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Vojnosanitetski pregled

80 years

Vol. 81, No. 9, 2024

VOJNOSANITETSKI PREGLED

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

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VOJNOSANITETSKI PREGLED

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

IZDAVAČ
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Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

This september, 80 years of continuous publication of the Vojnosanitetski pregled (Military Medical Review), a scientific journal of physicians and pharmacists of the Serbian Army will be marked. It is one of the leading medical journals in Serbia, indexed in well-known indexing databases including Science Citation Index Expanded (see Editorial, p. 531–540).

U septembru ove godine navršava se 80 godina kontinuiranog izlaženja „Vojnosanitetskog pregleda“ (VSP), naučnog časopisa lekara i farmaceuta Vojske Srbije. VSP je jedan od vodećih medicinskih časopisa u Srbiji, indeksiran u najpoznatijim svetskim bazama naučne publicistike, uključujući i *Science Citation Index Expanded* (SCIe) (vidi Uvodnik, str. 531–540).

E D I T O R I A L

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Vojnosanitetski pregled – 80th anniversary

Vojnosanitetski pregled – 80. godišnjica

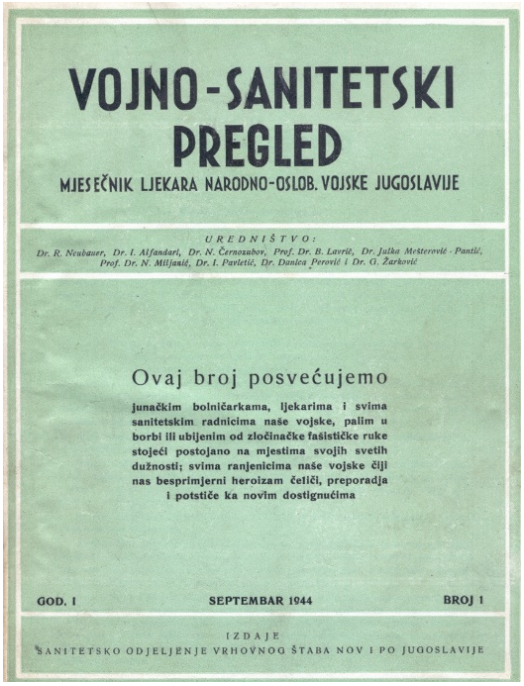
Dragana Vučević

**University of Defence, Faculty of Medicine of the Military Medical Academy,
Center for Medical Scientific Information, Belgrade, Serbia**

Introduction

Vojnosanitetski pregled (Military Medical Review), a journal of physicians, dentists, and pharmacists of the Serbian Army, one of Serbia's oldest scientific journals, was born in September 1944. The Second World War was raging when the first issue of *Vojnosanitetski pregled* was published (Figure 1). It was printed in Bari, Italy, and soon after, the second issue was released in liberated Belgrade, Serbia. *Vojnosanitetski pregled* continued the tradition of, at the time, a very respected pre-war journal – *Vojnosanitetski glasnik* (Military Medical Herald) (Figure 2) published from 1930–1941 as an expert newsletter of the Medical Department of the Ministry of Army and Navy of the Kingdom of Yugoslavia, with its concept, appearance, and content [1, 2].

Reflecting the spirit of the time when it began to be released, *Vojnosanitetski pregled* was focused on the issues of the military medical service during the war and on current problems of war medicine. At the time, the main goal of *Vojnosanitetski pregled* was to increase the physicians' level of knowledge in the fields of war surgery, war epidemiology, and war military medical service. Hence, the published papers predominantly covered these topics. In these articles, initial information regarding sulfamidime, penicillin, dichlorodiphenyltrichloroethane-powder, etc. was provided.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MJESEČNIK LJEKARA NARODNO-OSLOB. VOJSKE JUGOSLAVIJE
UREDNIŠTVO:
Dr. R. Neubauer, Dr. I. Alfandari, Dr. N. Černozubov, Prof. Dr. B. Lavrič, Dr. Julka Mešterović-Pantić, Prof. Dr. N. Miljanić, Dr. I. Pavletić, Dr. Danica Perović i Dr. G. Žarković
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junačkim bolničarkama, ljekarima i svima sanitetskim radnicima naše vojske, palim u borbi ili ubijenim od zločinačke fašističke ruke stojeći postojano na mjestima svojih svetih dužnosti; svima ranjenicima naše vojske čiji nas besprimjerni heroizam čelići, preporadja i potstiče ka novim dostignućima
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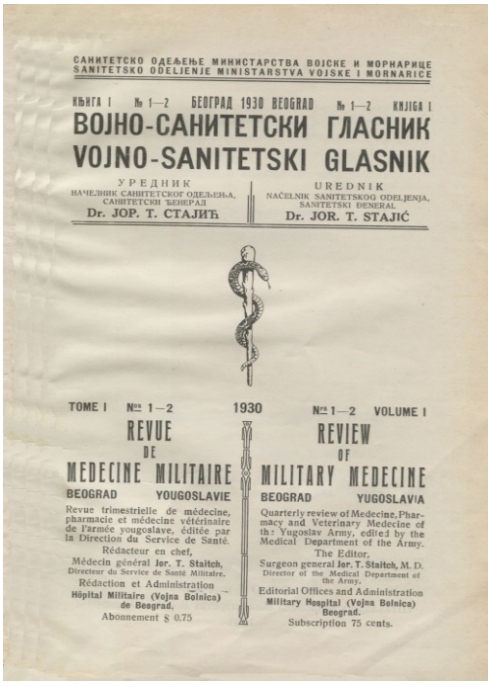
IZDAJE
SANITETSKO ODJELJENJE VRHOVNOG ŠTABA NOV I PO JUGOSLAVIJE

Fig. 1 – Cover page of the first issue of the *Vojnosanitetski pregled* (September 1944).


САНИТЕТСКО ОДЕЉЕЊЕ МИНИСТАРСТВА ВОЈСКЕ И МОРНАРИЦЕ
SANITETSKO ODELJENJE MINISTARSTVA VOJSKE I MORNARICE
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ВОЈНО-САНИТЕТСКИ ГЛАСНИК
VOJNO-SANITETSKI GLASNIK
УРЕДНИК
НАЧЕЛНИК САНИТЕТСКОГ ОДЕЉЕЊА, САНИТЕТСКИ ЋЕНЕРАЛ
Dr. JOP. T. СТАЈИЋ
UREDNIK
NAČELNIK SANITETSKOG ODELJENJA, SANITETSKI GENERAL
Dr. JOR. T. STAJIĆ
TOME I № 1—2 1930 № 1—2 VOLUME I
REVUE DE MEDECINE MILITAIRE
BEOGRAD YOUGOSLAVIE
Revue trimestrielle de médecine, pharmacie et médecine vétérinaire de l'armée yougoslave, éditée par la Direction du Service de Santé.
Rédacteur en chef,
Médecin général Jor. T. Staitch,
Directeur du Service de Santé Militaire.
Rédaction et Administration
Hôpital Militaire (Vojna Bolnica) de Beograd.
Abonnement $ 0.75
REVIEW OF MILITARY MEDECINE
BEOGRAD YUGOSLAVIA
Quarterly review of Medecine, Pharmacy and Veterinary Medecine of the Yugoslav Army, edited by the Medical Department of the Army.
The Editor,
Surgeon general Jor. T. Staitch, M. D.
Director of the Medical Department of the Army.
Editorial Offices and Administration
Military Hospital (Vojna Bolnica) Beograd.
Subscription 75 cents.

Fig. 2 – Cover page of the first issue of the *Vojnosanitetski glasnik* (1930).

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Although *Vojnosanitetski pregled* was focused from the start on the issues of military services, the Editorial Board was trying to contribute to solving the deficiencies of the health system both in the military service and outside of it. *Vojnosanitetski pregled* was striving to inform physicians and pharmacists of all significant accomplishments, scientific achievements, and expert work in various fields of medicine and pharmacy. The journal has always been open to reviewing all important innovations in medicine. Many significant successes in the development of medical science in our region have been published on the pages of our journal. New diagnostic and treatment methods were quite often described for the first time in our journal. Therefore, in those years, *Vojnosanitetski pregled* was considered "the herald and the oldest propagator of the new era of medicine in our country". In the first 40 years of its existence, *Vojnosanitetski pregled* had published 3,640 scientific articles written by 3,000 authors. Furthermore, over 1,300 reviews of significant results written by international authors in the field of biomedicine were published. The journal already gained a reputation not only with domestic but also with international readers. This is evidenced by the fact that our journal was exchanged with 90 medical journals from 28 countries, and printed in 2,500 copies with a growing tendency. This was the way for *Vojnosanitetski pregled* to become the bearer of our scientific thought into the world, which it has [3–5]. In the 1950s and early 1960s, *Vojnosanitetski pregled* was covered by several well-known bibliographic abstract and citation journals and, later, their electronic databases, such as *Index Medicus* (MEDLINE) (1950–2017), *Excerpta Medica* (EMBASE), *Biological Abstracts*, *International Pharmaceutical Abstracts*, and *Chemical Abstracts*. Since 2002, *Vojnosanitetski pregled* has been included in the EBSCO base, which provides a free online approach to the journal contents and downloads the articles in full form to its subscribers. On the occasion of its 25th publishing anniversary (1969), *Vojnosanitetski pregled* was awarded the Grand Star by the Order of Military Merit, which was a huge recognition for the Editorial Board, the authors, and everyone who contributed to this accolade (Figure 3) [1, 2].

From the first issue until today, *Vojnosanitetski pregled* has managed to maintain the continuity of publishing and overcome the occasional downfalls that were inevitable in the historical circumstances our region has faced in the last eight decades. The dynamic of publishing was changing, depending on the number of submitted manuscripts. In the first ten years, *Vojnosanitetski pregled* was occasionally published monthly or bimonthly. From 1954 until 1972, it was regularly published monthly, which implies a large number of submitted manuscripts [5]. From 1973 until 2005, six issues were published *per* year. The difficult period that our country underwent during the 1990s reflected on the journal as well. In the mid-1990s, the number of submitted articles was sixtyish [6]. After that period, the slow recovery and reformation of the journal commenced. In the early 2000s, articles selected by the reviewers as especially significant started to be published both in Serbian and English to be more "visible". This practice influenced a greater influx of articles, and in 2005, for the first time after 30 years, 12 issues of *Vojnosanitetski pregled* were published [1, 2]. Since 2005 until present, the journal has been published every month. Currently, *Vojnosanitetski pregled* is the only biomedical journal in Serbia that maintains this publishing dynamic.

From its foundation until today, the journal has been edited by 14 Editors-in-Chief (Table 1). All of them, each in their own way, often limited by circumstances in which they

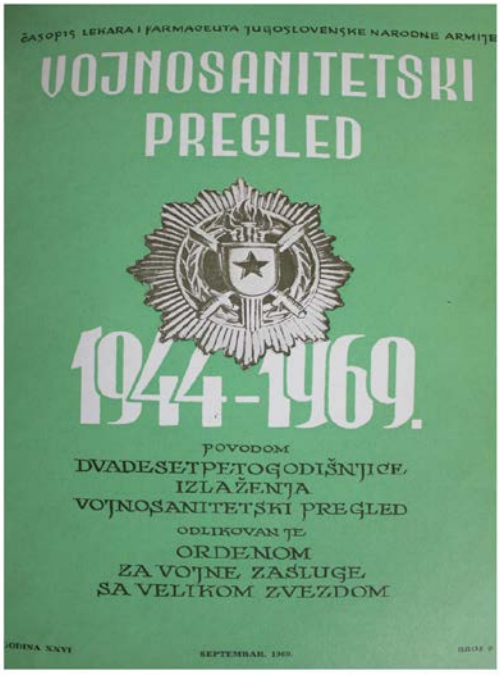


Fig. 3 – Cover page of the jubilee issue of the *Vojnosanitetski pregled* in 1969 dedicated to its 25th Anniversary.

Table 1

Editors-in-Chief of the *Vojnosanitetski pregled* from the foundation to present

1944–1952	Major General Dr. Gojko Nikoliš
1953–1954	Major General Dr. Đura Mešterović
1955–1960	Lieutenant General Dr. Gojko Nikoliš
1960–1962	Colonel Dr. Ivo Pavletić, surgeon
1963–1971	Major General Dr. Žarko Cvetković, surgeon
1971–1979	Colonel Dr. Božidar Nikolić, epidemiologist
1979–1990	Colonel Dr. Vladimir Đergović, surgeon
1990–1995	Colonel Dr. Čedomir Marković
1995–2000	Colonel Dr. Dušan Milić, anesthesiologist
2000–2005	Colonel Prof. Dr. Vladimir Tadić, pharmacologist-toxicologist
2005–2006	Colonel Assist. Prof. Dr. Mile Ignjatović, surgeon
2006–2020	Prof. Dr. Silva Dobrić, clinical pharmacist
2021–2022	Colonel Prof. Dr. Tihomir Ilić, neurologist
2022–	Prof. Dr. Dragana Vučević, immunologist

performed this responsible function, contributed to the development and improvement of the journal. The first Editor-in-Chief of *Vojnosanitetski pregled,* Lieutenant General Dr. Gojko Nikoliš (Editor from 1944–1952 and 1955–1960) (Figure 4), a member of the Serbian Academy of Science and Art, recognized the need for constant professional specialization of the military medical members. His contemporaries described the "astonishing fervor" he pleaded to initiate one scientific and professional military medical journal [3]. Another Editorial Board member who left an indelible mark on the development of the journal during his 25-year-long membership (1964–1990) was Colonel Professor Dr. Dragoljub Pantelić (Figure 5). According to his contemporaries, Dr. Pantelić, a man of high literacy and in-depth knowledge of the scientific method, was *de facto* Chief Editor. He invested his comprehensive efforts into the scientific literacy of the authors whose articles were published in the journal [6].

Major changes in the journal's development were initiated when Colonel Professor Dr. Vladimir Tadić (Figure 6) became the Editor-in-Chief. He was the *spiritus movens* of these changes. The domestic Editorial Board was expanded by joining renowned names of Serbian medicine. The number of peer-reviewers from different fields of medicine, pharmacy, and dentistry who reviewed submitted manuscripts increased (more than 100). Experts outside of military medical institutions are also included in the *Vojnosanitetski pregled* circle of reviewers [7]. Reviews of submitted manuscripts were at least double anonymous peer-reviews [2, 7]. All these changes resulted in improved quality of published articles. For the first time after the first issue in 1944, when among editors two women had been included,

Fig. 4 – Major General Dr. Gojko Nikoliš, founder and the first Editor-in-Chief of the *Vojnosanitetski pregled* (1944–1952 and 1955–1960).

Fig. 5 – Colonel Prof. Dr. Dragoljub Pantelić, member of the *Vojnosanitetski pregled* Editorial Board (1964–1990).

Fig. 6 – Colonel Prof. Dr. Vladimir Tadić, Editor-in-Chief of the *Vojnosanitetski pregled* (2000–2005).

Fig. 7 – Colonel Assistant Prof. Dr. Mile Ignjatović, Editor-in-Chief of the *Vojnosanitetski pregled* (2005–2006).

the Editorial Board was proud to include five women. The current Editorial Board consists of 13 women, including the Editor-in-Chief. Colonel Assistant Professor Dr. Mile Ignjatović (Figure 7) who came as an Editor-in-Chief to *Vojnosanitetski pregled* in 2005 is creditable for the second stage of reforms initiated in 2002. In the same year (2002), the process of establishing the International Editorial Board was completed, which created the formal conditions for "joining the society" of the international journal family [1]. The journal was enriched with new types of articles, e.g., Letters to the Editor, Comments, Critical Views, etc. During this period, the journal got a new logo and a modern look. This is the very first time the logo (Figure 8) was introduced into *Vojnosanitetski pregled*. The logo is a combination of a medical symbol (a snake curled around a stick) and a literacy symbol (a pen), including the significant years 1930 and 1944 (the launching years of *Vojnosanitetski glasnik* and *Vojnosanitetski pregled*, respectively) [8, 9]. The selection criteria for article acceptance have been tightened. The pre-review procedure was established during which authors were advised how to technically, professionally, and in English language improve the manuscript before it was officially sent to reviewers. Special focus was put on preventing plagiarism and self-plagiarism by implementing measures for their detection and sanctioning. Changes in the editorial policy of *Vojnosanitetski pregled* made in 2002 and 2005 contributed to increased quality of published papers and their better international visibility. There was an increase in the influx of manuscripts by authors from abroad and an increase in the number of articles published in English (e.g., in 2007 every fifth, and every fourth article in 2008) [2].

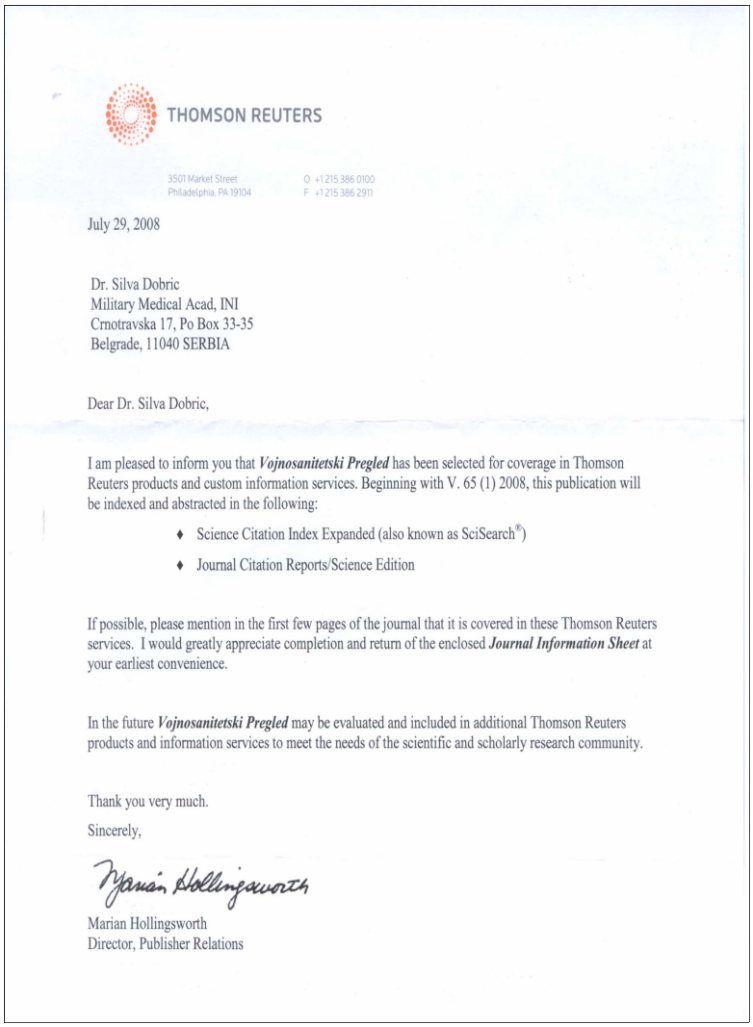
In 2006, Professor Dr. Silva Dobrić (Figure 9) was appointed as Editor-in-Chief and remained in this position until 2020. During this time, several key moments related to the journal occurred. Professor Dobrić continued with the changes her predecessors started in order to improve the quality of the journal. The first fruits of the mentioned changes were harvested in 2008 when *Vojnosanitetski pregled* was accepted into the *Science Citation Index Expanded* (SCIe), a famous base of scientific journals of the

Fig. 8 – A new logo of the *Vojnosanitetski pregled* (2006).

Fig. 9 – Prof. Dr. Silva Dobrić, Editor-in-Chief of the *Vojnosanitetski pregled* (2006–2020).

Institute for Scientific Information (ISI), now *Thomson Reuters*, from Philadelphia, USA (Figure 10) [2]. *Vojnosanitetski pregled* was the first domestic, clinically oriented medical journal that was included in the society of the most influential scientific journals [1]. This was an important event in the journal's history since ISI, through its basic network Web of Science (WoS), provides scientists worldwide with insights into the most recent scientific achievements. Additionally, entering the WoS ensured that the journal received an impact factor (IF), which is an indicator of the influence a journal has in its field [7]. A year later (2009), The Ministry of Science, Technological Development, and Innovation of the Republic of Serbia performed a categorization of domestic biomedical scientific journals and placed *Vojnosanitetski pregled* in the M24 category, a category of international journals (code M20) [10]. In 2008, the journal became available online *via* the website of the Military Medical Academy, Belgrade, (link) which additionally contributed to its better visibility and availability to interested readers [1]. *Vojnosanitetski pregled* received its first IF in 2011 (IF for 2010) and the value was 0.199. This IF placed *Vojnosanitetski pregled* at that moment in the 135th position among 153 most influential journals in the field of General and Internal Medicine [2], and in the same year the journal was placed in the M23 category [11] The highest IF of *Vojnosanitetski pregled* was recorded in 2018 (IF for 2017) and its value was 0.405 [12]. Unfortunately, the IF did not continue to grow. It started to decline, and today, the IF is 0.2 (IF for 2023). Therefore, one of the priority tasks of the Editorial Board in the future will be to increase the IF.

After obtaining the IF, the Editorial Board continued introducing innovations to improve the journal in all domains. In 2011, *Vojnosanitetski pregled* entered several novelties. The first was getting the new official email address vsp@vma.mod.gov.rs. The second was entering the journal into the so-called DOI (Digital Object Identifier) system. This system allows the identification of documents in electronic form and the establishment of a permanent link to the Internet site where the original document is located. Thanks to the DOI number, articles in electronic form (as *Online First*), can be found and cited even before the printed version of the journal is published. This way, accepted articles become accessible to the readers faster. Furthermore, since 2011, only manuscripts prepared in English have been accepted for publication in *Vojnosanitetski pregled*. The exceptions are certain articles in the category History of Medicine [2, 13].



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July 29, 2008

Dr. Silva Dobric
Military Medical Acad, INI
Crnotravska 17, Po Box 33-35
Belgrade, 11040 SERBIA

Dear Dr. Silva Dobric,

I am pleased to inform you that ***Vojnosanitetski Pregled*** has been selected for coverage in Thomson Reuters products and custom information services. Beginning with V. 65 (1) 2008, this publication will be indexed and abstracted in the following:

- Science Citation Index Expanded (also known as SciSearch®)
- Journal Citation Reports/Science Edition

If possible, please mention in the first few pages of the journal that it is covered in these Thomson Reuters services. I would greatly appreciate completion and return of the enclosed ***Journal Information Sheet*** at your earliest convenience.

In the future ***Vojnosanitetski Pregled*** may be evaluated and included in additional Thomson Reuters products and information services to meet the needs of the scientific and scholarly research community.

Thank you very much.

Sincerely,

Marian Hollingsworth
Director, Publisher Relations

Fig. 10 – Information on inclusion of the *Vojnosanitetski pregled* into the *Science Citation Index Expanded* – SCIe.

In 2012, *Vojnosanitetski pregled* moved to the system for electronic editing of journals (the so-called e-UR) which enables higher quality of editorial policy, including checking for plagiarism and self-plagiarism (using the software *iThenticate*). Starting from July 2012, *Vojnosanitetski pregled* has been using an improved version of this system named ASEESTANT [2]. It offers several benefits, such as checking for plagiarism/self-plagiarism, controlling reference accuracy, and selecting appropriate keywords according to the thesaurus of the keywords from the U.S. National Library of Medicine, which, as standardized terms, are used in all medical scientific publications [14]. In 2013, a request was sent to the authors, based on the recommendation of the *International Committee of Medical Journal Editors* – ICMJE, to provide a signed statement on the contribution to the research and manuscript preparation and on the absence of conflict of interests in order to ensure the transparency of information about authorship and funding and thereby strengthen ethical principles in scientific publications [15]. Activities in creating the journal's website started in 2016 [16]. We hope that the journal will have a more modern website by the end of this year. The purpose of all these changes was to increase the visibility of published content and citation of published articles, as well as to consequently increase the quality of the journal.

Shortly after, at the end of 2017, another important change in the history of the journal occurred. The Institute for Scientific Information of the Military Medical Academy in Belgrade, where the Journal's Editorial Office had been situated from 1961, became a member of the University of Defence, which took over all the duties related to the Journal. As a consequence of this change, instead of the Military Health Department of the Ministry of Defence of the Republic of Serbia, which was the publisher of *Vojnosanitetski pregled* from 1944, starting from 2018, the publisher became the University of Defence [17].

The main thematic structure of the journal did not change significantly, but its appearance was modified for the purpose of modernization and updating [1, 5]. Starting from 2006 until today, the front cover has been used to publish images related to the content of that issue or images of important international dates or persons in the history of medicine (Figure 11).

From the very beginning, great attention has been paid to the professional quality of the journal. Detailed Instructions for Authors were given in 1955, with attachments regarding language and spelling (issue 1–2), and in the Impressum, members of the Editorial Board were listed (issue 7–8), including the founder of our gastroenterology department, Lieutenant Colonel Dr. Antun Gašparov and Major Zlatko Binenfeld, BPharm, one of the most prestigious Yugoslav toxicologists. The proofreaders for foreign languages and Serbo-Croatian were Dr. Ines Wesley, the author of Yugoslav Scientific Informatics, and Srđa Petrović, respectively. International standards and conventions for scientific periodical publishing have been in use. Since the 1960s, apart from the abstract in Serbian, the articles published in *Vojnosanitetski pregled* also had an abstract in several foreign languages (English, French, Russian, and German). There was a practice of publishing so-called thematic issues (later supplements), dedicated to significant events or personalities, like the opening of the Military Medical Academy of the Yugoslav Army (issue 3–4 in 1950), the work of the founder and first Editor-in-Chief, Lieutenant General Dr. Gojko Nikoliš (issue 3–4 in 1953) [1, 2], abstracts from the scientific meeting "Serbian Military Health Care 1917–1918" (2008) [18]. On the 25th anniversary of the journal, a complete bibliography of original articles published from 1944–1969 was prepared [1, 6]. In September 2014, on the 70th anniversary of the journal, a bibliography of all articles published in *Vojnosanitetski pregled* was released in an electronic form (on CDs) (link), and a movie was made about the Journal (link) [19].

The disintegration and war in the former Yugoslavia at the beginning of the 1990s was a huge challenge to the survival of the journal as it was faced with a reduced inflow of manuscripts and difficulties in regular publication. In order to provide a large number of manuscripts, the *Vojnosanitetski pregled* Editorial Board and the Publisher introduced the "Author of the Year" award in 1995. Author(s) who published the most articles in the journal in the previous year were awarded [2]. In 1995, 144 authors, with 83 articles, were in the competition [20]. This prize was awarded as a recognition of the author's contribution to the development of biomedical science and increasing of the Journal's prestige. Selection criteria for the award were established in 1996 and include the number and category of articles published in the year for which the award is assigned, and the author's order in the byline. Besides the "Author of the Year" award, since 2013, the "Reviewer of the Year" award of the *Vojnosanitetski pregled* has also been assigned with the aim to emphasize the reviewer's important role in improving the quality of articles published in the journal. Criteria for this award include the number and quality of reviews, and their submission in due time. Author and Reviewer of the Year in 2016 were Assistant Profesor Dr. Branka Roganović, gastroenterologist, and Colonel Professor Dr. Slobodan Obradović, cardiologist, respectively. Dr. Roganović published two original articles as the first author and, among 768 authors, achieved the highest score – 24. Dr. Obradović reviewed 23 submitted papers in total. That was the third time that Dr. Obradović received this award [21]. These two awards were granted until 2017.

Due to inclusion in the SCIe database and later obtaining of IF, the number of papers received for publication had increased significantly (between 2005 and 2007, 225–235, and after 2008, there were about 350) [1, 2]. A record in the number of submitted manuscripts was noted during 2015 and 2016 (about 400) [16, 22]. The inflow of manuscripts in the later years had various dynamics. For comparison, the average number of submitted articles in the period 2020–2023 was 270. This year (until September 15), 201 manuscripts were submitted for consideration. Number of published articles was also changing. In 2005, 2008, and 2009, 145, 160, and 170 articles were published,

Fig. 11 – Cover pages of the *Vojnosanitetski pregled* related to important international dates or persons in the history of medicine.

respectively. Since 2011, the number of published articles has been over 180 [2]. The number of published articles in the last 10 years (2014–2023) spans from 151 (in 2023) to 280 (in 2020), with an average number of 189 articles.

One of the most significant indicators of the journal's position in the scientific community is the number of accesses to the journal and the number of downloaded articles. According to the data obtained from the EBSCO database, in 2008 when *Vojnosanitetski pregled* was included in the SCIe database, the number of accesses and downloads of articles published in *Vojnosanitetski pregled* was 2,340, and in 2009, it was already five times higher (11,562), with a continuous increase in the coming years (in 2012 the number was 38,376, in 2013, it was 39,436 or slightly more than 100 accesses daily) [2]. This number of views has been maintained until now. During 2023, the number of accesses and downloads of articles published in *Vojnosanitetski pregled* was over 41,300. More than 2,800 different universities and other academic institutions have accessed our journal. In the first two places were *Consejo Nacional de Universidades* (Nicaragua) and Stanford University (USA) with 3,345 and 2,305 downloads, respectively. It is particularly encouraging that over 37,500 full-text downloads were recorded in the period January–July 2024. Universities that followed us the most in the first seven months of this year are Pukyong National University (South Korea) with 8,085 downloads and Stanford University (USA) with 3,279 downloads, respectively. We hope that this trend will continue in the future.

By marking 80 years of continuous publishing of *Vojnosanitetski pregled* we should not forget all of those who invested their enthusiasm, creative persistence, and dedication to making *Vojnosanitetski pregled* a recognized name in the scientific community of our region. For many of them, *Vojnosanitetski pregled* was not just work but also an integral part of life. Editors-in-Chief and members of the Editorial Board managed to find the most valuable reviewers and encouraged the most talented authors, hence the published articles would always be of the highest scientific quality. Editorial Office members by knowledge, experience, and constant care made uninterrupted publishing possible. We should not forget all other members of the former Institute for Scientific Information of the Military Medical Academy (Figure 12), and the present Center for Medical Scientific Information of the Faculty of Medicine of the Military Medical Academy (Figure 13) who provided selfless assistance to the Editorial Office of *Vojnosanitetski pregled* in all areas of activity, wherever it was possible. We owe them all sincere gratitude. We are deeply grateful to the members of the publisher's advisory board of *Vojnosanitetski pregled* and all friends and venerators of the *Vojnosanitetski pregled* journal.

The value of each journal is based on the value of its authors and reviewers. Therefore, many thanks go to the authors who have chosen *Vojnosanitetski pregled* to present their professional results during these years. Special thanks go to eminent reviewers who invested their time and knowledge to *Vojnosanitetski pregled*. It is a great pleasure and strong encouragement that, in the last two years, the number of *Vojnosanitetski pregled* reviewers was over 200 annually (link). We owe special gratitude to foreign reviewers who joined the *Vojnosanitetski pregled* review team and made it recognizable beyond our country's borders.

Fig. 12 – Institute for Scientific Information of the Military Medical Academy (2011).

Fig. 13 – Center for Medical Scientific Information of the Faculty of Medicine of the Military Medical Academy (2024).

We entered the ninth decade of the journal's existence. The present Editorial Board and Editorial Office do not have an easy task. On one end, there is an enormous obligation toward the *Vojnosanitetski pregled* tradition, on the other is the vision of the future, and in between is the current state. Every effort to maintain the existing state (keeping the *status quo*) is an imminent beginning of an end. The only way is stepping forward. Moving forward is possible only if we improve the quality of published articles and the quality of work of the Editorial Board and Editorial Office.

This year, the Editorial Board of *Vojnosanitetski pregled* was expanded to 30 domestic and 15 international members (link). The aim was to have the most respected names from various fields of medicine, dentistry, and pharmacy and to rejuvenate the membership. The new Editorial Board has 4 members of the Serbian Academy of Science and Art whose acceptance speaks of the status that *Vojnosanitetski pregled* has achieved in our country. Members of the International Editorial Board are the most prestigious names from different fields of medicine and dentistry. It is not modest to say, but we believe that according to the names of its members, this Editorial Board is one of the most respectable in the history of *Vojnosanitetski pregled*.

On this occasion, when we proudly point out and glorify the undoubted successes of previous generations, we must state that there are still many difficult tasks ahead of us. First on the list of tasks will be to return to the PubMed database. The process will be longlasting and laborious but there is no other way just as there is no time to waste. Changes are necessary, both the major, essential ones and the small, "cosmetic" ones. Before us is the modernization of the journal, bringing the journal closer to the domestic and international scientific community, and motivating the best physicians, dentists, and pharmacists to publish in *Vojnosanitetski pregled*. The constant striving of the current and former Editors-in-Chief to publish the highest quality articles of domestic authors in *Vojnosanitetski pregled* does not meet sufficient response from the authors whose work results could be cited and which could, therefore, strongly influence the quality of the journal. For this reason, the Editorial Board of *Vojnosanitetski pregled* considers its permanent commitment to encourage such authors to publish in *Vojnosanitetski pregled*. The main goal of the Editorial Board is to strengthen the international position and reputation of *Vojnosanitetski pregled*. To accomplish this aim, we expect strong support from all former and future *Vojnosanitetski pregled* collaborators, editors, reviewers, authors, and publisher's advisory board members.

When all is said and done, on behalf of everyone who is in any way involved in the life of *Vojnosanitetski pregled* today, I would like the next journal jubilee to be filled with pride and joy, for, with united efforts, we have taken a step forward on the path built for us by generations of predecessors.

Finally, I would like to ask you all to make a toast to *Vojnosanitetski pregled*, to all its achievements over the past 80 years, and to wish the journal a very successful another 80 years. Happy anniversary!

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GENERAL REVIEW

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The importance of determining lactate dehydrogenase in laboratory and experimental work in oncology

Značaj određivanja laktat dehidrogenaze u laboratorijskom i eksperimentalnom radu u onkologiji

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Ključne reči:
biomarkeri; istraživanje, biomedicinsko; ćelije, kultura; laktat dehidrogenaza; nekroza; neoplazme.

Introduction

Among many other enzymes, lactate dehydrogenase (LDH) is an important metabolic enzyme widely used as a biochemical marker associated with aberrant glycolysis pathway [1–3]. Based on this consideration, LDH has long been used in the diagnosis of many diseases, such as tumors, but also in patients with tuberculosis, tissue necrosis in heart attacks, erythrocyte hemolysis, and inflammation, and has also shown a significant role in coronavirus disease 2019 (COVID-19), which was described in the recent period [4–10]. In a large number of scientific publications, reference values of the LDH enzyme in healthy people have been characterized [11]. Contrary to this, an enormously elevated LDH value in various diseases was reported [4, 7, 8]. Today, the LDH values are generally expressed in IU/mL in serum and are routinely used in laboratory devices that are mostly standardized and that can show the values for a large number of patients in different hospitals. The development of modern biochemistry techniques has made it possible to obtain findings in a short time from the moment the biological material is provided. All of these are very important for patients in emergency medicine as well as for confirmed early diagnosis [12–15]. Due to the availability and low prices of the analysis of this biochemical marker, it is tested in many countries around the world as well as in small or local clinics, almost the same as in large University Clinical Centers [15]. Bearing in mind the importance of LDH detection in tumors, we have explained in detail the possibilities of LDH analysis in various tissue sections as well as the use of LDH in laboratory work in order to demonstrate the possibilities of LDH testing using modern techniques.

LDH as a marker in clinical work

LDH tests are mainly created in clinical work based on the determination of its values in the serum of the patients [16]. The levels of the LDH enzyme in the serum depend on many factors, but mostly on the size of the tumor, localization of the tumor, the blood supply to the tumor, the presence of a capsule, as well as necrosis in the tissue [12, 17–19]. However, it is possible to determine LDH in pleural effusion, mostly used for confirmation of lung cancer or secondary pleural metastasis. Various cystic and inflammatory changes and their content obtained by biopsy are also suitable as an appropriate source for determining the enzyme LDH but also other mediators. For diverse types of tumors, LDH can also be measured in plasma. High LDH values are also described in several cystic fluids from ascites by puncture in different tumors or benign metaplasia [6]. It is important to confirm that LDH is not a specific marker for specific tumor types, and it is used in combination with some other biological tumor markers that are more specific for certain types of tumors. LDH can also be used to rule out other tissue changes and

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necrosis besides tumors, so it is not specific. At the same time, in the presence of certain types of tumors where its values are high and in the case of a confirmed tumor diagnosis, LDH can be used to monitor the effects of therapy and mostly monitor the reduction of the tumor mass during the application of various types of therapy, including chemotherapy, radiotherapy or immunotherapy individually or in their combination [20–22].

New directions for the application of LDH testing in experimental work

For a long time, the determination of LDH was significant only if the concentration of the enzyme was high enough to cross the threshold of measuring devices and reliably determine it in serum [23]. However, in some diseases where the serum value was low, it does not necessarily mean that there was no tissue necrosis or tissue damage, as is the case with myocardial infarction in certain parts of the tissues that are sufficiently blood-stained or with tumors at the very beginning of the disease where the tumor mass is small and where tissue necrosis did not occur. Therefore, the values are false negative in these cases. The problem of LDH analysis in small concentrations was successfully solved recently with the development of modern devices [24, 25]. During the '90s, the application of LDH enzyme determination *ex vivo* in cell cultures also began when it was noticed that cells with damaged membranes release enzymes [26]. Bearing in mind that the enzyme is intracellular, any change in the membrane leads to its passage through the cell membrane outwards and its detection extracellularly [23]. However, determining the size of the enzyme, elucidating its structure and determining its intracellular content helped better clarify the phenomena of cellular metabolism [27, 28].

Possibilities of determining LDH in tissue

For LDH, being an intracellular enzyme, the importance of its determination in tumor tissue has been demonstrated in the literature, not only in everyday clinical practice but more in scientific papers that describe tissue characteristics, especially in different tumor types [2, 28]. By applying new techniques in biomedicine, it is now possible to analyze and monitor changes in LDH levels and its isoforms in the tumor cell using various methods, which include classical biochemical methods and zymography, gel electrophoresis, two-dimensional electrophoresis immunohistochemistry, Western blotting methods, and, recently, the Polymerase Chain Reaction (PCR) [19, 29–32]. The PCR methods are used to prove the *LDH* gene as well as the *LDH* isozyme gene mutations and gene variations in tumor tissue [19]. To determine LDH in tumor tissue, procedures that are required in order to obtain material from the tumor patients by surgical procedure, biopsy or puncture, are carried out with as little damage to the tissue as possible [6, 29, 33, 34]. It is necessary to protect the tissue and mix it with certain protease inhibitors so that the destruction and digestion of the protein does not occur [29]. All these methods and procedures have greatly contributed to better clarifying the process of carcinogenesis, as well as to clarifying the biochemical changes associated with anaerobic metabolism [1, 35–38]. It has been shown that certain LDH fractions, such as LDH 5, correlate better with anaerobic metabolism as well as with genetic changes in tumors in hypoxia [24, 37, 39, 40]. Based on the results of such studies in tumor tissue and the knowledge obtained, in recent times, the application of enzyme blockers and enzyme system inhibitors has been tried in order to treat tumors because the anaerobic process of obtaining energy is predominant in tumor tissue [11, 41–43].

LDH assays in cell cultures as a new direction of application

Based on membrane permeability for LDH release, tests were conducted on many tumor cells for *ex vivo* conditions, where the mechanism of action of potential pharmaceutical compounds as potential drugs was shown (Figure 1). Whether it is a question of natural biological preparations isolated from numerous plants or a question of synthesized compounds, nanoparticles, or the application of recombinant proteins, it is possible after all these treatments of tumor cells to determine LDH values in laboratory and experimental work [26, 44–47]. What particularly attracted the attention of researchers is the fact that trials of various drugs can be performed and tested on tumor cells during cultivation in *ex vivo* conditions [48]. In this system, a wide range of applications can be achieved because LDH is released from the cell after damage to the cell membrane [49]. By clearly defining the process of apoptosis, necrosis, necroptosis, and autophagy, which are explained in detail and related to the process on the cell membrane, the LDH test was extremely useful [26, 50]. The process of apoptosis is defined as the shrinking of the cell membrane and changes in the nucleus but without clearly visible damage to the membrane [50]. In contrast,

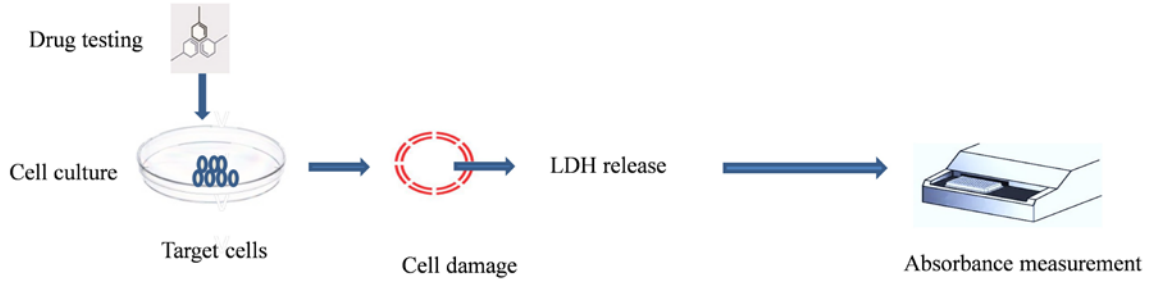

Fig. 1 – Principles of measuring cytotoxicity using the lactate dehydrogenase (LDH) test.

tissue necrosis is defined as the rupture of the cell membrane and the passage of intracellular contents outside of the cells [26]. When these findings were applied to cell cultures, many new phenomena were observed in the medium of cell cultures, including an increase of LDH enzyme values [48, 51]. Thus, a series of cytotoxicity tests were developed, which showed great success in laboratory work and replaced the radioactive tests used earlier for labeling cells in *in vitro* research with enzymatic test [15]. These tests are very simple for routine work, nontoxic, very sensitive, and easy to perform, and they are not expensive either [51, 52]. They can be applied in laboratories after taking the supernatant from cell cultures treated with preparations immediately or after a certain time [51, 53]. However, when storing samples, care should also be taken to freeze the samples immediately and store them until testing so that the enzymes are not destroyed. The possibilities are different, and in such systems, drugs or substances with potential antitumor effects are usually tested in various concentrations, in a large number of repetitions, and in various types of tumor cells [54, 55]. Today, in the modern system of science, it is possible to process such findings mathematically and to predict the effects of new or similar synthesized compounds based on previously conducted experiments, which creates mathematical models with the help of artificial intelligence [56].

Special characteristics of LDH testing in *ex vivo* conditions

LDH is usually determined biochemically simply by adding a substrate for the enzyme and in a biochemical reaction [26]. There are commercial LDH tests for the determination of cytotoxicity and custom assays for the determination of cytotoxic reactions that are incomparably cheaper, allow a large number of analyses to be performed with the help of reagents and substrates, and work perfectly, using microplates [54]. The following reagents are necessary for a custom LDH (colorimetric) assay as substrate or reagents: acetic acid (glacial), β-nicotinamide adenine dinucleotide sodium salt, iodonitrotetrazolium chloride, L-LDH, 1-methoxy phenazine methosulfate, sodium L-lactate, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris Base). All substances are easily available and not expensive [26, 33]. The absorbance is determined from each of the 96 microwell plates using a multi-plate absorbance reader at a wavelength that depends on the type of test, colorimetric (496 nm) or ELISA test [54]. In order to achieve exact results, the special culture media must be used during the cell cultures that do not contain phenol red, which can change the color of the medium itself and cover the background absorbance [26, 57]. Phenol red is added to the cell culture medium in order to control the reaction of the medium and changes depending on pH values following the cell cultivation and its monitoring [53, 54]. In order to reach the reaction threshold and for the reaction to be visible after the addition of the substrate, it is necessary to use a medium for growing cells that is transparent and without phenol red dye. It is also necessary to first standardize for each system the required concentration of cells and the optimal volume of the sample in which the cells are cultivated [53, 58]. It is best to have as many tested cells as possible in a smaller volume in order to release enough LDH in a measurable concentration in the cytotoxic test [58, 59]. However, an excessive number of cells is not desirable during cell cultivation because spontaneous necrosis of tumor cells occurs at a high concentration of cells, which leads to false positive findings. That is why it is preferable to always cultivate, as a separate, control in the identical concentration of cells without the addition of the tested substances and the same concentration of cells with the addition of substances, in order to observe the difference in the effects of a substance on the release of LDH, which is proportional to cytotoxicity [58]. In addition, it is possible to perform the total cell lysis in order to determine the maximum intracellular concentration of the LDH enzyme. Later, the enzyme release in the supernatant can be recalculated in relation to the total intracellular concentration, and the values can be standardized and expressed as the percentage of damage to the cell membrane and the percentage of LDH enzyme leakage, resulting in a much more precise and highly reliable finding in the given system [58, 59]. Based on such mathematical formulas, the percentage of cytotoxicity is obtained so that the result is easily used for simple comparison in various laboratories and various experiments.

Advantages and disadvantages of the cytotoxic test using LDH

In these biochemical reactions where additional substances are applied for testing as potential drugs, care must be taken when interpreting the results [60, 61]. In all cases where a chemical reaction occurs between the potential drug and the substrate to which LDH binds, false findings may occur. That is why the large application of the LDH test in laboratory work is reserved mainly for testing the effect of viral particles on the integrity of the cell membrane, where other methods are not simple, than testing the stability of the cell membrane after gene transfection of cells, which is very specific and reliable in order to show that there are no damaged cell membranes [62, 63]. Application is also of great importance when testing individual natural and herbal preparations on tumor cell lines [55]. However, when testing newly synthesized chemical compounds and potential drugs, one should always be careful, using several tests that show changes in several cell structures, including changes in the nucleus, cytoplasm, and cell membrane, so that the data obtained on tumor cells after treatment are as accurate as possible [64]. When testing newly synthesized compounds whose toxicity is being screened, it is possible that the interactions between enzymes and new drugs could potentially change biochemical reactions, hence the finding would not correspond to the death of the tumor cell but to the interaction of the drugs [65].

The comparison of cell membrane damage using the LDH cytotoxic test was compared with the findings obtained on the flow cytometer, and a significant correlation was shown [53]. However, the flow cytometer uses propidium iodide and annexin that mark the membrane and better indicate early and late changes of apoptosis in cell cultures, while the release of the LDH enzyme indicates total cell necrosis and gives

higher values [49, 54]. However, flow cytometry is not available in all laboratories, and the equipment is very expensive compared to the equipment necessary for biochemical analyses. It is similar to other tests based on the determination of proteins and not only on the examination of LDH in treated cells because drugs can break down proteins, and we can also get false results, even though the experiments are performed with expensive devices and by using proteomics techniques [58].

Conclusion

Lactate dehydrogenase assay is simple to perform, accurate enough, and can be used to determine total cell death as a screening when examining a large number of samples. However, many other more specific laboratory tests are recommended for a more precise investigation of cellular changes, especially at different cellular levels.

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Low-grade inflammation and inflammatory mediators in individuals with prediabetes

Inflamacija niskog stepena i medijatori inflamacije kod osoba sa predijabetesom

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Abstract

Background/Aim. Prediabetes is a condition that refers to the state of hyperglycemia not sufficiently high to reach the diagnostic values for type 2 diabetes mellitus (T2DM). This condition often precedes the appearance of T2DM. The association between the development of early glycoregulation disorders and the state of low-grade chronic inflammation is still not sufficiently well understood. The aim of the study was to assess the values of different inflammatory mediators and biomarkers in individuals with prediabetes. **Methods**. This cross-sectional, observational study included 60 respondents divided into two groups: the prediabetes group (PDG) with 31 patients and the healthy control group (HCG) with 29 respondents. Serum values of seven selected cytokines/biomarkers were compared between the two groups. Examined biomarkers were: interleukin (IL)-1β, IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, E-selectin, and vascular endothelial growth factor (VEGF)-A. In addition, the values of body mass index (BMI), waist circumference (WC), blood pressure (BP), serum triglyceride (TG), fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c) were also compared between the two groups. **Results**. PDG patients had statistically significantly higher TNF-α values compared to the HCG patients (73 pg/mL vs. 55 pg/mL, $p = 0.024$). A trend towards higher levels of IL-8 and IL-1β and lower levels of E-selectin, VEGF-A, and IL-18 was registered in PDG patients but without statistical significance. Furthermore, PDG patients had higher values of BMI, WC, systolic BP, serum TG, FPG, and HbA1c when compared to HCG. **Conclusion**. The results of our study suggest the importance of inflammation and some inflammatory mediators in the pathogenesis of early glycoregulation disorder. We believe that the main goal of future studies should focus on anti-inflammatory therapy in prediabetes.

Key words:
biomarkers; blood glucose; diabetes mellitus, type 2; prediabetic state.

Apstrakt

Uvod/Cilj. Predijabetes je stanje povišene vrednosti glukoze u krvi (hiperglikemije) ali nedovoljno visoke da bi se postavila dijagnoza dijabetes melitus tipa 2 (DMT2). Ovo stanje često prethodi pojavi DMT2. Povezanost razvoja ranog poremećaja glikoregulacije sa stanjem hronične upale niskog intenziteta još uvek nije dovoljno dobro shvaćeno. Cilj rada bio je da se ispitaju vrednosti različitih medijatora zapaljenja i biomarkera kod osoba sa predijabetesom. **Metode.** Opservacionom studijom preseka obuhvaćeno je 60 ispitanika, podeljenih u dve grupe: grupu od 31 bolesnika sa predijabetesom (PDG) i kontrolnu grupu (KG) od 29 zdravih osoba. Serumske vrednosti sedam izabranih citokina/biomarkera su upoređivane između dve grupe ispitanika. Ispitivani su biomarkeri: interleukin (IL)-1β, IL-6, IL-8, IL-18, faktor nekroze tumora (*tumor necrosis factor* – TNF)-α, E-selektin i faktor rasta vaskularnog endotela (*vascular endothelial growth factor* – VEGF)-A. Takođe, između ove dve grupe upoređivani su i indeks telesne mase (ITM), obim struka (OS), krvni pritisak (KP), trigliceridi (TG) u serumu, glukoza u plazmi (GP) natašte i glikozilirani hemoglobin (HbA1c). **Rezultati.** Bolesnici iz PDG imali su statistički značajno više vrednosti TNF-α u poređenju sa ispitanicima KG (73 pg/mL vs. 55 pg/mL, $p = 0,024$). Registrovan je trend viših nivoa IL-8 i IL-1β i nižih nivoa E-selektina, VEGF-A i IL-18 kod bolesnika iz PDG, ali bez statističke

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značajnosti. Takođe, bolesnici iz PDG imali su više vrednosti ITM, OS, sistolnog KP, serumskih TG, GP natašte i HbA1c, u poređenju sa ispitanicima KG. **Zaključak.** Rezultati našeg istraživanja ukazuju na značaj inflamacije i pojedinih medijatora inflamacije u patogenezi ranog poremećaja glikoregulacije. Verujemo da bi ključni cilj budućih istraživanja trebalo da bude usmeren na anti-inflamacijsku terapiju u predijabetesu.

Ključne reči:
biomarkeri; glukoza u krvi; dijabetes melitus, tip 2; predijabetes.

Introduction

Type 2 diabetes mellitus (DM) – T2DM, is a heterogeneous group of metabolic diseases characterized by chronic hyperglycemia associated with protein, lipid, and carbohydrate metabolic disorders. It is a direct consequence of a relative or absolute lack of insulin and insulin resistance. Prediabetes is a term for slightly elevated values of blood glycemia, but not high enough to reach the diagnostic criteria for DM [1, 2]. Prediabetes may be defined as either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). It is a state of high risk of developing T2DM. The progression from normoglycemia to prediabetes is thought to be directly caused by insulin resistance and the further progression to T2DM and later complications of the disease by a progressive decline in the secretory capacity of beta cells [3–5]. As a main characteristic of these disorders, chronic hyperglycemia with hyperinsulinism is followed by changes in sera levels of different cytokines [6]. In different ways, the immune system is involved in every single stage of T2DM development [7, 8].

There is a growing body of evidence that both inflammation and pro-inflammatory cytokines play a significant role in the occurrence of T2DM and the development of its complications. This is achieved by different pathophysiological mechanisms and influences on atherogenesis and endothelial dysfunction [9]. The connection between T2DM and chronic inflammation was first suggested more than a century ago when a high dose of sodium salicylate was noticed to reduce glycosuria in patients with a milder form of T2DM [10]. Numerous epidemiological and other studies showed the correlation between the occurrence of T2DM and increased values of certain inflammatory mediators and acute phase reactants [11–14]. It was proved that low-grade chronic inflammation precedes T2DM [15]. Some of the studies were focused on observing the pro-inflammatory markers in prediabetes [7, 8, 16–18]. A few of them were monitoring inflammatory markers during the progression of glycemic status, from normoglycemia through prediabetes to T2DM [8]. The concentration of some of the biomarkers [C-reactive protein (CRP), white blood cell count (WBC), interleukin (IL)-1β, IL-1 receptor antagonist (RA), IL-6, IL-8, IL-18, monocyte chemoattractant protein (MCP)-1, interferon-gamma-inducible protein 10 (IP 10), haptoglobin and fibrinogen] turned out to have been increased for many years before the occurrence of T2DM, which indicates the existence of chronic, subclinical inflammation [12, 19–21]. Increased gene expression of some pro-inflammatory agents has also been proven in people with prediabetes at the level of pancreatic endocrine islets [19].

Therefore, the aim of the study was to investigate the connection between chronic inflammation and glucose homeostasis in the early stages of the disease. Bearing this in mind, the focus of our study was on inflammatory mediators in prediabetes. We intended to determine the structure of inflammatory markers in the prediabetic population compared to healthy individuals.

Methods

Study population and design

We conducted a cross-sectional, observational study on 60 participants classified into two groups: 31 patients were in the prediabetes group (PDG) and 29 were in the healthy controls group (HCG). Prediabetes is defined through three clinical entities: IFG, IGT, and the combination of the two (IGT + IFG). Subjects were classified as having a normal glucose tolerance if fasting plasma glucose (FPG) was < 6.1 mmol/L and 2-hour oral glucose tolerance test (OGTT) < 7.8 mmol/L or glycated hemoglobin (HbA1c) < 6.0%. Prediabetes was defined if FPG ≥ 6.1 and IFG < 6.9 mmol/L or 2-hour OGTT ≥ 7.8 and IGT < 11.1 mmol/L. DM was classified with a FPG ≥ 7.0 mmol/L or 2-hour OGTT ≥ 11.1 mmol/L or HbA1c ≥ 6.5%. Body mass index (BMI) was calculated as body weight (in kg) divided by body height (in meters) squared. Participants were selected during regular visits or as part of systematic examinations in ambulances of the Cabinet for Endocrinology of the Military Medical Academy (MMA), Belgrade, Serbia. The study was approved by the Ethics Committee of the Faculty of Medicine MMA (No. 1494-2, from April 11, 2023), and every patient provided a signed consent form.

Exclusion criteria were: diagnosed T2DM, T1DM, ischemic cardiomyopathy, valvular heart disease, prior myocardial infarction, uncontrolled arterial hypertension, existing chronic kidney disease [estimate glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2], acute inflammation, malignant or systemic autoimmune diseases, pregnancy, people younger than 18 years and older than 70 years. Values of detected systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure (DBP) > 90 mmHg were considered as unregulated arterial hypertension. Demographic and clinical data of the patients were collected by conducting patient interviews or from hospital medical notes and hospital blood test results. The following serum biochemical parameters and inflammatory cytokines were analyzed from the morning venous blood sample: CRP [reference range (RR): 0.0–4.0 mg/L], fibrinogen (RR: 2.1–4.0 g/L), D-dimer (D-D) (RR: < 0.50 mg/LFEU), FPG (RR: 4.1–5.9 mmol/L), HbA1c (RR: < 6.0%), triglyceride (TG) (RR: < 1.7 mmol/L), total cholesterol (TC) (RR: < 5.2 mmol/L), low-density lipoprotein (LDL) (RR: < 3.5 mmol/L), high-density lipoprotein (HDL) (RR: > 1.3 mmol/L), IL-1β, IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, E-selectin and vascular endothelial growth factor (VEGF)-A.

Data collection

Anamnesis processing of patients, measurements, and clinical examination [waist circumference (WC), body height and weight, BMI, blood pressure (BP), heart rate (HR), blood sampling for the investigated laboratory parameters (after a minimum of 15 min of rest)] were done at the Clinic for Endocrinology. Two sitting BP and HR measurements were taken for each participant using a mercury sphygmo-manometer according to a standard protocol. The mean of these two BP measurements was used in the data analysis. Following the collection of 24 mL of peripheral blood from consenting fasting study participants between 8:30 a.m. and 10:30 a.m., various biochemical parameters and cytokines were measured. Standard laboratory analyses were performed on the same day at the Institute of Medical Biochemistry of the MMA. HbA1c, CRP, FPG, and lipid profile were measured using an Advia 1,800 automatic biochemical analyzer (Siemens). Coagulation screen, fibrinogen, and D-D were measured using a BCS XP coagulometer (Siemens). The cytokine concentrations were measured at the Institute for Medical Research (IMR), MMA. A peripheral blood sample was submitted to the IMR immunology laboratory within one hour after sampling, where serum was separated and stored at -70 °C until analysis. All collected serum samples were analyzed in the same act. The biomarker/cytokine concentrations (E-selectin, VEGF-A, TNF-α, IL-1β, IL-6, IL-8, and IL-18) were measured in the sera of patients using a Premixed Multiplex Kit-Human Custom 10 Plex (N. Orange Grove Ave., Pomona, CA 91767, USA), performed according to the manufacturer's instructions (flow cytometer Beckman Coulter Navios EX). Detection kits were produced by AimPlex Biosciences, Inc.

Statistical analysis

The differences in demographic, clinical characteristics, and laboratory analyses between patients with prediabetes and the control group were compared using the Chi-square test for categorical variables, the *t*-test for continuous variables with normal distribution, and the Mann-Whitney *U* test for non-normally distributed variables. Association between variables was tested using Pearson's or Spearman's correlation, where appropriate, according to the normality distribution. Statistical analyses were performed using IBM SPSS Statistics version 25 for Windows (IBM Corporation, Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$.

Results

Basic clinical parameters

The average age of the study participants was 47.48 ± 10.21 years, with 53.3% of the study population being male. The median BMI in the study cohort was 28.04 kg/m^2 (25.35–30.45). Patients with prediabetes, compared to the control group, had significantly higher levels of BMI (median: 29.0 kg/m^2 vs. 27.1 kg/m^2, $p = 0.010$), WC (mean: 103.0 cm vs. 93.2 cm, $p = 0.04$), SBP (mean: 126.0 mmHg vs. 119.8 mmHg, $p = 0.035$), FPG (mean: 5.6 mmol/L vs. 5.1 mmol/L, $p = 0.001$), HbA1c (mean: 5.6% vs. 5.1%, $p < 0.001$), and serum TGs (median: 1.75 mmol/L vs. 1.15 mmol/L, $p = 0.007$). There were no significant differences between the two groups for DBP, HR, TC, LDL, CRP, fibrinogen, and D-D. The mean levels of HDL in HCG were significantly higher compared with PDG (mean: 1.56 mmol/L vs. 1.35 mmol/L, $p = 0.046$) (Table 1).

Table 1

Baseline subject characteristics of study participants

Characteristics	HCG (n = 29)	PDG (n = 31)	*p*-value
Age (years)	44.07 ± 9.49	50.68 ± 9.96	**0.011**
Male gender	13 (40.6)	19 (59.4)	0.201
Body mass index (kg/m^2)	27.1 (22.6–29.5)	29.0 (26.6–32.5)	**0.010**
Waist circumference (cm)	93.2 ± 17.9	103.0 ± 16.0	**0.04**
Systolic BP (mmHg)	119.8 ± 11.8	126.0 ± 10.2	**0.035**
Diastolic BP (mmHg)	80 (70–85)	85 (80–85)	0.082
Heart rate (beats/min)	70 (65–85)	75 (70–85)	0.363
C-reactive protein (mg/L)	1.03 (0.40–3.22)	0.71 (0.10–3.15)	0.208
Fibrinogen (g/L)	3.4 ± 0.7	3.3 ± 1.4	0.686
D-dimer (mg/L FEU)	0.31 (0.22–0.41)	0.42 (0.22–0.66)	0.093
Fasting plasma glucose (mmol/L)	5.1 ± 0.5	5.6 ± 0.7	**0.001**
HbA1c (%)	5.1 ± 0.4	5.6 ± 0.4	**< 0.001**
Triglyceride (mmol/L)	1.15 (0.81–1.56)	1.75 (1.06–2.63)	**0.007**
Total cholesterol (mmol/L)	5.0 (4.61–5.73)	5.45 (4.68–6.18)	0.355
Low-density lipoprotein (mmol/L)	3.08 (2.54–3.61)	3.41 (2.61–3.72)	0.261
High-density lipoprotein (mmol/L)	1.56 ± 0.41	1.35 ± 0.41	**0.046**

HCG – healthy control group; PDG – prediabetes group; BP – blood pressure; HbA1c – glycated hemoglobin.
Results are given as mean ± standard deviation or median (interquartile range), except for male gender which is presented as numbers (percentages).
Bold values indicate the significance level of $p < 0.05$.

Cytokines

Baseline levels of cytokines are presented in Table 2. Median TNF-α levels in PDG subjects were significantly higher compared with HCG (73 pg/mL vs. 55 pg/mL, $p = 0.024$) (Figure 1). There was a trend towards elevated serum levels of IL-8 and IL-1β in PDG compared to respondents from HCG, although statistical significance could not be reached. On the contrary, a trend towards lower levels of E-selectin, VEGF-A, and IL-18 among the PDG subjects was seen compared to the HCG. Again, comparing serum levels of E-selectin, VEGF-A, and IL-18 between the groups showed no statistical significance.

Correlation between cardiovascular risk factors, cytokines, and basic clinical parameters

There was a statistically significant correlation between the majority of traditional cardiovascular (CV) risk factors (TC, TG, HDL, LDL, BMI, SBP, DBP) in both groups (Table 3). Furthermore, there was a significant correlation (positive or negative) between most inflammatory cytokines in both groups (Table 4). However, statistical significance in the correlation between traditional CV risk factors and inflammatory cytokines was rarely observed (Table 5). A significant positive correlation was found only between IL-18 and TG in HCG, PDG, and the total monitored population. Likewise, there was a statistically significant cor-

Table 2

Levels of serum biomarkers in healthy control group (HCG) and prediabetes group (PDG).

Biomarkers	HCG (n = 29)	PDG (n = 31)	*p*-value
IL-1β	9 (3.5–17)	11 (9–14)	0.534
IL-6	13 (10–18)	12 (10–18)	0.911
IL-8	336 (157–452)	457 (172–578)	0.258
TNF-α	55 (38.5–64.5)	73 (44–92)	**0.024**
E-selectin	1,665 (1,463–2,065)	1,165 (1,463–2,065)	0.446
VEGF-A	790 (506–1,307)	700 (590–1,391)	0.641
IL-18	170.4 ± 55.9	164.4 ± 54.3	0.672

IL – interleukin; TNF – tumor necrosis factor; VEGF – vascular endothelial growth factor.
Data are expressed as median (interquartile range) or mean ± standard deviation.
Bold value indicates the significance level of $p < 0.05$.
Units of measurement of all presented cytokine concentrations are given in pg/mL.

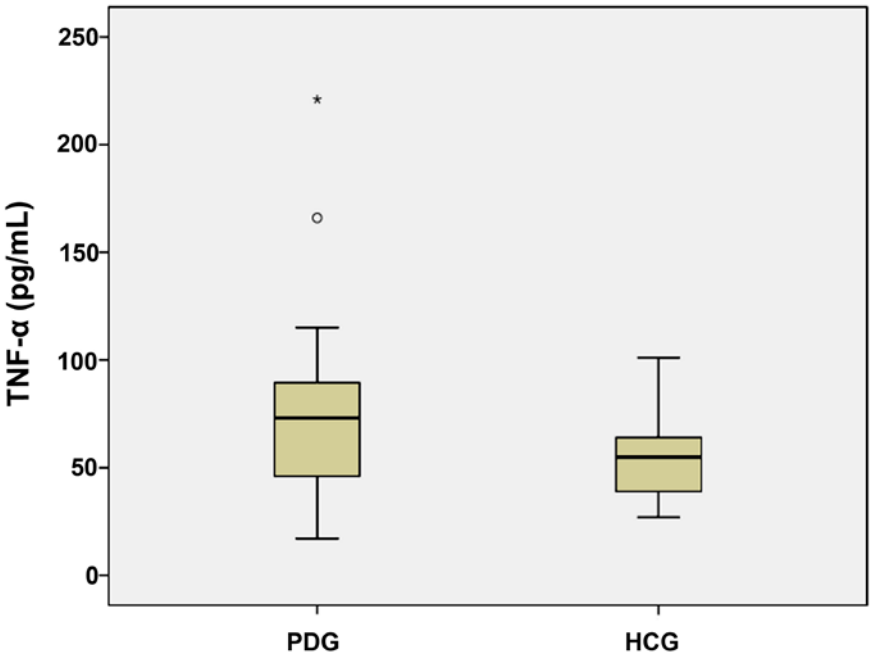

Fig. 1 – Levels of tumor necrosis factor (TNF)-α in the prediabetes group (PDG) and healthy control group (HCG). *$p<0.05$.

Table 3

Correlation coefficients between cardiovascular risk factors in healthy control group (HCG) and prediabetes group (PDG)

Parameters	TC	TG	HDL	LDL	BMI	SBP	DBP
HCG (n = 29)							
TC	1						
TG	0.487**	1					
HDL	-0.140	-0.501**	1				
LDL	0.941**	0.391*	-0.248	1			
BMI	0.475	0.538**	0.358	0.438*	1		
SBP	0.403	0.306	-0.479**	0.430*	0.722**	1	
DBP	0.453*	0.305	-0.371*	0.455*	0.695**	0.868**	1
PDG (n = 31)							
TC	1						
TG	0.465**	1					
HDL	0.319	-0.543**	1				
LDL	0.894**	0.377*	0.174	1			
BMI	-0.130	0.152	-0.399*	-0.119	1		
SBP	0.031	0.156	-0.059	-0.037	0.160	1	
DBP	0.243	0.244	-0.055	0.130	0.349	0.648**	1

TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure. *$p < 0.05$. **$p < 0.01$. Units of measurement of all presented parameters are given in Table 1.

Table 4

Correlation coefficients between inflammatory markers in the healthy control group (HCG) and prediabetes group (PDG)

Parameters	IL-1β	IL-6	IL-8	TNF-α	E-selectin	VEGF-A	IL-18
HCG (n = 29)							
IL-1β	1						
IL-6	0.695**	1					
IL-8	0.705**	0.649**	1				
TNF-α	0.571**	0.493**	0.569**	1			
E-selectin	0.362	0.419*	0.560**	0.216	1		
VEGF-A	0.444*	0.646**	0.540**	0.293	0.588**	1	
IL-18	0.658**	0.595**	0.819**	0.440*	0.748**	0.585**	1
PDG (n = 31)							
IL-1β	1						
IL-6	0.680**	1					
IL-8	0.751**	0.757**	1				
TNF-α	0.787**	0.700**	0.816**	1			
E-selectin	0.628**	0.451*	0.492**	0.684**	1		
VEGF-A	0.605**	0.574**	0.696**	0.743**	0.477**	1	
IL-18	0.683**	0.501**	0.656**	0.659**	0.393*	0.653**	1

For abbreviations, see Table 2. *$p < 0.05$. **$p < 0.01$.
Units of measurement of all presented cytokine concentrations are given in pg/mL.

Table 5

Correlation coefficients between inflammatory markers and cardiovascular risk factors in healthy control group (HCG) and prediabetes group (PDG)

Parameters	TC	TG	HDL	LDL	BMI	SBP	DBP
HCG (n = 29)							
IL-1β	-0.224	-0.074	0.203	-0.306	-0.178	-0.264	-0.360
IL-6	-0.154	0.015	0.038	-0.232	0.005	-0.169	-0.340
IL-8	0.069	0.319	0.094	-0.071	0.060	-0.210	-0.276
TNF-α	-0.019	0.072	-0.116	-0.049	-0.080	-0.263	-0.385*
E-selectin	0.151	0.337	0.153	0.020	-0.013	-0.230	-0.240
VEGF-A	-0.126	0.116	0.292	-0.219	0.086	-0.293	-0.330
IL-18	0.068	0.387*	0.036	-0.065	0.166	-0.073	-0.077
PDG (n = 31)							
IL-1β	0.058	0.104	0.114	-0.011	-0.218	0.014	-0.156

Table 5 (continued)

Parameters	TC	TG	HDL	LDL	BMI	SBP	DBP
IL-6	0.037	0.004	0.055	0.008	0.042	0.133	0.112
IL-8	0.176	0.130	0.095	0.095	-0.061	0.022	-0.005
TNF-α	0.078	0.146	0.023	0.020	-0.125	0.115	-0.101
E-selectin	0.302	0.003	0.354	0.227	-0.444*	0.102	-0.149
VEGF-A	-0.031	0.079	-0.071	-0.098	0.059	0.144	-0.107
IL-18	0.210	0.383*	-0.278	0.146	-0.019	0.052	0.077

For abbreviations, see Tables 2 and 3. ∗$p < 0.05$.
Units of measurement of all presented parameters are given in Tables 1 and 2.

Table 6

Correlation coefficients between inflammatory markers and WC, HbA1c, and FPG in the prediabetes group (PDG) and healthy control group (HCG).

Biomarkers	PDG (n = 31)			HCG (n = 29)		
	WC	HbA1c	FPG	WC	HbA1c	FPG
IL-1β	-0.329	-0.566**	0.271	-0.069	-0.313	-0.035
IL-6	-0.196	-0.636**	-0.195	0.132	-0.160	-0.036
IL-8	-0.174	-0.633**	0.175	0.027	-0.208	0.160
TNF-α	-0.276	-0.567**	-0.025	-0.080	-0.113	-0.098
E-selectin	-0.533**	-0.381*	0.089	0.131	-0.127	0.253
VEGF-A	-0.016	-0.429*	0.015	0.151	-0.095	0.095
IL-18	0.053	-0.496**	0.343	0.030	-0.430*	-0.014

IL – interleukin; TNF – tumor necrosis factor; VEGF – vascular endothelial growth factor; WC – waist circumference; HbA1c – glycated hemoglobin; FPG – fasting plasma glucose. ∗$p < 0.05$. ∗∗$p < 0$.
Units of measurement of all presented parameters are given in Tables 1 and 2.

relation between HbA1c, FPG, BMI, and WC (Table 1). There was a significant negative correlation between HbA1c level and all cytokines measured in PDG, which was, at the same time, absent in patients from HCG (Table 6).

Discussion

The inspiring results of epidemiological and other studies connected the occurrence of T2DM with elevated levels of some of the cytokines, mediators of inflammation, and reactants of acute phase [11–14]. Some studies took a step further and focused on elevated pro-inflammatory markers in prediabetes, then proved that low-grade chronic inflammation precedes T2DM [7, 15, 16]. Wang et al. [9] monitored inflammatory markers during the progression of glycemic status, from normoglycemia through prediabetes to T2DM. Numerous markers, both pro- and anti-inflammatory, have been linked to the process of prediabetes progressing to DM [7]. Evidence of a correlation between chronic inflammation and the development of severe, late complications in T2DM has led to the hypothesis that some specific inflammatory factors may serve as screening biomarkers for the early detection of patients with a poor prognosis.

Following the recruitment of the study participants, we comparatively assessed the inflammatory milieu of prediabetes study participants and healthy individuals. The results of our study showed an increased level of only TNF-α in subjects with prediabetes compared to the control group. In addition, our data showed increased BMI, WC, SBP, TG, FPG, and HbA1c levels in prediabetes subjects compared with controls.

TNF-α is a pro-inflammatory cytokine that increases insulin resistance *via* modulation of glucose transporter type 4 and phosphorylation of insulin receptor substrate-1 [22]. Our results regarding TNF-α are in agreement with many similar studies focusing on insulin resistance, obesity, prediabetes, or T2DM. Most studies found that subjects with some glucose impairment had increased levels of TNF-α [23–28]. Marques-Vidal et al. [23] noticed the increased values of TNF-α in people with insulin resistance, metabolic syndrome, and T2DM and CV diseases. In 2016, there was a study that indicated the possibility of using systemic inflammatory cytokines (TNF-α) as a screening tool to detect people with a higher risk of developing T2DM and CV diseases [24]. Guzmán-Flores et al. [25], in a study on the Mexican population with T2DM, explained the importance of ethnicity in relation to TNF-α values and glucose tolerance. Furthermore, certain gene alleles of the *TNF-α* factor promoter (-238A) increase the risk of developing T2DM in Mexican patients. In addition, the frequency of the GA haplotype (created by the -308G and -238A alleles) is significantly increased in T2DM patients compared to the controls [25]. The previous name of TNF-α was cachectin due to its significant role in the pathogenesis of cachexia in various diseases and its influence on lipid metabolism [27]. In 2017, while researching the role of adipose tissue in thrombosis, Vilahur et al. [28] discovered an interesting fact – in diabetic or obese patients, which is reflected by high levels of cytokines such as TNF-α and other inflammatory markers.

However, there are studies whose results point to a different aspect. Al-Shukaili et al. [29] showed decreased levels of IL-6 and TNF-α in T2DM compared to the healthy controls.

The author of the study mentioned the duration of the diseases, the small sample size, and the differences in age and sex of the studied groups as the possible reasons for this discrepancy. In the study of Wang et al. [4], there was no significant difference between TNF-α and IL-6 in the three monitored groups (the healthy, the prediabetic, and the T2DM one). Gupta et al. [18] reached a similar result in their analysis of inflammatory cytokines in prediabetes. Higher levels of TNF-α, IL-6, and interferon (IFN)-β were detected in PDG but without statistical significance. We do not exclude the possibility of a dual, pro-, and anti-inflammatory role of TNF-α in different stages of the disease. There is also a study hinting at the dual pro- and anti-inflammatory role of TNF or a self-limited inflammatory response in vascular smooth muscle cells [30].

Many studies indicated a connection between elevated values of IL-6 and the progression of glucose impairment. IL-6 is a pro-inflammatory cytokine produced by numerous cells such as activated leukocytes, endothelial cells, and adipocytes [31, 32]. This cytokine proved to induce hyperglycemia and compensatory hyperinsulinemia in murine models and humans [33, 34]. Pradhan et al. [12] study pointed out that elevated levels of IL-6 and CRP are connected to the risk of developing T2DM in the future. According to Spranger et al. [26], plasma IL-6 and TNF-α levels were connected to the forthcoming T2DM. Some studies have shown that HbA1c and IL-6 levels were significantly higher in IFG and IGT patients compared to healthy individuals [35, 36]. Such results are not in accordance with the results of our research, which showed no significant difference in IL-6, and CRP. IL-8 and IL-1β levels were slightly higher, while E-selectin, VEGF-A, and IL-18 levels were lower in PDG compared to HCG. Notably, the differences in cytokine levels among the groups failed to reach statistical significance.

Obtaining significant differences in BMI, WC, SBP, TG, HDL, FPG, and HbA1c values is in agreement with the results of earlier studies [4, 12, 29, 35, 36]. These significant correlations for most traditional CV factors (in both groups), as well as most cytokines (in both groups), were expected. Surprisingly, we found highly significant and inversive correlation values between HbA1c and all the cytokines in the PDG group. Neither the respondents from HCG nor the relations of cytokines with WC, BMI, and FPG (in both groups) contain such correlations. According to available data, one might expect a much higher probability of a positive correlation between HbA1c and various cytokines in T2DM patients. In 2018, a study was published regarding a significant positive correlation of IL-6, TNF-α, and CRP with FPG, HbA1c values, and blood pressure level [37]. In 2019, Sari et al. [38] published the results about the association between HbA1c and serum levels of IL-6 in patients with T2DM. In 2020, a group of scientists pointed out the correlation between the elevated HbA1c and IL-3, IL-4, IL-7, TNF-α, and IFN-α_2 values in obese Afro-American women [39]. However, there is not much evidence on the relation of HbA1c values and cytokines in the state of prediabetes [35, 36]. A question arises: "Is it possible that the same cytokines play a double role during the development of T2DM?" The first role might be anti-inflammatory, with a self-limited inflammatory response in the early stage of prediabetes, and the second, a pro-inflammatory one in the advanced stage of T2DM.

Conclusion

Reducing cardiovascular risk is a major goal in the treatment of patients with prediabetes and diabetes. Early diagnosis of glucose impairment, ideally during the prediabetes phase, as well as promptly starting the intensive therapy, is crucial when it comes to reducing cardiovascular risk. In that regard, the idea of inflammation as a therapeutic target, besides hyperglycemia, is quite interesting. Inflammation during prediabetes could be a useful target for clinicians in the future to prevent, or at least slow down, the progression of prediabetes to T2DM. These results highlight the importance of two items – firstly, the fact that prediabetes, as a precursor of diabetes, is an important clinical entity, and secondly, that inflammation and some inflammatory mediators have their own role (although still not completely understood) in the pathogenesis of prediabetes. Future studies are expected to provide answers to whether certain cytokines have a dual role in different stages of the disease (pro- and anti-inflammatory).

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Evidence of helminthic infestation and efficacy of anthelminthic treatment in children investigated for eosinophilia

Dokazi infestacije helmintima i učinak terapije antihelminticima kod dece ispitivane zbog eozinofilije

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Abstract

Background/Aim. The cause of eosinophilia often remains unelucidated. The aim of the study was to analyze causes and treatment approaches in children with eosinophilia in pediatric tertiary care hospital. **Methods.** The medical records of children investigated for eosinophilia (based on the International Classification of Diseases code D72.1) were retrospectively reviewed in the University Children's Hospital, Belgrade, Serbia, from December 2011 to December 2022. A total of 105 children (62 boys; male:female ratio was 1:4) aged one month to 16.5 years (median 7.7 years) were diagnosed with eosinophilia. After excluding 15 of them due to incorrectly assigned diagnosis based on relative eosinophil number only, the remaining 90 children were grouped according to the severity of eosinophilia (mild, moderate or severe). **Results.** Serological analysis confirmed toxocariasis in six (6.7%) patients, while two (2.2%) had a confirmed nematode infestation (*Ascaris lumbricoides* and *Enterobius vermicularis*, respectively). Thirty-two (35.6%) children with eosinophilia and three with no true eosinophilia were diagnosed with helminthiasis *ex juvantibus*. Eosinophilia was ultimately explained by allergic/atopic conditions [19 (21.1%)], drug reactions [four (4.4%)], bacterial infections [nine (8.9%)], hematological problems [five (5.5%)], autoimmune disorders [three (3.3%)], unrelated congenital disorders (one), or as an isolated finding [seven (7.8%)]. In addition, one of the children without an increased absolute eosinophil number was diagnosed with eosinophilic esophagitis. A total of 56 (53.3%) children received anthelminthic treatment: 9 (90.0%) with severe eosinophilia, 19 (51.4%) with moderate, 23 (53.5%) with mild, and 5 (33.3%) children with no true eosinophilia. Most (42) of the children were given mebendazole only, while the remaining 14 (eight with severe, three with moderate, and three with mild) were also initially treated with mebendazole but subsequently shifted to albendazole due to the persistence of eosinophilia. In all treated children, eosinophilia and other relevant findings (if any) subsided in a matter of a few days to a few weeks after initializing treatment. **Conclusion.** Our results support the recommendation that unexplained eosinophilia of all levels of severity requires a standardized diagnostic approach. The results also provide some support for a potential rational basis for *ex juvantibus* administration of anthelminthic drugs in a fraction of children with eosinophilia without an obvious etiological explanation.

Key words:
anthelmintics; child; diagnosis; diagnosis, differential; eosinophilia; tertiary care centers; treatment outcome.

Apstrakt

Uvod/Cilj. Uzrok eozinofilije često ostaje nerasvetljen. Cilj rada bio je da se analiziraju uzrok i terapijski pristup kod dece sa eozinofilijom u pedijatrijskoj bolnici tercijarnog stepena zbrinjavanja. **Metode.** Retrospektivno je analizirana medicinska dokumentacija dece koja su ispitivana zbog eozinofilije (naznačene šifrom D72.1 na osnovu Međunarodne klasifikacije bolesti) u Univerzitetskoj dečjoj klinici u Beogradu, Srbija, u periodu od decembra 2011. do decembra 2022. Dijagnozu eozinofilije imalo je ukupno 105 dece (62 dečaka; odnos dečaci:devojčice iznosio je 1:4) uzrasta od mesec dana do 16,5 godina (medijana 7,7 godina). Posle isključenja 15 dece zbog pogrešno postavljene dijagnoze samo na osnovu relativnog broja eozinofila, preostalih 90 dece grupisano je prema težini eozinofilije (blaga, umerena ili teška). **Rezultati.** Serološkom analizom potvrđena je toksokarijaza kod šest (6,7%) bolesnika, dok je kod dvoje dece (2,2%) dokazana infestacija nematodama (*Ascaris lumbricoides*, odnosno *Enterobius vermicularis*). Kod 32 (35,6%) dece sa eozinofilijom, kao i kod troje dece bez prave eozinofilije, helmintijaza je dijagnostikovana *ex juvantibus*. Eozinofilija je na kraju objašnjena

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alergijskim/atopijskim stanjima [19 (21,1%)], reakcijama na lekove [četiri (4,4%)], bakterijskim infekcijama [devet (8,9%)], hematološkim problemima [pet (5,5%)], autoimunskim bolestima [tri (3,3%)], nepovezanim urođenim stanjima (jedno dete) ili kao izolovan nalaz [sedam (7,8%)]. Pored toga, kod jednog deteta s dijagnozom eozinofilije ali ne i povišenim apsolutnim brojem eozinofila postavljena je dijagnoza eozinofilnog ezofagitisa. Ukupno 56 (53,3%) dece dobilo je terapiju antihelminticima: 9 (90,9%) sa teškom eozinofilijom, 19 (51,4%) sa umerenom, 23 (53,5%) sa blagom i 5 (33,3%) dece bez prave eozinofilije. Većina (42) dece dobila je samo mebendazol, dok je preostalih 14 (osmoro sa teškom, troje sa umerenom i troje sa blagom) takođe prvobitno lečeno mebendazolom, ali su kasnije, zbog perzistentnosti eozinofilije, lečeni albendazolom. Kod sve dece lečene antihelminticima, eozinofilija i ostali relevantni nalazi (ako ih je bilo) povukli su se u roku od nekoliko dana do nekoliko nedelja od početka lečenja. **Zaključak.** Naši rezultati u celini govore u prilog preporuke da neobjašnjena eozinofilija bilo kog stepena težine iziskuje standardizovani dijagnostički pristup. Takođe, rezultati potkrepljuju potencijalnu racionalnu osnovu za primenu antihelmintika *ex juvantibus* kod jednog broja dece sa eozinofilijom bez očiglednog etiološkog razjašnjenja.

Ključne reči:
antihelmintici; deca; dijagnoza; dijagnoza, diferencijalna; eozinofilija; zdravstvene ustanove, tercijarne; lečenje, ishod.

Introduction

Eosinophilia is defined as an absolute blood eosinophil count above 0.5×10^9/L [1]. As a rather nonspecific finding, eosinophilia can accompany a wide range of allergic, autoimmune, and infectious disorders, notably those caused by eukaryotic organisms – protozoa and multicellular parasites (helminths) [2]. Eosinophilia is also the hallmark of rare but highly significant primary eosinophilic syndromes [3]. This breadth of potential clinical implications of eosinophilia is in line with the wide array of roles played by eosinophil granulocytes – an evolutionarily ancient part of our immune system – in health and disease [4].

Even though eosinophilia is a common finding (and no less commonly incidental), its exact cause often remains unelucidated [5]. This is partly due to the transient and fluctuating nature of eosinophilia in many known disorders, as well as in the case of parasitic eosinophilia due to the limited sensitivity of routine parasitological tests [6]. However, at least a fraction of unexplained eosinophilia is likely to result from the absence of (or insufficient adherence to) standard guidelines or protocols for physicians investigating patients with eosinophilia, particularly in the pediatric population, where parasitic infestations are more prevalent relative to adults, and primary (genetically determined or influenced) causes of eosinophilia are more likely to present. Among helminthic infestations, toxocariasis appears to be particularly elusive, underdiagnosed, and prone to bring about potentially serious consequences [7–9].

The aim of the study was to present and analyze the diagnostic workup of patients with eosinophilia in a tertiary care pediatric institution, with particular emphasis on the evidence of potential helminthic infestation and the documentation of its specific treatment (including that administered *ex juvantibus*).

Methods

This retrospective study reviewed medical records of children investigated for eosinophilia in the University Children's Hospital, Belgrade, Republic of Serbia, from December 2011 to December 2022. This work has been approved by the Ethical Review Board of the University Children's Hospital (No. 16/9, from February 8, 2024).

All children who have been assigned the diagnosis of eosinophilia [based on the International Classification of Diseases (ICD) code D72.1] were included in the analysis. This criterion was met by a total of 105 children (62 boys and 43 girls, male:female ratio was 1:4) aged one month to 16.5 years (median 7.7 years). Fifteen (14.2%) children were excluded because they had been incorrectly assigned the diagnosis of eosinophilia based on the relative number of eosinophils, while the absolute number was below the cut-off value for this diagnosis (0.5×10^9/L). The patients were divided into subgroups according to the severity of their eosinophilia: mild [$0.5–1.5 \times 10^9$/L, n = 43 (47.8%)], moderate [$1.5–5.0 \times 10^9$/L, n = 37 (41.1%)], or severe [$> 5.0 \times 10^9$/L, n = 10 (11.1%)]. The patients were further grouped according to whether they were referred to our hospital from primary healthcare institutions due to eosinophilia *per se* or were diagnosed during diagnostic workups conducted for various clinical indications. These indications were then broadly grouped according to the organ system that was primarily affected.

All patients had an automatic complete blood count (CBC) with additional leukocyte differential count conducted non-automatically (using optical microscopy). The eosinophils were enumerated on a smear stained according to Leischman. In addition to CBC, data on relevant clinical parameters and laboratory findings, including parasitological investigations (stool examination for parasite ova, serological tests for toxocariasis), bone marrow examination (if performed), and other relevant findings were collected and analyzed. Information on anthelminthic treatment (AT) and its efficacy was also noted.

Results

Indications for investigation

In 44 (41.9%) children, CBC was performed upon referral from a primary healthcare institution due to

eosinophilia, while in 61 (58.1%) children, the indication for this investigation was set in the course of clinical examination in our hospital. The general types of these indications are presented in Table 1. The most prevalent indications were gastroenterological [22 (24.4%), e.g., abdominal pain], followed by hematological [18 (20.0%), e.g., leukocytosis, splenomegaly, enlarged lymph nodes], and immunological/allergological indications [9 (10.0%), e.g., asthma, urticaria].

Final diagnosis

We classified our patients (including those investigated based on relative eosinophile numbers only) into broad groups according to the final diagnosis (Table 2). Toxocariasis was serologically confirmed in six patients (6.7%), while two (2.2%) had a confirmed nematode infestation, *Ascaris* (*A.*) *lumbricoides* and *Enterobius vermicularis*, respectively. In addition, 32 (35.6%) children, or 35 (33.3%) of a combined series, including those with no true eosinophilia, were diagnosed with helminthiasis based on the success of tentative AT (*ex juvantibus*). Eosinophilia was explained by allergic/atopic conditions in 19 (21.1%) patients, while four (4.4%) had a drug reaction, three of whom satisfied some or all criteria for drug reaction with eosinophilia and systemic symptoms (DRESS). Eosinophilia was associated with bacterial infections in nine (8.9%) children: respiratory infections in four, streptococcal angina in two, and otitis media, urinary infection, and acute appendicitis in one child each. Five (5.5%) patients had a hematological problem (severe anemia in two, leukopenia in one, and isolated splenomegaly in one). Three children were found to suffer from autoimmune disorders (inflammatory bowel disease, diabetes mellitus type 1, and autoimmune uveitis, respectively). Two children with D72.1 designation (one of whom did not have eosinophilia) were diagnosed with congenital syndromes (not belonging to the group of hypereosinophilic syndromes): sodium voltage-gated channel alpha subunit 2A (SCN2A)-spectrum epileptic syndrome and familial adenomatous polyposis, respectively. One of the children excluded from the series was diagnosed with eosinophilic esophagitis. In seven (7.8%) children, eosinophilia turned out to be an isolated and transient finding, while three (3.3%) were never brought to us by their parents for a follow-up visit.

Table 1

Indications for eosinophilia investigation and severity of the observed eosinophilia

Parameter	Eosinophilia (severity)				Total eosinophilia	Total (D72.1)
	none	mild	moderate	severe		
Referred for eosinophilia	10	16	13	5	34	44
Indication set at UCH	5	27	24	5	56	61
Σ	15	43	37	10	90	105
Gastroenterologist	2	12	8	2	22	24
Hematologist	0	7	10	1	18	18
Immunologist/allergologist	2	5	3	1	9	11
Pulmonologist	0	1	1	1	3	3
Nephrologist	0	1	0	0	1	1
Infectologist	0	1	1	0	2	2
Other	1	0	1	0	1	2
Σ	5	27	24	5	56	61

UCH – University Children's Hospital. All values are given as numbers.

Table 2

Final diagnosis in patients with different degrees of eosinophilia

Parameter	Eosinophilia (severity)				Total eosinophilia	Total (D72.1)
	none	mild	moderate	severe		
Isolated eosinophilia	3	2	5	0	7	10
Helminthiasis *ex juvantibus*	3	15	12	5	32	35
Toxocariasis	0	1	2	3	6	6
Nematode infestation	0	0	1	1	2	2
Allergic/atopic disorders	5	9	10	0	19	24
Drug reaction/[1]DRESS	0	2	1	1	4	4
Bacterial infections	1	4	4	0	8	9
Hematological disorders	0	3	2	0	5	5
Autoimmune disorders	0	3	0	0	3	3
Congenital syndromes	1	1	0	0	1	2
Eosinophilic esophagitis	1	0	0	0	0	1
Lost to follow-up	1	3	0	0	3	4
Σ	15	43	37	10	90	105

[1] Three patients out of the total number of drug reactions fulfilled the criteria for drug reaction with eosinophilia and systemic symptoms (DRESS).
All values are given as numbers.

Parasite detection

Stool examination for parasite ova was performed in 34 (32.4%) patients: 10 (100.0%) patients with severe eosinophilia, 17 (45.9%) with moderate, 3 (7.0%) with mild, and 4 (26.7%) of those who turned out to have no eosinophilia at all. Findings were positive in two children overall (2.2% of those with eosinophilia or 1.19% of total children investigated under ICD code D72.1): a child with moderate eosinophilia was found to be infested with *Enterobius vermicularis*, while another child with severe eosinophilia had *A. lumbricoides* ova in the stool.

Serological testing for *Toxocara* (*T.*) *canis* was performed in 28 children (26.7% of the total or 31.1% of children who actually had eosinophilia): 8 (80.0%) with severe eosinophilia, 10 (27.0%) with moderate eosinophilia, and 10 (23.2%) with mild eosinophilia. Serological evidence of *T. canis* was found in 6 children (5.7% of the total number with ICD code D72.1 or 6.7% of those with actual eosinophilia). Three of the six children with documented *T. canis* infestation had severe eosinophilia (37.5% of those analyzed or 30.0% of the total number in this group), two had moderate eosinophilia (20.0% of those analyzed or 5.4% of group total), and one had mild eosinophilia (10.0% of those analyzed or 2.3% of group total).

Anthelminthic treatment

In total, 56 (53.3%) children received AT: 9 (90.0%) of those with severe eosinophilia, 19 (51.4%) with moderate eosinophilia, 23 (53.5%) with mild eosinophilia, and 5 (33.3%) children with no true eosinophilia. Most of the children were given mebendazole only (42, comprising 40.0% of all children in the series or 79.2% of all treated children): 1 with severe eosinophilia (10.0% of the group total or 11.1% of those treated within the group), 16 with moderate eosinophilia (43.2% of the group total or 84.2%, of those treated within the group), 20 with mild eosinophilia (46.5% of the group total or 87.0%, of those treated within the group), and 5 (33.3% of the group total or 100.0%, of those treated within the group) with no eosinophilia. The remaining 14 children (13.3% of the total or 26.4% of the treated children) were also initially treated with mebendazole but were subsequently shifted to albendazole due to the persistence of eosinophilia. Among those, 8 had severe eosinophilia (80.0% of the group or 88.9% of those treated within the group), 3 had moderate eosinophilia (8.1% of the group or 15.8% of those treated within the group), while 3 had mild eosinophilia (7.0% of the group or 13.0% of those treated within the group).

In all children treated with AT, eosinophilia and other relevant findings (if any) subsided in a matter of a few days to a few weeks after initializing treatment.

Patients with severe eosinophilia

Ten children met the criterion for severe eosinophilia (eosinophils above 5×10^9/L): seven boys and three girls (male:female ratio was 2:3) aged one year and three months to 12 years and two months (median age 9.9 years). The absolute number of blood eosinophils in these children ranged from 5.8 to 62.2×10^9/L (median 11.3×10^9/L). Five of the children had isolated eosinophilia (and were referred to us for this reason), two had gastrointestinal symptoms (abdominal pain, nausea, vomiting, diarrhea), while one had fever and cough, as well as a maculopapular rash apparently triggered by amoxicillin treatment. Another child also had a nonspecific rash, not associated with any other symptoms, while the remaining child had allergic rhinitis. Two children were found to have splenomegaly on initial clinical examination. A stool examination for parasites was performed in all 10 patients and was positive for *A. lumbricoides* in one. A serological test for *T. canis* was performed in eight children, yielding a positive result in three (30.0% of children in the group or 37.5% of those serologically tested). Bone marrow examination was performed in three patients, all three exhibiting a marked hyperplasia of eosinophilic granulocyte lineage.

In addition to the child with parasitologically confirmed *A. lumbricoides* infestation and three children with serologically confirmed toxocariasis, five children were clinically suspected (albeit not confirmed) to also harbor *T. canis*, even though one of these was also found to have IgM antibodies to *Aspergillus* as an alternative explanation for eosinophilia. Two of the five patients in the latter group (suspected helminthiasis without laboratory confirmation) were also genetically investigated for hypereosinophilic syndromes by sequencing an appropriate gene panel. However, no pathological or potentially pathological variants were found.

The child with fever and rash attributed to amoxicillin was diagnosed with an allergic (or pseudoallergic) drug reaction and treated accordingly. All other children with severe eosinophilia received AT: mebendazole alone in the one child known to be infested with *A. lumbricoides* and mebendazole followed by albendazole in the remaining eight children. In all 10 children, eosinophilia resolved upon treatment, and all other signs and symptoms abated, with the exception of persistent splenomegaly in one patient belonging to the group with confirmed toxocariasis.

Discussion

The main limitation of our study design was that it included only patients investigated under the direct diagnostic designation of eosinophilia with the corresponding ICD code. Thus, most or all patients who had been diagnosed with specific disorders featuring eosinophilia were not assigned the code for eosinophilia and were, therefore, not included in this analysis. That probably explains the absence of antineutrophil cytoplasmic antibody-associated vasculitides in our patient series, as well as the near-absence of eosinophilic gastrointestinal disorders in spite of their increasing global prevalence [10]. On the other hand, our choice of inclusion criterion places principal emphasis on the diagnostic workup performed in children with eosinophilia without a readily apparent cause.

The largest subgroup of our patients, comprising over a third of them (35.6%), were children with unexplained eosinophilia that cleared upon AT administered *ex juvantibus*. Allergic disorders (21.1%) were thus not the most prevalent etiological category, contrary to published data, including a recent large (n = 1,178) and well-documented pediatric patient series from Türkiye, where this group of disorders amounted to no less than 80% [11]. However, the prevalence of moderate and severe eosinophilia was much higher among our patients (41.1% vs. 17.8% and 11.1% vs. 1.4%, respectively). Given the approximately tenfold difference in series size, the fact that we discovered no children with a primary immune deficiency is not inconsistent with the recorded prevalence of 8.5% for this group of disorders in the aforementioned study. Allergy/atopy was also the most common cause of hypereosinophilia in a patient review from a tertiary care pediatric center in the United States, followed by graft-versus-host disease, drepanocytosis, and parasitosis [12].

Importantly, no hypereosinophilic syndromes were diagnosed among our patients. This was, admittedly, somewhat unexpected; however, hypereosinophilic syndromes have also been relatively rare in the above-cited study from Türkiye (0.3%) [11] and a Canadian review of one hundred consecutive patients (6.0%) [13], with the *caveat* that the latter included people of all ages. The same applies to a 17-year retrospective review of patient records from Leicester (United Kingdom), where myeloproliferative hypereosinophilic syndromes were found in 2.0% of all patients investigated for eosinophilia [14]. Three of our 10 patients with severe eosinophilia were investigated in this respect by bone marrow examination, yielding only apparently reactive hyperplasia of eosinophilic lineage. Two patients also underwent genetic testing for hypereosinophilic syndromes, with no pathological or suspect gene variants identified. Notably, parasitic etiology was confirmed in four of the 10 children with severe eosinophilia, while in five more the disorder effectively cleared after a course of AT. Only one child had a verified nonparasitological cause of severe eosinophilia (a drug reaction). Such reactions, however, must always be excluded by a thorough anamnesis and clinical examination because they can be severe and even life-threatening, particularly in the event of DRESS [15]. While these findings in no way preclude a comprehensive hematological workup aimed at early detection of hypereosinophilic syndromes in children with severe eosinophilia, the observed outcomes in our series do strengthen the case for making every possible effort to actively seek out potential parasites before proceeding with time-consuming, expensive and partly invasive investigations directed at hypereosinophilic syndromes. This argument may even justify the decision for empirical AT prescribed to our patients.

A rather low number of positive serological or parasitological findings for helminthiasis in our patient series is by no means an unexpected finding. Seroprevalence of *T. canis* in children in Serbia was found to be 10.0% in one study conducted by a group from the University of Niš in collaboration with the "Sapienza" University of Rome [16]. The prevalence was quite similar in a published patient series from Grenoble (6.6% for stool examination and 7.9% for serology) [17], even though this series differed from ours since it included patients of all ages with unexplained eosinophilia. On the other hand, a team from the Croatian National Institute of Public Health led by Sviben found an overall seropositivity rate for *T. canis* of as much as 31% among 142 asymptomatic children with eosinophilia aged 3–18 years [18]. It should be kept in mind, however, that this pertains to a high-risk population and does not necessarily reflect the overall seropositivity rate in Croatia or our region. The local seroprevalence of *T. canis* among children has been positively correlated with the contamination of their peridomiciles – particularly public squares and playgrounds – with parasite ova [19, 20]. It is also associated with a range of socioeconomic factors [21]. A recent study from Texas (United States of America) found that toxocariasis in general, and pediatric toxocariasis in particular, tends to be concentrated in certain epidemiological hotspots [22]. A number of such hotspots have been highlighted in worldwide publications so far, including Ahwaz in Iran [23], the southern seashore of Brazil [24], and Chungcheongnam-do in South Korea [25].

It is conceivable that some of the investigated children without confirmed parasitosis, in reality, harbored undetected parasites, even though this is obviously impossible to prove due to the frequently transient nature of eosinophilia *per se*, as well as the existence of a myriad of potential confounding factors [26]. However, it is reasonably safe to assume that the true incidence of parasite-associated eosinophilia among our patients is higher than that confirmed by parasitological tests since symptoms of such infestation may often be absent, unremarkable, or nonspecific [27]. Furthermore, *Toxocara cati*, a species not covered by currently available serological tests in Serbia, might plausibly account for some of such instances since its prevalence appears to be roughly comparable to that of *T. canis* [28]. It is also impossible to exclude the possibility that some children harbored other rare helminths or protozoa that are not routinely sought. Though all this assuredly speaks of the incomplete adequacy of our currently employed routines for detecting parasites, these results do offer a degree of justification for the practice of administering an *ex juvantibus* course of AT in children with unexplained long-standing eosinophilia. In this regard, it is notable that no adverse effects of such treatment were reported in our series.

Another important takeaway message of the present series is that no standard algorithm or set of guidelines appears to have been consistently employed in the search for the causes of eosinophilia in our institution; indeed, even the very definition of the condition was not applied rigorously, resulting in 15 children being – almost mindbogglingly – incorrectly deemed to have eosinophilia based on their relative eosinophil numbers only. Two-thirds (more precisely 10) of these children received their ICD code D72.1 in a primary healthcare institution, while the remaining (five children) were thus served in our tertiary center. Accordingly, there appears to be significant room

for improvement of existing practices, preferably in the direction of more consistent adherence to appropriate definitions and guidelines, as part of a comprehensive approach, such as that recently proposed by a large collaborative team in France [29].

Conclusion

Pediatric patients with unexplained eosinophilia of all levels of severity require a meticulous and standardized diagnostic approach, including, but not limited to appropriate parasitological investigations. True hypereosinophilic syndromes are rare but need to be carefully excluded. Due to the limited sensitivity of parasitological tests, administration of antihelminthic drugs *ex juvantibus* may be rational in a fraction of children with eosinophilia, particularly in high- or moderate-prevalence areas for *Toxoplasma* species.

Conflict of interest

The authors declare no conflict of interest.

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The impact of everyday usage of different dental implant torque wrenches on their performance accuracy and repeatability: an *in vitro* study

Uticaj svakodnevnog korišćenja različitih moment-ključeva za dentalne implantate na tačnost i ponovljivost njihovog učinka: *in vitro* studija

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Abstract

Background/Aim. The clinical success of prosthetic rehabilitation that commences after the attained implant osseointegration is dependent on the influence of several factors, of which screw loosening is a frequent one, and it is highly related to inadequate tightening (torquing) using torque wrenches. Although the wrenches are initially calibrated by the manufacturer, it is of great importance to evaluate their function after usage for a certain period of time. The aim of this study was to evaluate the accuracy and repeatability of the performance of implant torque wrenches in delivering necessary torque values before and after one year of usage. **Methods.** Two types of wrenches were used in the study: the beam-type and the toggle-type. Four various brands of beam-type wrench were marked as Beam 1 – Beam 4, and three various brands of toggle-type wrench were marked as Toggle 1 – Toggle 3, according to their design. Torque values delivered by wrenches were measured and analyzed using the One-Sample *t*-test, Independent-Samples *t*-test, and Mann-Whitney *U* test. The Bland-Altman bias test was used as an index of accuracy, whereas Forkman's comparison of datasets coefficients of variation (CV) served as an index of repeatability. **Results.** All wrenches except new Beam 2 and Beam 3 showed differences between the average measured torque value and target torque value. Differences were found in the average measured values between all used and new wrenches. Higher bias was observed in Toggle 1, Toggle 2, and Toggle 3 brands, whereas lower bias was recorded between used and new Beam 1 and Beam 3 wrenches. When comparing the CV for used and new wrenches, Beam 1, Beam 4, Toggle 1, and Toggle 2 revealed differences, whereas the CV for Beam 2, Beam 3, and Toggle 3 did not differ significantly. **Conclusion.** Compared to toggle-type, the beam-type wrenches offer greater accuracy in achieving the target torque value. The torque deteriorates in all wrenches after aging/usage and is more prominent in toggle-type devices.

Key words:
biomechanical phenomena; dental implantology; dental instruments; in vitro techniques; torque.

Apstrakt

Uvod/Cilj. Klinički uspeh protetske rehabilitacije nakon postignute oseointegracije implantata zavisi od uticaja nekoliko faktora, od kojih je razlabavljivanje šrafa čest faktor i u velikoj meri je povezano sa neadekvatnim zatezanjem upotrebom moment-ključeva. Iako je proizvođač inicijalno kalibrisao ključeve, veoma je važno proceniti njihovu funkciju nakon korišćenja tokom određenog vremenskog perioda. Cilj ove studije bio je da se proceni tačnost i ponovljivost učinka moment-ključeva za implantate u postizanju potrebnog stepena zatezanja, pre i posle njihovog korišćenja tokom godinu dana. **Metode.** Dva tipa moment- ključeva su korišćena u studiji: *beam*-tip i *toggle*-tip. Četiri različite marke *beam*-tipa ključeva koji su korišćeni u studiji su označeni kao *Beam* 1 – *Beam* 4, a tri različite marke *toggle*-tipa ključeva su označene kao *Toggle* 1 – *Toggle* 3 u skladu sa njihovim dizajnom. Vrednosti obrtnog momenta koje su postigli ključevi merene su i analizirane pomoću *One-Samples t*-testa, *Independent-Samples t*-testa i Mann-Whitney *U* testa. Bland-Altman-ov test odstupanja korišćen je kao indeks tačnosti, dok je Forkman-ov test poređenja koeficijenata varijacije (KV) skupova podataka korišćen kao indeks ponovljivosti. **Rezultati.** Svi moment-ključevi osim novih *Beam* 2 i *Beam* 3, pokazali su razlike između prosečne izmerene vrednosti i ciljne vrednosti obrtnog momenta. Utvrđene su razlike u prosečnim izmerenim vrednostima obrtnih momenata

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između svih korišćenih i novih ključeva. Veće odstupanje primećeno je kod *Toggle* 1, *Toggle* 2 i *Toggle* 3 modela, dok je manje odstupanje zabeleženo između korišćenih i novih ključeva *Beam* 1 i *Beam* 3. Kada su upoređivani KV korišćenih i novih ključeva, *Beam* 1, *Beam* 4, *Toggle* 1 i *Toggle* 2 su pokazali značajne razlike, dok se KV za *Beam* 2, *Beam* 3 i *Toggle* 3 nisu značajno razlikovali. **Zaključak.** U poređenju sa *toggle*-tipom, moment-ključevi *beam*-tipa nude veću tačnost u postizanju ciljne vrednosti obrtnog momenta. Vrednost obrtnog momenta se nakon starenja/korišćenja pogoršava kod svih ključeva i izraženiji je kod uređaja *toggle*-tipa.

Ključne reči:
biomehanika; stomatološka implantacija; stomatološka oprema; in vitro; obrtni moment.

Introduction

Prosthetic rehabilitation that commences after the attained implant osseointegration represents one of the milestones in implant therapy. Its long-term clinical success is highly dependent on the influence of distinctive mechanical and biological features. One of the most commonly reported in the literature that may have an impact on the clinical outcome is screw loosening [1–4]. Screw loosening is considered a substantial clinical problem caused by various factors. Most of them are related to inadequate tightening (torquing), incompatible screw alloy type or shape, dominant lateral occlusal loading and repeated bending, improper occlusal morphology, and misfit of implant-abutment components [5–11]. First, it is of crucial importance that the initial tightening force using the torque wrench (TW) applied to the screw is neither inadequate nor excessive but rather accurately applied as designated by the manufacturer. Hence, a proper fit of the abutment and implant without possible complications is achieved [12–14]. That consequently leads to a long-term integrity of the implant components assembly with functional loading [15, 16]. Different designs of TW are currently available on the market, classified as electrical and mechanical, whereas the latter are further divided into beam-type (BT) (spring) wrenches and toggle-type (TT) (friction) wrenches [15]. BT devices use the bending of an attached bar to the extent value readable on the scale, whereas the TT devices are designed to break away once the determined torque value (TV) is achieved [16, 17]. TWs are initially calibrated by the manufacturer and are ready to deliver an adequate torque value for specific implant components. Although the manufacturer calibration is undisputable, there are considerable differences between the target TV and achieved TV in brand-new TWs [18]. Moreover, each torque device is subjected to different clinical conditions in the oral environment and consequently requires proper maintenance [19]. Hence, various fluids such as saliva, blood, and saline solution or improper handling and dismantling are issues that are expected to have an impact on the accuracy of TWs. In support of this, a study implementing unused TWs and the used ones under normal clinical conditions showed significant fluctuations above and below the target TV [20]. According to previously published studies, BT TWs possess a more consistent range of TVs, whereas the variations are dependent on the wrench design and the obtained torque level [21, 22]. However, features like the accuracy and repeatability of mechanical oral TWs have not been fully evaluated, particularly considering the influence of aging deterioration due to metal fatigue, cleaning, and disinfection or wet conditions in the oral environment. Considering these issues, the present study aimed to evaluate the accuracy and repeatability of measurement of various TWs with different mechanical designs before and after annual clinical use. The null hypothesis was that regardless of the TWs' mechanical design (BT or TT) or condition (new or used), no significant difference would be found among them with regard to their measurement accuracy and repeatability while achieving the target TV proposed by the manufacturer.

Methods

Ethical standard

The procedures performed in the study were approved by the Ethics Committee of the Faculty of Dental Medicine, University of Belgrade, Serbia (No. 36/53), and were in line with the ethical standards of the 1964 Helsinki Declaration.

Selection of torque wrenches

The TWs selected for the study are representative of the two different torque mechanisms (BT and TT), as well as of various brands mostly presented in our country's market, designated for use with manufacturer-supplied implant component parts. Tested wrenches of seven brands, marked as Beam 1 (Straumann Group), Beam 2 (Neodent® Dental Implants System), Beam 3 (Nobel™ Biocare), Beam 4 (Bredent Group), Toggle 1 (Bredent Group), Toggle 2 (Astra Tech Implant System® – Dentsply Sirona), and Toggle 3 (Alpha-Bio Tec) according to their design, with their required target TVs adopted from the manufacturer's instructions, are presented in Table 1. TWs were divided into two groups. The first study group (new) consisted of new TWs in the "as-received" condition. The second study group (used) was comprised of TWs of the same brands that were used for one year in usual clinical practice (minimum 250 times a year) [23]. For each study group, one TW *per* brand was tested (n = 1).

Experimental procedure

The experimental procedure for this investigation was performed using a test assembly comprising a torque-measuring device (iSD900, NSK-Nakanishi International) operating at 230 V. Holding the torque-measuring device in one hand, the operator was able to apply the target TV using the tested TW in another one [24] (Figure 1).

Table 1

Brands of torque wrenches included in the study

Torque wrench	Manufacturer	Target torque value (Ncm)
Beam 1	Straumann Group	35
Beam 2	Neodent® Dental Implants System	20
Beam 3	Nobel™ Biocare	35
Beam 4	Bredent Group	25
Toggle 1	Bredent Group	25
Toggle 2	Astra Tech Implant System®, Dentsply Sirona	25
Toggle 3	Alpha-Bio Tec	30

Ncm – Newton x cm.

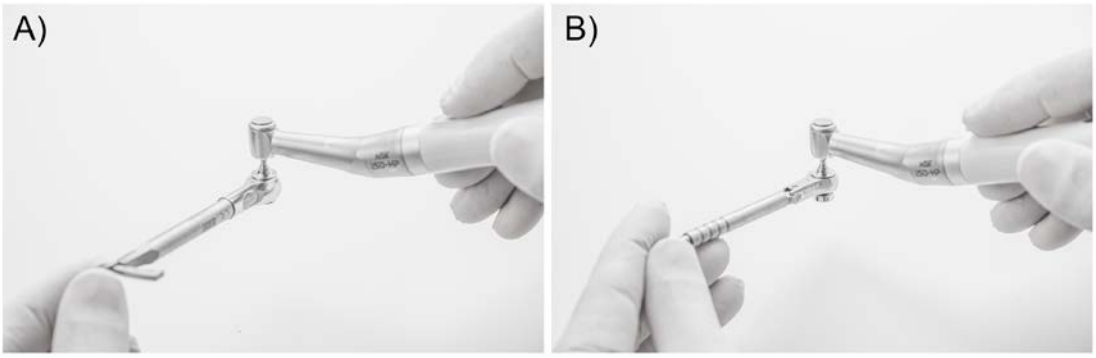

Fig. 1 – Measuring the applied target torque value by holding the torque-measuring device in one hand and the tested beam (A) or toggle (B) torque wrench in another hand.

The measurements were repeated 15 times for each TW and were performed by one investigator to avoid discrepancies resulting from the inclusion of multiple operators. The average measured TV of all the measurements was calculated and recorded accordingly.

The bias that represents the difference between the average measured TV and target TV divided by the target TV was used as the index of measurement accuracy. The coefficient of variation (CV) that represents the standard deviation (SD) of the measured TV divided by the average measured TV was used as an index of measurement repeatability.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 26.0, SPSS) and Prism 9 for macOS version 9.5.1, GraphPad Software, LLC. The difference between the target TV and the average measured TVs of the used and new TWs was analyzed using the One-Sample *t*-test. A pairwise comparison of the average measured TVs of the used and new TWs was performed using the Independent-Samples *t*-test and Mann-Whitney *U* test, according to the results obtained by the One-Sample Kolmogorov-Smirnov test for normal distribution. Mean ± SD and median (minimum-maximum) were used to describe the numeric data. The bias was determined using the Bland-Altman plot method [25]. This method was used to quantify the agreement between two quantitative measurements within the limits of the agreement by calculating the mean and SD of the differences between the two measurements. Therefore, the mean difference between the used and new TW (within its 95% limits of agreement) vs. the average of the two datasets is used to depict and quantify bias. The Forkman test [26] was implemented to compare the CVs. Differences were considered significant when the *p*-value was < 0.05.

Results

The results from the present study revealed that the majority of the tested TWs showed a significant difference ($p < 0.05$) between the obtained average measured TV and the target TV. The only TWs that showed an absence of statistical significance with regard to the same parameters were Beam 2 ($p = 0.257$) and Beam 3 ($p = 0.065$) wrenches, both in the new "as-received" condition (Figure 2).

Furthermore, the obtained data revealed a significant difference ($p < 0.05$) in the average measured TV when comparing the used and new TWs of all brands included in the research (Figure 3). The detailed descriptive statistics are given in Table 2.

Regarding the measurement accuracy, according to the presented data, a certain degree of bias was noticed in all tested implant brands (Figure 4). Descriptive statistics (Table 2) revealed the highest bias in Toggle 2, followed by Toggle 1 and Toggle 3. On the other hand, Beam 1 and Beam 3 exhibited the lowest bias when comparing the used and new TWs. Considering the repeatability of the measured, CVs are listed in Table 2. When comparing the CVs of the datasets for used and new TWs, Beam 1, Beam 4, Toggle 1, and Toggle 2 revealed significant differences ($p < 0.05$), whereas the CVs of Beam 2, Beam 3, and Toggle3 did not differ significantly ($p > 0.05$) (Figure 5).

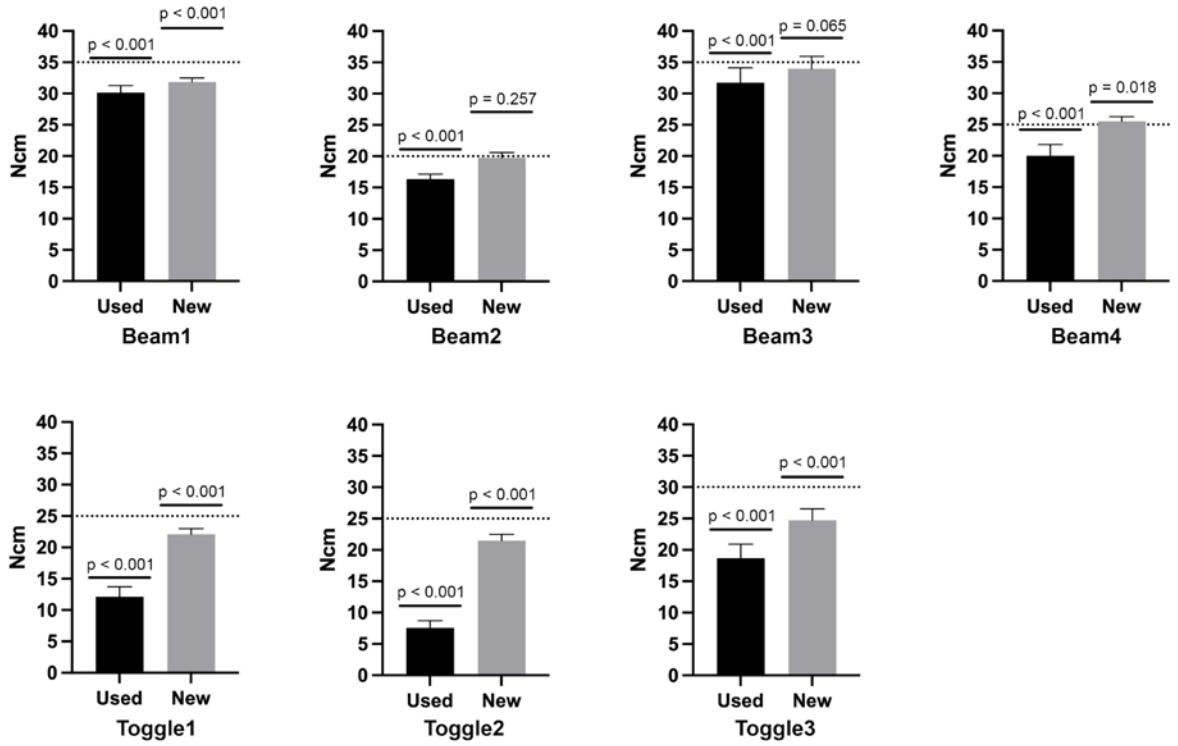

Fig. 2 – The difference between the target torque value (dotted line) and the average measured torque values of the used and new torque wrenches (One-Sample *t*-test).
Ncm – Newton x cm.

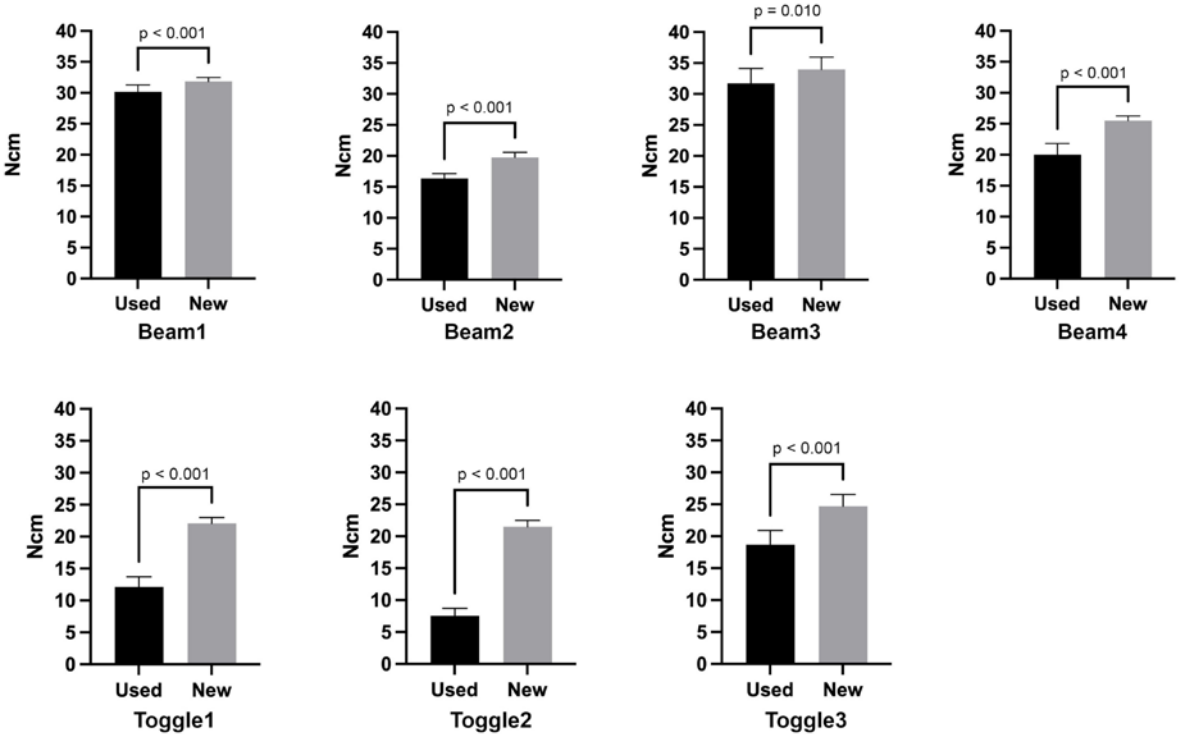

Fig. 3 – Pairwise comparisons of the average measured torque values of the used and new torque wrenches (Independent-Samples *t*-test and Mann-Whitney *U* test).
Ncm – Newton x cm.

Table 2

Descriptive statistics of datasets for torque values of various used and new torque wrenches

Torque wrench	Mean ± SD (Ncm)	Median (min–max) (Ncm)	CV (%)	Bias (Mean difference ± SD)
Beam 1				
used	30.14 ± 1.15	30.02 (28.26–32.67)	3.82	1.71 ± 0.71
new	31.85 ± 0.62	31.79 (30.91–32.67)	1.94	
Beam 2				
used	16.35 ± 0.80	16.68 (14.72–17.66)	4.89	3.40 ± 0.73
new	19.75 ± 0.82	19.62 (17.66–20.60)	4.14	
Beam 3				
used	31.73 ± 2.39	30.91 (28.26–35.32)	7.54	2.24 ± 1.24
new	33.97 ± 1.10	35.32 (30.91–36.20)	5.88	
Beam 4				
used	20.01 ± 1.81	19.62 (17.66–23.54)	9.03	5.50 ± 1.22
new	25.51 ± 0.74	25.51 (24.53–27.47)	2.90	
Toggle 1				
used	12.12 ± 1.59	12.6 (9.90–14.40)	13.12	9.96 ± 0.87
new	22.08 ± 0.89	21.6 (20.70–23.40)	4.04	
Toggle 2				
used	7.53 ± 1.20	7.95 (5.30–8.83)	15.90	13.95 ± 0.49
new	21.49 ± 0.98	21.19 (19.43–22.96)	4.58	
Toggle 3				
used	18.64 ± 2.23	17.66 (15.89–22.96)	11.96	6.08 ± 1.94
new	24.72 ± 1.83	24.72 (21.19–28.26)	7.40	

SD – standard deviation; CV – coefficient of variation; Ncm – Newton x cm; min – minimum; max – maximum.

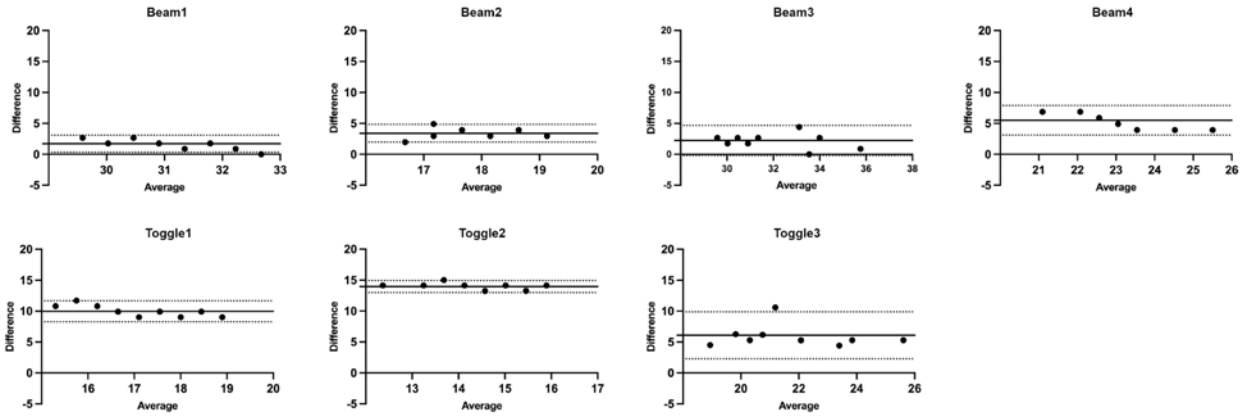

Fig. 4 – The bias (index of measurement accuracy) represents the difference between the average measured torque value and the target torque value, divided by the target torque value. The solid line represents the mean difference, and the dotted lines represent the 95% of limits of agreement (Bland-Altman plot method [25]).

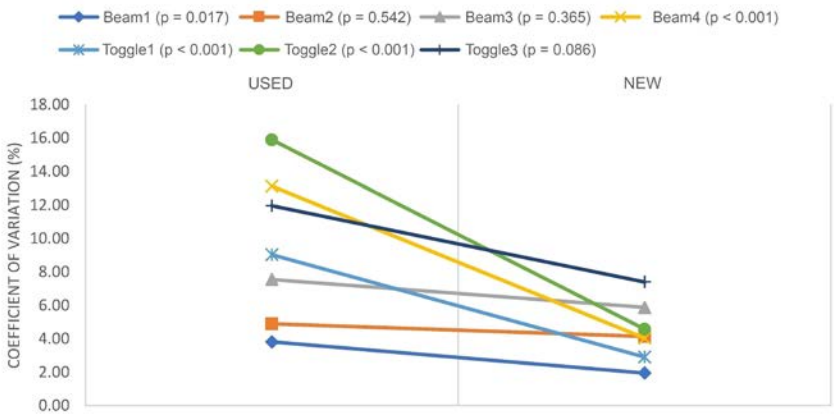

Fig. 5 – The coefficient of variation (CV) represents the standard deviation of the measured torque value, divided by the average measured torque value (Forkman test [26]). The CV was used as an index of measurement repeatability.

Discussion

It is of paramount importance to position and fix the implant-abutment complex in correct relation to the delivered manufacturer's recommended TV, obtaining in such a way specific stability and long-term functionality [27–30]. A prerequisite for achieving stability and screw preload is the clinician's knowledge of the amount of torque required for the specific implant brand employed. Each manufacturer provides its own recommendation of TV depending on various factors, including the type of implant, implant-abutment connection, abutment design, screw design, and screw material. Improper and inadequate screw torquing can result in various mechanical failures, including screw loosening or fracture, with consequent restoration loss [31]. On the other hand, overtorquing may initiate screw joint high preload with abutment screw complications such as screw fracture or flattening of the screw threads [32, 33]. Since hand-held screwdrivers do not deliver sufficient torque force and are not able to provide adequate abutment tightening [34], mechanical torque-limited devices are considered standard tools for precise and accurate torquing in everyday clinical practice. This study aimed to evaluate the measurement accuracy and repeatability of different used and new mechanical torque devices (wrenches) from implant brands that are most common in our country's market today.

The results from the present study are in agreement with the outcomes of previous research that revealed that even in the new, "as-delivered" condition, there is variation among TWs in their ability to deliver specific values of torque [35]. Discrepancies between the target and obtained TVs were found for the majority of the tested wrenches. These findings indicate that clinicians should be aware that each new wrench unwrapped from the factory package carries some torque errors. A possible explanation for this may be that new, "as-delivered" mechanical components of the wrenches are still stiffened and require some manipulation prior to everyday use. However, new, unused devices with BT (spring) mechanical design, such as Beam 2 and Beam 3 wrenches, delivered TVs without significant difference compared to the target value, whereas only a new Beam 4 wrench managed to achieve the exact target value. Furthermore, the highest bias, index of accuracy, was observed for all three representative wrenches of the TT.

In addition, after comparing wrenches with different mechanical designs (BT and TT) from the same manufacturer (Beam 4 and Toggle 1), a higher TV was obtained for the BT device. One may speculate that the ability of the oral implant TWs to deliver the target TV is most likely influenced by the design of the wrench components. Thus, the results of this study confirm that the BT (spring) wrenches offer greater accuracy regarding the target values compared to the TT (friction) wrenches, which is in agreement with previous findings [21]. The results suggest that the prerequisite for TW accuracy is BT mechanical construction.

In comparing the average measured TVs between the used and new TWs, a significant difference was observed within all tested wrenches. The results of the present research support that the observed TVs tended to be lower than the target values for both mechanical designs [35], which is the opposite of the studies where greater TVs in both used and new wrenches compared to the target values were found [17, 22]. Furthermore, the obtained TVs of the TT wrenches were less consistent compared to those of the BT, which were associated with a lower risk of disagreement between repeated measurement values, which is in favor of a previously reported statement [36]. However, some studies did not find that the design of the wrenches and their limiting mechanism had any impact on the repeatability and confidence interval [16]. According to our results, the CVs of the datasets for the used and new wrenches of Beam 2, Beam 3, and Toggle 3 did not differ significantly. On the other hand, a large discrepancy in the results of the average measure TVs between the used and new devices was observed for Toggle 1 and Toggle 2 wrenches, supporting the speculation that TT mechanical design is more susceptible to inaccurate values due to aging and reuse. Considering all the aforementioned, the results of the present study revealed some degree of error between the used and newly tested devices. However, Beam 1 and Beam 3 wrenches were the most consistent throughout all measurements. In other words, the reported data imply that TVs for used and new Beam 1 and Beam 3 wrenches can be expected to differ by no more than 3 Ncm or no more than 10%, which could be regarded as insignificant from a clinical point of view and still lead to accepted clinical target values [24].

Taking into consideration all of the results from the present study, the null hypothesis – regardless of the TWs' mechanical design (BT or TT) or condition (new or used), no significant difference would be found among them with regard to their accuracy and repeatability while achieving the target TV proposed by the manufacturer – was rejected.

The major limitation of the current study might be the fact that only one TW device from each manufacturer was used for the analyses. Therefore, any differences between individual wrenches were omitted. In order to strengthen the study, although a similar conceptualization was reported previously [15, 37], we incorporated a relatively large number of repeated measurements for each tested TW, thus indicating greater assurance of whether the device delivered its target TV. Despite the aforementioned limitations, the generalized applicability of the present study may be that the accuracy of wrenches deteriorates during use, which implies the importance of calibration of the wrenches from time to time, as recommended by the International Organization for Standardization (ISO) 6789-2 [38]. Moreover, clinicians must be aware that handling during clinical use and maintenance must be performed mindfully because TWs are prone to misfit owing to their regular use. The latter is of much more importance for TT devices since it has been shown that their accuracy loss is more expected after prolonged clinical use. Therefore, the results from the present study may be

beneficial for selecting implant and implant components assembly systems and achieving clinical functionality.

Conclusion

Based on the findings of the present study, two conclusions could be drawn. First, compared with the toggle-type (friction), the beam-type (spring) of torque wrenches offers greater accuracy while delivering the target torque value proposed by the manufacturer. Second, the ability to torque after aging and prolonged clinical use deteriorates in all tested torque wrenches and is more prominent in those with toggle-type mechanical design than beam-type devices.

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Conflict of interest

The authors declare no conflict of interest.

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Mental health and quality of life of female breast cancer survivors in Southeast Serbia

Mentalno zdravlje i kvalitet života žena koje su preživele karcinom dojke u jugoistočnoj Srbiji

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Abstract

Background/Aim. Quality of life (QoL) is one of the outcome measures for patients with chronic diseases. Mental health issues often impact the QoL of cancer patients. Cancer patients with a good QoL have a longer life expectancy and are able to lead a more productive and social life. The aim of the study was to determine the association of mental health with the QoL, as well as to determine significant predictors of QoL in patients with breast cancer (BC). **Methods.** The study included 118 patients treated at the Specialized Breast Cancer Department of the Oncology Clinic of the University Clinical Center Niš, Serbia. The study's inclusion criteria were the presence of early-stage nonmetastatic BC and completed surgical treatment. The following questionnaires were used: the general sociodemographic questionnaire, Hospital Anxiety and Depression Scale (HADS), Buss-Perry Aggression Questionnaire, Early Maladaptive Schema questionnaire - short form (SQ-SF), Flanagan's Quality of Life Scale (QoLS), Holmes and Rahe Stress Scale, Berlin Social Support Scales (BSSS), Health Locus of Control. **Results.** A statistically significant negative correlation was found with emotional state, i.e., anxiety score, depressiveness score, total stress score, and physical aggression score, while QoL correlated positively with all subscales of the perceived social support (PSS) questionnaire. QoL was significantly different for patients with the following early maladaptive schemas: emotional deprivation, emotional inhibition, and a sense of entitlement/narcissism. Moreover, QoL differed significantly depending on partnership status, the presence of clinically significant anxiety, the presence of hormone-sensitive cancer, the presence of human epidermal growth factor receptor 2 (HER2)-positive cancer, and time since diagnosis. Multiple linear regression was performed, and depression and PSS had the highest share. **Conclusion.** In our study, the presence of depressiveness and PSS were the best predictors of QoL of BC survivors.

Key words:
breast neoplasms; emotions; mental health; prognosis; quality of life; social factors; surveys and questionnaires; women.

Apstrakt

Uvod/Cilj. Kvalitet života (KŽ) je jedna od mera ishoda za bolesnike sa hroničnim bolestima. Problemi mentalnog zdravlja često utiču na KŽ bolesnika obolelih od karcinoma. Bolesnici oboleli od karcinoma koji imaju dobar KŽ imaju duži životni vek i sposobni su da vode produktivniji i društveniji život. Cilj rada bio je da se utvrde povezanost mentalnog zdravlja i KŽ i prediktori KŽ kod obolelih od karcinoma dojke (KD). **Metode.** U istraživanje je uključeno 118 bolesnica koje su lečene na specijalizovanom Odeljenju za karcinom dojke Klinike za onkologiju Univerzitetskog kliničkog centra Niš, Srbija. Kriterijumi za uključivanje u studiju bili su prisustvo nemetastatskog KD u ranoj fazi i završeno hirurško lečenje. Korišćeni su sledeći upitnici: opšti socijalno demografski upitnik, Bolnička skala za procenu anksioznosti i depresije (*Hospital Anxiety and Depression Scale* - HADS), Bas-Perijev Upitnik agresije (*Buss-Perry Aggression Questionnaire*), Upitnik o ranim maladaptivnim šemama – skraćena verzija *(Early Maladaptive Schema Questionnaire-Short Form* – SQ-SF), Flanaganova Skala za procenu kvaliteta života (*Flanagan's Quality of Life Scale* – QoLS), Holms-Raheova skala stresa (*Holmes and Rahe Stress Scale*), Berlinska skala so-cijalne podrške (*Berlin Social Support Scales* – BSSS), Zdravstveni lokus kontrole (*Health Locus of Control).* **Rezultati.** Utvrđena je statistički značajna negativna korelacija sa emocionalnim stanjem, odnosno skorom anksioznosti, skorom depresivnosti, ukupnim skorom stresa

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i skorom fizičke agresije, dok je KŽ pozitivno korelisao sa svim subskalama upitnika percipirane socijalne podrške (PSP). Značajno drugačiji KŽ imale su bolesnice sa sledećim ranim maladaptabilnim shemama: emocionalna deprivacija, emocionalna inhibicija i osećaj za pravo/narcizam. Takođe, KŽ se značajno razlikovao u zavisnosti od partnerskog statusa, prisustva klinički značajne anksioznosti, prisustva hormon-senzitivnih karcinoma, prisustva *human epidermal growth factor receptor 2* (HER2)-pozitivnog karcinoma i vremena od postavljanja dijagnoze. Urađena je višestruka linearna regresija, a najveći udeo imale su depresivnost i PSP. **Zaključak.** U našoj studiji, prisustvo depresivnosti i PSP bili su najbolji prediktori KŽ žena koje su preživele KD.

Ključne reči:
dojka, neoplazme; emocije; mentalno zdravlje; prognoza; kvalitet života; socijalni faktori; ankete i upitnici; žene.

Introduction

Breast cancer (BC) is one of the most common types of cancer and is associated with a high mortality rate. According to the results of the International Agency for Research on Cancer of the World Health Organization for the year 2020, BC is the second most common cancer in Serbia, while it ranks first in the female population. The mortality rate for BC is also high and ranks second compared to all other cancers [1].

It is well known that BC affects women's mental health (MH) [2]. Certain types of BC treatment can affect a woman's MH as much as the cancer itself. For instance, surgical treatments, chemotherapy, and radiotherapy can lead to important deterioration of the quality of life (QoL) and adverse psychological effects, such as depression, anxiety, and body dysmorphic disorder [3].

Long-term MH problems that can occur in patients with BC include sadness, helplessness, anxiety, discomfort, grief, fatigue, difficulty concentrating, sleep disturbances, cognitive problems, sexual dysfunction, psychological problems, body image problems, and psychiatric disorders [3].

The prevalence of psychiatric disorders in cancer patients ranges from 29% to 47%. The most commonly diagnosed psychiatric disorders are adjustment disorders, depressive disorders, neurotic disorders, and severe stress disorders. They can affect disease prognosis, treatment adherence and success, QoL, social functioning, and survival rate [3]. Many women treated for recently diagnosed BC report clinically relevant symptoms of depression and/or anxiety and impairment in all areas of QoL [4]. In one study, results showed that only 11.5% of those women had an optimal QoL [5].

QoL measures are crucial for patients with BC. They help us assess the impact of health impairments and oncologic interventions on patients' lives [6]. A good QoL also ensures a longer life expectancy for cancer patients and enables them to lead a more social and productive life [7].

Considering the abovementioned facts, our primary research objective was to determine the contribution of MH to the QoL of patients with early BC. Bearing in mind that other factors, such as socioeconomic factors and oncologic parameters, can also influence the QoL, our secondary aim was to compare all the factors mentioned and determine those that had a significant impact on the QoL of these patients. In addition, we wanted to determine the profile of breast tumor patients considering the mentioned parameters, especially the MH profile. If we find that certain factors are more important for the QoL than others and are among the factors we can change, the results of our research could help us plan preventive measures to maintain and/or improve the QoL of BC patients.

Methods

This observational, cross-sectional study of adult women with early, nonmetastatic BC was performed from October 2019 to October 2020. We obtained research approval from the Ethics Committee of the Clinical Center Niš, Serbia (No. 23679/50, from July 2, 2019).

Participants

The patients were recruited from a specialized BC Department at the Oncology Clinic, University Clinical Center Niš. The presence of early, nonmetastatic ductal or lobular BC and completion of surgical treatment were the inclusion criteria for the study. At the time, all patients were on ongoing adjuvant therapy (chemotherapy, hormonotherapy, targeted molecular or radiaton therapy). Patients with comorbid conditions, including uncontrolled cardiovascular, metabolic, pulmonary, or renal disease, which may seriously affect QoL, were excluded from the study.

The patients were recruited during the control examination. The purpose, reason, and plan of the research were explained to the patient, and they gave their consent for participation by signing an informed consent form. The consent was obtained by an oncologist who is part of the team of authors of this article.

Procedures

BC diagnoses have been made and based on the biopsy of the abnormality seen during clinical examination, ultrasound, and magnetic resonance imaging or mammography, depending on the age of the patient. Staging based on other systems was done to exclude the presence of metastases. After the biopsy, the pathohistological (PH) assessment showed the presence of either ductal or lobular BC, which was one of the inclusion criteria. The type of therapy was decided based on

prognostic parameters – the size of the tumor, lymph node status, and receptor status, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Patients with positive ER and/or PR were in the group of hormone-sensitive cancers, while the ones who were ER and PR negative were in the group of non-hormone-sensitive cancers.

After the control examination, the patients filled out questionnaires at the Oncology Clinic (20–25 min). The patients' completed questionnaires were returned in closed envelopes to ensure anonymity and put in a closed box with other envelopes. After opening the envelopes, nine of the 127 questionnaires were incomplete, so the data obtained from 118 respondents entered the final data processing.

Information on whether they were currently receiving chemotherapy, hormonotherapy, targeted therapy, or radiation therapy, information about HER2 or hormonal receptor positivity, PH type, and stage of BC were given by the clinicians.

We used several evaluation instruments. The first one was the general sociodemographic questionnaire, a standardized questionnaire for obtaining information on patient's place of residence, age, partnership/marital status, level of education, occupational status, and satisfaction with their relationships. The second was the Hospital anxiety and depression scale (HADS) used to assess anxiety and depressiveness in patients with somatic diseases. It is designed to avoid questions about somatic correlates of emotions, as these could be part of a somatic illness or part of the clinical presentation of depression and anxiety. For this reason, the results of this questionnaire are more appropriate and reliable. The scale consists of fourteen questions, of which seven refer to anxiety and seven to depression. A score ≤ 7 indicates the absence of pathological depression or anxiety, a score of 8–10 suggests a possible presence of anxiety and depression, and a score ≥ 11 indicates the presence of clinically significant depression and anxiety [8]. HADS has been used extensively in the field of cancer, but some authors suggested lower cut-off scores for this population. The best balance between sensitivity and specificity for cancer patients is ≥ 5 [HADS - depression subscale (HADS-D)], ≥ 7 [HADS - anxiety subscale (HADS-A)], and ≥ 13 [HADS for the total score (HADS-T)] [9]. Therefore, we used these cut-off scores. The third evaluation instrument we used was the Buss-Perry Aggression Questionnaire, developed by Arnold Buss and Mark Perry [10]. It is a self-report instrument that has become the gold standard for measuring aggression [11]. It consists of four subscales: physical aggression, verbal aggression, anger, and hostility. A total aggression score was also calculated [10]. The purpose of the questionnaire is to provide a comprehensive assessment of aggression, with an average score calculated to compare the subscales. The fourth instrument we used, the Early Maladaptive Schema Questionnaire-short form (SQ-SF), is comprised of 75 questions and was developed to measure 15 early maladaptive cognitive schemas. The score in each schema is calculated by adding the scores of five questions of the same schema. A high score indicates a strong presence of dysfunctional schemas; the minimum score for each schema is 5, and the maximum is 25. Young and Brown [12] consider a schema with two or more items scoring 5 or 6 clinically significant. Early maladaptive schemas (EMS) are the patterns most commonly formed in childhood and develop throughout a person's life. These patterns, which relate to the individual and their relationships with others, determine how they perceive and interpret their behavior and the world around them. They can be the result of traumatic experiences and unmet basic needs in the early stages of development. They are self-reinforcing and resistant to change [13, 14]. In the fifth instrument we used, QoL was estimated using the 15-item Flanagan's QoL Scale (QoLS) [15, 16], modified by adding a 16th item for independence [17, 18]. All items were scored on a 7-point scale [19]. A total score with a possible range of 16 to 112 was used for this study. The sixth evaluation instrument was the Health Locus of Control. It is a questionnaire that measures where patients locate the center of control over the illness – internally (the patient considers himself/herself predominantly responsible for his/her health and the course of the illness) or externally (the patient believes that external factors such as chance, fate, doctors, and other people are responsible for the course of his/her illness and the outcome of treatment) [20]. A higher total score means a higher external locus of control. Holmes and Rahe Stress Scale is the seventh questionnaire we used in order to assess stress levels over the past year. It consists of 43 stress situations or items; each item brings a certain number of points. A score of 150 indicates low stress, which is less likely to affect physical health. A score ≥ 300 indicates very high stress, which represents a high risk for the later occurrence of somatic disorders [21]. The final instrument we used was the Berlin Social Support Scales (BSSS). It is an instrument for measuring multidimensional social support [22]. The six subscales of the BSSS (perceived support, provided support, received support, need for support, support seeking, and protective buffering) measure both cognitive and behavioral aspects of social support. They can be used in different clinical and healthy adult populations. Our study used four subscales based on patients' subjective estimation of support: Perceived Social Support (PSS), Need for Support, Seeking Support, and Received Social Support. The remaining two scales have to be filled by some family members or some other people who give the patient social support. Subjective estimation is more important for our psychological well-being, even if the real support is the opposite.

Statistical analysis

Categorical variables were shown using percentages and frequencies, and continuous variables were shown using arithmetic means and their standard deviations. Student's *t*-tests and ANOVA were used to examine possible differences in an overall QoL score within the variables studied. A correlation analysis of QoL with the continuous variables was performed, and a multiple regression analysis was used to examine the predictors of QoL.

Results

Group structure

The group consisted of 118 patients with ductal or lobular BC. The average age of participants was 53.55 ± 11.435 years. The average score of satisfaction with partnership was 8.04 ± 2.2. The average total aggression score was 56.07 ± 14.465. The average score was calculated for subscales as well. The participants had the highest average score on the subscale of verbal aggression (2.36 ± 0.729) and the lowest average score on the subscale of physical aggression (1.45 ± 0.361). The average stress score in the previous year was 62.59 ± 66.218. The average value of the locus of control was 41.12 ± 10.017. Patients with BC had the lowest scores on "need for support" (11.14 ± 2.413) and "support seeking" (14.66 ± 2.421). The highest score was on "received social support" (50.67 ± 11.491) (Table 1).

The highest percentage of patients lived in the city area (93.2%), were employed (56.8%), were in a relationship (84.7%), had two children (42.40%), and had a bachelor degree (42.40%). The highest percentage of patients with clinically significant schemas had the "unrelenting standards" schema (45.80%), followed by the "self-sacrifice" schema (39.00%) (Table 2).

The average intensity of anxiety was 6.37 ± 3.404, and clinically significant anxiety was present in 40.70% of participants. The average intensity of depression was 4.34 ± 2.996, and clinically significant depression was present in 42.40% of participants (Tables 1 and 2).

Oncological heredity was present in only 12.70% of participants. Approximately two-thirds of patients had hormone-sensitive cancers (62.70%), negative HER2 (67.80%), and time since diagnosis was shorter than six months (63.55%). The highest percentage of patients had ductal BC (83.05%) (Table 2).

Table 1

Characteristics of patients with ductal or lobular breast cancer

Parameter	Values	
	Mean ± SD	Min–Max
Age (years)	53.55 ± 11.435	28–80
Anxiety	6.37 ± 3.404	0–16
Depression	4.34 ± 2.996	0–13
Total distress	10.71 ± 5.651	1–25
Stress	62.59 ± 66.218	0–273
Aggression – total	56.07 ± 14.465	28–91
Anger – mean score	2.06 ± 0.915	0.14–4.57
Physical aggression – mean score	1.45 ± 0.361	0.22–2.22
Verbal aggression – mean score	2.36 ± 0.729	1.2–4.80
Hostility – mean score	2.1 ± 0.726	0.63–3.75
Locus of control	41.12 ± 10.017	13–61
QoL – total score	80.54 ± 12.7	54–107
Perceived social support	27.72 ± 3.518	15–32
Need for support	11.14 ± 2.413	6–15
Support seeking	14.66 ± 2.421	9–20
Received social support	50.67 ± 11.491	16–60
Satisfaction with partnership	8.04 ± 2.2	0–10

QoL – quality of life; SD – standard deviation; Min – minimum; Max – maximum.

Table 2

Sociodemographic and clinical characteristics of patients ductal or lobular breast cancer

Parameter	Values, n (%)
Place of residence – city area	110 (93.20)
Relationship status – in a relationship	100 (84.70)
Number of children	
without	14 (11.90)
one	40 (33.90)
two	50 (42.40)
three or more	14 (11.90)
Level of education	
primary	10 (8.50)
secondary	40 (33.90)
bachelor	50 (42.40)
master/doctoral	18 (15.20)
Employment status	
employed	67 (56.80)
unemployed	32 (28.00)
retired	18 (15.20)

Table 2 (continued)

Parameter	Values, n (%)
Clinically significant anxiety	48 (40.70)
Clinically significant depression	50 (42.40)
Clinically significant EMS	
emotional deprivation	10 (8.50)
abandonment	20 (16.90)
distrust	18 (15.30)
social isolation	2 (1.70)
defectiveness	2 (1.70)
failure in achievement	2 (1.70)
dependence	0 (0)
vulnerability	6 (5.20)
symbiosis	12 (10.20)
subjugation	2 (1.70)
self-sacrifice	46 (39.00)
emotional inhibition	8 (6.80)
unrelenting standards	54 (45.80)
entitlement/narcissism	10 (8.50)
over control	8 (6.80)
Positive oncological heredity	15 (12.70)
[1]Hormone-sensitive cancer	74 (62.70)
Time since diagnosis < 6 months	75 (63.55)
Positive HER2	38 (32.20)
Pathohistological type	
ductal	98 (83.05)
lobular	20 (16.95)

EMS – early maladaptive schema; HER2 – human epidermal growth factor receptor 2.

Note: [1]estrogen receptor positive.

Differences in the QoL of BC patients – influence of psychological, oncological, and sociodemographic factors

QoL was significantly different in persons who had clinically significant following schemas: emotional deprivation ($p = 0.002$), emotional inhibition ($p = 0.007$), and a sense of entitlement/narcissism ($p = 0.001$). In addition, QoL significantly differed depending on the partnership status ($p < 0.001$), the presence of clinically significant level of anxiety ($p = 0.028$), whether the cancer was hormone-sensitive or not ($p = 0.004$), whether the cancer was HER2 positive or not ($p = 0.034$), and the time since the diagnosis ($p = 0.017$) (Table 3).

Table 3

Differences in the quality of life (QoL) of breast cancer patients depending on the sociodemographic, psychological, and oncological characteristics

Parameter	Mean	SD	SE	*t*-test/F-test	*p*-value
Place of residence					
city area	80.64	12.341	1.199	0.369	0.713
rural area	78.67	19.439	7.936		
Partnership/marital status					
single	82.38	11.572	1.181	4.000	< 0.001
in a relationship	69.50	13.924	3.481		
Clinically significant anxiety					
yes	77.55	13.108	1.892	-2.230	0.028
no	82.81	11.988	1.498		
Clinically significant depression					
yes	79.46	13.549	1.956	-0.776	0.439
no	81.34	12.070	1.509		
EMS – emotional deprivation					
yes	86.80	4.917	1.555	3.400	0.002
no	79.92	13.071	1.294		
EMS – abandonment					
yes	82.60	15.935	3.563	0.801	0.425
no	80.09	11.941	1.245		

Table 3 (continued)

Parameter	Mean	SD	SE	*t*-test/F-test	*p*-value
EMS – distrust					
yes	80.89	17.872	4.212	0.089	0.930
no	80.50	11.705	1.220		
EMS – vulnerability					
yes	75.00	8.198	3.347	-1.095	0.276
no	80.88	12.985	1.273		
EMS – symbiosis					
yes	87.33	15.406	4.447	1.964	0.052
no	79.73	12.298	1.242		
EMS – self-sacrifice					
yes	82.38	14.712	2.270	1.171	0.244
no	79.44	11.461	1.390		
EMS – emotional inhibition					
yes	69.00	10.876	3.845	-0.743	0.007
no	81.42	12.436	1.219		
EMS – unrelenting standards					
yes	81.12	13.626	1.890	0.423	0.671
no	80.07	12.139	1.594		
EMS – entitlement/narcissism					
yes	93.20	14.673	4.640	3.428	0.001
no	79.30	11.979	1.198		
EMS – over control					
yes	79.00	7.521	2.659	-0.357	0.722
no	80.69	13.156	1.303		
Oncological heredity					
yes	81.23	13.111	1.977	0.462	0.645
no	80.09	12.505	1.516		
Hormone-sensitive cancer					
yes	78.75	12.480	1.471	-2.955	0.004
no	86.92	10.407	2.139		
Time since diagnosis, months					
< 6	78.93	11.638	1.503	-2,437	0017
≥ 6	85.73	14.032	2.502		
Pathohistological type					
ductal	79.53	11.597	1.330	0.525	0.601
lobular	77.67	9.903	2.859		
HER2					
positive	82.59	11.899	2.041	2.158	0.034
negative	76.53	11.902	1.931		
Number of children					
without	77.43	6.630	1.772	1.185	0.319
one	82.60	12.792	2.023		
two	78.83	13.405	1.977		
three or more	83.83	14.440	4.168		
Level of education					
primary	73.00	13.357	5.453	1.161	0.328
secondary	79.25	11.718	1.853		
bachelor	81.75	14.839	2.142		
master/doctoral	82.67	6.633	1.563		
Occupational status					
employed	79.93	12.271	1.584	1.953	0.147
unemployed	78.28	10.839	2.013		
retiree	84.96	15.242	3.178		

EMS – early maladaptive schema; HER2 – human epidermal growth factor receptor 2; SD – standard deviation; SE – standard error.

Correlation analysis of QoL and the tested variables

A statistically significant negative correlation was found with the emotional state indicators, i.e., anxiety score ($p = 0.022$), depression score ($p < 0.001$), total stress ($p = 0.011$), and physical aggression score ($p = 0.024$), while the positive correlation of QoL was found with all subscales of PSS (Table 4).

Multiple linear regression

Multiple linear regression was done, and after the exclusion of co-linear variables in the final analysis, the following was included: depression score, total stress score, the average score of physical aggression, a score of perceived support, and satisfaction with the partnership. The model was

Table 4

Correlation analysis of quality of life and the tested variables

Parameter	Pearson's correlation/ Spearman's rho	*p*-value
Age	-0.068	0.479
Anxiety	-0.216	0.022
Depression	-0.358	< 0.001
Stress	-0.238	0.011
Aggression – total	-0.059	0.541
Anger – mean score	0.002	0.987
Physical aggression – mean score	-0.214	0.024
Verbal aggression – mean score	0.076	0.432
Hostility – mean score	-0.070	0.470
Locus of control	-0.108	0.257
Perceived social support	0.505	< 0.001
Need for support	0.358	< 0.001
Support seeking	0.263	0.006
Received social support	0.258	0.007
Satisfaction with partnership	0.450	< 0.001

Table 5

Multiple linear regression

Parameter	Standardized coefficients beta	*p*-value	F	*p* - value	Adjusted R^2
Depression - score	-0.356	< 0.001			
Total stress - score	-0.193	0.022			
Average score of physical aggression	-0.245	0.004	16.425	<0.001	0.459
Score of perceived support	0.305	< 0.001			
Satisfaction with partnership	0.216	0.010			

statistically significant $F(5,86) = 16.425$, $p < 0.001$, and based on adjusted R square, explains 45.9% of the variance of QoL in women with BC. Depression and PSS had the highest share (Table 5).

Discussion

Considering the results of our study, the average person from our sample is female, between 53 and 54 years old, with a bachelor degree, employed, in a relationship, mother of two children, and satisfied with her partnership. She is verbally rather aggressive, without anxiety or depression. She perceives herself as a strong and independent woman (low need for support, low level of support seeking, rarely present schema "dependence on others"). She tends towards self-sacrifice (frequent schema "self-sacrifice") and perfectionism, rigid rules, "having to do something", and is concerned with efficiency (frequently present schema "unrelenting standards"). However, when it comes to her health, she predominantly sees her illness as something that is "a set of external circumstances" such as good or bad luck and fate, and that predominantly depends on others and not on herself (high average score of locus of control). The time since diagnosis is less than six months, and she has ductal, hormone-sensitive BC that is HER2 negative. There is no family inheritance of BC.

Other studies have reported similar profiles of women with BC. In a recent study [23], the average age of women was 52.4 years; most of them lived in cities and were in a relationship (66.1%), with an average (35.2%) and higher education (33.3%). Age, relationship status, education level, and financial situation influenced the QoL of women with BC. A higher QoL was found in patients in partnerships, with higher education, and with a subjective assessment of a very good financial situation [23].

In our group, patients with higher scores for depression, physical aggression, stress, and anxiety, as well as clinically significant anxiety scores, had a poorer QoL. Our results are primarily in line with other studies dealing with the QoL of BC patients. In a study of 120 patients aimed at identifying risk factors for lower QoL in nonmetastatic BC patients, it was found that lower global QoL was associated with major depressive disorder, presence of personality disorder, greater pain, self-blame, lower levels of positive reframing coping strategies, and lack of hormone therapy. The authors also pointed out that lower QoL was strongly associated with variables related to the person's premorbid psychological characteristics and how the person was coping with cancer (e.g., depression, personality, and coping) than with cancer-related variables (e.g., type of treatment, cancer severity) [24].

If we want to take personality into consideration, a better QoL was observed in patients with a positive early maladaptive schema "emotional deprivation". This schema includes the general expectation that basic emotional needs are not met or perceived. The three main forms of emotional deprivation include deprivation of care, protection, and empathy [14]. The same is true for respondents who exhibited an early maladaptive schema of "a sense of entitle-

ment/narcissism". This schema is related to the belief in a person's superiority over others or the general belief that one is entitled to special privileges, rights, or exemptions. There may be a tendency to exert power over others, to impose one's views on others, or to try to control the behavior of others in a self-serving way [14]. In contrast to the maladaptive schemas mentioned above, the presence of the "emotional inhibition" schema was associated with poorer QoL. A limited number of studies have been made on the relationship between the QoL of BC patients and EMS [25, 26]. As a result of the investigation, Katebi et al. [26] stated that dysfunctional schemas and personality traits predict the QoL in women with BC. Based on multiple correlation coefficients, 54% of changes in the QoL among women with BC were dependent on EMS and their personality traits. At the same time, when we discuss EMS in BC patients, we should bear in mind that there is a particular link between EMS and the immune response. In BC patients with a poor immune profile, two schemas were found to be activated: a schema of mistrust and a schema of emotional inhibition [27].

Strong social relationships and support among patients with BC can reduce stress and improve the effectiveness of treatment, psychological functioning, survival, and QoL. Furthermore, it prevents cancer recurrence. In the opposite direction, long-term psychological distress increases the risk of BC progression as well as recurrence and mortality. For patients with BC, spouses or intimate partners and family members are perceived as the most essential persons in social support [28]. In our study, QoL was worse if the patient had a partner. Even more important was whether the woman was satisfied with her relationship with her partner – the lower the satisfaction, the worse the QoL. At the same time, QoL was positively correlated with PSS. The lower the PSS, the poorer the QoL of these patients. In one of the studies, information on social support status was collected from 1,160 women newly diagnosed with BC. The results were consistent with our findings, i.e., adequate social support from family members, friends, and neighbors and higher scores on PSS were associated with better QoL in BC patients [29].

Lower QoL scores were associated with specific oncological parameters – patients with hormone-sensitive cancer, the time since diagnosis of less than six months, and HER2-negative tumors had poorer QoL.

However, in our research, five variables proved to be of particular importance for the final QoL score in subjects with BC. Those are depression score, PSS, physical aggression score, satisfaction with partnership, and stress score. This model explains 45.9% of variances in QoL in women with BC. It means that the selected predictors accounted for a substantial amount of the variance in QoL. Depression (negative correlation) and PSS (positive correlation) have a particularly significant share.

If we look at the results of our research, i.e., the variables related to QoL, we will see that some of them cannot be changed (e.g., if the tumor is hormone-sensitive and HER2 positive). However, some variables can be influenced and changed (e.g., anxiety, depression, aggression, PSS). Considering the information we received during the current research, it is important to carry out a psychological exploration during clinical work with patients who have BC in order to find potential MH problems that are connected to the QoL of the examinees [30]. If changeable MH issues are present, we can apply some of the psychological or psychiatric interventions and influence the improvement of the QoL by controlling those variables [31].

For instance, interventions for depression (cognitive-behavioral therapy and/or medications), improvement of PSS (psychotherapy, social skills training, assertiveness training), reduction of aggression (dominantly psychotherapy, possibly medications), improvement of satisfaction with partnership (couples therapy, assertiveness training), and stress reduction (cognitive-behavioral therapy, relaxation training, assertiveness training), or even stress prevention (stress inoculation training) could be included [32–36].

Study limitations

The present study has some limitations. We did not collect information on actual therapy and the time elapsed since the last therapy, as well as the breast reconstruction after mastectomy. These factors may impact QoL in BC survivors.

Conclusion

Quality of life in breast cancer patients, as one of the important predictors of the outcome in chronic illnesses, is associated with numerous variables, predominantly with emotional state and patient's PSS. The highest percentage of the tested variables is changeable and can be influenced. For that reason, and to adequately assist these patients, the introduction of screenings that would identify those patients who are at risk or already have problems that could have a negative impact on the quality of life, as well as targeted work with them, is proposed.

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Conflict of interest

The authors declare no conflict of interest.

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Theta burst stimulation promotes nestin expression in experimental autoimmune encephalomyelitis

Stimulacija teta praskovima pojačava ekspresiju nestina kod eksperimentalnog autoimunskog encefalomijelitisa

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Abstract

Background/Aim. Multiple sclerosis (MS) is an immune-mediated disease of the nervous system in which the myelin sheath is destroyed during the process of neurodegeneration. Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS in which preservation of myelin and remyelination of axons can improve neuron survival. The aim of the study was to evaluate the activation capacity of neuronal tissue by autoimmune inflammation and treatment with intermittent (i) theta burst stimulation (TBS)-iTBS or continuous TBS (cTBS) based on the expression profiles of nestin in astrocytes, oligodendrocytes, and neurons. **Methods.** Two forms of TBS – iTBS and cTBS – were used to extend the period during which axons can be remyelinated. It was investigated how iTBS or cTBS protocols affect the expression profiles of nestin with glial fibrillary acidic protein, myelin basic protein (MBP), and neuronal nuclear protein in rat spinal cord. Changes at the molecular level were monitored using the immunofluorescence method. **Results.** The obtained results showed that both protocols (iTBS and cTBS) increased the expression of nestin and MBP and reduced astrogliosis in the spinal cord of EAE rats. **Conclusion.** The therapeutic potential of TBS in EAE contributes to the improvement of the intrinsic ability to recover from spinal cord injury.

Key words:
encephalomyelitis, autoimmune, experimental; multiple sclerosis; nerve regeneration; nestin; rats; spinal cord; transcranial magnetic stimulation.

Apstrakt

Uvod/Cilj. Multipla skleroza (MS) je bolest nervnog sistema posredovana imunskim mehanizmima u kojoj dolazi do oštećenja mijelinskog omotača u toku procesa neurodegeneracije. Eksperimentalni autoimunski encefalomielitis (EAE) je animalni model MS, u kome obnavljanje mijelina i remijelinizacija aksona mogu poboljšati preživljavanje neurona. Cilj rada bio je da se proceni aktivacioni kapacitet neuronskog tkiva pod uticajem autoimunskog zapaljenja i intermitentne (i) stimulacije teta praskovima (*theta burst stimulation* – TBS) ili kontinuirane (*continuous* – c) TBS (cTBS), na osnovu profila ekspresije nestina u astrocitima, oligodendrocitima i neuronima. **Metode.** Dva oblika TBS – iTBS i cTBS su korišćena za produženje perioda tokom koga aksoni mogu biti remijelinizovani. Ispitivan je uticaj iTBS i cTBS protokola na ekspresiju nestina sa glijalnim fibrilarnim kiselim proteinom, mijelin baznim proteinom (MBP) i neuronalnim nuklearnim proteinom u kičmenoj moždini pacova. Promene na molekulskom nivou su praćene primenom imunofluorescentne metode. **Rezultati.** Dobijeni rezultati su pokazali da oba protokola (iTBS i cTBS) povećavaju ekspresiju nestina i MBP i redukuju astrogliozu u kičmenoj moždini EAE pacova. **Zaključak.** Terapijski potencijal TBS u EAE doprinosi poboljšanju intrinzične sposobnosti za oporavak od povrede kičmene moždine.

Ključne reči:
encefalomijelitis, autoimunski, eksperimentalni; multipla skleroza; živac, regeneracija; nestin; pacovi; kičmena moždina; stimulacija, magnetna, transkranijalna.

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Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) characterized by repeated loss and regeneration of myelin sheaths. Pathological accumulation of degradation products during inflammation, neurodegeneration, and demyelination is thought to contribute to the onset and progression of the disease [1].

Experimental autoimmune encephalomyelitis (EAE) is a neurological disease characterized by disruption of blood-brain barrier, perivascular neuroinflammation, and neuronal damage due to progressive axon sheath destruction [2]. It is one of the most commonly used models of MS. Disruptions in the glial-neuronal network between astrocytes, oligodendrocytes, and neurons lead to metabolic deficits in all cell types, resulting in cellular dysfunction and death. Remyelination of CNS axons is essential for functional recovery after demyelinating injury. The reduction of astrogliosis has a positive effect on the remyelination process [1].

Various inhibitory signals in inflammatory demyelination prevent the required differentiation of oligodendrocytes and suppress the expression of myelin basic protein (MBP), which is required for the onset of healing [3]. The intense activity of oligodendrocytes is supported by a high metabolic rate, considering the high turnover in the formation of myelin sheaths [4]. Loss of integrative relationships between neurons, astrocytes, and oligodendrocytes leads to metabolic disturbances in these cells, permanent damage, and even death [5]. Stimulation of remyelination of axons in the CNS is crucial as it promotes the return of axons to a competent state suitable for myelination, regeneration, and thus, restoration of function and reduction of astroglia contribute to this [6].

Intermediate filaments influence cell type and developmental stage. Nestin is a class VI intermediate filament protein transiently expressed in adult neural stem cells (NSCs) and immature neural progenitor cells and disappears when the cells enter differentiation. It is commonly used as a marker for NSCs in both the embryo and the adult brain. Nestin is the main marker for immature astroglial cells and multipotent progenitor cells while maturing astrocytes contain glial fibrillary acidic protein (GFAP) [7]. Nestin expression has been shown to be reinitiated in reactive astrocytes [8]. Nestin-expressing cells indicate an active stage of embryonic progenitor cells and are involved in the repair of damaged CNS tissue during the course of EAE [9].

Nestin is mainly distributed in the cytoplasm, and its expression is an indicator of cell growth and proliferation. The level of nestin messenger ribonucleic acid is more pronounced in damaged tissue than in intact tissue [10]. Various factors regulate the expression of nestin. It was originally thought to be exclusively associated with NSCs [11].

The neuron-specific nuclear protein (NeuN) is a small soluble protein mainly localized in the nucleus [12]. It is considered a relevant marker for *post*-mitotic neurons [13]. NSCs compete for a central role in the recovery process after tissue injury [14]. Consequently, the changes in NSCs in EAE might be reflected in the expression of nestin. NeuN, a specific marker for mature neurons, is present in most neurons of adult neurogenesis [15].

Repetitive transcranial magnetic stimulation (TMS) enhances neuronal activity through synaptic potentiation induced by high stimulation frequency and subsequent suppression of EAE-induced tissue damage [16]. Our studies have shown that two different TMS stimulation protocols, intermittent theta burst stimulation (TBS) – iTBS and continuous TBS (cTBS), are structured types of repetitive TMS that induce changes in redox homeostasis in both healthy and EAE animals [17, 18]. While iTBS induces cortical facilitation similar to long-term potentiation (LTP), cTBS causes depression of cortical activity reminiscent of long-term depression (LTD) [19]. Our previous report indicated that neuroplasticity induced by LTP and LTD could lead to local changes in the brain at the molecular level, such as increased availability of brain-derived neurotrophic factor, which can reduce inflammation and support myelin repair [20].

The aim of this study was to evaluate the activation capacity of neuronal tissue by autoimmune inflammation and treatment with iTBS or cTBS using the expression profiles of nestin in astrocytes, oligodendrocytes, and neurons.

Methods

Animals

The experimental animals were handled in accordance with the ethical guidelines for the use of animals in research. The experimental procedures were approved by the Ethics Committee from the Ministry of Agriculture and Environmental Protection – the Veterinary Directorate of the Republic of Serbia No. 323-07-00622/2017-05.

Female Dark Agouti rats aged 10–14 weeks weighing 150–200 g were used for the experiments. The animals were held in cages under constant environments (light-dark cycle of 13/11 hrs, temperature of 23 °C ± 2 °C, and humidity of 55% ± 3%). Food and water were provided *ad libitum*, while water was offered manually in cases of severe paralysis.

EAE induction and TMS treatment

The rats were anesthetized intraperitoneally (i.p.) with ketamine (50 mg/kg) and xylazine (10 mg/kg) before EAE induction by subcutaneous (s.c.) injection of 0.1 mL suspension of rat spinal cord tissue homogenate (50% weight/volume – w/v in saline) dissolved in Complete Freund's Adjuvant (CFA) containing 1 mg/mL *Mycobacterium tuberculosis* (Sigma, St. Louis, MO, USA) into the right posterior footpad [21].

TMS was performed with a specific coil (25 mm figure-of-eight) using a MagStim Rapid [2] device (The MagStim Company, Whitland, Dyfed, UK). The center of the coil was located directly above the bregma. The iTBS pattern (iTBS group) consisted of 20 trains of ten bursts of three pulses at a frequency of 50 Hz repeated at 5 Hz (duration 192 s with 10 s

pauses between series) [17]. The cTBS pattern (cTBS group) consisted of a single 40 s train of bursts repeated at 5 Hz. Each pattern consisted of 600 pulses. The intensity of the stimulus was 30% of the maximal stimulator output, which was currently below the motor threshold (expressed as apparent upper limb contraction in all TBS-treated rats).

Experimental procedure

The experimental animals were randomly divided into seven groups: Control group (C), n = 3; CFA treated group (CFA), n = 3; EAE-immunized animals group (EAE), n = 3; two groups of EAE-immunized animals treated with iTBS (EAE+iTBS), n = 3, or cTBS (EAE+cTBS), n = 3, and two groups of healthy animals stimulated with iTBS (iTBS), n = 3 or cTBS (cTBS), n = 3. Healthy animals were treated with iTBS or cTBS for ten days, while both treatment protocols were applied to EAE animals for ten days from day 14 post-immunization (p.i.).

Clinical evaluation of EAE was performed daily as a part of a double-blind study up to day 24 p.i. [22]. All animals were anesthetized i.p. with sodium/pentobarbital 45 mg/kg body weight and decapitated 24 hrs after the last TBS protocol.

Immunofluorescence

For fluorescence staining, spinal cords were quickly isolated and fixed for 12 hrs at 4 °C in 4% paraformaldehyde. For cryoprotection, the tissues were placed in a graded concentration of sucrose (10%, 20%, and 30% sucrose, pH 7.4). The spinal cord was frozen in 2-methyl butane and stored at -80 °C before sectioning with the cryotome (Leica CM 1850, Germany). The 25 µm thick tissue sections were mounted on glass slides, dried at room temperature for 2 hrs, and stored at -20 °C before staining. The primary mouse monoclonal anti-Nestin antibody (1:100; Abcam, Germany) was applied overnight at 4 °C. The secondary donkey anti-mouse antibody Alexa Fluor 555 (1:250; Invitrogen, Carlsbad, CA, USA) was used for 2 hrs in the dark (at room temperature). The slides were then washed several times before being incubated overnight at 4 °C with a new primary rabbit polyclonal antibody: anti-GFAP (1:1,000; Abcam, Germany), anti-MBP (1:1,000; Abcam, Germany), or anti-NeuN (1:1,000; Abcam, Germany). To complete the staining, the slides were incubated with the secondary goat anti-rabbit Alexa Fluor 488 antibody (1:500; Invitrogen, Carlsbad, CA, USA) for 2 hrs in the dark (at room temperature). All antibodies were diluted in phosphate-buffered saline-PBS with 1% bovine serum albumin-BSA. Slides were mounted on microscope slides with Mowiol medium (Sigma Aldrich) and analyzed under a confocal microscope (Zeiss Axiovert 200M, LSM 510 laser module).

Immunofluorescence quantification

Three sections of the ventral horn *per* animal were used to define fluorescence intensity using the Fiji version of ImageJ software. Five random photomicrographs of the section of interest were taken at one magnification (×40) and analyzed using the Coloc2 program processor of the ImageJ software [23].

Image processing and calculation of a combined colocalization coefficient

Quantitative colocalization analysis is an advanced digital imaging tool for the observation of antigens in immunofluorescence images obtained by confocal microscopy. It uses specialized algorithms that calculate many coefficients from which colocalization can be quantitatively estimated [24].

Colocalization is presented in the form of a plate with three images consisting of fluorescence images for red and green channels and a third merged image in which the channels are combined (overlapping pixels turn yellow). The analysis is done using computer software based on the evaluation of the color of the selected pair of channels.

The images were imported into the Fiji version of the free image processing software – ImageJ. Fiji contains several pre-installed plugins, including a colocalization analysis method called Coloc 2, which calculates several colocalization parameters, such as Pearson's and Manders' coefficient, based on correlation measurements of pixel intensity. In addition to the numerical correlation parameters, a 2D intensity histogram is also generated to visualize the correlation between the two channels. For more information, see Stevanovic et al. [20].

Statistical analysis

A one-way ANOVA and Tukey's *post hoc* multiple tests (GraphPad Prism software, version 6.0) were used for statistical data analysis. Values are presented as mean ± standard deviation. The correlation coefficients were determined using the Spearman test, whereby the differences were considered statistically significant if $p < 0.05$.

Results

The microscopic images of dual fluorescent immunoreactivity of nestin with GFAP (Figure 1), nestin with MBP (Figure 2), or nestin with NeuN (Figure 3) in spinal cord tissue were used for quantitative colocalization analysis (Figures 4 and 5).

In the EAE group, the expression of nestin and GFAP increased (Figure 1), while MBP decreased (Figure 2). Compared to the EAE group, the application of both the iTBS and cTBS protocols resulted in increased nestin expression with a simultaneous decrease in GFAP and increased MBP. The expression of NeuN showed no difference between the groups, independent of the increased nestin expression (Figure 3).

The increase in nestin immunoreactivity was measured in the EAE group compared to control values (Figure 4A, $p < 0.05$). Increased nestin expression was also measured after

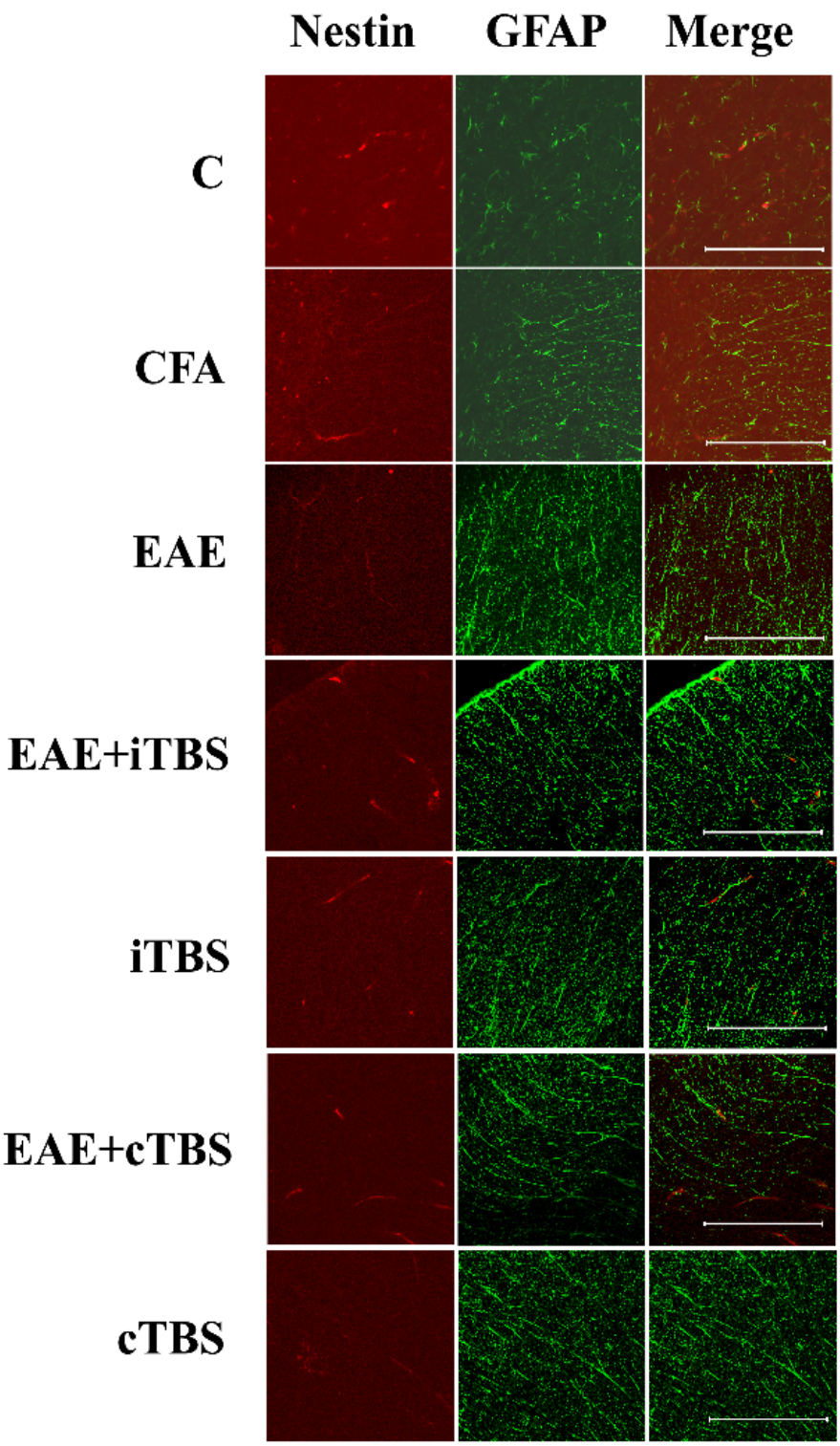

Fig. 1 – Double fluorescence staining showing immunoreactivity for nestin and GFAP in rat's spinal cord. Red represents nestin positivity, and green in the photograph represents GFAP positivity. C – control group; EAE – rats with EAE; CFA – rats treated with CFA; EAE+iTBS – iTBS treatment on EAE animals; iTBS – iTBS treatment on healthy animals; EAE+cTBS – cTBS treatment on EAE animals; cTBS – cTBS treatment on healthy animals. Photomicrographs of the stained spinal cord sections were taken at a magnification of ×40. The scale bar represents 200 μm. GFAP – glial fibrillary acidic protein; CFA – Complete Freund's Adjuvant; EAE – experimental autoimmune encephalomyelitis; iTBS – intermittent theta burst stimulation; cTBS – continuous theta burst stimulation.

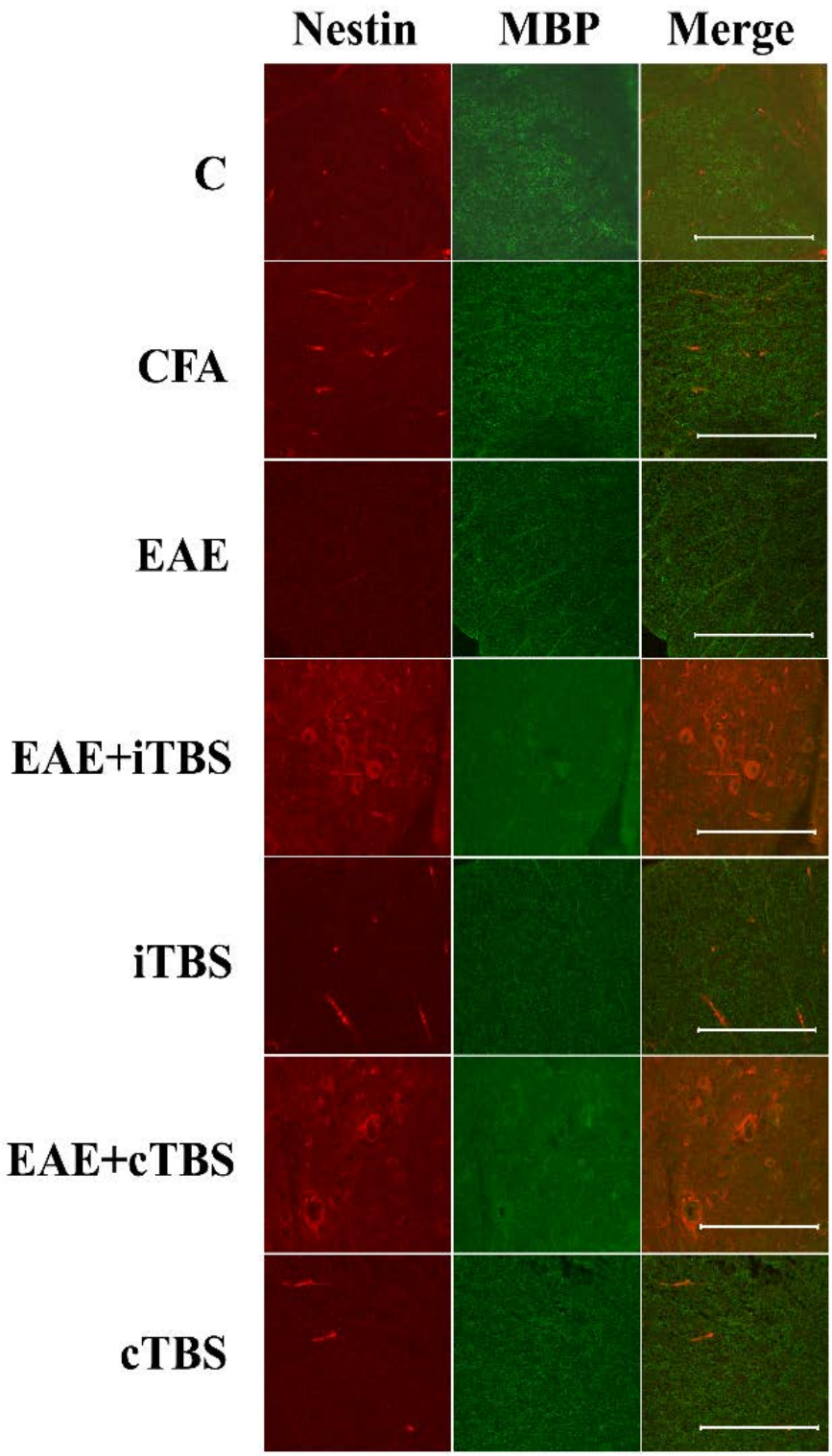

Fig. 2 – Double fluorescence staining showing immunoreactivity for nestin and MBP in rat's spinal cord. Red represents nestin positivity, and green represents MBP positivity. Photomicrographs of the stained spinal cord sections were taken at a magnification of ×40. The scale bar shows 200 μm.
MBP – myelin basic protein. For other abbreviations, see Figure 1.

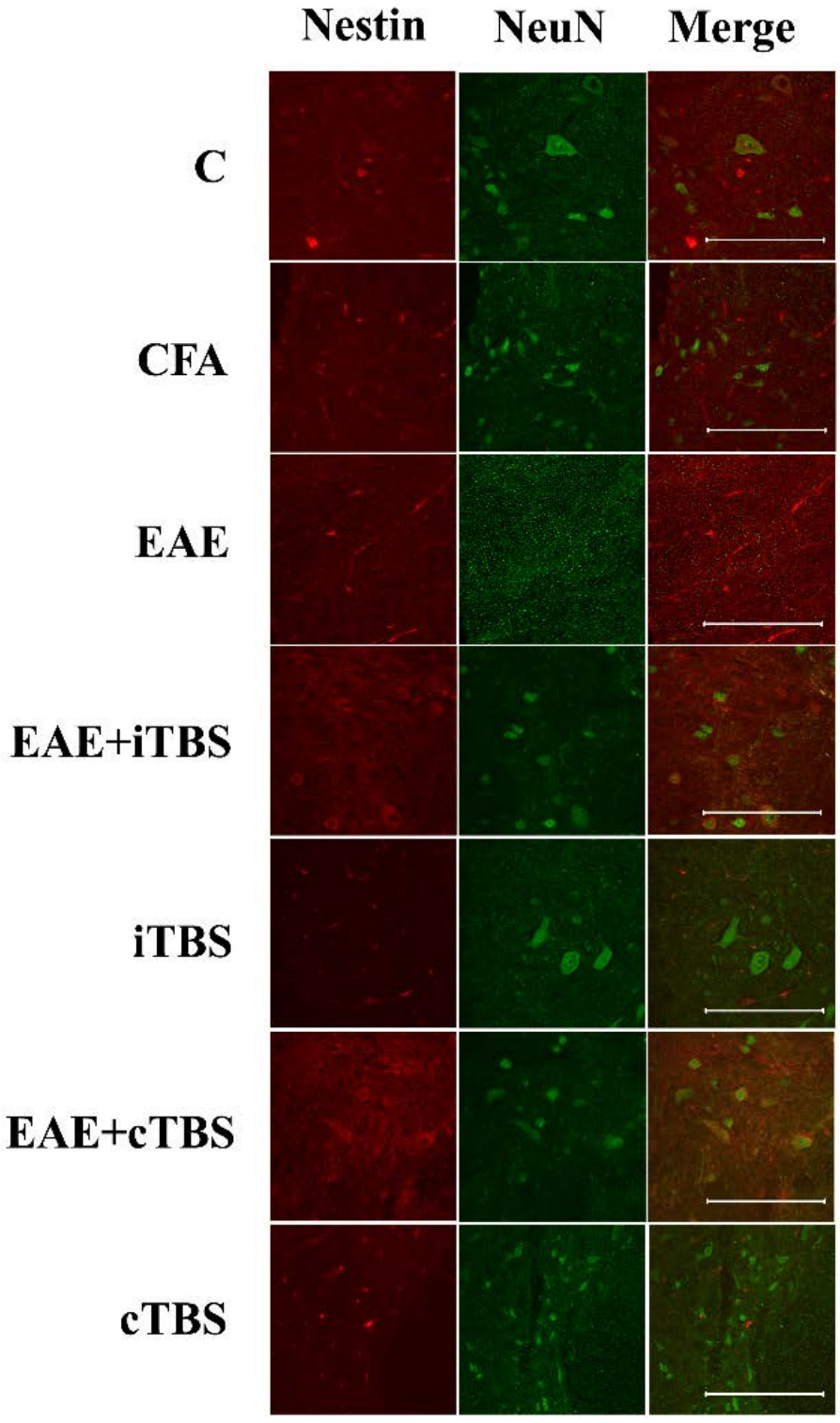

Fig. 3 – Double fluorescence staining showing immunoreactivity for nestin and NeuN in rat's spinal cord. Red represents nestin positivity, and green represents NeuN positivity. Photomicrographs of the stained spinal cord sections were taken at a magnification of ×40.
The scale bar shows 200 μm.
NeuN – neuronal nuclear protein. For other abbreviations, see Figure 1.

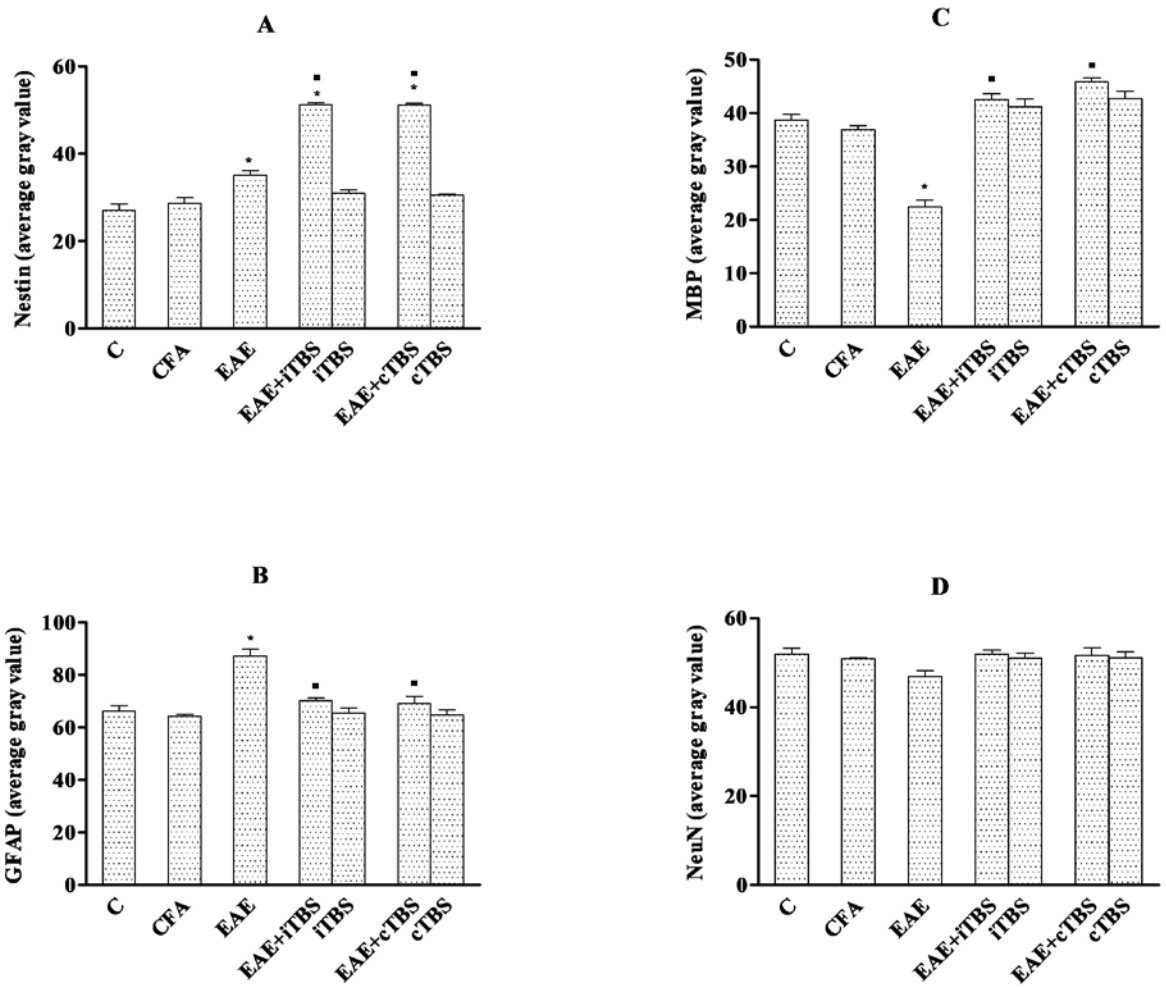

Fig. 4 – Effects of iTBS and cTBS on double staining of nestin (A), nestin with GFAP (B), nestin with MBP (C), or nestin with NeuN (D) in the rat spinal cord. Results were expressed as average grey value ± SD (n = 3). Analysis of variance (ANOVA) was performed followed by Tukey's multiple comparison test with GraphPad Prism 6.0.
MBP – myelin basic protein; NeuN – neuronal nuclear protein; SD – standard deviation. For other abbreviations, see Figure 1.
***$p < 0.05$ compared to the control group; ▪ $p < 0.05$ compared to the EAE group.**

both iTBS/cTBS treatments in the EAE animals compared to controls and compared to the EAE group (Figure 4A, $p < 0.05$). In contrast to nestin, GFAP expression increased in EAE animals compared to the controls (Figure 4B, $p < 0.05$). However, iTBS/cTBS treatment significantly decreased the expression of GFAP in the EAE animals (Figure 4B, $p < 0.05$).

A significant decrease in MBP immunoreactivity was observed in the EAE group compared to controls, and then both protocols (iTBS and cTBS) applied to EAE animals significantly increased MBP expression compared to the EAE group (Figure 4C, $p < 0.05$). There was no significant difference between nestin and NeuN in any of the experimental groups (Figure 4D).

We found that the nestin and GFAP molecules had high numerical colocalization values, according to the intensity-based coefficients (Pearson's and Manders' correlation parameters) (Figure 5A, 5D). High correlation values were found after labeling the same compartments with nestin and GFAP in the EAE, EAE+iTBS, and EAE+cTBS groups compared to controls. In contrast, Pearson's correlation was lower in the EAE+iTBS and EAE+cTBS groups compared to the EAE group. The M2 Manders' coefficient (GFAP) increased in EAE and EAE+iTBS compared to the controls, while it decreased in the EAE+cTBS group compared to the EAE animals (Figure 5D).

Pearson's correlation coefficient of colocalizations between nestin and MBP showed increased values in the EAE+cTBS group compared to the control and in both TBS protocols in the EAE animals compared to the EAE group (Figure 5B, $p < 0.05$). The Manders' coefficients of correlations and colocalizations between nestin and MBP were not significant (Figure 5E).

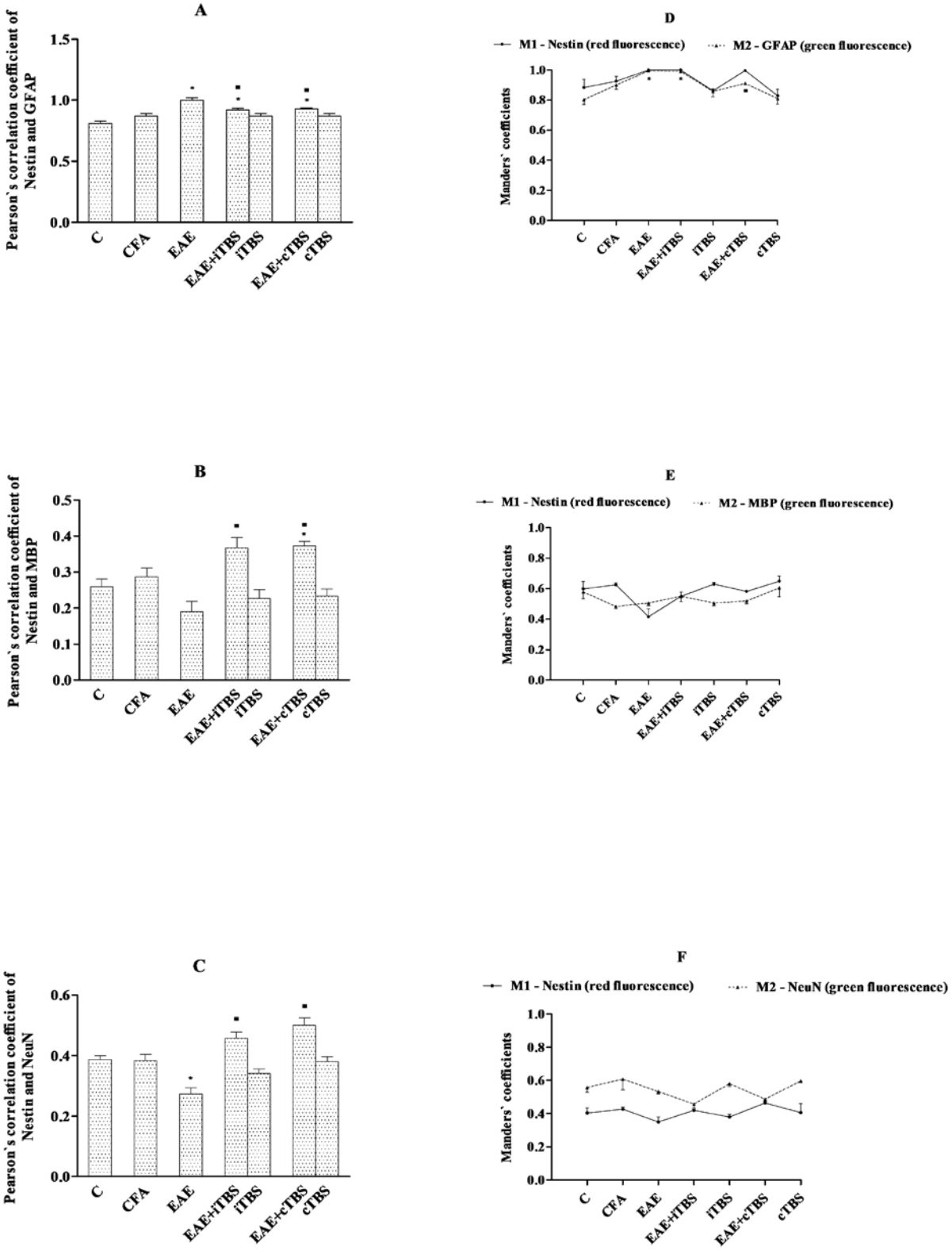

Fig. 5 – Colocalization parameters proposed as Pearson's coefficient (A, B, C) and Manders' coefficient (D, E, F): M1 (nestin) and M2 (GFAP, MBP, NeuN) after double labeling of nestin with GFAP (A, D), nestin with MBP (B, E), or nestin with NeuN (C, F) in the rat spinal cord. Results were expressed as average grey value ± SD (n = 3). Analysis of variance (ANOVA) was performed followed by Tukey's multiple comparison test with GraphPad Prism 6.0.

MBP – myelin basic protein; NeuN – neuronal nuclear protein; SD – standard deviation. For other abbreviations, see Figure 1.

***$p < 0.05$ compared to the control group; ▪ $p < 0.05$ compared to the EAE group.**

The Pearson's coefficients between nestin and NeuN showed increased values, albeit at a low level, in the EAE group compared to the controls (Figure 5C, $p < 0.05$), while both TBS treatments increased the same coefficient in the EAE animals compared to the EAE group (Figure 5C, $p < 0.05$). The Manders' coefficients between these two proteins were not significant (Figure 5F).

Discussion

Both applied TBS protocols in a model of actively induced EAE showed comparable effects of the expression of nestin, GFAP, and MBP in the spinal cord.

During recovery from EAE inflammation, nestin changed dynamically. Previous studies have shown weak nestin immunoreactivity in vascular endothelial cells but not in the neural parenchyma of the spinal cord of healthy rats [25]. EAE immunization can induce the appearance of progenitor cells/radial glia, which is supported by increased nestin expression. Neuronal damage is confirmed by the detected GFAP overexpression in and around the lesions [26].

Proper regulation of NSCs in injured neural tissue is a prerequisite for CNS remodeling. This study revealed increased reactivity of nestin throughout the spinal cord along with GFAP. In the EAE group, nestin immunoreactivity was detected in gray and white matter astrocytes on day 24 p.i. EAE underscores the accumulation of enlarged, multipolar GFAP immunoreactive astroglial cells within inflammatory demyelinating lesions. The accumulation of astroglia at the edges of lesions is followed by depletion of oligodendroglia, which may indicate that marked gliosis is not immediately followed by infiltrating oligodendrocytes near the lesions [27]. This is consistent with the decreased MBP immunoreactivity in the EAE group compared to controls.

In the mature CNS, it appears that glia have multipotent capabilities that can be triggered by a noxious attack such as neuroinflammation and can transform into neurons, oligodendroglia, or astrocytes [28]. The data on unaltered NeuN presentation in the spinal cord on day 24 p.i. suggest that nestin-positive multipotent cells in the spinal cord convert their phenotype into oligodendrocytes or astrocytes. This speculation is supported by the discovery of nestin-positive cells with long processes that resemble both preoligodendrocytes and mature oligodendrocytes [29].

Following EAE-immunization, several complexes of secondary injury cascades develop, offering great potential for therapeutic intervention. Nestin-positive NSCs could be induced by TBS treatments and help replace lost cells, raising the prospect of effective integration into neural circuits. Therefore, we hypothesize that TBS treatment contributes to functional recovery and pathophysiological changes by increasing the number of nestin-positive cells. Remarkably, the number of nestin/GFAP double-positive cells in the spinal cord sections decreased after iTBS or cTBS treatment in the diseased animals compared to the EAE group, indicating a beneficial effect of stimulation. Our study provides evidence that TMS promotes the formation of nestin-positive cells in the spinal cord of adult rats, suggesting that this may be related to the potential for functional recovery.

In contrast to the increase in the number of GFAP-positive cells in the EAE animals, which is indicative of astrogliosis, treatment with iTBS or cTBS reduced the proliferation of astrocytes in the EAE animals. Compared to the EAE animals, some of the cells with intense nestin labeling in the diseased animals treated with iTBS or cTBS gradually showed weak GFAP reactivity along with increased MBP expression, indicating the considerable therapeutic potential of TMS in functional recovery. These data complement our previous study, which confirmed that TMS treatment has a positive impact on astroglia and microglia, along with a better clinical outcome, including disease duration and exposed paralysis [30]. The therapeutic effect is likely to be a modulation of astrocyte activity in the zone of neural tissue damage [31].

Conclusion

The current study suggests that both theta burst stimulation protocols improve histologic recovery. These treatments appear to lead to an increase in nestin- and myelin basic protein-positive cells with decreasing astrogliosis in EAE animals, promising reconstructed neuronal differentiation. The therapeutic potential of theta burst stimulation is thus recommended in EAE as it helps improve the intrinsic ability to recover from spinal cord injury.

Conflict of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR

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Glaucoma Weeks and Glaucoma Screening/Prevention – part 2

Nedelje glaukoma i skrining/prevencija glaukoma – 2. deo

To the Editor:

Glaucoma is a common name for a group of eye diseases also called optic neuropathy. It is a chronic, incurable disease that can, nevertheless, be kept under control with regular monitoring and treatment. Glaucoma represents a unique, currently significant social health problem of all societies [1]. Glaucoma is the second most common cause of blindness in the world and affects about 80 million people. About 118 million people have a predisposition to get glaucoma by 2040, of which over four and a half million are blind. Even 50% of people do not know they have glaucoma [2]. In Serbia, about 150,000 people have this disease [2–5]. The Glaucoma Weeks are an opportunity to draw the attention of the public to the seriousness of this disease, which leads to blindness if it is not diagnosed and treated on time, as well as to the risk factors for the occurrence of glaucoma, methods of prevention, and treatment options [3].

The primary objective of fighting against glaucoma is to take action in order to recognize and acquaint the general population with the emphasized importance of regular, ophthalmological controls in the mission of preventing blindness, to which untreated and uncontrolled glaucoma surely leads [4]. The second objective, no less important, is to recall the numerous, archived, and successfully held actions in the past – the Weeks of the Fight against Glaucoma as part of the worldwide action and the Weeks of the Fight against Glaucoma at our University Clinical Center Kragujevac, Serbia, at the Clinic for Ophthalmology (each year in March, in the duration of five days) [5].

To achieve our objectives, we observed a 16-year period, from 2008 to 2024, and collected the data we needed. We separated the observation period into two parts: 2008–2017, the first part, and 2018–2024, the second part. The program of Glaucoma Screening at the University Clinical Center Kragujevac included 1,392 people in the first part and 2,417 in the second part. If we compare the two parts of the observation period, we can see that glaucoma was more present in women (n = 907 vs. n = 1,691) than in men (n = 485 vs. n = 726). Concerning age, there were people aged 15–49 years (n = 326 vs. n = 635) and 50–84 years (n = 1,030 vs. n = 1,782). Previously, the largest increase in newly discovered glaucoma cases was recorded in 2017 for men and persons aged 49, and in 2024, the increase reached 429 persons (261 women and 168 men), 67 of them aged 15–49, and 362 of them aged 50–84 years (Figure 1). Older age is statistically significantly associated with glaucoma ($p = 0.018$). Female gender is also statistically significantly associated with glaucoma ($p = 0.045$). During the year 2023,

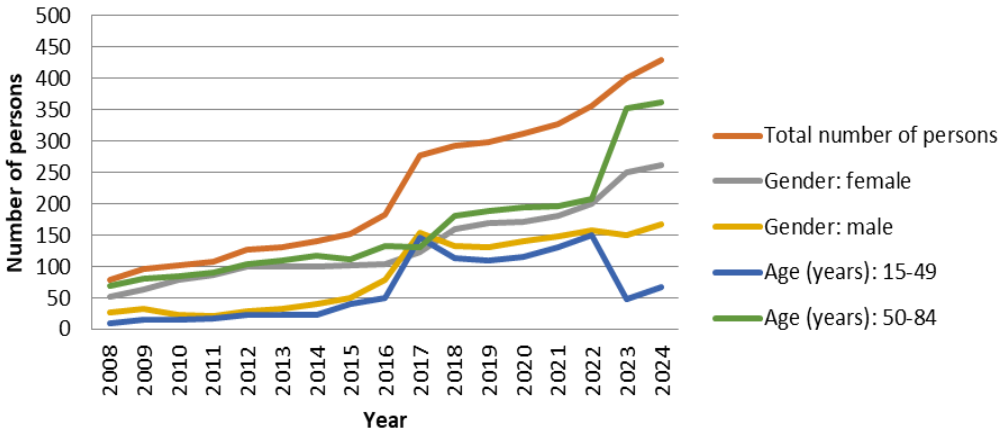

Fig. 1 – Number of persons by gender and age screened for glaucoma during the Glaucoma Weeks in the previous 17 years (2008–2024).

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400 individuals were examined, mostly women. The prevailing age group was 50–84 years, with ten newly diagnosed glaucomas. During the first half of 2024, a total of 429 individuals were observed, predominantly females also, most of them in the age group from 50–84 years (median age 55.1 years), and 14 new glaucoma cases were diagnosed, with further necessary diagnostic and therapeutic procedures at the Clinic for Ophthalmology, University Clinical Center Kragujevac. The prevalence of newly discovered or newly diagnosed glaucoma was 2.113%. Every calendar year, the number of persons for glaucoma screening increases. Our results showed that the number of persons increased linearly every year, from 79 persons in 2008 to 270 persons in 2017 (a 3.4-fold increase), and in 2023/24, there was a 3.7–4.1-fold increase of persons compared to 2008. The number of newly discovered glaucomas in the initial phase, compared to the number of newly discovered ones in the terminal stages of the disease, has increased significantly every year, especially in the last seven years of the current program and actions of the Glaucoma Weeks in our country. A total of 869 (20.19%) individuals were identified as having suspected glaucoma (2008–2024), of which 317 (16.25%) had to undergo a further and more detailed ophthalmological examination in order to diagnose glaucoma. During the year 2024, the following were recorded: 261 glaucomas in the initial stage of development, 147 glaucomas in the developmental (manifest-compensatum) stage of development, 13 glaucomas in the neglected (inverteratum) stage, 8 glaucomas in the stage of pre-blindness (fereabsolutum) and blindness (absolutum) (Figure 2). The number of glaucoma types confirmed and/or recorded during the first half of 2024 by gonioscopy and fundus examination were as follows: a) primary open-angle glaucoma (POAG) – 302 patients, b) pseudoexfoliative (PEX) glaucoma – 56 patients (separated from PEX syndrome), c) primary closed-angle glaucoma (PCAG) – 59 patients, and d) secondary glaucoma – 12 patients (Figure 3).

In conclusion, the weeks of the fight against glaucoma in our country stood out and were confirmed through many years of practice, profession, and science, justifiably

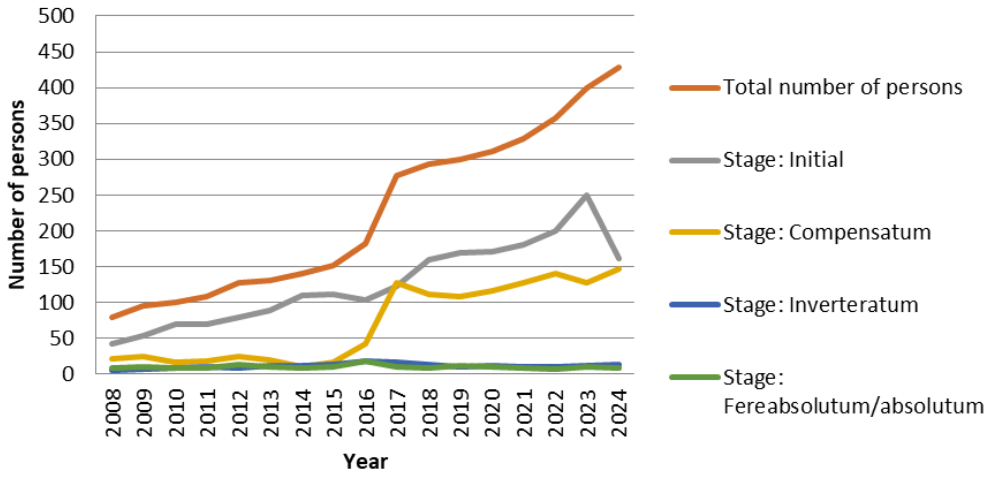

Fig. 2 – Number of persons classified by stage of glaucoma during the Glaucoma Weeks in the previous 17 years (2008–2024).

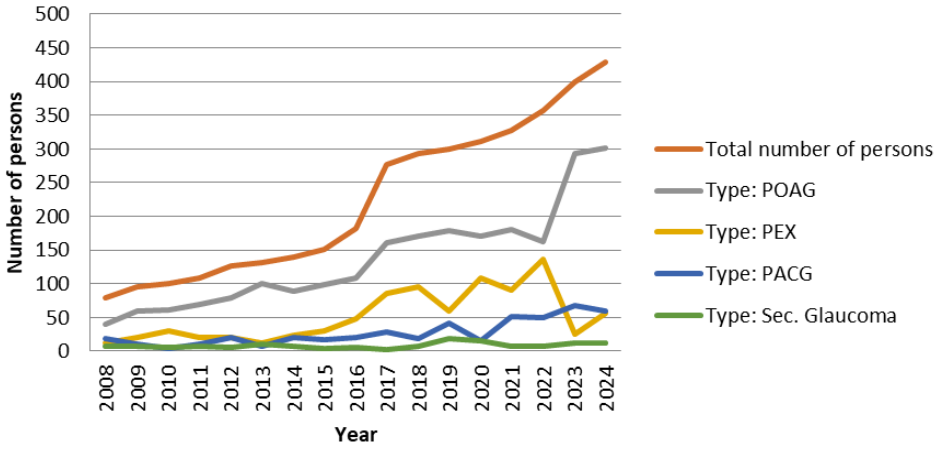

Fig. 3 – Number of persons by gonioscopy (type of glaucoma) screened for glaucoma during the Glaucoma Weeks in the previous 17 years (2008–2024).
POAG – primary open-angle glaucoma; PEX – pseudoexfoliative; PCAG – primary closed-angle glaucoma; sec. glaucoma – secondary glaucoma.

highlighting the public importance of the necessary knowledge and skills that can be applied today in the prevention, diagnosis, and treatment of glaucoma. Successful cooperation between healthcare institutions and the media can be a useful way of increasing awareness and detecting individuals with an increased risk of developing glaucoma, as well as the necessary controls of already diagnosed glaucoma. Screening and preventive examinations by an ophthalmologist will help detect the disease at the right time, keep it under control, and focus it in a targeted manner through actions of Weeks of Fight Against Glaucoma. Continuous medical implementation of these programs would benefit our society as a whole based on health, social, and economic levels.

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CASE REPORT

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Transmetacarpal replantation

Transmetakarpalna replantacija

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Abstract

Introduction. Transmetacarpal amputation (TA) distal to the superficial palmar arch is one of the most difficult procedures in replantation surgery because it requires the reconstruction of blood vessels, muscles, tendons, bones, nerves, and skin. We present a case of a patient with TA of the left hand and microsurgical replantation. **Case report.** A 23-year-old left-hand dominant male worker with a positive smoking history sustained TA from a radial saw blade and avulsion injury of the palmar side of the thumb with no other apparent injuries. The time between the amputation and replantation was 4 hrs. The patient sustained the amputation at the level of metacarpophalangeal joints. The successful replantation included: wound and intrinsic muscles debridement, microvascular anastomoses of the blood vessels, internal fixation of the metacarpal bones, neurorrhaphy, tenorrhaphy, and wound closure with local skin flap for thumb soft tissue defect. The patient was followed for seven years postoperatively. **Conclusion.** The replantation surgery requires a specialized department and well-educated teams of hand microsurgeons, orthopedic surgeons, and anesthesiologists. The most important factors that influenced the outcome after the replantation in the presented case included the type and level of injury, ischemia time, comorbidities, age, smoking history, and physical therapy.

Key words:
amputation, traumatic; hand injuries; metacarpal bones; plastic surgery procedures; quality of life; replantation.

Apstrakt

Uvod. Transmetakarpalna amputacija (TA) distalno od površinskog dlanskog luka je jedna od najtežih procedura u replantacionoj hirurgiji jer zahteva rekonstrukciju krvnih sudova, mišića, tetiva, kostiju, nerava i kože. Prikazan je slučaj bolesnika sa TA leve šake i mikrohirurškom replantacijom. **Prikaz bolesnika.** Radnik star 23 godine, pušač, dominantno levoruk, zadobio je TA cirkularom i avulzionu povredu palmarne strane palca bez drugih, vidljivih povreda. Vreme između amputacije i replantacije iznosilo je 4 sata. Bolesnik je zadobio povredu, amputaciju u nivou metakarpofalangealnih zglobova. Uspešna replantacija je uključivala: debridman rane i intrizičnih mišića, mikrovaskularne anastomoze krvnih sudova, unutrašnju fiksaciju metakarpalnih kostiju, neurorafiju, tenorafiju i zatvaranje rane lokalnim kožnim režnjem za defekt mekog tkiva palca. Bolesnik je praćen sedam godina posle operacije. **Zaključak.** Replantaciona hirurgija zahteva specijalizovani centar i dobro obučen tim mikrohirurga šake, ortopedskih hirurga i anesteziologa. Najznačajniji faktori koji su kod prikazanog bolesnika uticali na rezultat posle replantacije bili su tip i nivo povrede, vreme ishemije, komorbiditeti, životno doba, anamneza pušenja i fizikalna terapija.

Ključne reči:
amputacija, traumatska; šaka, povrede; kosti, metakarpalne; hirurgija, rekonstruktivna, procedure; kvalitet života; replantacija.

Introduction

Transmetacarpal replantation (TR) is a challenging procedure, but it is the patient's only chance to restore the function of the hand. The first successful replantation of the thumb was performed by Komatsu and Tamai [1] in 1965. Until today, many techniques have been developed to improve the function of the hand, and good results were

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associated with the replantation at several levels. Reports of the functional outcome of TR have been mixed because transmetacarpal amputation (TA) always injures the intrinsic muscles, rendering the amputated part ischemic. In TRs, the distal portions of any transected intrinsic muscles must be debrided [2, 3]. Vascular thrombosis is the most feared complication and a common cause of TR failure. Considering the numerous anastomoses of the blood vessels in TR, the thrombosis of one or more arterial anastomoses may lead to losing one or all fingers [3, 4].

We present a case of TR with the reconstruction of soft tissue defect of the palmar side of the left thumb and good postoperative outcomes at 2-year clinical follow-up and 7-year telemedicine follow-up.

Case report

A 23-year-old man, left-hand dominant, was transferred to our hospital 4 hrs after a left-hand TA from a radial saw blade. He was working at the factory as a carpenter. The patient was a smoker. Examination of the left hand showed a total amputation of the left hand at the level of metacarpophalangeal joints (MPJ) and avulsion injury with soft tissue defect of the palmar side of the left thumb. The surgery was performed according to ischemic protocol. The microbiological samples were taken from the stump and amputated part of the left hand. We identified superficial dorsal veins, common palmar digital arteries, radial artery of index finger, ulnar artery of little finger, common palmar nerves, superficial and deep flexor tendons, and metacarpal bones. The articular cartilage of all MPJ was destroyed. Metacarpal bones were shortened to avoid tension in microvascular anastomoses after osteosynthesis (Figure 1). We used four Kirschner wires (K-wires) for the osteosynthesis of metacarpal fractures (Figure 2). The distal portions of all interosseous muscles were debrided. We performed five microvascular anastomoses of palmar arteries with 9–0 monofilament sutures after washing with heparinized saline solution. Neurorrhaphy of common palmar digital nerves II–V was made with 8–0 monofilament with epineural sutures. We used modified Kessler sutures 3–0 and 4–0 for suturing superficial and deep flexor tendons for the index, middle, ring, and little finger. After examining the dorsal side of the left hand, we performed tenorrhaphy of extensor tendons for fingers II–V and three microvascular anastomoses of superficial dorsal veins. The skin was primarily closed. The soft tissue defect of the palmar side of the left thumb was closed with the local skin flap. The surgery was performed under general anesthesia and tourniquet control. A postoperative replant dressing was applied immediately after the surgery. The wrist was placed in 10° to 15° of extension and MPJ in 70° of flexion. Total ischemia time was 9 hrs. Total procedural time was 5 hrs. Vascular flow in the replanted hand was correct, and saturation was 96%. We administered ceftriaxone 2 g intravenously (iv) daily for seven days, enoxaparin 4,000 IU for two weeks, and acetylsalicylic acid 100 mg daily for two weeks. The K-wires were removed at the clinic six weeks after the replantation. Soft massage of the hand and passive physiotherapy of the proximal and distal interphalangeal joints started ten days following the replantation surgery to prevent scar adhesions. In the sixth week postoperatively, the patient began muscle-strengthening exercises, hydromassage, and

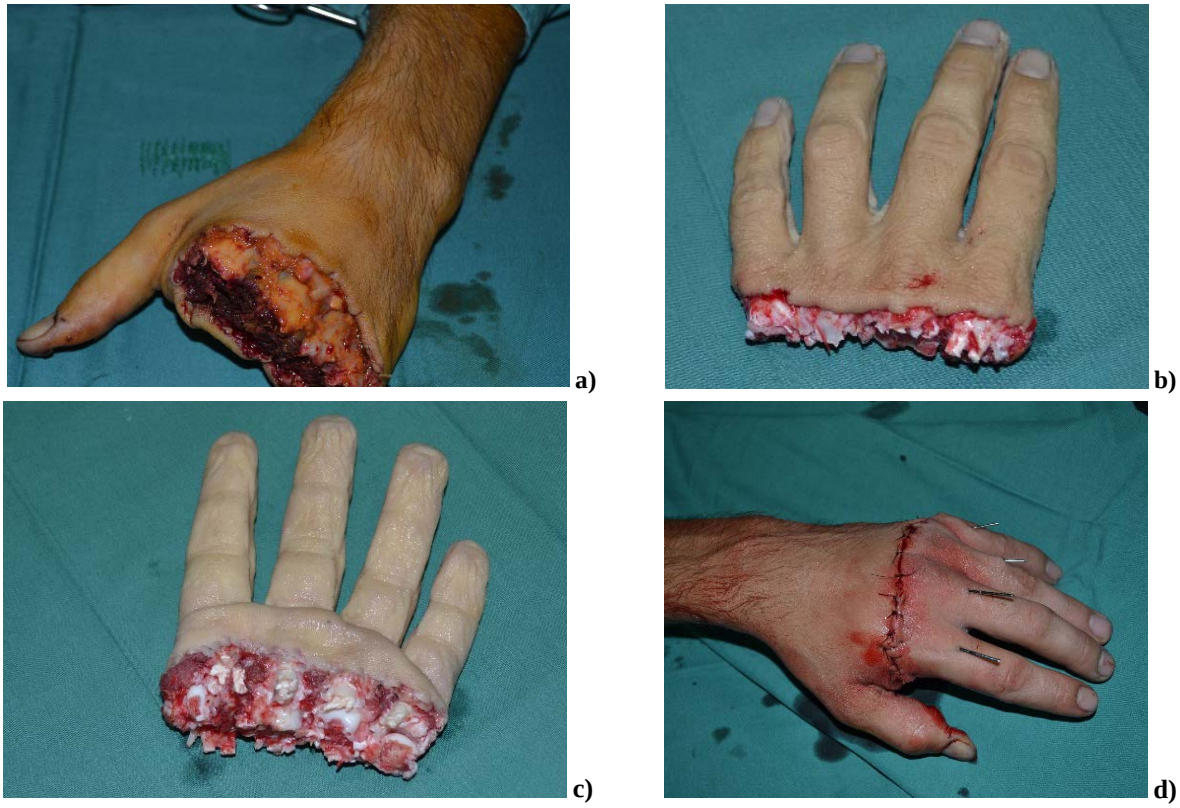


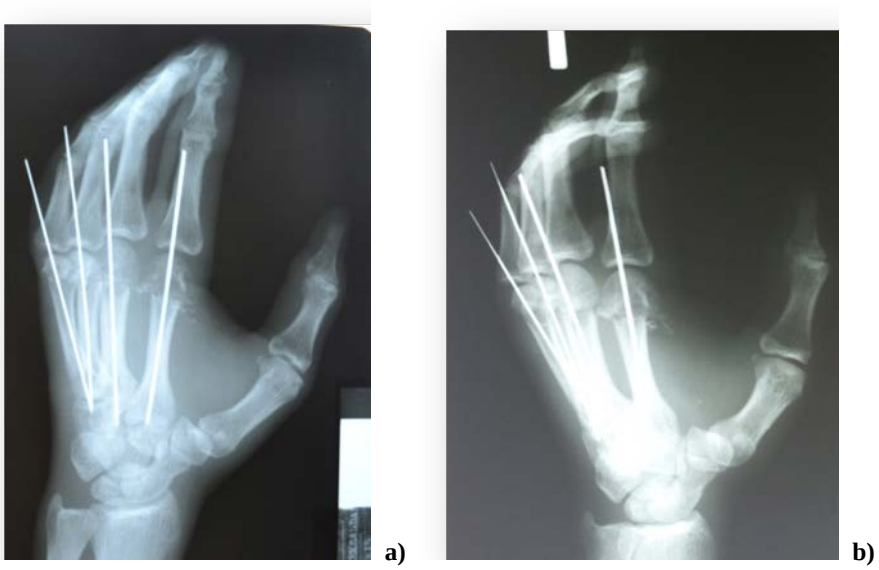
Fig. 1 – a) left-hand stamp; b) amputated hand – dorsal side; c) amputated hand – palmar side; d) intraoperative result after replantation.

electrical stimulation. Transcutaneous and neuromuscular electrical stimulation were used as well. At six months, the Tinel sign hand reached his fingertips. Then, he was admitted to the hospital for tenolysis of flexor tendons of the index and middle finger of the left hand five months after the replantation. The patient's follow-up was done one month, six months, two years, and seven years after the surgery. Figure 3 shows the postoperative result after TR (two-year follow-up).

After a period of occupational therapy, the patient regains good cosmetic and functional results with two-point discrimination equal to 10 mm. At seven months, grip strength was 12 kg, and key pinch strength was 3 kg. He was employed full-time, reported no difficulty or mild difficulty with most daily activities, and preferred using his replanted hand.

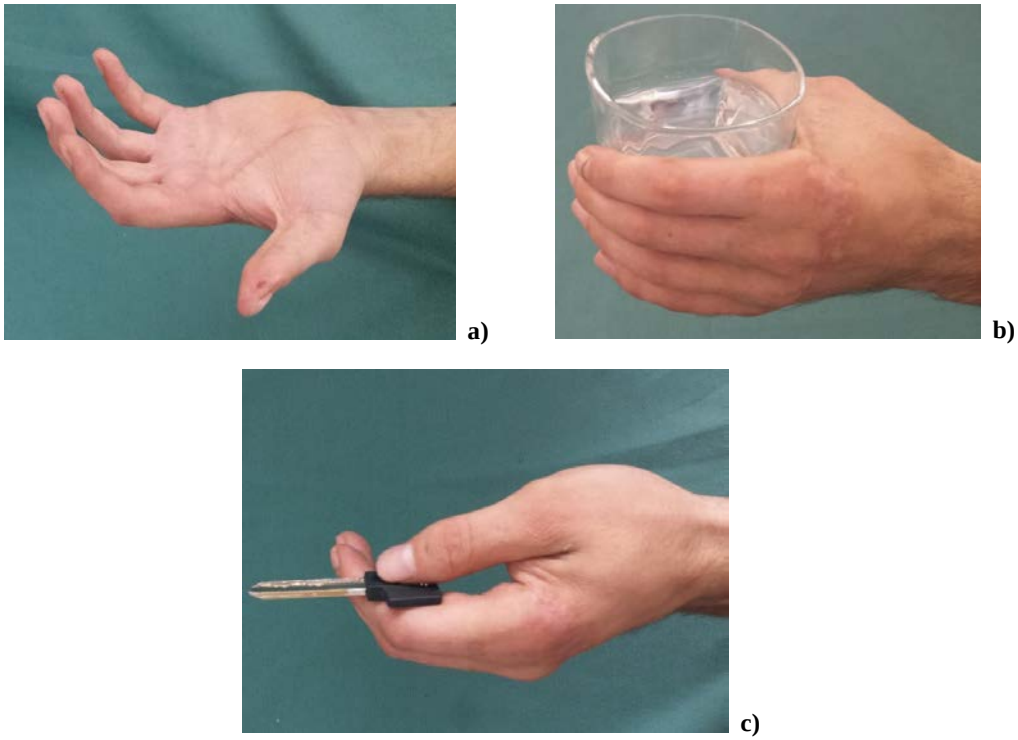
Discussion

The goals of TR are to restore circulation and reestablish the function and sensation of the amputated hand. The vascular supply to the palmar surface of the hand and



a) **b)**

Fig. 2 – a) and b) Postoperative plain radiograph showing the Kirschner wires (K-wires) fixation after transmetacarpal replantation.



a) **b)** **c)**

Fig. 3 – a), b), and c) Postoperative results after transmetacarpal replantation (2-year follow-up).

atypical anatomical variants of the arteries must be well-known as they are essential for the successful result [5–7]. Suppose the volar metacarpal vessels are not ligated. In that case, they may allow continued bleeding into the palm following the reattachment of TA. Still, they can also cause compression and hematoma on the microvascular anastomoses of the arterial blood vessels [8]. For this reason, the key to the survival of the replanted fingers is close monitoring of vascular patency in the immediate postoperative period. It can be achieved by monitoring the perfusion of fingers and evaluating the color, pulp turgor, capillary refill, and temperature of the replanted digits [8, 9]. Furthermore, the most important factors influencing the long-term results of TR are the type and level of amputation, ischemia time, history of diabetes, age, gender, smoking, and postoperative care [10]. According to the literature [11, 12], the survival rate in metacarpal level replantation varied between 66% and 100%, showing that laceration and guillotine-type injuries had the best survival rate. They reported that the success of the surgery was good because the arterial system of the fingers is anatomically well described, and there are no difficulties when preparing and performing microsurgical unions. However, achieving an efficient venous union is challenging because microclots forming in an incompetent union may add to thrombosis of the replanted hand [13].

Based on our experience and numerous reports [2, 3, 11, 12], we see that the main factors contributing to the poor functional results after TA are the type and level of amputation and intrinsic muscle ischemia. In crush injuries with an extensive zone of injury, the intrinsic muscles are usually irreparably damaged. For this reason, vast numbers of authors recommend the debridement of injured intrinsic muscles and early protective active mobilization with anti-claw splinting initiated 72 hrs after the replantation [3, 9, 11, 14]. Furthermore, prolonged ischemia time when performing TR can compromise the functional results. In this sense, the amputated hand should be brought to the operating room as soon as possible, and all vital structures must be identified to save time and minimize the ischemia of the fingers. During the replantation procedure, osteosynthesis of the fractured metacarpal bones, tendons repair, arterial and venous anastomoses, neurorrhaphy, debridement of interosseous muscles, and skin coverage must be performed [8].

In this case, we performed TR at the level of MPJ with soft tissue reconstruction of the left thumb. TA destroyed the articular cartilage, capsule, and ligaments of MPJ II–V. This level of amputation with the destruction of joint structures may cause contractures of MPJ and impair the functional result. Furthermore, this type of replantation is most complicated since we need to restore many blood vessels, soft tissue structures, and metacarpal bones. In addition, it is well-known that the reconstructive method depends on the level of the injury related to the superficial palmar arch. If the amputation is at the level of the superficial palmar arch, only the superficial palmar arch must be reconstructed. On the other hand, if the amputation level is distal to the superficial palmar arch, each common palmar digital artery must be repaired [15]. In our case, since the amputation occurred distally, each common palmar digital artery was restored. Likewise, we tried to achieve some recovery of the intrinsic function by shortening the bone and resection of devitalized intrinsic muscles. This concept was described by Paavilainen et al. [16], showing that four of ten patients with intrinsic tendon repair achieved some finger abduction and MPJ flexion.

The patient's level of functional recovery was assessed as follows: the ability to return to work, recovery of sensibility and muscle power. According to Zhong-Wei et al. [17] criteria, pinch and grip strengths, return of sensibility, and functional recovery were good in our case. Moreover, Zhong-Wei et al. [17] reported that the functional result after TR was satisfactory in 59% of patients, while Scheker et al. [18] had poorer results. The discouraging results have been primarily linked to the inadequate recovery of intrinsic muscle function, which is due to direct intrinsic muscle injury, ischemia, or postoperative scarring [12, 18].

Conclusion

The replantation surgery requires a specialized department and a well-educated team of hand microsurgeons, orthopedic surgeons, and anesthesiologists. Our patient was very happy with his replanted hand, which helped him return to everyday activities and have a good quality of life.

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

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

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

Literatura

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

Primeri referenci:

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

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

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

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

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

Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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