight loss, night sweats, or respiratory symptoms. The patient's training regimen required intense physical activities; he did not notice fatigue and difficulty performing activities.

Physical examination revealed a mass in the left supraclavicular area with a diameter of about 5 cm. The change was dense and fixed to the base with hyperemic skin above. Mild pain was felt during palpation (Figure 6).

Routine laboratory investigations were within normal range, except for a mildly elevated erythrocyte sedimentation rate. Laboratory tests for viruses, including HIV, were negative. Chest X-ray and CT scan revealed no significant abnormalities in the lungs and mediastinum (Figure 7). We decided to perform an FNA biopsy of the neck mass. HP find-

ings revealed granulomas indicative of TB. The Ziehl-Neelsen staining confirmed the presence of acid-fast bacilli, which confirmed the diagnosis of TBL.

Given the patient's military background, strict infection control measures were implemented to prevent transmission to other trainees. The patient was started on a standard ATT regimen, including H, R, E, and Z (2HRZE/4HR). Directly observed therapy was facilitated within the Military Academy setting to ensure treatment adherence.

The patient faced unique challenges related to academic commitments and physical training. Special considerations were taken to modify the training schedule, allowing the student to continue academic obligations while accommodating the physical limitations imposed by the disease and its treatment. The importance of bed rest and non-pharmacological therapy in the treatment of TB needs to be emphasized. Close collaboration between the medical team and military authorities was essential to strike a



Fig. 6 – Initial appearance of the patient: supraclavicular mass with changes in the skin above.

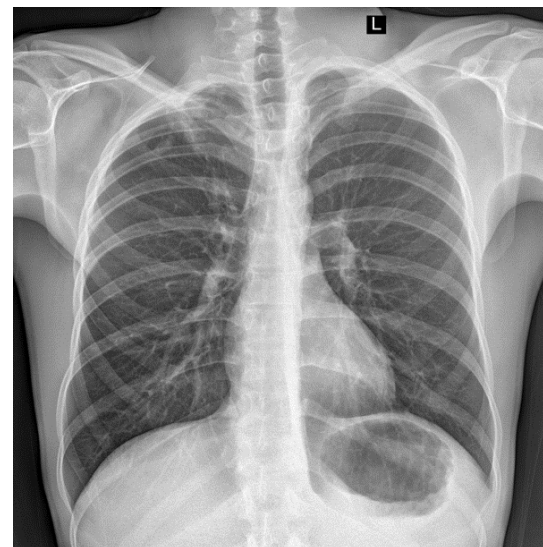


Fig. 7 – Chest X-ray: a suspicious infiltrate at the top of the right lung on the computed tomography scan has not been confirmed – no abnormalities in the lungs were found.

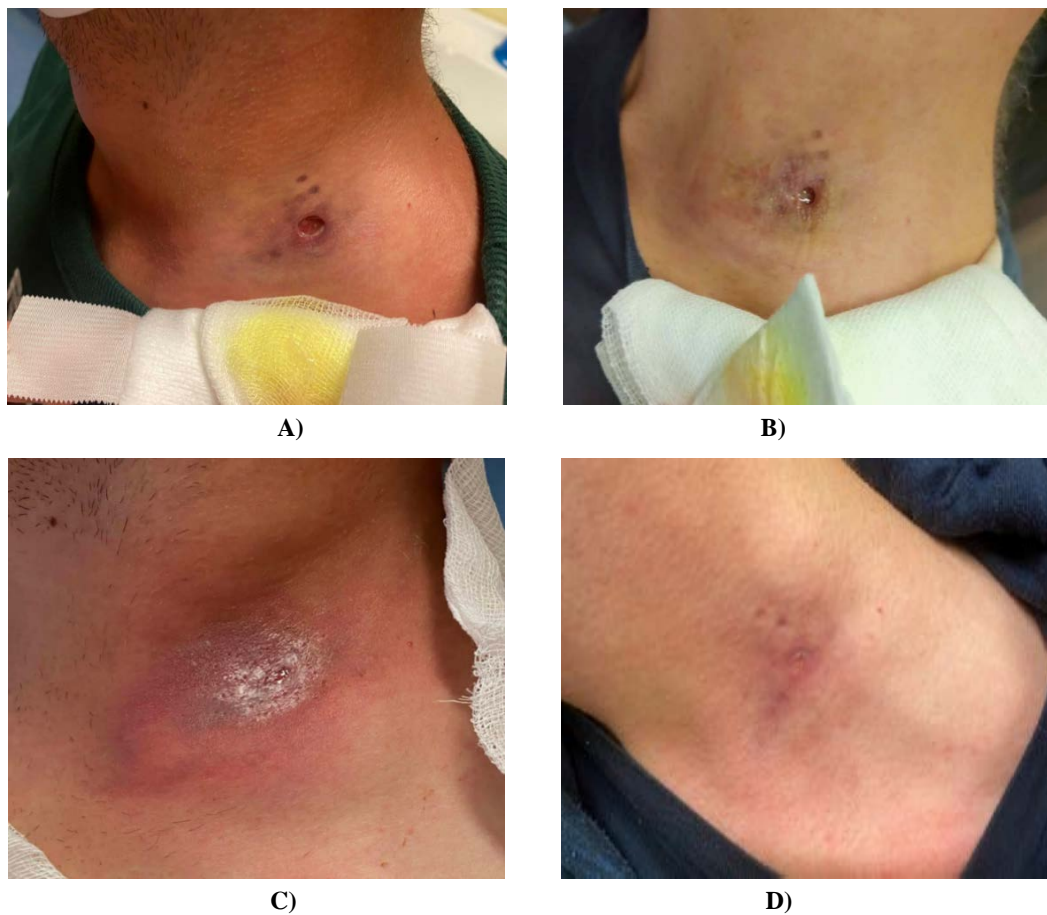


Fig. 8 – Evolution of cutaneous fistula: development (A and B), closure (C), and residual scar (D).

balance between academic responsibilities and medical requirements.

After one month of starting ATT, a complication occurred—a cutaneous fistula. We continued ATT with local wound care. In the course of the disease, in four months, the fistula closed, with the presence of a small skin scar (Figure 8).

Regular clinical and radiological assessments were conducted throughout the treatment. The patient responded well to ATT, with a reduction of LN size and resolution of symptoms. The modified training regimen allowed the student to fulfill academic requirements without compromising his health.

Discussion

Clinical presentation of TBL can vary widely, making diagnosis challenging. Common manifestations include painless, gradually enlarging LNs, usually in the cervical region. These nodes may become fluctuant and form abscesses, leading to the formation of a cutaneous fistula. Constitutional symptoms such as fever, weight loss, and night sweats may also be present¹³.

Early detection of TBL is crucial for several reasons. Timely diagnosis allows for prompt initiation of appropriate treatment, minimizing the risk of disease progression and complications. Considering the localization of LNs, there was a possibility of major blood vessel lesions and fatal bleeding¹⁴.

Early intervention also reduces the risk of transmission to others, contributing to the control of TB spread within communities. Furthermore, the identification of TB cases at an early stage facilitates contact tracing and preventive therapy for individuals at high risk of infection¹⁵.

Several conditions can present with similar clinical features, making the differential diagnosis crucial for accurate management. Other causes of lymphadenopathy, such as bacterial or viral infections, lymphoma, and autoimmune diseases, must be considered. FNA biopsy for cytological and bacteriological examination or surgical biopsy of the affected LN is often required for definitive diagnosis, allowing for the identification of acid-fast bacilli and confirming TB. The first presented patient also had mediastinal lymphadenopathy and lymphoma strongly suspected. We decided on a surgical biopsy due to the size of the tissue sample for HP examination. The treatment of TBL typically involves a combination of anti-tubercular drugs, most commonly H, R, E, and Z. However, challenges in the treatment of this condition may arise due to factors such as drug resistance, treatment non-adherence, and the development of adverse drug reactions^{16,17}.

TBL has broader implications for public health. Active surveillance and early detection contribute to the overall control of TB transmission within communities. If there is no simultaneous lung localization, this form of TB is not the most infectious. Prompt treatment reduces the reservoir of

infectious individuals, limiting the potential for further spread.

Additionally, effective management of TBL prevents the development of drug-resistant strains, which could pose a more significant threat to public health. This particularly applies to individuals with specific occupations that involve constant presence in large groups. The second presented patient is a professional soldier, which involved numerous measures taken both towards the patient and the members of the military community¹⁸.

Conclusion

Tuberculous lymphadenitis is a significant public health challenge that necessitates a comprehensive understanding of its pathogenesis, clinical presentation, diagnos-

tic modalities, and management strategies. Those cases highlight the importance of considering tuberculous lymphadenitis in the differential diagnosis of neck masses, even in regions with low disease incidence. Timely diagnosis and treatment are essential for transmission, preventing complications, and ensuring a successful outcome. This paper aims to contribute to the collective knowledge base, fostering continued advancements in diagnosing and treating tuberculous lymphadenitis. As the global fight against tuberculosis persists, a thorough grasp of tuberculous lymphadenitis is essential for healthcare professionals and researchers.

Conflicts of interest

The authors declare no conflict of interest.

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The influence of two Milan Jovanovićs on the establishment of civil and military medical service in Montenegro

Uticaj dva Milana Jovanovića na osnivanje civilne i vojne zdravstvene službe u Crnoj Gori

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Key words:

delivery of health care; history, 19th century; history of medicine; military medical school; montenegro; organization and administration; physician.

Ključne reči:

zdravstvena zaštita; istorija, 19. vek; istorija medicine; vojna škola, sanitetska; crna gora; organizacija i rukovođenje; lekari.

Introduction

Montenegro preserved its independence through constant struggles and conflicts. The warrior tradition has become a characteristic of everyday life. Scarce natural resources and a relatively small area surrounded by enemies prevented significant economic and other development and, therefore, the absence of organized medical care. In constant battles, the soldiers themselves, i.e., able-bodied men and their tribal organizations, carried the basic logistics of weapons, clothing, and food. The main supply line was through women, who brought food to their relatives on the battlefield and often extracted the wounded and dead. For centuries, they were the only and the main military medical service (MMS), which took care of its closest ones. Due to the small population, all able-bodied men participated in the fighting, so there was no room to deal with the logistic, extracting the wounded or caring for them, which required a significant commitment and a reduction in the available number of fighters. Old men were often on the front battle lines, but also pupils and students. In addition to women, traditional healers, i.e., healers and herbalists (balm healers), also provided significant help. These are individuals who, from generation to generation, passed on knowledge of the treatment and care for the sick, dealing with folk medicine and medicinal herbs and balms, which are nowadays considered quackery. As such, they were extremely valued and respected in society, both in war and peace¹, and they summarized their knowledge in special books – *ljekaruša*, which represented

collections of knowledge and experiences acquired up to that point. The most famous folk healers/wound healers were from the Iličković brotherhood² from Crmnica, Montenegro, who had a great reputation among the people. About a dozen of them served Montenegro at all times. The Ministry of Military Affairs treated them as “domestic military medical personnel”, and in 1880, the Montenegrin Senate awarded them the title “people’s physicians”. Even after the establishment of MMS and the official ban on their work, they continued with their mission. There were no permanent and educated doctors, except for those who served as “externals” at the court, and, therefore, no permanent medical service that would take professional and organized care of the wounded and sick at the front. Medical service was the last thing that was taken into account in the Montenegrin Armed Forces, not only because of the low level of awareness of its importance but also because of the scarcity of material resources and the lack of food, weapons, and ammunition, the acquisition of which was a priority. As a result, the Montenegrin soldiers had bad general health and hygiene¹.

The year 1873 is considered the turning point in the development of modern Montenegrin medicine, including military medicine. That year, the construction and equipping of the hospital in Cetinje, Montenegro, was completed. In 1875, the outbreak of the Herzegovina Uprising occurred when a large number of civilians and wounded arrived in Montenegro. In order to take care of these people, the Committee to Aid Herzegovinian Fighters and Their Refugee Families (Committee for Suffering Herzegovinians) was formed in

Cetinje, through whose efforts the Senate ratified the Geneva Convention on November 29, 1875 (this date is taken as the establishment date of the Montenegrin Red Cross). In addition, numerous international committees were formed to help the Herzegovinian insurgents, and with the help of these committees, the first hospitals outside Cetinje were opened. The most significant and large-scale activities were carried out in Russia, where a large Red Cross campaign was organized to help the insurgents with money and equipment. Moreover, medical teams with experts, hospitals, and outpatient units were formed. They arrived in Montenegro in stages and were the backbone of medical care³. During the war, there were five to six hospitals in Montenegro, and there was no medical service within the army. From the uprising in Nevesinje, Bosnia and Herzegovina, to the establishment of peace made in San Stefano, that is, during the three years of hostilities, interrupted by brief truce periods, the number of wounded reached 8,000, and the number of sick 12,000, and all of them were on the shoulders of voluntary help because there was no separate MMS. Thus, for the first time, aid was provided to the Montenegrin Armed Forces at the front in an organized and professional manner, and from the beginning of 1876, we can conditionally speak of an organized and established MMS comprised of doctors from outside of Montenegro and members of medical missions, predominantly from Russia⁴.

The first organizational steps towards the establishment of MMS were made when doctor and military officer Nikola Kovačević was promoted to the rank of medical captain and appointed as the first military doctor of the newly established Standing Army in 1896. By assuming this duty, he began work on the organizational structure of the professional medical service in the Montenegrin Armed Forces⁵, so that on December 19, 1903, with the passing of the Law on the Organization of the Ministry of Military, the Medical Department was established within the General Military Department. Under his order, the Military Medical School program for military medical assistants, i.e., military paramedics, was established in 1906⁶. In this school, located in Cetinje, three classes with 56 cadets in total were educated⁷. With this program, one medical non-commissioned officer in each infantry battalion was trained. By decree on the formation of the Montenegrin Armed Forces in 1908, divisions of the Montenegrin Armed Forces were formed, which included one medical company⁷. In 1912, before the Balkan wars, the Montenegrin Armed Forces had four medical companies¹.

A special contribution to the establishment and development was made by two Serbian doctors with the same first and last name. Those two doctors were Milan Jovanović Morski (Bombajac) and Milan Jovanović Batut.

The influence of Milan Jovanović Morski (Bombajac) on the development of medicine and military medical service in Montenegro

Numerous doctors came to Montenegro during the war period after the beginning of the Herzegovina uprising, but Dr. Milan Jovanović Morski (Bombajac) played a notable

role. He was born in 1834 in a place named Jarkovac in Banat, Serbia, which was then former Austria. He studied lower secondary school in Vršac, Serbia, and higher secondary school in Timisoara, Romania, and Pest, Hungary, (1854). He started his studies in medicine in Vienna, Austria, (1861–1865) but finished them in Leipzig, Germany, (1867). He received his doctorate in the field of malaria in Leipzig (August 1867). Besides being the founder of the modern science of hygiene in Serbia and a lecturer of forensic medicine and dialectics at the Great School in Belgrade, Serbia, Morski was also extremely research- and travel-oriented⁸. Because of his frequent travels, he got the nicknames Morski (sea, transl. note) and Bombajac (Bombay, India, transl. note). He wrote several travelogues and literary works and did a considerable number of translations. Since 1869, he had been a member of the Serbian Learned Society. In addition to the above, before coming to Herceg Novi, Montenegro, he worked as a doctor in Bogoslovija (Seminary) (1865–1869), a private doctor in Novi Sad, Serbia, (1871–1875), and as a high school principal in Novi Sad⁹.

All of the above influenced that, upon his arrival in Montenegro, not only was he the court physician of the prince's family but also the teacher of the heir to the throne, Danilo Petrović Njegoš, from 1876 to 1879. Before serving as a court physician (from 1877), at the beginning of the Herzegovina uprising, he organized a special clinic for the reception and treatment of the wounded, and he was also a municipal doctor (physicist) in Herceg Novi in 1875¹⁰.

Undoubtedly patriotic Morski came to Herceg Novi in the beginning of 1875 to provide medical assistance to a large number of refugees who found refuge in this city¹¹. At the invitation of Prince Nikola Petrović, he moved from Herceg Novi to Cetinje in 1876. In this period, there was no permanent doctor or medical staff with a university degree in Cetinje, except for one Frenchman (medical major Gustav Friley). Morski first worked on providing medical aid to the wounded and refugees during the Montenegrin-Ottoman War (1876–1878). His role was particularly important in the first months of the war when he tried to organize a medical service in Cetinje in a modern way despite numerous problems and shortcomings. With the arrival of the Russian medical mission in Montenegro, his work became somewhat easier. In the period of stabilization of war conditions, in 1877, he was appointed court physician of the Petrović Njegoš dynasty⁶. In the winter of 1877/78, as a court physician, he stayed in Naples with Princess Milena and Crown Prince Danilo Petrović Njegoš, taking care of their health on the way¹⁰. Through his service with the Petrović Njegoš dynasty, he influenced the promotion of the need to establish a health service on a modern basis. Although he spent a relatively short period in Montenegro, from 1876 to the end of September of 1878, his role was great because it was a period of war in which the development of certain services was faster than in peacetime⁶.

Morski stayed in Montenegro in difficult war conditions, although his activity as an individual was quite limited. He was important in the work of MMS in the army and with refugees from Herzegovina. His presence in Montenegro as

an expert had an impact on the organization, method, and implementation of sanitary measures not only in the Montenegrin Armed Forces but also in refugee shelters. In mid-1876, he organized an improvised hospital for the reception of seriously wounded people who came from the front. He organized volunteer paramedics, mostly Montenegrin women, to work under his instructions and provide medical assistance. He was especially dedicated to maintaining hygiene and health culture, as well as preventing infectious diseases. Without any previous catches and medical personnel, he managed to start the development of military health when the Montenegrin Armed Forces were fighting many battles from Herzegovina to Podgorica. He left Cetinje for Herceg Novi in September 1878 and soon got a job as a doctor in the shipping company "Lloyd" on whose ships he sailed from the Adriatic to Bombay (1878–1882), which is how he got his nickname. Since 1892, he had been a regular member of the Serbian Royal Academy. After leaving Montenegro, he worked as a military doctor (second class medical captain) in the Šumadija Cavalry Corps, Serbia, (1885–1886) and as a professor of military hygiene and doctor (medical major) at the Military Academy (1889–1896). He died in Belgrade on June 6, 1896⁹.

The influence of Milan Jovanović Batut on the development of medicine and military medical service in Montenegro

Milan Jovanović Batut was born on October 10, 1847, in Sremska Mitrovica, Serbia,¹² He attended high school both in Pančevo, Serbia, and Sremski Karlovci, Serbia, but finished it in Osijek, Croatia, (1865). He completed his medical studies in Vienna (1878). Until 1880, he worked as a practical doctor in Sombor, Serbia¹³. On August 17, 1880, he received an invitation from the Montenegrin government (from the then Minister of Education and personal friend, Jovan Pavlović) and was appointed the head of the health department at the Ministry of Internal Affairs. Taught by the experience from his previous position, the Montenegrin government entrusted him with the task of organizing and managing Montenegrin health care. He was soon appointed as the chief physician of the Cetinje Hospital and also the fourth manager of the hospital "Danilo I". He stayed in Montenegro until October 1882¹¹. At his suggestion, a competition was announced in 1881 for ten district doctors in Andrijevića, Bar, Grahovo, Danilovgrad, Podgorica, Rijeka Crnojevića, Kolašin, Nikšić, Šavnik, and Ulcinj (Montenegro). The competition was also announced for a veterinarian working in Cetinje. Only two doctors (Petar Miljanić in Podgorica and Đorđe Babić in Bar) were registered for the competition⁶.

In 1881, the entire medical staff, apart from Milan Jovanović Batut, included Dr. Petar Miljanić, Jovo Mišić, Filip Salambros, assistant Janko Teodorides, four paramedics, one bandager, administrator Popović, porter, cook and cook assistant, employee in charge of water, and assistant administrator¹⁴. With the arrival of a young doctor Jovanović from Sombor to the manager position of the hospital "Danilo I" and "state physician", the period of establishment of modern

MMS and its organization began. Until his arrival, Montenegrin medicine relied on foreign doctors, mostly French, at the court. Milan Jovanović Batut worked in extremely difficult conditions, where there were no educated doctors, with a low level of awareness of the importance of health and education, with quack doctors as the dominant, and, it can be said, the only doctors outside the court. Immediately after his arrival, he began raising awareness of the need for the development of medicine, primarily by training the staff; hence, he started by providing practical training for the clinical staff. He pointed out to the authorities the need to establish a Medical Council (the forerunner of the Ministry of Health) and that raising awareness about diseases, hygiene, and medicine must be part of the general emancipation of citizens⁴. After arriving in Cetinje, he started with the reform, solved numerous problems in the operation of the hospital and the functioning of the medical service, introduced rules and work procedures, and strived to establish a medical system based on modern medical science, for which he received undivided support from the Prince and other authorities. In the hospital "Danilo I", he established and published rules. The rules applied first to patients who come for examination, as well as to those who visit them¹⁵. Over time, resistance to his ideas and aspirations grew stronger, especially in rural parts of Montenegro, where individuals felt their personal positions were threatened, and the low level of understanding and importance of medicine did not favor radical reforms. Determined to put an end to quackery, he succeeded, despite public and open resistance, especially from the folk doctors of the time, in passing the law banning the practice of quackery in Montenegro, with the threat of strict punitive measures. Special resistance, as expected, was shown by "domestic military medical personnel" and "folk doctors" from the Iličković brotherhood. Two years after November 1891, the collection of rules, regulations, and orders in the medical profession was published, prohibiting quackery. The Iličkovićs turned to Prince Nikola, who expressed understanding for their work, and, at the suggestion of the Minister of Defence, Ilija Plamenac, on December 1, 1895, the Iličkovićs were praised, rewarded, and assigned to the service in military units, with *stotinaš* (centurion) ranks "by which they will be distinguished from other peasants for their merits and be proud among Montenegrins"⁴.

His activity led to the adoption of rules for the operation of the Medical Service in coastal ports in 1891, after which the port medical captain's office was formed with equipment for steam disinfection following international sanitary standards, and sanitary passports were introduced¹⁶.

He was also involved in the procurement of books for the patients of the hospital "Danilo I". In one of his letters, Dr. Jovanović states: "We are asking the famous societies of Serbian newspapers, fiction and educational content, and writers and publishers of such books, to send us one copy of their publications, so that a smaller library can be created in the Montenegrin hospital "Danilo I" for the use of convalescent patients"¹⁷. The fact how carefully he took care of the hospital, but also what kind of problems he faced, is best shown in one of his letters: "...I take care of the hospital's

needs with great effort. Whenever I ask for something, they do not have it. For the smallest thing, I have to go from Pontius to Pilate. ... I got upset – all for that. And I am willing to help these people as much as I can”¹⁸.

He was the editor of the journal *Zdravlje*, which contained medical advice to the people. The first issue was printed on January 15, 1880, in Sombor, where 13 more issues were printed. After a short break due to Batut's departure to Montenegro, the journal began being printed again in Cetinje, where six more issues were printed¹⁹.

In addition to general medicine, Batut was also involved in examining the health condition of the Montenegrin people, for which purpose he traveled to Montenegro in 1881 and 1882, but only a small part of his observations remained written down¹⁹. Before the research, he defined the goals and content of the study of the people's health status. His main goals as a doctor were to examine each family individually and to calculate the following from the examination: the overall health of the people; what the most common disease was; how many people were wounded, maimed, blind, mute, insane, paralyzed, present, etc.; how many disabled, partially disabled were there; how many accidents from rifles and other weapons, from fire, frostbite, animals, etc. Apart from this, he recorded and examined infectious diseases, marital and family history, the influence of healers, priests, and other persons on treatment, the response of state institutions to treatment, what the attitude of the people toward their health was, as well as surveying the land and determining the places of healing springs, lakes, waters, caves, and areas. He examined the influence of age and the direction of progress of the population when it comes to their state of health. He paid attention to food, clothing, place of residence and stay, as well as the health of livestock. In addition to all the examinations, he did not forget, as he says: “as a doctor, to help in urgent cases, either with surgery, medicine, advice, or any other way”²⁰.

Thus, on July 16, 1881, after the first analysis, he submitted a report to the Minister of Internal Affairs on the general state of health and recommendations for better work and organization of MMS. It is the first systematized document on the state and conditions of health in Montenegro. In it, he stated that “the health and physical strength of Montenegrins are far worse than could be expected” and that the greatest need was for doctors since there was only one doctor *per* 200,000 inhabitants²¹, compared to America, where there was one doctor *per* 600 people, one *per* 1,000 in France, one *per* 2,000 in Austria, and one *per* 10,000 citizens in Serbia. He further stated that the hospital in Cetinje examined about 5,000 patients *per* year on an outpatient basis and that the existence of one hospital meant that only the most seriously ill patients made the difficult and costly trip (several days of walking) to the doctor only as a last resort²¹. For this reason, he proposed that a central health administration under the Ministry of Internal Affairs be established. That central health administration would have the authority to propose sanitary laws to the Senate for confirmation, manage doctors and medical personnel, propose doctors to the Senate for confirmation, report on the state of health in the country,

conduct health visits, keep health statistics, etc.²². He also advocated for the establishment of district hospitals and the hiring of doctors in Bar, Ulcinj, Nikšić, Podgorica, and Kolašin. The report mentioned above had a significant resonance and influence, so a competition was soon announced for the admission of ten district doctors, to which only two candidates applied, among them Dr. Petar Miljanić, the successor of Dr. Batut. The importance of his reporting is also evidenced by the fact that during his second and more detailed tour of Montenegro in 1882, the authorities ordered all tribal captains to help Dr. Batut and provide all the necessary assistance, even coercion, if the people refused to undergo examinations²³. On the order of Prince Nikola, Dr. Jovanović was sent with a special recommendation from voivode Mašo Vrbica, the then Minister of Internal Affairs of the Principality of Montenegro²⁴. He set out on his journey around Montenegro on July 4, 1882. He set out with a small entourage – from Rijeka Crnojevića to Andrijevića. On his way from Morača, Montenegro he was accompanied by Josef Holeček, a famous Czech writer and journalist²⁵. He visited every part of Montenegro and wrote about it in his occasional letters to Minister Vrbica, apart from his detailed reports.

He concluded that “the health and physical strength of Montenegrins is far worse than what could be expected”²¹. It is interesting to note that Batut, in his address to Prince Nikola, among other things, talks about being wounded in the war and states: “and there are more of them in Montenegro than anywhere else. There are few adults in our country who have not been wounded at least once, and of those severely wounded, there are quite a large number of those who suffer eternally from the unfortunate consequences”²¹. In his actions, work, and reporting, Dr. Jovanović was precise and systematic, but one cannot fail to state that his reporting was partly exaggerated, but with the main goal of influencing decision-makers to improve the health situation in Montenegro²⁶. This undisputed humanist, professional and lover of his profession made detailed assumptions about modern medicine and the further development of medical service in Montenegro.

The reasons for his sudden departure from Montenegro in October 1882, at a time when he was planning a tour of the southern region of Montenegro, are not fully known. It is assumed that it was an agreement with the head of the medical service of Serbia and his referral to training at the state's expense in the most important European centers, which he crowned in 1919 with the establishment of the Faculty of Medicine in Belgrade. The ministerial council and Prince Nikola I accepted the submitted resignation. During his stay in Montenegro, despite his many obligations, he wrote the booklet “Marriage and the People”¹². From 1882 to 1885, he specialized in Munich, Berlin (Germany), Paris, France, and London, Great Britain, and after that, he worked in Novi Sad as a private physician. In 1887, he was elected full professor at the Great School in Belgrade. He wrote a large number of books and textbooks. He was a great educator in the field of healthcare, and he collected practices from folk medicine, demonstrated bacteriological analyses, and did anthropomet-

ric measurements. For a long time, he was the president of the Main Health Association of the Yugoslav Medical Association and the Society for the Protection of Public Health¹².

After his short but successful career, he was succeeded by Petar Miljanić³, a doctor and lawyer who laid the professional and legal foundations of the Montenegrin medical system and who remained the head of the Medical Department until his death in 1897.

Conclusion

Milan Jovanović Morski (Bombajac) acted in wartime circumstances when the priority was taking care of the wounded. He had an impact on the development of the medical service during the war. In addition to the above, working as a doctor for the Montenegrin dynasties, he also influenced the spread of health culture and awareness of the development of the medical service. The first hospital in Cetinje was named after his student, Prince Danilo. In Montenegro, he played an important role in raising and creating awareness about the need for the development and organization of healthcare. Unlike his predecessor, Milan Jovanović Batut worked in Montenegro during the peacetime period (August 17, 1880 – October 1882), when the focus was on overall social development, including the development of the health service. In the absence of adequate local staff, the job of organizing the medical service was left to Batut. In the manner of a great professional, he conducted a detailed analysis of the health conditions and needs in Montenegro. He organized health statistics based on which it was possible to get an overall picture of the health status of the people and the

measures that needed to be taken. Although he did not remain for a long time, his influence on the development of health care in Montenegro and the creation of assumptions for the organization of military health care is very significant. During his stay in Montenegro, he held important state functions and had complete freedom in the development of healthcare. In a short period, he went from being a practical doctor to becoming the organizer and manager of the Montenegrin health system. In this position, he showed perseverance, organizational ability, persistence, dedication, and erudition. He did not act only in one direction but covered a wide range of development areas, from health statistics, institution organization, staffing, field research, and health studies. His contribution to the development of health care in Montenegro is of great importance and, therefore, also important to the development of military health care. On the other hand, the experience he gained in Montenegro was great and represented an important period for his further development – he could show his abilities, and it served him in his later medical career. Although he planned to return to Montenegro, his obligations and training led him elsewhere. In the end, it can be concluded that the stay of this great doctor and intellectual in Montenegro was mutually beneficial and satisfying. Two prominent Serbian doctors, Milan Jovanović Morski and Milan Jovanović Batut, independently of each other in the second half of the nineteenth century, with a gap of several years, stayed in Montenegro and participated in the treatment of sick and wounded soldiers and civilians, but also significantly influenced the formation of organized civil and military healthcare, health education, and the beginnings of hygiene, epidemiology, and health statistics.

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3. Tekst članka

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

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

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

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

Literatura

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

Primeri referenci:

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Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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www.vsp.mod.gov.rs

