YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

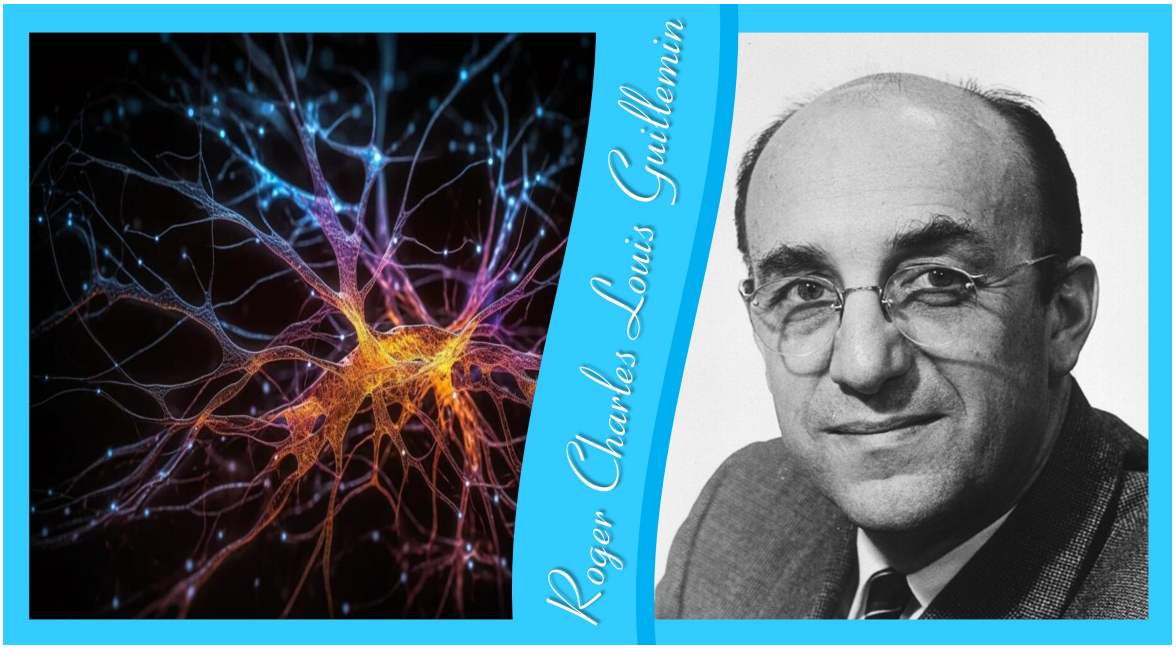
Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2024; January Vol. 81 (No. 1): pp. 1–64.

Vojnosanitetski Pregled 2024 January Vol. 81 (No. 1): pp. 1–64.



https://en.wikipedia.org/wiki/Roger_Guillemin

Vol. 81, No. 1, 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

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

ISSN 0042-8450
eISSN 2406-0720

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

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

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

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 81, br. 1, 2024

VOJNOSANITETSKI PREGLED

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

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

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

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

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

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

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

EDITORIAL / UVODNIK

Dragana Vučević
***Vojnosanitetski pregled* in 2024 - 80 years of existence**
Vojnosanitetski pregled u 2024 - 80 godina postojanja 5

GENERAL REVIEW / OPŠTI PREGLED

Julija Radojičić, Branislav Trifunović, Aleksandra Radojičić, Tatjana Čutović
Orthodontic therapy in children with bilateral cleft lip and palate: presurgical orthopedic aspects in the newborn period
Ortodontska terapija kod dece sa bilateralnim rascepom usne i nepca: prehirurški ortopedski aspekti u periodu novorođenčeta..... 9

ORIGINAL ARTICLES / ORIGINALNI RADOVI

Nora Mihalek, Dragana Radovanović, Sanja Starčević, Jelena Vukoje, Daniel Juhas
Hyperoxia therapy for prevention of postoperative nausea and vomiting after breast cancer surgery
Terapija hiperoksijom u prevenciji postoperativne mučnine i povraćanja nakon operacije karcinoma dojke 18

Nemanja Stevanović, Aljoša Mandić, Slobodan Maričić, Gabriel Stefan Nadj, Tamara Maksimović, Nevena Stanulović, Vladimir Čančar, Milan Rodić
Survival analysis of patients with rare tumors of the uterine corpus – carcinosarcomas
Analiza preživljavanja bolesnika sa retkim tumorima tela materice – karcinosarkomima 27

Nataša Kovač, Maja Samardžić Lukić, Nataša Kaćanski, Aleksandra Kovač, Tijana Latinović, Jovanka Kolarović
Urinary tract infections in children with cancer and febrile neutropenia – single center experience
Infekcije mokraćnih puteva kod dece obolele od malignih tumora sa febrilnom neutropenijom – iskustvo jednog centra.. 34

Vidna Karadžić Ristanović, Selena Gajić, Ana Bontić, Jelena Pavlović, Aleksandra Kezić, Jovana Radovanović, Milan Radović
Evaluating the renoprotective effectiveness of sodium-glucose co-transporter 2 inhibitor therapy in patients with chronic kidney disease: a prospective study
Procena renoprotektivne efikasnosti terapije inhibitorima natrijum-glukoznog kotransportera tipa 2 kod bolesnika sa hroničnom bolešću bubrega: prospektivna studija..... 39

Dragan Djurdjević, Aleksandra Nikolić, Sanja Mazić, Sandra Šipetić-Grujičić
Association between eating habits and low physical activity in adolescents
Povezanost između navika u ishrani i nedovoljne fizičke aktivnosti među adolescentima..... 45

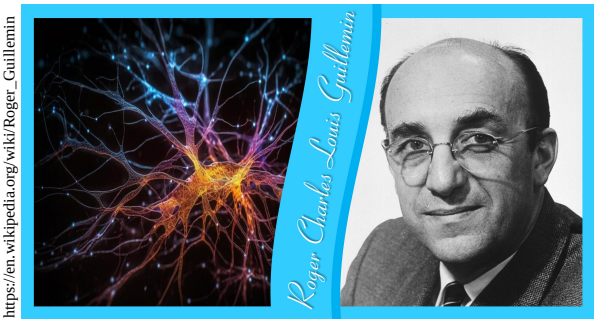
CASE REPORTS / KAZUISTIKA

Tanja Tirnanić, Tatjana Radević, Andrea Djordjević, Nenad Petrov, Željko Mijušković
Pancreatic panniculitis associated with periampullary duodenal diverticulum
Panikulitis pankreasa udružen sa periampularnim divertikulumom duodenuma..... 52

Isidora Arsenović, Danijela Leković, Dijana Šefer, Jelena Ivanović, Mihajlo Smiljanić, Andrija Bogdanović
Thymic hyperplasia as a rare etiology of pure red cell aplasia
Hiperplazija timusa kao retka etiologija čiste aplazije eritroidne loze..... 56

BOOK REVIEW / PRIKAZ KNJIGE 61

INSTRUCTIONS TO THE AUTHORS / UPUTSTVO AUTORIMA..... 63



https://en.wikipedia.org/wiki/Roger_Guillemin

In January this year, one of the founders of the field of neuroendocrinology, Roger Charles Louis Guillemin (January 11, 1924, Dijon, France), celebrated his 100th birthday. Roger Guillemin has received numerous awards and recognitions for many decades of fruitful work. In 1977, he received the Nobel Prize in Physiology or Medicine (which he shared with Andrew V. Schally and Rosalyn Yalow) for their discoveries concerning the peptide hormone production of the brain. Among the hypothalamic hormones that Roger Guillemin and his associates discovered and isolated were thyrotrophin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), and somatostatin.

U januaru ove godine, jedan od utemeljivača oblasti neuroendokrinologije, Rože Šarl Luj Gijman (11. Januar 1924, Dižon, Francuska), proslavio je 100. rodjendan. Za svoj višedecenijski plodotvoran rad, Rože Gijman dobio je mnogobrojne nagrade i priznanja. Godine 1977. dobio je Nobelovu nagradu za fiziologiju ili medicinu (koju je podelio sa Endru Šelijem i Rozalin Jelou) za otkrića koja su povezana sa produkcijom peptidnih hormona u mozgu. Medju hormonima hipotalamusa koje su Rože Gijman i njegovi saradnici otkrili i izolovali su oslobađajući hormon tirotropina (TRH), oslobađajući hormon gonadotropina (GnRH) i somatostatin.

E D I T O R I A L

UDC: 655.41
DOI: https://doi.org/10.2298/VSP2401005V

Vojnosanitetski pregled in 2024 - 80 years of existence

Vojnosanitetski pregled u 2024 - 80 godina postojanja

Dragana Vučević

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

Introduction

The year 2024 marks the 80th anniversary of the *Vojnosanitetski pregled* (VSP), an official scientific journal of physicians, dentists, and pharmacists of the Serbian Army. The first issue of the VSP was published in September 1944, continuing the tradition of *Vojnosanitetski glasnik*, which was published between 1930 and 1944. VSP, along with the *Serbian Archive for entire medical staff*, is the oldest professional medical journal in the entire region. For this multidecade continuous existence, the gratitude goes to the editors, reviewers, and authors, as well as to all the members involved in the process of publishing. This year is significant for the Medical Services of the Serbian Army, not only because of the VSP jubilee, but also because we are celebrating 180 years of the establishment of the Central Military Hospital, whose tradition has been continued by the Military Medical Academy, 185 years from the first organized action of the Military Medical Service, and 15 years since the foundation of the Medical Faculty of the Military Medical Academy, University of Defence in Belgrade. For the above-mentioned jubilees, various manifestations will be organized where it will be spoken of these significant health, scientific, and educational institutions that make our country recognizable worldwide. Our journal will also be presenting these events throughout the year. The special focus will be on the development of our journal in the previous 80 years and the possibilities of further improvement of its content.

As usual, in this issue, we will make a brief overview of the Editorial Board and Editorial Office performance in the previous year. Unfortunately, in 2023, we did not manage to complete all that was planned, but the energy and enthusiasm that we invested daily were at the highest level. Therefore, I would like to use this opportunity to express my gratitude to all the members of the Editorial Office for their efforts to publish on time all 12 journal issue numbers planned for 2023.

I would like to express my enormous respect for all the members of the Editorial Board who did not save their time and knowledge to provide their opinion on all the papers received at the Editorial Office during 2023. Special gratitude goes to Professor Silva Dobrić, BPharm PhD, a member of the Editorial Board, for her much-appreciated first-line screening regarding the scientific value of the manuscripts in pre-review manuscript selection as well as the suggestion of the names of those reviewers who guaranteed the quality of the published articles.

It was a great honor to have Academician, Full-Time Professor of Research, and Emeritus Professor of Transfusion Medicine Bela Balint, a member of the Editorial Board, who published in the November issue a review paper from his field of expertise related to basic concepts and "operating design" of cryopreserved cells (click here).

Furthermore, we were fortunate to have Research Assistant Professor Srdja Janković, MD, PhD, immunologist, and a member of the COVID-19 advisory task force of the Republic of Serbia, who shared with our readers his opinion on RNA vaccines in the author's paper titled: *RNA vaccines: a milestone toward a new era* (click here). We are especially grateful that Dr. Janković's paper was published the same month the Noble Committee awarded The Nobel Prize in Physiology or Medicine 2023 to Katalin Karikó and Drew Weissman for the discoveries critical for developing effective mRNA vaccines against COVID-19. This paper and all other published in VSP can be found on our website (https://www.vsp.mod.gov.rs/).

During the last year, VSP received fewer manuscripts for consideration of publication compared to 2022, 226 vs. 264. As usual, the largest number was from the categories "Original Articles" (147 or 65%) and "Case Reports" (43 or 19%) (Table 1).

Correspondence to: Dragana Vučević, University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: vsp@vma.mod.gov.rs

Table 1

Categories and the number of manuscripts submitted to the *Vojnosanitetski Pregled* in 2023

Category	Manuscripts n (%)
Original Articles	147 (65.0)
Case Reports	43 (19.0)
General Review	14 (6.2)
Current Topics	4 (1.8)
Letter to the Editor	4 (1.8)
History of Medicine	4 (1.8)
Book Review	3 (1.3)
Clinical Practice	2 (0.9)
Meta-analysis	2 (0.9)
Editorial	1 (0.4)
In Focus	1 (0.4)
Short Communications	1 (0.4)
Total	226 (100.0)

Analysis of submitted papers by the institutions of their authors showed that 88.6% of papers came from the so-called civilian health and academic institutions, of which about 35.6% were from abroad, and the authors of the remaining 11,4% of the submitted manuscripts were from the University of Defence in Belgrade. As before, we are especially proud of foreign authors for submitting their manuscripts, from 12 countries, not only from our region, but also from all around the world: Bosnia and Herzegovina, Bulgaria, China, Croatia, India, Iran, Montenegro, North Macedonia, Norway, Poland, Saudi Arabia, and Turkey (in alphabetical order). Thank you for your trust.

In the pre-review phase, we declined 44% of manuscripts, and 56% entered the review stage. Among reviewed manuscripts, until January 1, 2024, we accepted 42.5% for publication (after correction) in electronic form (as online first with an assigned DOI number) and rejected 28.3%. The remaining 29.2% are under the reviewing process. The total number of articles that were in the process of receiving DOI numbers in 2023 (including the ones submitted for consideration before 2023) was 79. They will be published in printing form in future issues of the Journal.

In 2023, we published 151 articles, including 2 book reviews (Table 2). The articles addressed various topics related to clinical and experimental studies obtained in the fields of medicine, dentistry, and pharmacy. As before, the majority of the articles were classified as “Original Articles” (87 or 57.6%) and “Case Reports” (43 or 28.5%), which is proportional to the number of manuscripts received from these categories in the previous years. Considering the authors’ affiliations, when analyzing the published articles, the largest number of authors were from the so-called civilian institutions, domestic and foreign (79.6%), of which about 10.4% were from abroad, followed by authors from the University of Defence in Belgrade (20.3%). Once again, I would like to thank most sincerely all the authors who chose VSP to present their results, especially the foreign ones. It is with your great support that we expect to be able to raise the academic rank of VSP.

According to the Center for Evaluation in Education and Science database, in 2023, the average time from submission to first decision was 51 days, and from submission to online ahead-of-print publication was 209 days.

We received our impact factor (IF) ranking in July 2023, and we experienced a decrease from 0.245 to 0.2. A decrease in the IF obliges us to identify areas for improvement and implement changes to ensure the ascending path of VSP in the coming years.

Table 2

Categories and the number of articles published in the *Vojnosanitetski Pregled* in 2023

Category	Articles n (%)
Original Articles	87 (57.6)
Case Reports	43 (28.5)
General Review	7 (4.6)
Current Topic	3 (2.0)
History of Medicine	3 (2.0)
Book Review	2 (1.3)
Letter to the Editor	2 (1.3)
Editorial	1 (0.7)
In Focus	1 (0.7)
Meta-analysis	1 (0.7)
Short Communications	1 (0.7)
Total	151 (100.0)

Table 3

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

Article Title	Corresponding author	N
Risk factors for cerebral palsy (DOI: https://doi.org/10.2298/VSP220209039D).	Čila Demeši Drljan	145
Prevalence and factors associated with depressive symptoms among medical students in their first and final year of study (DOI: https://doi.org/10.2298/VSP220209046C).	Dušan Čanković	137
Gender differences in suicide in Serbia within the period 2016–2020 (DOI: https://doi.org/10.2298/VSP210909015D).	Gordana Dedić	126
The occurrence of depressive symptoms in rheumatoid arthritis: a cross-sectional study (DOI: https://doi.org/10.2298/VSP211125019G).	Sonja Golubović	117
Practical analysis of the impact of social marketing strategies on attitudes of potential reproductive cell donors in the Republic of Serbia (DOI: https://doi.org/10.2298/VSP221115006D).	Biljana Đorđević	116
Analgesic protocol for procedural pain treatment of second-degree burns in children (DOI: https://doi.org/10.2298/VSP220305043K).	Aleksandar Komarčević	113

According to the EBSCO database, the number of downloads of the VSP articles published in 2023 was 3,639. In total, 6 articles were downloaded more than 100 times (Table 3), and 53 articles were downloaded more than 20 times. The highest number of downloads of a single article is 145, and the title of that article is *Risk factors for cerebral palsy* (click here). In total, 2,827 universities, colleges, libraries, and other institutions worldwide accessed the papers published in VSP more than 41,300 times. We hope that we will continue to publish educational and interesting papers for all our readers.

The greatest challenge this year will be to maintain the quality of published papers on the necessary level for the SCI list journal. The crucial part of accomplishing this task will be on the authors and their desire to publish their most valued results in our journal. Regardless of that fact, this year, as was for all the previous ones, achieving our key challenge will not be possible without the support of our reviewers. Therefore, I would like to express my deepest gratitude and respect to all our reviewers. A list of reviewers who invested their time in 2023 and who, with their expert opinion, made the selection of the highest quality papers possible is presented in Table 4.

This year, as was for the previous ones, huge challenges are awaiting. The enormous enthusiasm of the Editorial Office and Board members, the sincere support we feel from the publisher's advisory board of VSP, and the belief that our reviewers team will grow, give us hope for the bright future of our journal.

We wish each one of you and your families a happy New Year 2024 filled with good health, success, and many mesmerizing moments, and we warmly welcome submissions of excellent new articles in 2024.

Table 4

Reviewers of the *Vojnosanitetski pregled* in 2023

Alster Piotr	Brdareski Zorica	Dostanić Jelena	Ivanović Mirjana
Amornyotin Somchai	Budić Ivana	Dotlić Jelena	Ivetić Dražen
Andrić Miroslav	Budinčević Hrvoje	Dragojević Simić Viktorija	
Aničin Aleksandar	Burić Nikola	Dragović Tamara	Jakšić Vesna
Arbanas Juraj			Janković Jelena
	Colak Edis	Folić Miljan	Janković Slobodan
Babić Nikola			Jeremić Knežević Milica
Bajkin Branislav	Čolić Jelena	Gajić Veljić Mirjana	Ješić Miloš
Baletić Nenad	Čolić Miodrag	Georgievski Brkić Biljana	Jovandarić Miljana
Baljošević Ivan	Čolic Snježana	Glamočlija Sofija	Jovanović Milan
Barichello Tatiana	Čolović Hristina	Glišović Jovanović Ivana	Jovanović Simić Jelena
Basarić Dragan	Čukić Ognjen	Gomez-Lira Macarena	Jovanović Vesna
Baščarević Zoran		Grubović Rastvorčeva Rada	Jović Rajko
Begović Vesna	Dacić Sanja		Jović Zoran
Ben Saad Helmi	Dedić Gordana	Hasanbegović Ilvana	Jovičić Jelena
Biočanin Vladimir	Delić Dragan	Hladiš Andjelko	
Bogdanović Aleksandar	Dimitrijević Milovan	Hočevar-Boltežar Irena	Kalezić Nevena
Bojić Suzana	Dinčić Dragan	Huppertz-Thyssen Markus	Kandolf Lidija
Bojović Ksenija	Djenić Nemanja		Karamarković Aleksandar
Božić Marija	Djukanović Ljubica	Ilić Tihomir	Klisić Aleksandra
Brajušković Goran	Djukić Svetlana	Iqbal Mohammed	Knežević Aleksandra

Table 4 (continued)

Knežević Predrag Kocić Gordana Končar Igor Kopacheva Barsova Gabriela Kosanović Maja Kosanović Rade Kostić Dejan Kostić Milena Kostić Smiljana Kovačević Gordana Kovačević Mila Kozomara Ružica Krejović Sanja Krivošejev Vladimir Labudović Borović Milica Lazić Vojkan Lazić Zoran Leković Danijela Lepić Milan Lepić Toplica Ležaić Višnja Maglić Rastko Maksimović Miloš Mandraš Ana Manojlović Nebojša Marić Nadja Marin Saša Marković Aleksa Marković Evgenija Marković Ljiljana Marković Srdjan Marković Vasiljković Biljana Martinez Antonio Mašulović Dragan Mathis Gebhard Meera Sahib Mohammed Abdul Kader Mesaroš Šarlota Meštrović Senka Mihajlović Goran	Mijušković Mirjana Mijušković Željko Mikić Dragan Milašinović Danko Milenković Branislava Milenković Saša Milenković Zoran Milić Lemić Aleksandra Milić Rade Milinković-Srećković Mirjana Milivojević Saša Miloradović Vladimir Milosavljević Marko Mirošević Nikica Mitković Milan Mitrović Katarina Mladenović Katarina Mladenović Raša Nedeljković Predrag Nejković Lazar Nešković Vojislava Nikolić Branka Nikolić Jelena Nikolić Ljubiša Obradović Rada Obradović Slobodan Ostanek Barbara Palibrk Ivan Panjković Milana Pavlović Jasna Pažin Vladimir Pekmezović Tatjana Perrault Louis Petrović Pajić Sanja Plavšić Aleksandra Popadić Svetlana Popović Milica Puranik Chaitanya	Rabrenović Violeta Radenković Sandra Radjen Slavica Radoičić Dragan Radosavljević Aleksandra Radosavljević Davorin Rakočević Jelena Rančić Nemanja Rasulić Lukas Ristanović Elizabeta Ristić Aleksandar Ristić Andjelka Ristić Arsen Ristić Dragana Ristić Ljubiša Ristić Medić Danijela Riwanto Ignatius Rodić Predrag Roganović Branka Roš Tatjana Safiye Teodora Sarač-Hadžihalilović Aida Sentruk Mert Simić Sanja Simović Aleksandra Sotirović Jelena Srećković Miodrag Srzentić Snežana Stamenković Bojana Stamenković Dragoslav Stanković Nebojša Stanojević Ivan Starčević Srđan Stevanović Ivana Stevanović Momir Stijak Lazar Stojanović Dušica Stojanović Jasmina Stojković Mirjana Stojković Siniša Svetel Marina	Šarenac Tatjana Šćepan Ivana Škrlec Ivana Šuljagić Vesna Šurbatović Maja Tepšić Ostojić Vesna Tiodorović Danica Todorović Ljubomir Todorović Zoran Tomanić Milena Tomić Aleksandar Trifunović Zoran Unić Dragana Urošević Ivana Vasović Miroslav Vezmar Kovačević Sandra Vlahović Zoran Vrzić-Petronijević Svetlana Vučić Miodrag Vujčić Isidora Vukomanović Aleksandra Vuković Natalija Vulović Maja Wang Liang Witkowska-Zimny Malgorzata Zamurović Milena Zdraveska Nikolina Zeba Snježana Zuccatosta Lina Zvezdin Biljana Živadinović Radomir Živaljević Vladan Živanović Dragoljub Živanović Željko Živković Igor Živković Slavoljub Žuljević Dragan

GENERAL REVIEW

UDC: 616.315-007.254-089.23
DOI: https://doi.org/10.2298/VSP230728056R

Orthodontic therapy in children with bilateral cleft lip and palate: presurgical orthopedic aspects in the newborn period

Ortodontska terapija kod dece sa bilateralnim rascepom usne i nepca: prehirurški ortopedski aspekti u periodu novorođenčeta

Julija Radojičić*, Branislav Trifunović†, [1]Aleksandra Radojičić*, Tatjana Čutović‡

***University of Niš, Faculty of Medicine, Department of Orthodontics, Niš, Serbia; †University Children's Hospital "Tiršova", Belgrade, Serbia; ‡Military Medical Academy, Department of Orthodontics, Belgrade, Serbia**

[1]PhD Student

Key words:
cleft lip; cleft palate; craniofacial abnormalities; infant, newborn; orthopedic procedures; palatal obturators; treatment outcome.

Ključne reči:
usna, rascep; nepce, rascep; kraniofacijalne anomalije; novorođenče; ortopedske procedure; opturatori, palatinalni; lečenje, ishod.

Introduction

Bilateral cleft lip and palate (BCLP) represent the most severe clinical form of clefts. A child born with BCLP has a characteristic facial appearance that is noticeable immediately upon birth (Figure 1). The clinical picture is variable, and the degree of deformity can vary from mild to severe; the protrusion of the premaxilla determines the degree of its severity. Regardless of the morphological variations within this type of cleft, the clinical picture is always dramatic and severe [1] (Figure 2). BCLP are reported with a 9.2% incidence of all clefts [2]. Within this group of clefts, there is a certain

Fig. 1 – Facial appearance of one-day-old newborn with bilateral cleft lip and palate.

Correspondence to: Julija Radojičić, University of Niš, Faculty of Medicine, Department of Orthodontics, Bul. dr Zorana Đinđića 81, 18 000 Niš, Serbia. E-mail: dr.julija.radojicic@gmail.com

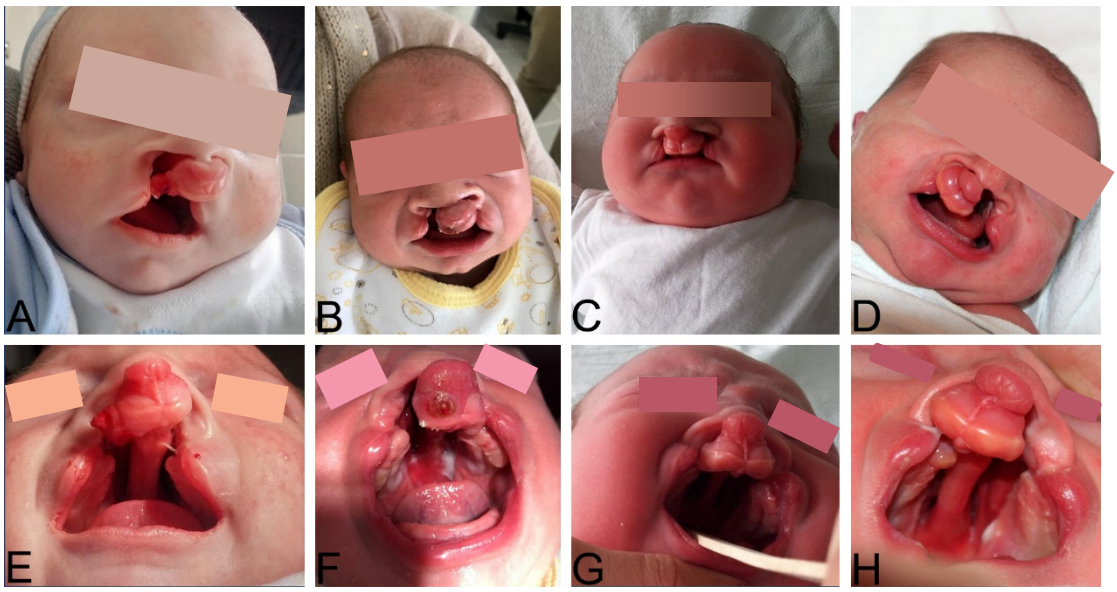


Fig. 2 – Clinical picture of bilateral cleft lip and palate (BCLP) in newborns: extraoral (A, B, C, and D) and intraoral (E, F, G, and H) presentation of BCLP in one-day-old newborns.

number of patients in whom the premaxilla is further protruded. The frequency of this subgroup of bilateral clefts is 4% of all orofacial clefts on average [3]. BCLP can be non-syndromic or part of over 400 described syndromes [4, 5]. The therapy represents a challenge, both from an aesthetic and functional perspective. It begins from the very birth of a person with a cleft and lasts for a long time. The necessity of starting therapy from the very beginning of life is justified because, otherwise, the risk of morbidity and mortality increases [6]. Surgical reparation of the lip is one of the priorities, and besides improving functionality, the goal is to achieve a more aesthetically acceptable appearance of the patient. The possibility of performing a surgical intervention and achieving good aesthetic results largely depends on the clinical picture of the patient; therefore, presurgical orthopedic care for the baby is extremely important. Despite the existence of a large number of treatment protocols, the ideal protocol has not yet been adopted either on the European or global level, although the difficulties caused by this type of cleft are significant. This paper provides an overview of different treatment protocols for infants with BCLP worldwide, as well as the treatment protocol using the RBJ (Radojičić Božidar and Julija – authors of the device) stimulator that is implemented in our country.

Bilateral cleft lip and palate

BCLP are considered the most severe clinical form of cleft lip and palate [1] and a reflection of severely violated morphological relationships of the split upper jaw. The entire upper jaw is divided into three parts so that the frontonasal process, which carries the premaxilla and the two maxillary extensions, is clearly visible. It seems that the frontonasal process protrudes out of the deepest parts of the nasal cavity, which makes the appearance of the newborn dramatic. Therefore, the procumbent and the rotated premaxilla is the stigma of this anomaly. There is also a significant increase in the width of the alar cartilage base, broadly separated lip segments, and a very short columella. Due to the disturbed physiological attachments of the muscles, the anomaly tends to worsen with growth and take on the feature of abnormality in untreated patients as they grow.

Functional, aesthetic, and therapeutic problems of patients with BCLP

The problems children with BCLP face manifest immediately after birth. Apart from other problems (malnutrition, infections, etc.), the two most significant ones are the inability to be fed through breastfeeding and significant aesthetic problems [7, 8]. Bearing in mind that they appear on the face, on the part of the body that is exposed to the views of other people and which cannot be hidden, the persons with this type of cleft also develop many psychological problems [9]. Severe aesthetic problems present at birth can become even more severe if the surgical reparation of the lip is not done perfectly. The therapy is highly complex, and the period prior to the first surgical intervention is crucial. During this time, growth is the most intense. Growth as a biological process does not differ from the growth of healthy individuals; therefore, any mistake in therapy can disrupt its course and have consequences for a lifetime.

The therapy depends on the development of the premaxilla, its size, shape, and rotation. Besides this, it also depends on the development of palatinal segments, the width of the cleft, both at the level of palatinal segments and alveolar ridges, the thickness, development, and shape of the vomer, the development of the prolabium, and the length of the columella. However, protrusion of the premaxilla remains the main stumbling block in the therapy of BCLP patients.

Protrusion of the premaxilla

The retraction of the premaxilla in its appropriate position to achieve a more functional and aesthetic reparation is the main goal in BCLP therapy [10, 11].

It is almost impossible to perform a surgical lip closure due to the considerable distance that exists between the prolabium and the muscular tissue of the lateral parts of the lip. Forced closure of these elements causes great tension in the sutures, which can lead to dehiscence. If dehiscence does not occur immediately, the consequences of such closure may become evident around the age of two. They are manifested by the vertical dropping of the premaxilla and its appearance outside the oral cavity at the level of the chin. Often, during this period, the incisors on the premaxilla have erupted, giving the entire clinical picture a very unaesthetic and undesirable appearance [3]. Such children are often teased about their "hot dog sticking out of their mouths" [12]. Correcting and returning the premaxilla to its normal position at this stage is even more difficult than in infancy. The reaction of the vomero-premaxillary suture (VPS) and the septomaxillary ligament is responsible for the increased sagittal growth of the premaxilla; thus, these two factors should always be taken into consideration from the beginning of therapy planning.

Vomero-premaxillary suture

The first evidence of the factor of protrusion was set by Veau (1934), who first correlated the excessive protrusion of the premaxilla with the excessive growth of the vomero-palatine structure resulting from the continuous formation of the bone or cartilage, but he did not histologically prove it [13]. The most convincing explanation of the factor contributing to the protrusion was established by Friede [14] in his study on the growth of VPS. It is very important for persons with BCLP because it is responsible for the rapid growth in the first postnatal months and is very fragile. Its activity is often referred to as "excessive growth" in both senses: the growth is greater than in people without a cleft, and it coincides with the protrusion of the premaxillary segment. It is a strategically important suture, as it is the only suture that can move the premaxilla to grow forward. Based on histological and X-ray analyses, he indicates the appearance of the secondary cartilage in the posterior part of the VPS, which occurs in response to mechanical loading in the period of rapid growth after birth. The secondary cartilage appears on both sides of the suture.

The septomaxillary ligament

Besides the VPS, the septomaxillary ligament contributes to the increased protrusion of the premaxilla. At the level of the VPS, this ligament connects the premaxilla to the nasal septum. At this level, a new bone is formed as a result of the tension appearing with the growth of the nasal septum and "carrying the premaxilla forward" [15]. The accelerated formation of the new bone requires the urgency of orthopedic therapy. Around the age of three, the newly formed bone, together with the surrounding bony structures and reduced tissue elasticity, compromises the success of therapeutic intervention. Verwoerd and Verwoerd-Verhoef [16] also point out the importance of caution regarding vomeral structures during therapy, highlighting their significance in the context of surgery. The part of the vomer in front of the premaxillary-vomeral suture is very fragile, and its damage can lead to irreversible consequences. For these reasons, the vomer osteotomy performed in patients with a protruded premaxilla should be done strictly distally from the VPS. In addition, with growth, there is a progressive ossification of the cartilaginous septum, extending to the perpendicular plate towards the alas of the vomer in the cranio-caudal direction. The loss of cartilage or damage to the vomer can occur as a result of surgical intervention performed between the vomer and the cartilage, which can lead to growth disturbances and deformities of the midface.

Age

It is extremely important to carry out the appropriate therapy at the very beginning of life because each mistake has consequences that cannot be corrected by any therapy.

Whether to operate on an infant or to initiate early orthodontic therapy is the question that continually raises a debate. The surgical intervention of connecting the lips in children with a highly protruding premaxilla has its specific risks. An incorrect therapy at the very beginning of life in a person with BCLP can have consequences, and the first signs of the wrong treatment can be visible at about the age of two [11]. First of all, they are related to malocclusions of dentofacial nature that can occur due to poorly performed surgical interventions of protruded premaxilla and vertically lowered premaxilla [17], or retrusion of the premaxilla and the entire middle floor of the face [13, 18]. A potential necrosis of the premaxilla is also possible. The significance of starting presurgical orthopedic therapy as early as possible is linked to the fact that the level of circulating estrogen, which the baby inherited from the mother, drastically decreases after the third month [19], as well as the contractility of the VPS [20]. After the eighth month, the contractility of the cartilaginous nasal structures is also reduced [21].

The shape and the size of the premaxilla

El-Kassaby et al. [22] study suggest a new descriptive classification of BCLP based on the characteristics of the premaxilla and indicates the dependence of the choice of therapy and the size of the premaxilla. Namely, the size of the premaxilla influences the results of both orthodontic and surgical therapies. Therefore, not all BCLP cases can be pooled under the same category or treated following the same rules, nor can we expect the same outcomes. All BCLPs were divided into the P group (protrusion of the premaxilla, longer prolabium) and the R group (resilient premaxilla, short prolabium). Small premaxilla occurs in people with a badly developed nasal septum, but the relationship of the premaxilla and the vomer is much more

flexible and better responds to orthodontic therapy (a more significant reduction in anteroposterior relationships of the premaxilla) and surgical therapy compared to those with the protruding premaxilla.

Therapy

Highly complex therapy starts at an early age and involves both presurgical orthopedic therapy and surgical therapy. These two are not mutually exclusive but rather interdependent.

Surgical therapy

In the past, the therapy for people with BCLP was solely focused on surgical lip reparation. As a consequence, a scope of different surgical techniques was developed, ranging from the osteotomy of the premaxilla, as the most radical and harmful method [18], to various surgical techniques. Some of these methods had an adverse effect on the development of the middle part of the face, which was indicated by orthodontists [23, 24]. The general impression was that after surgical procedures, patients resembled each other – they had a short columella, a stiff nasal tip, and widened nostrils. Better aesthetic results were achieved in the late 1980s. The study by McComb and Coghlan [25] showed that early neonatal surgery did not harm nasal growth, as previously thought. Consequently, the first operations, including nasal interventions, led to better aesthetic results.

Afterward, new surgical techniques were developed, aiming to improve the appearance of the nose while correcting the lip deformity, and the "columella in the nose" became a new approach in the surgical treatment of newborns with BCLP [26, 27].

Modern surgical therapy involves a large number of surgical modifications that vary among cleft centers.

Presurgical orthopedic therapy

Early on, it was realized that it was necessary to perform a retrusion of the premaxilla between previously aligned maxillary segments so as to create preconditions for good surgical therapy results, including a stable upper alveolar arch and the absence of oronasal fistulas.

Different presurgical procedures such as extraoral traction, oral pinning, premaxillary setback, and premaxillary excision [28] have been described. However, in all of the described methods, the impact on the development of maxillary growth was possible in the sense that they could have a negative impact. In the literature, two main techniques for presurgical manipulation of the premaxilla have been described – passive and active. Passive plates did not affect the reduction of the cleft size between the alveolar ridges and palatinal segments, and the retraction of the premaxilla was performed by an external force that was not part of the appliance.

Active intraoral appliances were described by Reisberg et al. [29]. Some authors directed the growth of the premaxilla in the downward and backward direction by application of force on the premaxilla [30–32]. However, some opposed presurgical orthopedic therapy. Millard et al. [33] suggest that any application of force could have a restriction impact on the growth of the premaxilla. Hotz et al. [34] thought that passive intraoral appliances should direct skeletal growth in the desired direction or even stimulate growth, which Weil [35] and Nolst et al. [36] confirmed in their studies. Both active and passive appliances incorporated into the lateral segments provide a stabilizing effect on them. All the devices described so far had different types of fixations that helped retain the apparatus in the mouth of the newborn. On the other hand, they caused certain complications to the development of the upper jaw. These complications included appliances where insertion of trans-premaxillary pins [30, 31] was performed, but this raised the probability of damaging the developing tooth buds and was not suitable for use in older age groups apart from being technique sensitive.

Georgiade technique

This technique involves the application of an active dentofacial orthopedic appliance [31]. A manually produced appliance based on the upper jaw impression is inserted into the baby's mouth under general anesthesia. Acrylic plates are secured (screwed) to the palatinal segments. A wire loop is passed through the premaxilla's neck at the level of the VPS. One elastic chain on each side connects the transmaxillary wire, goes to the posterior part of the plate, passes underneath, and then returns to the front edges of the appliance on each side. Parents turn the screw positioned in the middle of the appliance, increasing the distance between the palatinal segments only in the anterior, alveolar width of the cleft. The orthodontist reduces the length of the elastic chain, retracting the premaxilla in this way. It takes six to eight weeks to align the premaxilla between the separated palatinal segments.

The Latham technique

The Latham appliance was the most effective in correcting the premaxilla position in the anteroposterior direction. However, the movement was mostly retroinclination rather than retroposition. The teeth became retroinclined, and the vomer was slightly curved. This appliance also corrected premaxillary rotation but had a small impact on the vertical position [15]. Nasoalveolar molding (NAM) is a newer presurgical orthopedic appliance in the development of presurgical orthopedic therapy. It arose from the need to correct nasal cartilage deformity and columella tissue deficiency before a surgical lip closure, thus eliminating the need for later surgical columella lengthening. Mazaheri et al. [37] emphasize that the main advantage of NAM is the possibility of performing lip and nasal surgery in a single procedure, by which better aesthetic results are achieved.

Grayson's nasoalveolar molding technique

Grayson et al. [38] started their technique in 1990. The appliance first performs alveolar molding, reducing the alve-

olar cleft to less than 5 mm. Nasal molding is then conducted through a nasal molding element added to the basic palatal passive plate. Stretching of the short columella is done using two acrylic nasal stents on the acrylic extension attached to the passive plate. For further columella stretching, a horizontal soft strip is used, while a vertical strip is placed from the prolabium to the appliance to provide a counterforce. At the same time, the retraction of the premaxilla is gradually carried out through the serial application of the strips going to the bottom of the cheeks or the lips. The strips are changed daily by the parents. The appliance must be adjusted every week in order to modify the alveolar molding plate.

Figueroa's nasoalveolar molding technique

The appliance described by Bennun and Figueroa [39] does not use nasal extension strips. The acrylic intraoral appliance rests loosely in the mouth. During sucking and swallowing, lingual movements transmit force *via* a flexible spring to the nasal extension with silicone stops. All techniques with external traction applied directed pressure to the lower basal part of the premaxilla, causing lingual inclination and curving of the vomer. Furthermore, it was difficult to centralize a severely rotated premaxilla with passive plates and external traction.

Liou's nasoalveolar molding technique

This technique uses dental adhesive to provide retention of the acrylic plate to the maxilla. The additional nasal component consists of a curved steel wire with balls made of soft acrylic, which appear on both sides of the dental plates. They are positioned in the nostrils and make movements forward and upward. The dental plate molds the maxillary segments, while the added acrylic balls positioned under the surface of the nasal cartilages mold the nasal cartilages. The technique also uses strips over the lips in order to retract the premaxilla, bring the alar bases closer together, and reduce the alveolar cleft area in this way. That contributes to a good nose configuration. The columella is lengthened by simultaneously pushing the premaxilla backward and the nose tip forward [40]. Numerous centers have replaced their protocols in the treatment of patients with BCLP and applied this method [12, 41]. However, in order to adopt this method, further research is needed to confirm its long-term positive effects [7]. The latest method applied in patients with BCLP involves NAM, followed by simultaneous surgical lip reparation and primary rhinoplasty. That results in a columella of approximately normal length until young adulthood. However, secondary nose corrections will be necessary for one-third of the operated patients since larger widths of all nasal characteristics are present when compared to individuals without a cleft [42].

RBJ stimulator

Presurgical orthopedic therapy *via* RBJ stimulator is one of the attempts to solve the problem of a severe clinical picture of a newborn with BCLP in the best possible way [43]. The aim is to bring the premaxilla and the lateral, palatinal segments to a most proper relationship prior to the first surgical intervention, thus creating the conditions for achieving a high success rate of future surgery. This therapy is based on the biological concept and the aspect of individuality. It starts from the earliest age, at the very beginning of life, by manufacturing an obturator and a stimulator. The procedure of obtaining a two-phase impression – anatomical (Figure 3A–C) and functional (Figures 3D–F and 4A), is of crucial importance for the precision and quality of the plaster model (Figure 4B), based on which the RBJ stimulator is manually produced (Figure 4C). Great knowledge of the embryonic development of the orofacial region, as well as the "attentiveness" to the craniofacial region in persons with BCLP, the recognition of the type of growth and the experience of doctors are the key to cleft care in persons with BCLP. An orthodontist should guide and direct the growth from birth to adolescence according to the very characteristics of the growth and the development of the cleft. Although they are

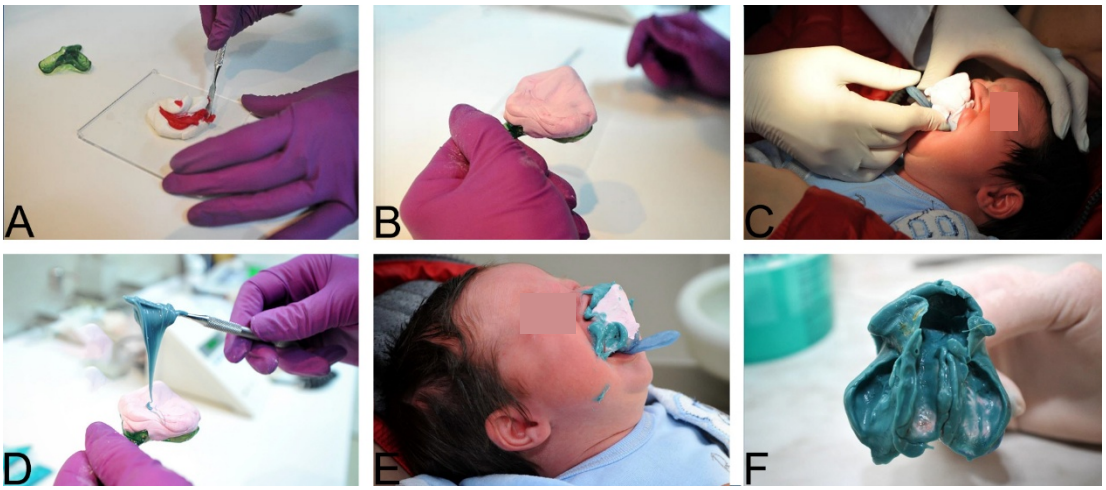


Fig. 3 – A two-phase process of obtaining the impression of the upper jaw in a newborn with bilateral cleft lip and palate: phases of obtaining the anatomical (alginate) impression (A, B and C); phases of obtaining the functional (corrective) impression (D, E and F).

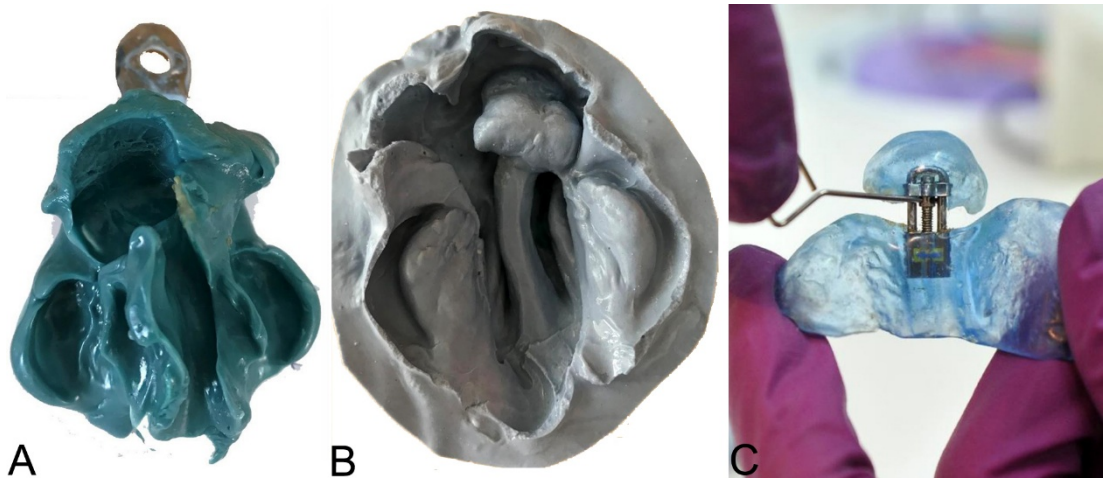


Fig. 4 – Impression (A), plaster model (B), and RBJ stimulator (C) of one-day-old newborn with bilateral cleft lip and palate. RBJ – Radojičić Božidar and Julija

similarly classified, they are not the same. Therefore, each case requires differential diagnosis and specific treatment planning. What may be the chosen treatment for one patient may be different for another, even if they have the same cleft type. Our results and attitudes regarding tracking and directing the growth are in line with Meazzini et al. [7].

Among the multitude of the described apparatuses, RBJ has its own place. For the stimulator to show its positive effect, it must have a well-insured base. Only the stimulator that intimately adheres to the upper jaw mucosa can be effective. Each shaking in the mouth, securing with the denture adhesive, various elastic strips, caps, etc., lead to the failure of the presurgical therapy [44]. A good base is established only by a precisely taken impression, which is, in fact, a key factor. A well-secured base (the secondary palate completely covered with acrylate: alveolar edges, palatine extensions with maxillary tubers included, up to the border with soft palate) is an ideal pressure site, which allows a smaller segment, premaxilla, to direct the path backward between the laterally set palatine segments using the orthodontic screw. It is best to start the therapy immediately after birth because, even after eight months, premaxillary-vomerine juncture, as well as maxillary segments, become stiff [21]. A rigid bond of acrylate that covers the premaxilla and the activation of the screw lead the premaxilla backward, affecting the premaxillary-vomerine juncture but also the nasal septum, not allowing it to continue to grow forward.

The question that arises is about the significance of the application of therapy with these devices. The stimulator does not use strong force for a short period of two to three weeks as Latham appliance, thus retracting the anterior nasal spine in conjunction with the alveolar region of the premaxilla [15].

The strategy for the development of the stimulator is in agreement with the studies of Friede and Morgan [13], who argue that an inadequate mechanical load at the VPS level can provoke border cells on both sides of the suture to form the secondary cartilage, which is responsible for excessive growth of the premaxilla, both in the sagittal and vertical directions. The most common type of such mechanical load is the inadequate extraoral or intraoral fixation of the appliance. The stimulator, as indicated, has excellent retention in the mouth of the newborn, so this kind of unpleasant pressure on the premaxilla is not present. The pressure acting on the above-mentioned structure of the damaged jaw and the force acting on the premaxilla is controlled by the stimulator design and by the speed and the number of rotations of the screw. The reduction of the protrusion of the premaxilla is performed by the stimulator through bone contraction by gradual compression, which is closely related to the mechanism of operation of the device by Liou et al. [40], and there is no resorption of the posterior part of the VPS. After the early orthodontic treatment with a stimulator and by achieving retrusion of the premaxilla, the vertical lowering of the premaxilla is avoided, which occurs if the lip surgery is performed without prior bringing the premaxilla in a corresponding sagittal position in relation to the lateral extensions. In that case, first, the resorption occurs at the posterior border (oral surface) of the vomeropalatine suture, and only then is the secondary cartilage that leads the premaxilla vertically downward created [12]. In addition, the stimulator limits the mobility of the premaxillary segment, which is also stated as one of the favorable factors in the induction of secondary cartilage – intermittent pressure. It occurs due to the absence of normal musculature, the absence of connective tissue over the split [24], and the presence of anterior pressure by the lower lips and the tongue [45]. The stimulator prevents increased mobility not only by rigidly securing the segment of the mentioned complex but also by disabling the pressure from the tongue and the lower lip. The great advantage of the RBJ stimulator in the sense of the absence of an extraoral fixation in its design is that there are no complications such as skin injuries caused by the adhesion tape, the stuffy appearance with the headband, or the patient's and parent's compliance. Furthermore, the results are not highly predictable [46].

Early presurgical therapy through stimulators is in agreement with the studies that advocate the thesis that, apart from a great knowledge of embryonic development, the

knowledge of craniofacial growth is also very important for the therapy [47]. Boo-Chai [47] stated that if the surgery has not been performed on the jaw, it will grow normally (normal relationships with other parts of the face), which means that the presence of the cleft is not the factor in the inhibition of the growth process. These findings are also consistent with Capelozza Filho et al. [48], who state that in the absence of surgery, the jaw will be able to reach the normal anteroposterior dimension. In addition, the findings are consistent with the recent study by Shetye and Evans [49] including the significance of the protrusion of point A in comparison to the control sample with the mandibular retrusion, and a much wider cranial base angle. All of this confirms our view that if the growth potential exists, it is possible to influence the life of the newborn with BCLP in the earliest days with appropriate devices, such as the stimulator. The therapy through the RBJ stimulator is conceived so that it acts safely on the VPS. The VPS is very sensitive, and poor therapy can cause its damage (severe retrusion of the middle floor of the face). Its growth can be influenced, but one has to be cautious. The therapy with the stimulator avoids complications that occur later in childhood. Nevertheless, by comparing them with the results of Oosterkamp et al. [50], in whose research a considerable retrusion of the premaxilla occurred, the retrusion through the RBJ stimulator was twice as high. The total value of the retrusion of the premaxilla was statistically significantly reduced in the course of six months due to the activation of the orthodontic screw and the construction of a stimulator that prevented the unrestricted growth of the septum and premaxilla from further sagittal "rampage". In order to avoid all the complications that can occur during the growth of a person with BCLP, it is necessary to orthodontically make an effect on the upper jaw damaged by a cleft at the earliest possible age, i.e., immediately after birth. Therefore, the results of this study show that it is possible to achieve good clinical effects in newborns with BCLP with the stimulator, although McNeil [51] proved in 1950 that it is difficult to design an apparatus that can simultaneously close the cleft and reposition the premaxilla. The application of the stimulator is considered irreplaceable in the period immediately after birth (it provides nutrition), and with all its characteristics, which primarily originate in its design without extraoral fixation, it makes excellent preparation for future successful surgical interventions, corrects severe aesthetic problems without harming subsequent growth, and reduces the need for implementing other treatment techniques (e.g., bone grafting) during growth.

If we compare the effects of the RBJ stimulator with the current NAM, its main goal is columella elongation and improvement of the aesthetic results of lip and nose surgery. A skeletal effect on the palatinal segments and protrusion of the premaxilla has not been demonstrated, except that it does not seem to have negative effects on skeletal development after craniofacial growth has been completed compared to the group of patients treated without NAM [52]. Radojičić [53] showcases the positive effects of the RBJ stimulator in a study based on a three-dimensional analysis of the effects of the early orthodontic therapy *via* stimulator of a specific construct by taking impressions, conventionally, of 50 newborns with different types of clefts (unilateral cleft lip and palate, BCLP, cleft palate) immediately after birth, and then continuously each month. Active stimulator effects are based on the application of basic biomechanical principles adapted to the individualized cleft anatomy [19, 36], excellent retention of the stimulator achieved without extraoral or intraoral fixation [20] (and thus avoiding harming of subsequent maxilla growth [54–58], effects from the first hours of life), maximal use of biological potentials – suppleness of cartilaginous structure [59], and the elimination of the complications of the ossified protruding premaxilla [21]. Until the conventional method of making RBJ stimulators is replaced by intraoral 3D scanning and the manufacturing of stimulators or feeding appliances by Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) technique, we consider that this method of manufacturing greatly influences the achievement of excellent presurgical therapy because the strategy of treatment and the design of our stimulator are identical to 3D CAD/CAM stimulators [53].

Conclusion

The presented overview of the main techniques for conducting presurgical orthopedic therapy in patients with BCLP provides the basic characteristics of their actions, positive effects, as well as drawbacks that require further modifications to achieve even better results. Thus, a definitive therapeutic treatment that could be recommended as the treatment of choice has not been selected yet. The RBJ stimulator applied in Serbia as a unique therapeutic solution is particularly highlighted. Its advantages compared to the other therapeutic techniques include the following characteristics: it is the only appliance whose palatal plate has an active function; by moving the palatinal segments, the entire zygomatic complex is moved through the sutures they are connected to, and by retracting the protruded premaxilla, the nasal complex is moved, thus influencing the orthopedics of the entire face; using the active effect skeletal base, balance is achieved, and the need for bone grafting is eliminated. Owing to these characteristics, the drawbacks of other described appliances have been overcome, and the surgical results of lip reparation after the application of orthopedic therapy using the RBJ stimulator in newborns with BCLP are of high aesthetic and functional quality. Therefore, considering all the mentioned, the application of the RBJ stimulator can be suggested as the recommended form of presurgical orthopedic therapy in patients with BCLP.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Radojicic J*, *Trifunovic B*, *Cutovic T*, *Radojicic A*. Presurgical Orthopedic Treatment of a 3-Year-Old Child with Unoperated Complete Bilateral Cleft Lip and Palate and Protruding Premaxilla. Cleft Palate Craniofac J 2023; 60(5): 627–34.
2. *Mahmood R*, *Flood T*, *Robinson S*, *Al-Gholmy M*. Early orthopedic retraction of the premaxilla in bilateral complete cleft lip and palate: an innovative approach to a difficult problem. Cleft Palate Craniofac J 2016; 53(3): 363–7.
3. *Latief BS*, *Lekkas C*, *Kuijpers MA*. Maxillary arch width in unoperated adult bilateral cleft lip and alveolus and complete bilateral cleft lip and palate. Orthod Craniofac Res 2010; 13(2): 82–8.
4. *Radojičić J*. The application of stimulator in the treatment of cleft lip and palate in Goldenhar syndrome, trisomy 13 and lobar holoprosencephaly with a median cleft lip. FU Med Biol 2018; 20(1): 24–9.
5. *Radojicic J*, *Tanic T*, *Pesic Z*, *Jovic N*, *Cutovic T*, *Filipovic G*. Rare disease: lobar holoprosencephaly with a median cleft lip-case report. Cleft Palate Craniofac J 2016; 53(1): 109–17.
6. *Shkoukani MA*, *Chen M*, *Vong A*. Cleft lip - a comprehensive review. Front Pediatr 2013; 1: 53.
7. *Meazzini MC*, *Rossetti G*, *Morabito A*, *Garattini G*, *Brusati R*. Photometric evaluation of bilateral cleft lip and palate patients after primary columella lengthening. Cleft Palate Craniofac J 2010; 47(1): 58–65.
8. *Vyas RM, Kim DC, Padwa BL, Mulliken JB*. Primary Premaxillary setback and repair of bilateral complete cleft lip: indications, technique, and outcomes. Cleft Palate Craniofac J 2016; 53(3): 302–8.
9. *Persson M*. Cleft lip and palate in adolescence identifying variables relating to psychosocial concerns [Ph.D. Thesis]. Lund, Sweden: Lund University Faculty of Medicine; 2007.
10. *ECPCA*. ECPCA Craniofacial Orthodontics Course 2022-2023. Craniofacial Anomalies and Cleft Lip and Palate: Clinical Principles and Orthodontics Management [Internet]. Milan: ECPCA; 2023 [updated 2023 June 24; cited 2023 June 26]. Available from: https://ecpcainternationalcourse2022-2023.it/
11. *Meazzini MC*, *Cohen N*, *Autelitano L*, *Radojicic J*. Non-surgical treatment of vertical excess of the premaxilla in growing bilateral cleft lip and palate patients. Int J Oral Maxillofac Surg 2021; 50(12): 1576–82.
12. *Meazzini MC*, *Brusati R*, *Bozzetti A*, *Mazzoleni F*, *Felisati G*, *Garattini G*, et al. Craniofacial Anomalies: Surgical-orthodontic Management. Bologna: Edizioni Martina; 2011.
13. *Friede H*, *Morgan P*. Growth of the vomero-premaxillary suture in children with bilateral cleft lip and palate. A histological and roentgencephalometric study. Scand J Plast Reconstr Surg 1976; 10(1): 45–55.
14. *Friede H*. Histology of the premaxillary=vomerine suture in a bilateral cleft case. Cleft Palate J 1973; 10: 14–22.
15. *Latham RA*. Development and structure of the premaxillary deformity in bilateral cleft lip and palate. Br J Plast Surg 1973; 26(1): 1–11.
16. *Verwoerd CD*, *Verwoerd-Verhoef HL*. Rhinosurgery in children: developmental and surgical aspects of the growing nose. GMS Curr Top Otorhinolaryngol Head Neck Surg 2010; 9: Doc05.
17. *Meazzini M*, *Lematti L*, *Mazzoleni F*, *Rabbiosi D*, *Bozzetti A*, *Brusati R*. Vertical excess of the premaxilla in bilateral cleft lip and palate patients: a protocol for treatment. J Craniofac Surg 2010; 21(2): 499–502.
18. *Millard DR*. Cleft Craft: The Evolution of its Surgery. II. Bilateral and Rare Deformities. Volume 2. Boston: Little Brown and Co; 1977. p. 922.
19. *Semb G*, *Shaw WC*. Facial growth related to surgical methods. In: *Berkowitz S,* editor. Cleft lip palate: Diagnosis and Management. 3rd ed. Heidelberg, Berlin: Springer; 2013. p. 325–43.
20. *Georgiade NG*, *Latham RA*. Maxillary arch alignment in the bilateral cleft lip and palate infant, using pinned coaxial screw appliance. Plast Reconstr Surg 1975; 56(1): 52–60.
21. *Mulliken JB*, *Wu JK*, *Padwa BL*. Repair of bilateral cleft lip: review, revisions, and reflections. J Craniofac Surg 2003; 14(5): 609–20.
22. *El-Kassaby MA*, *Abdelrahman NI*, *Abbass IT*. Premaxillary characteristics in complete bilateral cleft lip and palate: A predictor for treatment outcome. Ann Maxillofac Surg 2013; 3(1): 11–9.
23. *Heidbuchel KL*, *Kuijpers-Jagtman AM*, *Freihofer HP*. An orthodontic and cephalometric study on the combined surgical-orthodontic approach of the protruded premaxilla in bilateral clefts. J Craniomaxillofac Surg 1993; 21(2): 60–6.
24. *Pruzansky S*. The growth of the premaxillary-vomerine complex in complete bilateral cleft lip and palate. Tandlaegebladet 1971; 75(12): 1157–69.
25. *McComb HK*, *Coghlan BA*. Primary repair of the unilateral cleft lip nose: completion of a longitudinal study. Cleft Palate Craniofac J 1996; 33(1): 23–30; discussion 30–1.
26. *Mulliken JB*. Bilateral complete cleft lip and nasal deformity: an anthropometric analysis of staged to synchronous repair. Plast Reconstr Surg 1995; 96(1): 9–23; discussion 24–6.
27. *Trott JA*, *Mohan N*. A preliminary report on one stage open tip rhinoplasty at the time of lip repair in bilateral cleft lip and palate: the Alor Setar experience. Br J Plast Surg 1993; 46(3): 215–22.
28. *Suri S*, *Tompson BD*. A modified muscle-activated maxillary orthopedic appliance for presurgical nasoalveolar molding in infants with unilateral cleft lip and palate. Cleft Palate Craniofac J 2004; 41(3): 225–9.
29. *Reisberg DJ*, *Figueroa AA*, *Gold HO*. An intraoral appliance for management of the protrusive premaxilla in bilateral cleft lip. Cleft Palate J 1988; 25(1): 53–7.
30. *Bitter K*. Latham's appliance for presurgical repositioning of the protruded premaxilla in bilateral cleft lip and palate. J Craniomaxillofac Surg 1992; 20(3): 99–110.
31. *Georgiade NG*, *Mason R*, *Riefkohl R*, *Georgiade G*, *Barwick W*. Preoperative positioning of the protruding premaxilla in the bilateral cleft lip patient. Plast Reconstr Surg 1989; 83(1): 32–40.
32. *Papay FA*, *Morales L*, *Motoki DS*, *Yamashiro DK*. Presurgical orthopedic premaxillary alignment in cleft lip and palate reconstruction. Cleft Palate Craniofac J 1994; 31(6): 494–7.
33. *Millard DR*, *Latham R*, *Huifen X*, *Spiro S*, *Morovic C*. Cleft lip and palate treated by presurgical orthopedics, gingivoperiosteoplasty, and lip adhesion (POPLA) compared with previous lip adhesion method: a preliminary study of serial dental casts. Plast Reconstr Surg 1999; 103(6): 1630–44.
34. *Hotz M*, *Perko M*, *Gnoinski W*. Early orthopaedic stabilization of the premaxilla in complete bilateral cleft lip and palate in combination with the Celesnik lip repair. Scand J Plast Reconstr Surg Hand Surg 1987; 21(1): 45–51.
35. *Weil J*. Orthopaedic growth guidance and stimulation for patients with cleft lip and palate. Scand J Plast Reconstr Surg Hand Surg 1987; 21(1): 57–63.
36. *Nolst TG*, *Weil J*, *de Roos P*. Observation: a comment on "A discussion of presurgical orthodontics in patients with clefts". Cleft Palate J 1990; 27(4): 419–23: discussion 423–4.
37. *Mazaheri M*, *Harding RL*, *Cooper JA*, *Meier JA*, *Jones TS*. Changes in arch form and dimensions of cleft patients. Am J Orthod 1971; 60(1): 19–32.

38. *Grayson BH*, *Santiago PE*, *Brecht LE*, *Cutting CB*. Presurgical nasoalveolar molding in infants with cleft lip and palate. Cleft Palate Craniofac J 1999; 36(6): 486–98.
39. *Bennun RD, Figueroa AA*. Dynamic presurgical nasal remodeling in patients with unilateral and bilateral cleft lip and palate: modification to the original technique. Cleft Palate Craniofac J 2006; 43(6): 639–48.
40. *Liou EJ*, *Chen PK*, *Huang CS*, *Chen YR*. Orthopedic intrusion of premaxilla with distraction devices before alveolar bone grafting in patients with bilateral cleft lip and palate. Plast Reconstr Surg 2004; 113(3): 818–26.
41. *Meazzini MC*, *Chiavenna C*, *Autelitano L*, *Garattini G*, *Brusati R*. Photometric Evaluation in Adolescence of Patients With Bilateral Cleft Lip and Palate Treated With Nasoalveolar Molding and Primary Columella Lengthening. Cleft Palate Craniofac J 2018; 55(4): 568–73.
42. *Meazzini MC*, *Parravicini F*, *Donati V*, *Brusati R*, *Biglioli F*, *Autelitano L*. Photometric Evaluation of Adult Patients With Bilateral Cleft Lip and Palate Treated With Nasoalveolar Molding and Primary Columella Lengthening. Cleft Palate Craniofac J 2022; 59(7): 852–8.
43. *Radojičić J*, *Tanić T*, *Jović N*, *Cutović T*, *Papadopoulos K*. Presurgical orthodontic treatment of patients with complete bilateral cleft lip and palate. Vojnosanitet Pregl 2014; 71(7): 693–9.
44. *Nemes B*, *Fábián G*, *Nagy K*. Management of prominent premaxilla in bilateral cleft lip and alveolus. Cleft Palate Craniofac J 2013; 50(6): 744–6.
45. *Glass D*. The early management of bilateral cleft of lip and palate. Br J Plastic Surg 1970; 23(2): 130–41.
46. *Kiya K*, *Oyama T*, *Sone Y*, *Ishii N*, *Hosokawa K*. A novel active intraoral appliance for presurgical orthopaedic treatment in patients with complete bilateral cleft lip and palate. J Plast Reconstr Aesthet Surg 2015; 68(5): 632–7.
47. *Boo-Chai K*. The unoperated adult bilateral cleft of the lip and palate. Br J Plast Surg 1971; 24(3): 250–7.
48. *Capelozza Filho L*, *Normando AD*, *da Silva Filho OG*. Isolated influences of lip and palate surgery on facial growth: comparison of operated and unoperated male adults with UCLP. Cleft Palate Craniofac J 1996; 33(1): 51–6.
49. *Shetye PR*, *Evans CA*. Midfacial morphology in adult unoperated complete unilateral cleft lip and palate patients. Angle Orthod 2006; 76(5): 810–6.
50. *Oosterkamp BC*, *van Oort RP*, *Dijkstra PU*, *Stellingsma K*, *Bierman MW*, *de Bont LG*. Effect of an intraoral retrusion plate on maxillary arch dimensions in complete bilateral cleft lip and palate patients. Cleft Palate Craniofac J 2005; 42(3): 239–44.
51. *McNeil CK*. Orthodontic procedures in the treament of congenital cleft palate. Dent Rec (London) 1950; 70(5): 126–32.
52. *Meazzini MC, Parravicini F, Cohen N, Rossetti G, Autelitano L*. Nasoalveolar molding and skeletal development in patients with bilateral cleft lip and palate: A retrospective cephalometric study at the completion of growth. J Craniomaxillofac Surg 2022; 50(5): 400–5.
53. *Radojicic J*. Cleft Care: Intraoral 3D Scanning. Cleft Palate Craniofac J. 2018: 55(9): 1330.
54. *Berkowitz SA*. Comparison of treatment results in complete bilateral cleft lip and palate using a conservative approach versus Millard-Latham PSOT procedure. Semin Orthop 1996; 2(3): 169–84.
55. *Berkowitz S*. Apoplectic or apocalyptic? J Oral Maxillofac Surg 2001; 59(10): 1252–3. Erratum in: J Oral Maxillofac Surg 2001; 59(12): 1516.
56. *Berkowitz S*. Primary repair of cleft lip and nasal deformity. Plast Reconstr Surg 2002; 109(6): 2158–61.
57. *Henkel KO*, *Gundlach KK*. Analysis of primary gingivoperiosteoplasty in alveolar cleft repair. Part I: facial growth. J Craniomaxillofac Surg 1997; 25(5): 266–9.
58. *Uzel A*, *Alparslan ZN*. Long-term effects of presurgical infant orthopedics in patients with cleft lip and palate: a systematic review. Cleft Palate Craniofac J 2011; 48(5): 587–95.
59. *Scott JH*. The cartilage of the nasal septum. Br Dent J 1953; 95: 37–40.

Received on July 28, 2023
Accepted on October 3, 2023
Online First October 2023

ORIGINAL ARTICLES

UDC: 618.19-006-089:[617-089.5-06:616.33-008.3-084
DOIhttps://doi.org/10.2298/VSP230522059M

Hyperoxia therapy for prevention of postoperative nausea and vomiting after breast cancer surgery

Terapija hiperoksijom u prevenciji postoperativne mučnine i povraćanja nakon operacije karcinoma dojke

Nora Mihalek*†, Dragana Radovanović*†, Sanja Starčević*†, Jelena Vukoje*, Daniel Juhas*

***Oncology Institute of Vojvodina, Department of Anesthesiology with Reanimatology, Sremska Kamenica, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia**

Abstract

Background/Aim. Postoperative nausea and vomiting (PONV) are one of the most common causes of patient dissatisfaction in the postoperative period after general anesthesia. Hyperoxia may prevent PONV after abdominal surgery, but the effectiveness of intraoperative and early postoperative hyperoxia in preventing PONV after breast cancer surgery has not been fully elucidated. The aim of this study was to assess if the application of intraoperative hyperoxia during surgery could prevent PONV. **Methods.** Forty female patients with breast cancer were recruited for the study, all of whom underwent surgical treatment of breast cancer with axillary sentinel node sampling or axillary lymph node dissection. A balanced general anesthesia was conducted, which was induced with propofol and maintained with sevofluran. Out of the 40 patients, 20 (intervention group) received a volatile gas mixture with a fraction of inspired oxygen (FiO_2) of 0.8 L/min intraoperatively and, afterward, 3 L/min of oxygen *via* face mask for two hours after surgery. The other 20 patients (control group) received a FiO_2 of 0.4 L/min during the surgery without further administration of oxygen in the early postoperative period. The presence and severity of PONV were assessed at 30 min, 4, 24, 32, 48, and 56 hrs after surgery with the use of the PONV numerical Intensity Scale by Wengritzky for evaluating clinically significant PONV in the first six hours after surgery. Data were collected in an Excel spreadsheet and analyzed using the independent Student's *t*-test. **Results.** The overall incidence of PONV during the 30 min after the surgery was 17.5% (15% in the group of patients receiving FiO_2 of 0.8 L/min and 20% in the group of patients receiving FiO_2 of 0.4 L/min intraoperatively). There was no statistically significant difference between the two groups in the frequency of PONV, as well as in the severity of PONV, measured with the PONV Intensity Scale by Wengritzky ($p \geq 0.05$). **Conclusion.** We found no benefit of intra- and post-operative hyperoxia in reducing the incidence of PONV. The data do not support routine administration of hyperoxia, in addition to antiemetics, for the prevention of PONV in patients undergoing breast cancer surgery.

Key words:
anesthesia, general; breast neoplasms; hyperoxia; nausea; oxygen inhalation therapy; postoperative period; surgical procedures, operative; vomiting.

Apstrakt

Uvod/Cilj. Postoperativna mučnina i povraćanje (*postoperative nausea and vomiting* – PONV) su jedan od najčešćih razloga nezadovoljstva bolesnika u postoperativnom periodu posle opšte anestezije. Hiperoksija može sprečiti PONV posle abdominalne hirurgije, međutim, efikasnost intraoperativne i rane postoperativne primene hiperoksije u prevenciji PONV posle operacije karcinoma dojke nije do kraja razjašnjena. Cilj rada bio je da se proceni da li primena hiperoksije tokom operacije može sprečiti PONV. **Metode.** U studiju je bilo uključeno 40 bolesnica sa karcinomom dojke, podvrgnutih operativnom lečenju, kojima su uzorkovani aksilarni limfni čvorovi "stražari" ili disektovani aksilarni limfni čvorovi. Sprovedena je balansirana opšta anestezija, koja je indukovana propofolom, a održavana sevofluranom. Od 40 bolesnica, njih 20 (grupa sa intervencijom) je intraoperativno primilo inhalacionu smešu gasova sa udahnutom frakcijom kiseonika (*fraction of inspired oxygen* – FiO_2) od 0,8 L/min i zatim još 3 L/min kiseonika putem maske za lice, tokom dva sata nakon operacije. Drugih 20 bolesnica (kontrolna grupa) primilo je FiO_2 od 0,4 L/min tokom operacije, bez dalje primene kiseonika u ranom postoperativnom periodu. Prisustvo i težina PONV bili su procenjivani 30 min, 4, 24, 32, 48 i 56 sati nakon operacije primenom Wengritzky-jeve PONV numeričke skale za procenu inteziteta klinički značajne PONV, tokom prvih šest sati nakon intervencije. Podaci su bili prikupljeni u *Excel* tabeli i analizirani pomoću nezavisnog Studentovog *t*-testa. **Rezultati.** Ukupna incidenca PONV tokom

Correspondence to: Nora Mihalek, Oncology Institute of Vojvodina, Department of Anesthesiology with Reanimatology, Put doktora Goldmana 4, 21 208 Sremska Kamenica, Serbia. E-mail: noramihalek@gmail.com

30 min nakon intervencije iznosila je 17,5% (15% u grupi bolesnica koje su primale FiO_2 od 0,8 L/min i 20% u grupi bolesnica koje su primale FiO_2 od 0,4 L/min intraoperativno). Nije bilo statistički značajne razlike između dve grupe ispitanica u učestalosti PONV i težini PONV, merenih pomoću Wengritzky-jeve numeričke skale za procenu intenziteta PONV ($p \geq 0,05$). **Zaključak.** Nije dokazana korist od intraoperativne i rane postoperativne primene hiperoksije u prevenciji PONV. Dobijeni rezultati ne podržavaju rutinsku primenu hiperoksije pored antiemetskih lekova, u cilju smanjenja učestalosti PONV kod bolesnica posle hirurškog lečenja karcinoma dojke.

Ključne reči:
anestezija, opšta; dojka, neoplazme; hiperoksija; mučnina; lečenje inhalacijom kiseonika; postoperativni period; hirurgija, operativne procedure; povraćanje.

Introduction

The subjective feeling of nausea or vomiting in the first 48 hrs after surgery (postoperative nausea and vomiting – PONV) represents one of the most common reasons for patient dissatisfaction in the postoperative period. Vomiting is assumed to be the most undesirable outcome after anesthesia [1], and many patients would prefer pain over PONV postoperatively [2]. The overall incidence of PONV is approximately 30% after balanced general anesthesia [3, 4]; however, in high-risk patient populations, it can be as high as 80% [5]. Although PONV seldom leads to any serious medical conditions (such as aspiration pneumonia, wound dehiscence, or even anastomotic leak formation due to emetic strains) in the modern clinical setting, severe vomiting can cause dehydration, electrolyte imbalance and negatively impact patient satisfaction with anesthesia care providers, as well as generate increased hospital costs (delayed postsurgical mobilization and longer hospital stay, restricted ambulatory surgery) [6].

Numerous drugs (e.g., opioid analgesics, volatile anesthetics, nitrous oxide) applied during general anesthesia affect the release of neurotransmitters (e.g., acetylcholine, dopamine, histamine, substance P, serotonin) contributing to the development of nausea and vomiting and the function of receptors in the central emetogenic areas (*area postrema*, *nucleus* in the solitary tract). Opioids also determine the gastrointestinal tract mechanics by decreasing gastric emptying, intestinal motility, and peristalsis [7].

The results of several randomized controlled trials prove that volatile anesthetics and opioid analgesics potentiate the development of PONV [8–10]. Therefore, total intravenous (IV) and regional anesthesia (if applicable) are superior to balanced general anesthesia in the prevention of PONV. It is established that anesthesia (and surgery) duration plays an important role in inducing PONV. The most common patient-specific risk factors are female gender, younger age, non-smoking status, and history of PONV or motion sickness [5]. Moreover, intense preoperative anxiety, insufficient perioperative fluid administration [11], or obesity can also enhance the development of PONV [12]. Morita et al. [13] reported a significantly higher incidence of PONV in patients undergoing breast cancer surgery with desflurane anesthesia, especially in the early postoperative period. According to the Enhanced Recovery After Surgery – ERAS protocol [14, 15], all patients, even those with no existing risk factors for PONV, should receive monoprophylaxis; however, patients with the Apfel Simplified Risk Score (ASRS) of 1 or 2 should be administered two antiemetics, and patients with high-risk of developing PONV should receive three to four antiemetics for prophylaxis [16, 17]. In modern clinical settings, clinicians are encouraged to pay special attention to lowering the risk for PONV by preferring propofol-based, i.e., total IV anesthesia, avoiding volatile analgesics and nitrous-oxide exposure, or applying opioid-sparing analgesia. Nevertheless, for pharmacologic prevention, a multimodal approach with drugs that act differently is recommended [18] (Figure 1).

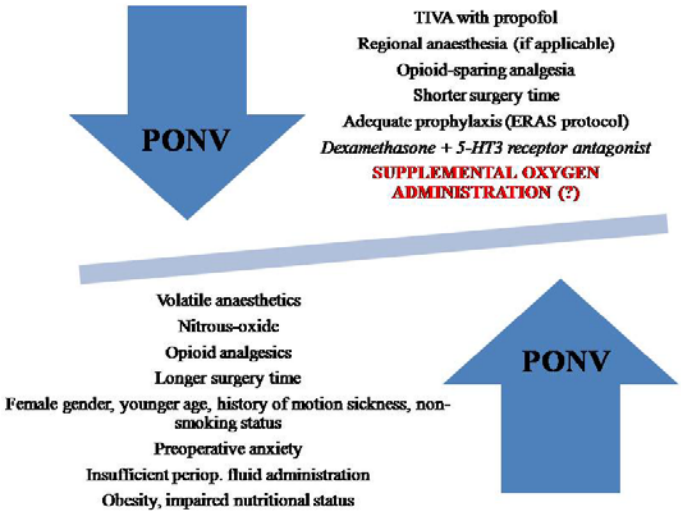


Fig. 1 – Summary of factors contributing to postoperative nausea and vomiting (PONV) and preventing PONV.
TIVA – total intravenous anesthesia; ERAS – enhanced recovery after surgery; 5-HT3 – 5-hydroxytryptamine (serotonin) 3 receptor.

According to the recommendations of the World Health Organization (WHO), supplemental oxygen administration in the intraoperative and early postoperative period may reduce the occurrence of surgical site infection (SSI) and the incidence of PONV [19]. Although the routine use of hyperoxia in the anesthesiology practice is controversial because of the possible adverse effects (increased oxidative stress, lung atelectasis, and hyperoxia-related vasoconstriction in the coronary arteries, with a sequential decrease in myocardial perfusion), the results of a recently published meta-analysis show, that the administration of fraction of inspired oxygen (FiO_2) of 0.8 L/min in the perioperative setting is free of complications [20]. Intestinal tissue is highly metabolically active and has poor tolerance for hypoxia; therefore, even short periods of insufficient perfusion in the intestinal tract can lead to the release of serotonin – an emetogenic substance [21]. It is supposed that supplemental oxygen administration may prevent intestinal hypoxia and reduce the incidence of PONV; thus, hyperoxia is a potentially highly available and cost-effective alternative treatment modality for the prevention of PONV.

In some highly vulnerable patient populations, more tremendous PONV prevention is required. In the cases of oncological patients' nutritional status may be modified due to chemotherapy-induced nausea and vomiting, decreased caloric intake, malabsorption, or even cachexia [22]. These factors lead to a higher incidence of PONV in cancer surgery and the patients undergoing balanced general anesthesia frequently require an intense multimodal approach for the prevention of PONV. Therefore, the aim of this study was to assess whether the intraoperative and early postoperative administration of supplemental oxygen reduces the incidence of PONV and thus evaluate the potential of hyperoxia as an alternative additional treatment modality in cancer patients.

Methods

The study was conducted between February and June 2022 at the Department of Anesthesiology with Reanimatology of the tertiary referral, high-volume center for cancer treatment in Vojvodina, Serbia. Ethical approval of the study protocol was obtained from the institutional Ethics Committee of the Oncology Institute of Vojvodina (approval No. 4/21/2-2611/2-3, from September 08, 2021). Written informed consent was obtained from all patients.

Forty female patients older than 18 years undergoing breast cancer surgery (quadrantectomy, segmentectomy, amputation, or mastectomy) with axillary sentinel node sampling or axillary lymph node dissection were approached for this study. Exclusion criteria were the following: patients receiving neoadjuvant chemotherapy prior to this surgery, the impossibility of completing the surgical procedure (metastasis, neighboring organ infiltration), anesthesia time shorter than 60 min, and patients who declined study participation. The Revised Cardiac Risk Index (RCRI) [23] was used to assess the patient's risk of developing perioperative cardiac complications. All potential subjects who had more than one risk factor and, hence, risk for cardiac death, nonfatal myocardial infarction, or nonfatal cardiac arrest greater than 6% were excluded from the study. According to the non-cardiac surgery guidelines of the European Society of Cardiology and European Society of Anesthesiology, the surgical procedures conducted in this study are associated with a low risk of developing major adverse cardiac events (30-day risk of cardiovascular death and myocardial infarction lower than 1%) [24].

Balanced general anesthesia with propofol induction (1.5–2.5 mg/kg) and sevoflurane maintenance (1.2%–1.4% end-tidal concentration or minimum alveolar concentration of 1–1.2) was conducted. Non-depolarizing muscle relaxant rocuronium was administered with the induction dose of 0.6 mg/kg IV and boluses of 0.1–0.3 mg/kg IV according to clinical requirements. Analgesia was provided with boluses of fentanyl (50–100 μg IV) – first dose at anesthesia induction (50 μg IV) and intraoperative boluses according to hemodynamic response to pain. At the end of the surgery, all the patients received metamizole (2.5 g IV) and ondansetron (4 mg IV), and the 8-hour dosing of these analgesic and antiemetic drugs was continued for two days. A laryngeal mask was used for airway management, and pressure-regulated volume control ventilation was ensured in all examined patients.

The patients were randomly assigned to one of the two investigated groups. In the intervention group, the patients received intraoperatively a volatile mixture with FiO_2 of 0.8 (80%) and 3 L/min of oxygen *via* face mask in the post-anesthesia care unit and the surgical ward for two hours after the surgery. Patients assigned to the control group received a volatile mixture with FiO_2 of 0.4 (40%) during surgery without further administration of oxygen in the early postoperative period. Except for the FiO_2 in the volatile mixture administered intraoperatively and the early postoperative administration of oxygen in patients in the intervention group, the entire perioperative and intraoperative treatment and administered medications (anesthesia drugs, muscle relaxants, antimicrobial, analgesic, antiemetic drugs, and thromboprophylaxis) were identical in the two groups.

The following intraoperative monitoring was conducted in all patients: electrocardiogram (leads II and V_5), non-invasive blood pressure measurement in 5-minute intervals, pulse oximetry, monitoring of ventilation parameters, capnography, and anesthesia gas monitoring. Non-invasive blood pressure monitoring and pulse oximetry were conducted in the postanesthetic care unit as well as in the surgical ward three times a day.

The ASRS [5] was used to estimate the patient's risk for developing PONV. It includes four risk factors: female gender, non-smoking status, history of PONV or motion sickness, and postoperative use of opioid analgesics. Patients with ASRS of 2 are considered medium-risk patients for developing PONV, and ASRS ≥ 3 is associated with a high risk of PONV.

The presence and severity of PONV were assessed at 30 min, 4, 24, 32, 48, and 56 hrs after surgery, using a five-grade scale, which was earlier applied [25] for estimating PONV in cancer surgical patients. The grading system was used as follows: grade 1 – no signs of PONV; grade 2 – minor nausea; grade 3 – mild nausea and vomiting; grade 4 –

severe nausea and vomiting; grade 5 – incoercible vomiting. A numerical PONV Intensity Scale [26] was applied to evaluate clinically significant PONV in the first six hours after surgery. The key scored features were intensity, pattern and duration of nausea, as well as the number of vomiting. A clinically important PONV is defined as a total score ≥ 50 at any time throughout the study period. PONV scores, patient's vital parameters, and eventually observed postoperative complications were recorded in the examination sheet.

Sample size calculation was used in order to determine the adequate total number of included patients. The confidence level was set to 95% and the margin of error to 5%. The incidence of PONV is 20% in patients treated according to institutional standards at our clinic who have not received neoadjuvant chemotherapy, undergoing general anesthesia for breast cancer surgery; therefore, the expected population proportion was set to 20%. We applied the adjusted sample size calculation, taking into consideration the examined population size. The total number of patients fulfilling the inclusion criteria during the study period in our hospital was 48. Hence, according to the adjusted sample size calculation, the adequate number of included subjects for obtaining statistical relevance was 40.33. This sample size was divided into two equal proportions, and the included patients were randomly assigned into one of the two groups (intervention group and control group). Forty female patients undergoing breast cancer surgery were recruited for this study – 20 (50%) patients were assigned to the intervention group and 20 (50%) to the control group. The total number of eligible patients to enter the investigation after screening was 48; however, one patient was excluded because of canceled surgery. Five patients were not included in the investigation because of rejection to participate in the study or because of the temporary absence of the investigators at the time of the surgery. The total number of randomized patients was 42, but two patients were excluded because of intraoperative hemodynamic instability and deviation from the study protocol through additional nitrous-oxide administration; therefore, the total number of included patients was 40 (Figure 2).

Data was collected in Excel and SPSS Software (IBM, Chicago, USA) was used for data analysis. Categorical data were analyzed with Pearson's Chi-square test or Fisher's exact test and quantitative data were analyzed using the independent Student's *t*-test for significance. Data were presented as numbers (percentages) or median (interquartile range – IQR) and statistical significance was set at $p \leq 0.05$.

Results

The median age of the included patients was 60 (IQR:19.5) years; most of them (90%) were classified as physical status II according to the American Standards Association (ASA), and 10% were in status ASA III. The most common comorbidities were arterial hypertension (n = 21; 52.5%), hypothyroidism (n = 8; 20%), and varicose veins of the lower extremities (n = 6; 15%). The most frequently performed surgical procedure was quadrantectomy with axillar sentinel node sampling (n = 27; 67.5%) or with axillar lymph node dissection (n = 5; 12.5%) and subcutaneous mastectomy (n = 6, 15%). The median duration of the surgical procedure was 60 (IQR:17.5) min, with a median anesthesia time of 75 (IQR:20) min. The median length of hospital stay was 4 (IQR:1) days. There were no statistically significant differences ($p \geq 0.05$) in surgery time, anesthesia time, and length of hospital stay between the intervention group and the control group (Table 1). In the postoperative period, 85% of the subjects had no complications, but 7.5% of the patients experienced pain, 5% somnolence, and 2.5% vertigo in the first four hours after surgery.

The median value of the RCRI was 3.9 (IQR:0) in both groups. Most (92.5%) of the patients had no existing risk factors for the development of perioperative cardiac complica-

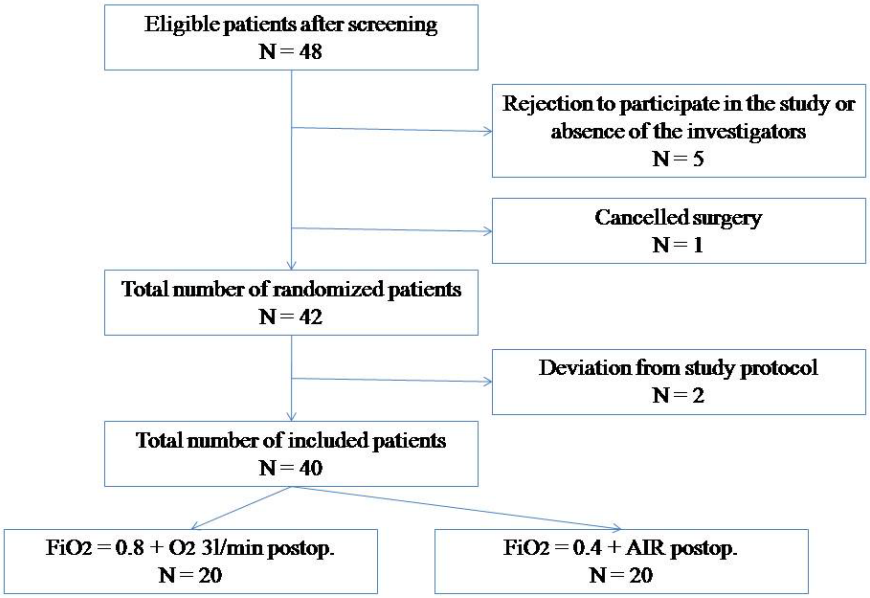


Fig. 2 – Flowchart of patient recruitment.
FiO_2 – fraction of inspired oxygen; AIR – patients without supplemental oxygen administration postoperatively; N – number of patients.

tions (estimated risk is 3.9%); nevertheless, one person in the intervention group and two people in the control group reported the presence of one risk factor (the estimated risk for developing cardiac death, nonfatal myocardial infarction or nonfatal cardiac arrest is 6%). According to the ASRS, 87.5% of the subjects had two risk factors for the development of PONV; hence, they were considered medium-risk patients. Ten percent of the patients (two people in each group) were classified as low-risk, and 2.5% (one person in the control group) as high-risk patients for the development of PONV. There were no statistically significant differences ($p \geq 0.05$) in the values of RCRI and ASRS between the two groups of patients (Table 1).

The median value of Wengritzky score was 0 (IQR:0) in both groups. As clinically important PONV was defined as a total Wengritzky score ≥ 50, there was only one person in the control group who developed clinically significant PONV. There was no statistically significant difference ($p \geq 0.05$) in the Wengritzky score between the two groups of patients.

The overall incidence of PONV was 17.5% during the first 30 min after surgery: two (10%) patients in each group experienced severe nausea and vomiting, two (5%) patients in the control group reported mild nausea, and one (2.5%) per-son in the intervention group developed minor nausea. Fifteen percent of the examined subjects reported the presence of PONV four hours after surgery: severe nausea and vomiting occurred in one (2.5%) person in the control group, two (10%) people in each group developed mild nausea, and one (2.5%) patient in the control group reported minor nausea. Twenty-four hours after the surgery, four (10%) patients experienced PONV: one (2.5%) person in the control group re-ported mild nausea, but two patients in the control group and one person in the intervention group (7.5%) experienced minor nausea. Thirty-two hours after surgery, only one (2.5%) person in the control group developed minor nausea. All the patients were without any signs of PONV 48 and 56 hrs after the surgery. There was no statistically significant difference ($p \geq 0.05$) in the incidence and severity of PONV between the two investigated groups of patients at any time point during the study period (Figures 3 and 4).

Table 1

Demographic and clinical characteristics of patients

Parameters	Intervention group	Control group	*p*-value
Age (years), median (IQR)	60 (14)	60 (21.5)	> 0.05
Physical status, n (%)			
ASA II	18 (45)	18 (45)	> 0.05
ASA III	2 (5)	2 (5)	> 0.05
Comorbidities, n (%)			
Arterial hypertension	12 (30)	9 (22.5)	> 0.05
Hypothyroidism	5 (12.5)	3 (7.5)	> 0.05
Varicose veins of lower extremities	3 (7.5)	3 (7.5)	> 0.05
Obesity	2 (5)	1 (2.5)	> 0.05
Depressive disorder	2 (5)	3 (7.5)	> 0.05
Surgery time (min), median (IQR)	62.5 (22.5)	60 (15)	> 0.05
Anesthesia time (min), median (IQR)	75 (27.5)	72.5 (17.5)	> 0.05
Hospital stay (days), median (IQR)	4 (1)	4 (0.5)	> 0.05
RCRI, median (IQR)	3.9 (0)	3.9 (0)	> 0.05
Apfel Simplified Risk Score, n (%)			
1	2 (5)	2 (5)	> 0.05
2	18 (45)	17 (42.5)	> 0.05
3	0 (0)	1 (2.5)	> 0.05

IQR – interquartile range; ASA – American Standards Association; RCRI – Revised Cardiac Risk Index; n – number of patients.

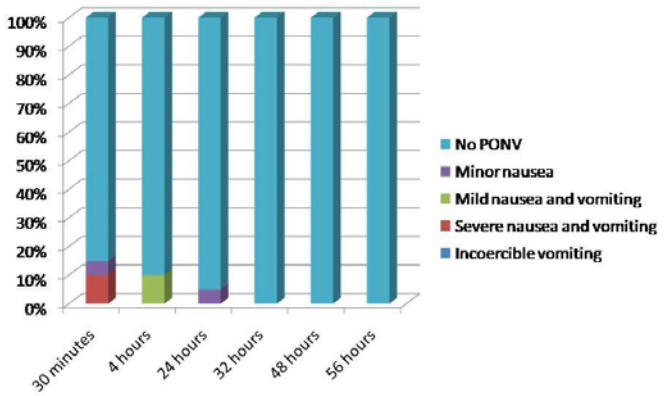


Fig. 3 – Incidence of postoperative nausea and vomiting (PONV) in the intervention group.

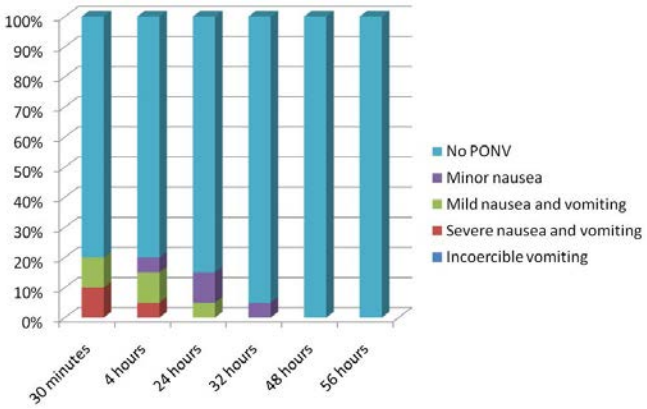


Fig. 4 – Incidence of postoperative nausea and vomiting (PONV) in the control group.

Discussion

The results of our study showed no statistically significant difference in the incidence and severity of PONV between the two groups of investigated patients. There was one person in the control group who developed clinically remarkable PONV according to the Wengritzky score, and that person was the only high-risk patient with an ASRS of 3. The incidence of PONV was the highest 30 min after surgery, and, in most of the cases, the symptoms did not persist longer than 24 hrs. There were no cardiovascular or respiratory adverse effects due to supplemental oxygen administration reported during the entire study period.

So far, no generally accepted agreement about the optimal FiO_2 concentration during general anesthesia has been made. However, in 2016, the WHO recommended that adult patients undergoing general anesthesia be ventilated intraoperatively using a volatile mixture with FiO_2 of 80%. It was supposed that hyperoxia may contribute to lowering the incidence of SSI and play an important role in the prevention of PONV [19]. The results of a recently conducted large-scale meta-analysis were heterogeneous, but the most wide-reaching included clinical trial failed to prove the effectiveness of hyperoxia in reducing the incidence of SSI. Therefore, numerous highly actual literature sources do not support the routine administration of high concentrations of FiO_2 intraoperatively, and the impact of hyperoxia on the prevention of PONV also remained unjustified [27]. Interestingly, another meta-analysis examining the hyperoxia-induced impact on SSI and PONV suggests that high FiO_2 has a beneficial effect on PONV and does not increase the risk of postoperative atelectasis. Analyzing the results of 11 trials, it was concluded that the incidence of PONV was reduced from 24.8% in patients receiving normal FiO_2 to 19.5% in patients undergoing abdominal, gynecological, breast, and thyroid surgery in high FiO_2 conditions. In patients receiving volatile anesthetics without prophylactic antiemetic drugs, hyperoxia seemed to have a strong protective effect against PONV. Moreover, the incidence of SSI also decreased in patients who, besides prophylactic antibiotics, also received high concentrations of FiO_2 [28]. According to the findings of the mentioned studies and currently available literature, the role of supplemental oxygen in the prevention of PONV remained controversial.

There are numerous meta-analyses conducted to observe the effect of perioperative administration of hyperoxia, and some of them, first of all, focus on reducing the PONV incidence. Orhan-Sungur et al. [29] found no clear benefit of the administration of high FiO_2 during general anesthesia in reducing the occurrence of PONV. On the other hand, Rincón and Valero [30] reported a decrease in the incidence of PONV due to the perioperative administration of high FiO_2, and they concluded that supplemental oxygen therapy has the potential to reduce PONV incidence but cannot replace the currently available pharmacologic treatment modalities. The largest meta-analysis, including 12 clinical trials and 5,583 patients, found no clear difference in PONV incidence between the groups of patients undergoing surgery in high (FiO_2 = 0.8) vs. low (FiO_2 = 0.3) fraction of oxygen in the inspired gas mixture. However, according to the subgroup analysis, there was a reduced incidence of PONV in patients having abdominal surgery, in contrast to the group of subjects undergoing laparoscopic gynecological intervention or some other type of non-abdominal surgery [31]. Our results correlate with the results of the newest meta-analysis, including all relevant previous trials on this topic, published in 2023. According to the findings of Markwei et al. [32], supplemental oxygen administration does not reduce the incidence of PONV after abdominal or non-abdominal surgery. Furthermore, it does not affect the severity of PONV, the number of rescue antiemetic doses given, or the time to the first rescue antiemetic drug administration.

Some earlier conducted case-control studies emphasize the impact of hyperoxia on the reduction of PONV incidence in cancer patients [33–35]. According to these studies, the incidence of nausea and vomiting was significantly lower in the group of patients with various oncological diseases who received 80% of oxygen intraoperatively and six hours after surgery, compared to the control group, and these results are in contrast to our findings. We can suppose that the main reason for the difference in the outcomes between the previous studies and our study is the relatively short exposure time in our patients' cases. Moreover, the patients in our study received a single dose of ondansetron intraoperatively, which

represents a standard treatment in our hospital. The administration of an antiemetic drug may have interfered with the results, but because of ethical issues, it was not justified to withdraw the best standard treatment. Nevertheless, Chiu et al. [36] highlight the effectiveness of a combined approach in cancer patients, i.e., total IV anesthesia with regional anesthesia (paravertebral and pectoral nerve blocks), in reducing opioid consumption and lowering the incidence of PONV after total mastectomy. Tripathy et al. [37] reported equally prolonged postoperative analgesia and low intra- and postoperative opioid requirements in the groups of patients undergoing breast cancer surgery receiving isoflurane-maintained anesthesia with pectoral or paravertebral nerve block.

According to the enhanced recovery after surgery protocol, all patients with existing risk factors for the development of PONV should receive a combination of two antiemetics for PONV prophylaxis [14]. The most commonly applied first-line treatment is a combination of dexamethasone and 5-hydroxytryptamine 3 receptor antagonist. Still, the routine administration of dexamethasone in cancer patients is controversial, as the effect of dexamethasone on the long-term oncological outcome has not been fully studied yet; therefore, a single treatment was applied in our study. Tabrizi et al. [38] reported a lower incidence of PONV following gynecological and breast surgery after routinely assessing the ASRS for PONV and implementing PONV guidelines. However, Krieser et al. [39] showed in their retrospective cohort study that female patients undergoing general anesthesia are affected by failure to adhere to PONV prevention guidelines to a disproportionately greater extent than male patients.

Several factors could affect the results of our study. Most importantly, the routine administration of ondansetron has probably highly interfered with the results. As we mentioned, we could not withdraw a single dose of antiemetic medication from our patients due to ethical issues. That being the case, we could examine if hyperoxia could be an effective additional treatment modality to the routine administration of ondansetron, but the antiemetic potential of supplemental oxygen without the usage of any other pharmacologic agent could not be assessed. Second, intraoperative analgesia was provided with boluses of fentanyl, according to the hemodynamic response to pain. This is a relatively subjective method, which, to some extent, depends on the individual practice of the anesthesia care providers. On that account, there could appear differences in opioid administration between the included subjects based on patient age, body constitution, pain response, and subjective evaluation of the anesthesiologist. Third, preoperative anxiolysis and fluid administration could affect the results. All patients received a single dose of an anxiolytic drug (bromazepam 3 mg *per os*) the evening prior to surgery; however, that is a standardized dose, which cannot reduce anxiety to the same extent in all patients. Moreover, after admission to the hospital, patients were allowed to eat until evening and drink water until midnight prior to surgery. Therefore, there were individual differences in preoperative hydration between the included patients, which could affect the incidence and severity of PONV. Fourth, anesthesia time, and thus the exposure time to volatile anesthetics, was slightly different between the included subjects, which could influence the study outcome. Fifth, neostigmine in a dose of 2.5 mg IV was administered to all patients at the end of the procedure for reversal of neuromuscular blockade. Neostigmine has a parasympathomimetic effect, increasing intestinal motility and secretion, thus potentially contributing to the development of PONV [40].

Our study has some limitations. First of all, the sample size and the occurrence of the examined phenomenon were low. According to the sample size calculation based on the prevalence of the examined clinical condition in our institution, the number of patients was sufficient, but the incidence of PONV was not high enough. Hence, to obtain stronger statistical evidence, a greater sample size is required. Furthermore, only patients were trial-blinded, but trial personnel were not. Third, the administration of a single dose of an antiemetic drug represents an indispensable part of the best standard treatment in our hospital, which could highly affect our results. Fourth, the anesthesia time and overall exposure time to hyperoxic conditions was relatively short.

Taking into consideration the systemic effects of oncological disease, cancer treatment, and a higher incidence of PONV in this population, we aimed to investigate PONV in a specific, highly sensitive patient population – breast cancer patients. Our study is unique in patient selection, and the strength of our study is in obtaining a relatively homogenous patient population with a specific disease undergoing surgical intervention and general anesthesia under identical conditions. We included only low-risk patients for the development of postoperative cardiovascular or respiratory complications as a consequence of supplemental oxygen administration. Summarizing our results and the findings of the previous years, we can suggest that supplemental oxygen should not be routinely administered intraoperatively with the expectation of reducing PONV incidence. Most of the studies reporting a favorable effect of hyperoxia in the prevention of PONV were conducted a decade ago [28, 30, 33–35]. Bearing in mind that anesthesia practice has been remarkably changed since that time, our results provide up-to-date information about the perioperative use of hyperoxia in the current clinical setting.

Conclusion

We found no benefit of intra- and postoperative hyperoxia in reducing the incidence of PONV. Results obtained do not support routine administration of hyperoxia in addition to antiemetics for the prevention of PONV in patients undergoing breast cancer surgery. For that reason, we can suggest that supplemental oxygen should not be administered routinely during general anesthesia for breast cancer surgery to prevent PONV.

Acknowledgement

This study was supported by a Collegium Talentum scholarship from the Ministry of Human Capacities (Hungarian Government) for the 2021/2022 and 2022/2023 academic years.

R E F E R E N C E S

1. *Macario A, Weinger S, Carney S, Kim A.* Which clinical anesthesia outcomes are important to avoid? The perspective of patients. Anesth Analg 1999; 89(3): 652–8.
2. *Sizemore DC, Singh A, Dua A, Singh K, Grose BW.* Postoperative Nausea [updated 2022 Nov 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK500029/
3. *Franck M, Radtke FM, Apfel CC, Kuhly R, Baumeyer A, Brandt C,* et al. Documentation of post-operative nausea and vomiting in routine clinical practice. J Int Med Res 2010; 38(3): 1034–41.
4. *Pierre S, Whelan R.* Nausea and vomiting after surgery. Cont Edu Anaesth Crit Care Pain Med 2013; 13(1): 28–32.
5. *Apfel CC, Läärä E, Koivuranta M, Greim CA, Roewer N.* A simplified risk score for predicting postoperative nausea and vomiting: conclusions from cross-validations between two centers. Anesthesiology 1999; 91(3): 693–700.
6. *Elvir-Lazo OL, White PF, Yumul R, Cruz Eng H.* Management strategies for the treatment and prevention of postoperative/postdischarge nausea and vomiting: an updated review. F1000Res 2020; 9: F1000 Faculty Rev-983.
7. *Stoops S, Kovac A.* New insights into the pathophysiology and risk factors for PONV. Best Pract Res Clin Anaesthesiol 2020; 34(4): 667–79.
8. *Vari A, Gazzanelli S, Cavallaro G, De Toma G, Tarquini S, Guerra C,* et al. Post-operative nausea and vomiting (PONV) after thyroid surgery: a prospective, randomized study comparing totally intravenous versus inhalational anesthetics. Am Surg 2010; 76(3): 325–8.
9. *Apfel CC, Kranke P, Katz MH, Goepfert C, Papenfuss T, Rauch S,* et al. Volatile anaesthetics may be the main cause of early but not delayed postoperative vomiting: a randomized controlled trial of factorial design. Br J Anaesth 2002; 88(5): 659–68.
10. *Gecaj-Gashi A, Hashimi M, Sada F, Baftiu N, Salihu S, Terziqi H,* et al. Propofol vs isoflurane anesthesia-incidence of PONV in patients at maxillofacial surgery. Adv Med Sci 2010; 55(2): 308–12.
11. *Jewer JK, Wong MJ, Bird SJ, Habib AS, Parker R, George RB.* Supplemental perioperative intravenous crystalloids for postoperative nausea and vomiting. Cochrane Database Syst Rev 2019; 3(3): CD012212.
12. *Gan TJ.* Risk factors for postoperative nausea and vomiting. Anesth Analg 2006; 102(6): 1884–98.
13. *Morita T, Yamamoto M, Sakamoto A.* What are the factors affecting postoperative nausea and vomiting following breast cancer surgery with inhalation anesthesia? J Nippon Med Sch 2020; 88(5): 418–22.
14. *Temple-Oberle C, Shea-Budgell MA, Tan M, Semple JL, Schrag C, Barreto M,* et al. Consensus Review of Optimal Perioperative Care in Breast Reconstruction: Enhanced Recovery after Surgery (ERAS) Society Recommendations. Plast Reconstr Surg 2017; 139(5): 1056e–71e.
15. *Xie J, Huang X, Gao M, Wei L, Wang R, Chen J,* et al. Surgical Pharmacy for Optimizing Medication Therapy Management Services within Enhanced Recovery after Surgery (ERAS®) Programs. J Clin Med 2023; 12(2): 631.
16. *Rajan N, Joshi GP.* Management of postoperative nausea and vomiting in adults: current controversies. Curr Opin Anesthesiol 2021; 34(6): 695–702.
17. *Gan TJ, Belani KG, Bergese S, Chung F, Diemunsch P, Habib AS,* et al. Fourth Consensus Guidelines for the Management of Postoperative Nausea and Vomiting. Anesth Analg 2020; 131(2): 411–48. Erratum in: Anesth Analg 2020; 131(5): e241.
18. *Kienbaum P, Schaefer MS, Weibel S, Schlesinger T, Meybohm P, Eberhart LH,* et al. Update on PONV-What is new in prophylaxis and treatment of postoperative nausea and vomiting? Summary of recent consensus recommendations and Cochrane reviews on prophylaxis and treatment of postoperative nausea and vomiting. Anaesthesist 2022; 71(2): 123–8. (German)
19. *Wenk M, Van Aken H, Zarbock A.* The new World Health Organization recommendations on perioperative administration of oxygen to prevent surgical site infections: A dangerous reductionist approach? Anesth Analg 2017; 125(2): 682–7.
20. *Weenink RP, de Jonge SW, van Hulst RA, Wingelaar TT, van Ooij PAM, Immink RV,* et al. Perioperative Hyperoxyphobia: Justified or Not? Benefits and Harms of Hyperoxia during Surgery. J Clin Med 2020; 9(3): 642.
21. *Akça O, Sessler DI.* Supplemental oxygen reduces the incidence of postoperative nausea and vomiting. Minerva Anesthesiol 2002; 68(4): 166–70.
22. *Mercadante S.* Nutrition in cancer patients. Support Care Cancer 1996; 4(1): 10–20.
23. *Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF,* et al. Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery. Circulation 1999; 100(10): 1043–9.
24. *Norderud K, Egholm G, Thim T, Olesen KKW, Madsen M, Jensen LO,* et al. Validation of the European Society of Cardiology and European Society of Anaesthesiology non-cardiac surgery risk score in patients treated with coronary drug-eluting stent implantation. Eur Heart J Qual Care ClinOutcomes 2019; 5(1): 22–7.
25. *Motamed C, Weil GG, Bourgain JL.* Impact of extending prevention of postoperative nausea and vomiting for cancer surgical patients in the PACU: a before and after retrospective study. Braz J Anesthesiol 2022; 72(6): 762–7.
26. *Wengritzky R, Mettho T, Myles PS, Burke J, Kakos A.* Development and validation of a postoperative nausea and vomiting intensity scale. Br J Anaesth 2010; 104(2): 158–66.
27. *Fasquel C, Huet O, Ozier Y, Quesnel C, Garnier M.* Effects of intraoperative high versus low inspiratory oxygen fraction (FiO_2) on patient's outcome: A systematic review of evidence from the last 20 years. Anaesth Crit Care Pain Med 2020; 39(6): 847–58.
28. *Hovaguimian F, Lysakowski C, Elia N, Tramér MR.* Effect of intraoperative high inspired oxygen fraction on surgical site infection, postoperative nausea and vomiting, and pulmonary function: systematic review and meta-analysis of randomized controlled trials. Anesthesiology 2013; 119(2): 303–16.
29. *Orhan-Sungur M, Kranke P, Sessler D, Apfel CC.* Does supplemental oxygen reduce postoperative nausea and vomiting? A meta-analysis of randomized controlled trials. Anesth Analg 2008; 106(6): 1733–8.
30. *Rincón DA, Valero JF.* Supplemental oxygen for the prevention of postoperative nausea and vomiting: a meta-analysis of randomized clinical trials. Rev Esp Anestesiol Reanim 2008; 55(2): 101–9. (Spanish)
31. *Holst JM, Klitholm MP, Henriksen J, Vallentin MF, Jessen MK, Bolther M,* et al. Intraoperative respiratory and hemodynamic strategies for reducing nausea, vomiting, and pain after surgery: Systematic review and meta-analysis. Acta Anaesthesiol Scand 2022; 66(9): 1051–60.
32. *Markwei MT, Babatunde IO, Kutlu-Yalcin E, Essber HA, Mascha EJ, Liu L,* et al. Perioperative Supplemental Oxygen and Postoperative Nausea and Vomiting: Subanalysis of a Trial, Systematic Review and Meta-analysis. Anesthesiology 2023; 138(1): 56–70.
33. *Greif R, Laciny S, Rapf B, Hickle RS, Sessler DI.* Supplemental oxygen reduces the incidence of postoperative nausea and vomiting. Anesthesiology 1999; 91(5): 1246–52.
34. *Purhonen S, Niskanen M, Wüstefeld M, Mustonen P, Hynynen M.* Supplemental oxygen for prevention of nausea and vomiting after breast surgery. Br J Anaesth 2003; 91(2): 284–7.

35. *Zhou Q, Cai M, Gou J, Ning N*. Effect of Postoperative High-Concentration Oxygen on Recovery After Thyroid Surgery: A Prospective, Open, Randomized, Controlled Study. Front Endocrinol (Lausanne) 2021; 12: 595571.
36. *Chiu C, Aleshi P, Esserman LJ, Inglis-Arkell C, Yap E, Whitlock EL*, et al. Improved analgesia and reduced post-operative nausea and vomiting after implementation of an enhanced recovery after surgery (ERAS) pathway for total mastectomy. BMC Anesthesiol 2018; 18(1): 41.
37. *Tripathy S, Mandal I, Rao PB, Panda A, Mishra T, Kar M*. Opioid-free anesthesia for breast cancer surgery: a comparison of ultrasound guided paravertebral and pectoral nerve blocks. A randomized controlled trial. J Anaesthesiol Clin Pharmacol 2019; 35(4): 475–80.
38. *Tabrizi S, Malhotra V, Turnbull ZA, Goode V*. Implementation of postoperative nausea and vomiting guidelines for female adult patients undergoing anesthesia during gynecologic and breast surgery in an ambulatory setting. J Perianesth Nurs 2019; 34(4): 851–60.
39. *Krieser KA, Riley JB, Baus JE, Hoffman JT, Sullivan JN, Lobato RL*. PONV Prophylaxis failure disproportionately affects female patients, despite intraoperative computerized decision support guidance. Graduate Med Edu Res J 2020; 2(1): 6.
40. *Jin Z, GanTJ, Bergese SD*. Prevention and Treatment of Postoperative Nausea and Vomiting (PONV): A Review of Current Recommendations and Emerging Therapies. Ther Clin Risk Manag 2020; 16: 1305–17.

Received on May 22, 2023
Revised on July 8, 2023
Revised on October 4, 2023
Accepted on October 17, 2023
Online First October 2023

ORIGINAL ARTICLE

UDC: 618.14-006-036
DOI: https://doi.org/10.2298/VSP230901063S

Survival analysis of patients with rare tumors of the uterine corpus – carcinosarcomas

Analiza preživljavanja bolesnika sa retkim tumorima tela materice – karcinosarkomima

Nemanja Stevanović*, Aljoša Mandić*†, Slobodan Maričić*†, Gabriel Stefan Nadj*†, Tamara Maksimović*†, Nevena Stanulović*†, Vladimir Čančar‡, Milan Rodić†

***Oncology Institute of Vojvodina, Sremska Kamenica, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; ‡University of East Sarajevo, Faculty of Medicine, Foča, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Uterine carcinosarcoma (UCS), formerly known as malignant mixed Müllerian tumor, is a rare, aggressive malignancy of the female genital tract. The aim of this study was to analyze the most important clinical and pathohistological characteristics of UCSs on operated patients, as well as to determine which of those factors are affecting progression-free survival (PFS) and overall survival (OS) of patients. **Methods.** The study was conducted as a retrospective analysis of medical data documentation of patients with a diagnosis of UCS who were surgically treated at the Department of Gynecology, Clinic for Operative Oncology, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia, in 10 years' period (from the beginning of 2009 to the end of 2018). The analysis included data for a total of 31 patients. **Results.** Of all the examined parameters (age of the patient, clinical stage of the disease, histological grade, depth of myometrial invasion, and lymphovascular invasion – LVI), the greatest influence on the choice of therapeutic procedure had a histological tumor grade. **Conclusion.** Our research showed the joint influence of the examined clinical and pathohistological factors on PFS and OS of patients with UCS. The only independent parameter that showed a statistically significant impact on survival is LVI.

Key words:
carcinosarcoma; progression-free survival; risk factors; survival analysis; uterine neoplasms.

Apstrakt

Uvod/Cilj. Karcinosarkom materice (KSM), ranije poznat kao maligni mešoviti Milerov tumor, je redak i agresivan malignitet ženskog genitalnog trakta. Cilj rada bio je da se analiziraju najvažnije kliničke i patohistološke karakteristike KSM operisanih bolesnica, kao i da se utvrde faktori značajni za preživljavanje bez progresije (PBP) bolesti i ukupno preživljavanje (UP) obolelih. **Metode.** Studija je rađena kao retrospektivna analiza dokumentacije medicinskih podataka bolesnica sa dijagnozom KSM, koje su hirurški lečene na Odeljenju ginekologije Klinike za operativnu onkologiju Instituta za onkologiju Vojvodine, Sremska Kamenica, Srbija, u periodu od 10 godina (od početka 2009. do kraja 2018. godine). Analizirani su podaci ukupno 31 bolesnice. **Rezultati.** Od svih ispitivanih parametara (starost bolesnica, klinički stadijum bolesti, histološki gradus, dubina invazije miometrijuma i limfovaskularna invazija – LVI), najveći uticaj na izbor terapijske procedure imao je histološki stepen tumora. **Zaključak.** Naše istraživanje pokazalo je zajednički uticaj ispitivanih kliničkih i patohistoloških faktora na PBP bolesti i UP bolesnica sa KSM. Jedini nezavisni parametar koji je pokazao statistički značajan uticaj na preživljavanje je LVI.

Ključne reči:
karcinosarkom; preživljavanje, bez progresije; faktori rizika; preživljavanje, analiza; materica, neoplazme.

Introduction

After several decades of scientific debate, it is known today that uterine carcinosarcoma (UCS), a malignant neoplasm, has both an epithelial and a mesenchymal part [1–4]. Incidence ranges from 0.5 to 3.3 cases *per* 100,000 women; depending on the studies, they make up from 1–2% to 5% of all uterine malignancies. These are highly aggressive tumors whose contribution to the total mortality from uterine malignancies is about 16.4% [1, 5–7]. According to the recent find-

Correspondence to: Nemanja Stevanović, Oncology Institute of Vojvodina, Put dr Goldmana 4, 21 204 Sremska Kamenica, Serbia.
E-mail: nemanja.p.stevanovic@gmail.com

ings, the greatest role in the clinical behavior and prognosis of this malignancy is attributed to the epithelial component due to a higher mitotic index, the frequent presence of lymphovascular invasion (LVI), and expression of endothelial growth factors [3, 4, 8–13]. In addition to age, other risk factors for its occurrence were observed, such as obesity, previous tamoxifen therapy, long-term exposure to estrogen, previous therapeutic irradiation of the pelvis, nulliparity, positive BRCA1 gene mutation, socioeconomic factor in African American women, etc. [12, 14, 15]. In patients with tumors in the early stages of the disease, in stage I according to the International Federation of Gynecology and Obstetrics (FIGO), survival reaches up to 50%, which is still significantly lower than the five-year survival rate in early endometrioid carcinoma, which exceeds 80% [16]. In the last stage of the disease (FIGO IV), the five-year survival rate is below 10%, and the occurrence of recurrence is less frequent locally compared to the occurrence of distant metastases [17, 18]. For now, the most significant prognostic predictors are the stage and histological grade of the disease, the depth of myometrial invasion, LVI, and the age of the patient [5]. Surgical treatment remains the most effective and commonest treatment for patients with localized disease, and chemotherapeutics commonly used are ifosfamide, cisplatin, carboplatin, paclitaxel, and doxorubicin [18, 19].

Methods

The study was conducted as a retrospective analysis of medical data documentation of patients with a diagnosis of UCS who were surgically treated at the Oncology Institute of Vojvodina, Serbia, in a period of 10 years, from the beginning of 2009 to the end of 2018. The research was approved on December 23, 2021, by the Ethics Committee of the Oncology Institute of Vojvodina (No. 4/21/2-4093/2-4).

The analysis of medical records included a total of 31 female patients. For one patient it was not possible to find information about the FIGO stage of the disease and she could not be included in the presented results regarding the spread of the disease and survival depending on the FIGO stage of the disease.

From the pathohistological parameters of the tumor, data were collected on: a) histological grade, whereby grades 1 and 2 were considered as well differentiated, while grade 3 was considered as poorly differentiated carcinosarcoma; b) histological type, depending on the characteristics of the sarcoma component – homologous and heterologous; c) presence or absence of LVI; d) the thickness of the involvement of the myometrium by tumor tissue (more or less than ½ of total thickness of the myometrium); e) tumor size in the largest diameter measured in mm.

Data were also collected on the applied therapeutic procedures. Survival of patients was analyzed based on progression-free survival (PFS), as well as the overall survival (OS) of the patient, expressed in months from surgery to death or until the end of 2021. Every patient was followed for at least three years from the moment of the operation.

Statistical analysis

Descriptive statistics were performed separately for continuous and ordinal variables. Age data were classified into two categories, with patients divided into two age groups (younger than the median value and older than the median value). For other categorical variables (homologous/heterogenous type, LVI, depth of invasion, lymphadenectomy, additional therapy, PFS code, OS code, three-year survival), frequency analysis was performed. A Cox regression model was used to test the significance of predictors on OS and PFS. This model determines the significance of the influence of individual predictor variables on the dependent variable (univariate analysis) and their joint influence (multivariate analysis). The cumulative survival of patients was shown using the Kaplan-Meier analysis. IBM-SPSS 21 and Statistica 14.0.0.15 software were used for statistical data processing; a significance threshold of 0.05 was used.

The aim of this study was to analyze the most important clinical and pathohistological characteristics of UCSs on operated patients, as well as to determine which of those factors are affecting PFS and OS of patients.

Results

Analyzing the ages of the patients showed that the youngest patient at the time of the operation was 50, and the oldest was 76 years old. The median age of patients was 67 years.

At the time of surgery, 80% of patients were in the early stages of the disease (FIGO I and II), of which the largest number were in the first stage of the disease (56.7%) (Table 1).

Table 1

Stage of the spread of the disease according to the FIGO classification

FIGO stage	Value
I	17 (56.70)
II	7 (23.30)
III	5 (16.70)
IV	1 (3.30)
Total	30 (100.00)

FIGO – International Federation of the Gynecology and Obstetrics.
All values are given as numbers (percentages).

Observing the histological grade of the tumors, more than 3/4 (78.6%) of patients in the observed group had poorly differentiated tumor grades 2 and 3 (high grade) (Figure 1a). Regarding the histological type of the tumor, 4/5 (80.8%) was homologous (made of tissues native to the uterus) and 1/5 (19.2%) was heterologous (made of tissues non-native to the uterus) (Figure 1b).

The tumor size ranged from 35 to 90 mm, while the median value was 61 mm.

LVI was present in 55.2% of patients (Figure 1c). Myometrial invasion of more than 50% was observed in 2/3 (73.3%) of patients (Figure 1d).

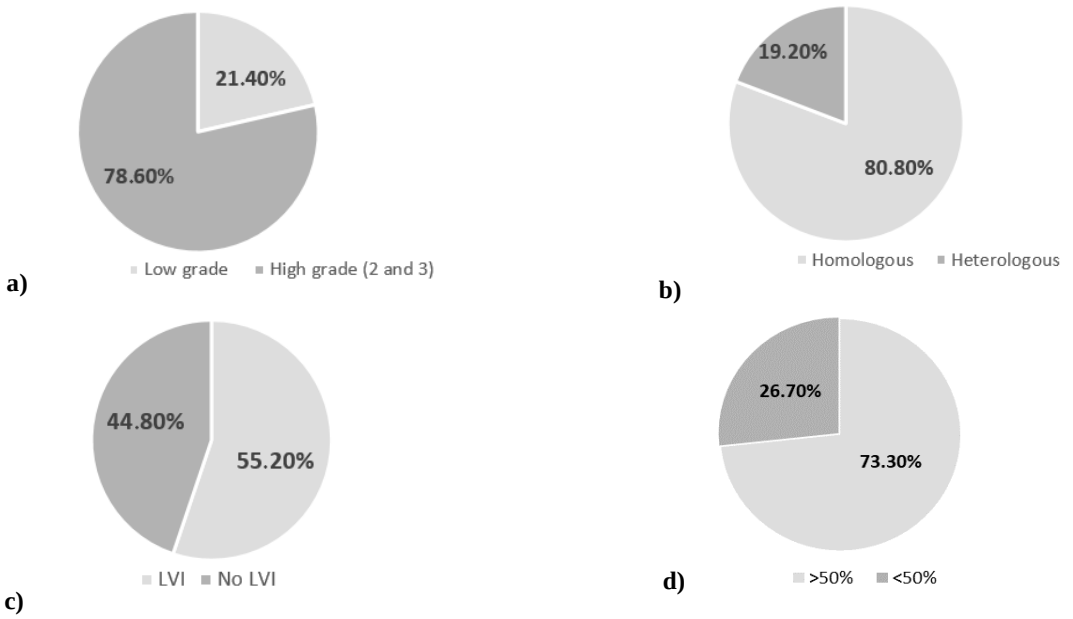


Fig. 1 – a) Histological grade of the tumor; b) Histological tumor subtype; c) Presence of lymphovascular invasion (LVI); d) Thickness of myometrium.

The median value of progression-free survival (PFS) in all stages of the disease was 28 months, while the median value of OS was 39 months (Table 2).

Median length of PFS and OS depended on whether the disease was in early (FIGO I and II) or advanced stages (FIGO III and IV) (Table 3).

Half (51.6%) of the patients experienced disease progression during the follow-up period, while three-year survival was 64.3%. At the end of 2021, 41.9% of those treated died (Figure 2).

Only in 16.1% of patients, all of whom were in the FIGO I stage of the disease, no adjuvant therapy was

Table 2

Parameters of progression-free survival and overall survival

Survival (months)	Mean	Med	Min–Max	SD
Progression-free survival	40.94	28.00	2.00–138.00	36.07
Overall survival	45.61	39.00	3.00–138.00	35.08

Med – median; Min–Max – minimum-maximum; SD – standard deviation.

Table 3

Parameters of progression-free survival and overall survival depending on the stage of the disease

Survival (months)/Stage	n	Mean	Med	Min–Max	SD
Progression-free survival					
FIGO I and II	24	45.17	45.50	2.00–138.00	38.98
FIGO III and IV	6	29.17	23.50	11.00–59.00	19.32
Overall survival					
FIGO I and II	24	48.88	50.00	3.00–138.00	36.88
FIGO III and IV	6	37.67	28.00	11.00–91.00	28.55

n – number of patients. For other abbreviations, see Tables 1 and 2.

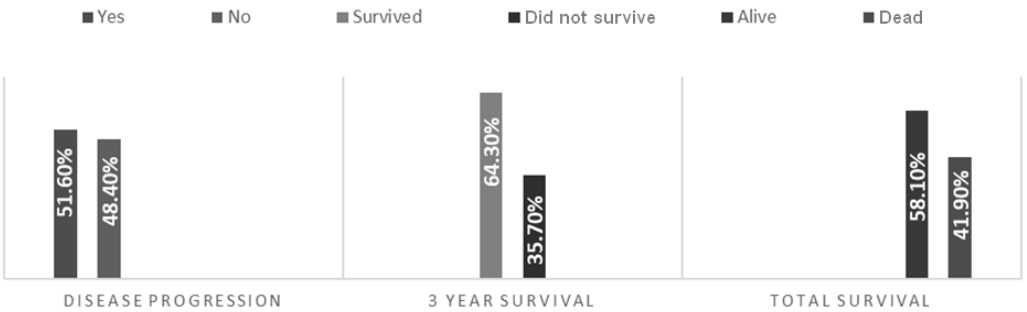


Fig. 2 – Disease progression, 3-year survival, and total survival.

applied in addition to surgery (Table 4).

Lymphadenectomy was performed in 54.8% of patients.

There were 15 (48.39%) patients in the first group (older than median 67 years) and 16 (51.61%) in the second. The results of the performed χ^2 test (Table 5) show the existence of statistically significant differences between younger and older patients when it comes to LVI, which was more often present in the group of younger patients (χ^2 = 3.892, p = 0.049), while in the other examined parameters, no significant differences were found between these two groups.

Of all the examined parameters, the greatest influence on the choice of therapeutic procedure had a histological grade (Table 6).

To evaluate the significance of the impact of individual predictors on PFS and OS, two Cox regression models were formed (Table 7).

When looking at the individual predictors of PFS in the Cox regression analysis, only LVI stands out as a statistically significant predictor ($p < 0.05$). However, the Cox multivariate regression model is overall statistically significant

Table 4

Applied therapeutic procedures

Parameter	Value
Surgery only	5 (16.10)
Adjuvant radiation therapy	4 (12.90)
Adjuvant chemotherapy	10 (32.30)
Adjuvant chemoradiation therapy	12 (38.70)
Total	31 (100.00)

All values are given as numbers (percentages).

Table 5

Differences in observed parameters between younger and older patients

Parameter	χ^2	p
FIGO	1.402	0.705
Histological type	1.704	0.192
Lymphovascular invasion	3.892	**0.049**
Depth of tumor invasion	0.386	0.544
Histological grade	0.039	0.843
Lymphadenectomy	0.457	0.491
Therapeutic procedure	5.707	0.127
Survival without a progression	0.819	0.366
Total survival	1.551	0.213

FIGO – International Federation of the Gynecology and Obstetrics. Bolded value is statistically significant.

Table 6

Influence of observed parameters on the choice of therapeutic procedure

Parameter	χ^2	p
Age (years)	0.068	0.127
FIGO	5.723	0.767
Histological grade	6.410	**0.043**
Depth of tumor invasion	0.697	0.264
Lymphovascular invasion	0.142	0.342

FIGO – International Federation of the Gynecology and Obstetrics. Bolded value is statistically significant.

Table 7

Influence of individual predictors on progression-free survival and overall survival

Parameters	Progression-free survival			Overall survival		
	df/b¤	F-value/SE b♦	p-value	df/b¤	F-value/SE b♦	p-value
FIGO	3	0.64	0.5959	3	0.49	0.6924
FIGO I & II vs. III & IV	1	0.93	0.3419	1	0.48	0.4952
Homologous/heterologous	1	0.71	0.4070	1	0.69	0.4147
Lymphovascular invasion	1	4.98	0.0342	1	3.07	0.0908
Depth of tumor invasion	1	2.81	0.1051	1	2.09	0.1597
Histological grade	1	0.98	0.3321	1	0.41	0.5255
Lymphadenectomy	1	1.71	0.2013	1	1.86	0.1838
Therapeutic procedure	3	0.47	0.7085	3	0.58	0.6318
Age (years)	-0.41¤	1.36♦	0.7629	-0.51¤	1.34♦	0.7115
Tumor size (mm)	-0.13¤	0.53♦	0.8012	-0.15¤	0.52♦	0.7731

FIGO – International Federation of the Gynecology and Obstetrics; ¤ – value of coefficient b; ♦ – standard error (SE) of coefficient b.

($\chi^2 = 21.59$, $p = 0.0103$), which means that all predictors considered together significantly affect PFS. If the selected predictors are observed individually, none of the observed factors is a statistically significant predictor of OS, but in this case, LVI stands out for its predictive significance (Table 7). However, in this case, as well, the multivariate regression model is entirely statistically significant ($\chi^2 = 22.18$, $p = 0.0083$), i.e., all examined parameters considered together significantly affect the overall length of survival.

Discussion

Although UCS was first described in 1852, there is currently no scientific consensus on which one of these factors could be used as a reliable prognostic predictor [4, 20]. The largest number of patients is at the end of the seventh or the beginning of the eighth decade of life [2, 16, 21, 22]. In some studies, age was shown as a bad predicting factor [4]. However, in our study, there was no statistically significant difference between younger and older patients in OS and PFS. The prognostic significance of age was not proven by some other authors either [23]. Traditionally, the stage of the disease, according to the FIGO classification, is one of the most important parameters when making decisions about therapeutic procedures and conclusions about outcomes [12, 24]. In our study, 80% of patients were in the early stages (FIGO I and II), and 20% were in the advanced stages of the disease (FIGO III and IV). Initially, a higher frequency of the early stages of the disease was recorded by some other researchers, although the diagnosis in the advanced stages is much more frequent compared to endometrioid tumors, which is attributed to the aggressive nature of the tumor [19, 24–26]. The high number of patients in the early stages can be partially explained by the early onset of symptoms that bring the patient to the doctor, such as painless postmenopausal bleeding. Most researchers have proven that there are differences in the length of survival depending on the FIGO stage [12, 24]. Hapsari et al. [24] determined even three times higher OS in patients in the early stage of the disease. The results of our study did not show a statistically significant effect of disease stage on the PFS and OS. The same conclusion was reached by Şükür et al. [13]. This can be partially explained by the small number of patients in the advanced stages of the disease, especially in FIGO stage IV in our study group.

In our studied group, the homologous type of tumor was significantly more frequent (80.8%), even though the ratio of these two types was more uniform in some other studies [25]. In the biological behavior of tumors, the mesenchymal component is given less and less importance, which is in accordance with our findings, where there is no significant difference in either OS or PFS depending on the characteristics of this component. The same conclusions were reached by other authors [10]. According to current knowledge, the clinical behavior and prognosis of the disease are primarily attributed to the epithelial component of the neoplasm, which is most often a histologically poorly differentiated carcinoma [10]. In the largest number (78.6%) of cases, a high grade, i.e., poor differentiation, was present in our research. It was similar in other comparative studies [16, 27]. In our study, the histological grade did not prove to be a significant predictor of PFS and OS, which was also the conclusion reached by Pautier et al. [28] and Yilmaz et al. [29]. The aggressive nature of the investigated neoplasm is indicated by the frequent presence of LVI and significant tumor invasion into the myometrium [10, 19], which were also recorded in the patients in our study. Thus, as many as 73.3% of examined patients had myometrial invasion greater than 50% of its total thickness, which is in accordance with the data on the predominance of poorly differentiated tumors in the examined group. The presence of LVI and the greater depth of myometrial invasion by the tumor are attributed in the literature to a greater potential for metastasis and recurrence [4]. In line with this is the fact that the presence of LVI is significantly more frequent in carcinosarcoma compared to other types of endometrial cancer [30]. Although some authors determined that both mentioned parameters are significant predictors of the reduction of PFS and OS length, in our research, statistical significance was proven only when it comes to the existence of LVI [2, 8, 29, 31, 32].

Operative treatment, primarily hysterectomy with bilateral salpingo-oophorectomy is still the primary modality of treatment and is used for curative purposes in FIGO stages I-III, while in FIGO stage IV, it is used for palliative purposes [2, 19]. All patients included in our research were treated operatively. Although operative treatment remains the “gold standard”, the high rate of recurrence and metastases, as well as available literature data, indicate the need for multimodal treatment [31]. In our research, adjuvant therapy (radiotherapy, chemotherapy, or their combination) was prescribed for 83.9% of the treated patients, which is more than the data of some other authors, sometimes almost double [19]. In our study, when choosing additional treatment methods, the histological grade of the tumor proved to be the most influential factor. Although in practical work, the stage of the disease remains one of the most significant factors influencing the choice of therapeutic modality, the absence of statistical significance of the influence of this factor in our study can be explained by the significant predominance of patients in the early stages of the disease in the examined group. Data on the effectiveness of radiotherapy differ in the literature and remain a subject of debate [2, 4, 17, 24, 33]. Gunther et al. [34] point out that its application reduces the possibility of local recurrence by as much as 50%. However, Callister et al. [35] point to the absence of significant differences regarding the length of OS of patients. In our research, no significant influence of the application of radiotherapy as the only adjuvant modality on the length of either overall or PFS was proven, as some other authors who dealt with this issue also came to [19, 24, 36]. In our study, no statistically significant relationship between the use of adjuvant chemotherapy and survival parameters was observed. Although the application of adjuvant chemotherapy leads to a prolongation of both PFS and OS according to a large number of authors, in some studies, it was observed that in patients in the FIGO I and II stages (to which our patients mainly belonged), there is no significant prolongation of life regardless of the applied chemothera-

py [19]. Nevertheless, regardless of the contradictory results that the researchers reached in their research, the use of chemotherapy is recommended in all stages of the disease after complete resection of the tumor [31]. The combination of radiotherapy and chemotherapy was the most common treatment modality for patients in our study (38.7%). Today, it is considered that this type of therapy is the most effective, especially in patients with advanced disease, and that it prolongs OS more than any modality used alone [19, 22]. However, both in our research and in the research of some other authors, such conclusions were not confirmed [24]. The role of pelvic and paraaortic lymphadenectomy in patients with UCS is still not completely clear. In our observed group, lymphadenectomy was performed in 54.8% of patients in all FIGO disease stages. This is somewhat lower than the findings of other authors [13, 25]. The performed testing did not show a statistically significant difference in the length of OS and PFS between patients who did and those who did not undergo lymphadenectomy. In the group of observed patients, progression of the disease, i.e., occurrence of metastases or recurrence, occurred in 51.6% of examined patients. This result is consistent with the findings of other studies, according to which recurrence rates are usually between 47–67% [4]. The median value of the follow-up period in which disease progression did not occur in our study group was 28 months. Some authors also recorded significantly shorter periods. Thus, in the study by McEachron et al. [22], the median PFS was only 13 months, while it should be considered that the proportion of patients with advanced disease in their study group was triple higher than in our study group. Patients in the early stages of the disease (FIGO I and II) had the median PFS in the follow-up period of 45.5 months, while in the late stages (FIGO III and IV), it was 23.5 months. Other authors also found significant differences in PFS depending on the stage of the disease. In their study, Hapsari et al. [24] recorded a median PFS of 39 months in FIGO I and II, while it was only nine months in FIGO III and IV. The median value of OS of all patients during the follow-up period is 39 months. In the early stages of the disease (FIGO I and II), the median OS was 50 months, while in the late stages (FIGO III and IV), the median OS was 28 months. Data on OS in the literature vary, depending on the length of the follow-up period and the proportion of patients in different stages of the disease in the examined samples. Kurnit et al. [25] recorded a length of total medial OS of 39 months, the same as in our study, while some researchers recorded lower values, i.e., 23 months in the study by Matsuzaki et al. [37]. The three-year survival of patients in our study was 35.7%, which is slightly higher than the values obtained in other studies, but within the expected values considering the predominant presence of early stages of the disease in the examined sample. By the end of the follow-up period, 41.9% of patients died, which shows data similar to those recorded by other authors. Thus, Yilmaz et al. [29], for example, recorded a mortality rate of 35% within the follow-up period.

Conclusion

Our research showed the joint influence of the examined clinical and pathohistological factors parameters on PFS and OS of patients with UCS. The only independent parameter that showed a statistically significant impact on survival is LVI. In order to improve the survival of patients suffering from UCS, additional multicenter randomized trials are needed to reach a consensus regarding therapeutic methods and prognostic parameters.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Cantrell LA, Blank SV, Duska LR.* Uterine carcinosarcoma: A review of the literature. Gynecol Oncol 2015; 137(3): 581–8.
2. *Pezzicoli G, Moscaritolo F, Silvestris E, Silvestris F, Cormio G, Porta C,* et al. Uterine carcinosarcoma: An overview. Crit Rev Oncol Hematol 2021; 163: 103369.
3. *Van der Horst RL, Van der Hel O, Lutgens L, Van der Aa M, Slangen B, Kruitwagen R,* et al. The role of multimodal adjuvant therapy for FIGO I-II carcinosarcoma of the uterus: a systematic review. Crit Rev Oncol Hematol 2022; 175: 103701.
4. *Kathan R, Senger JL.* Uterine carcinosarcomas (malignant mixed müllerian tumours): a review with special emphasis on the controversies in management. Obstet Gynecol Int 2011; 2011: 470795.
5. *Chandrasekaran A, Kumar A, Kumar T, Chauhan A, Kaur P, Khurana A.* Malignant mixed Mullerian tumour (MMMT) of uterus: Rare and aggressive tumor. J Clin Med Res 2019; 7(3): 267–9.
6. *Denschlag D, Ulrich UA.* Uterine Carcinosarcomas - Diagnosis and Management. Oncol Res Treat 2018; 41(11): 675–9.
7. *Yamada SD, Burger RA, Brewster WR, Anton D, Kohler MF, Monk BJ.* Pathologic variables and adjuvant therapy as predictors of recurrence and survival for patients with surgically evaluated carcinosarcoma of the uterus. Cancer 2000; 88(12): 2782–6.
8. *Matsuo K, Takazawa Y, Ross MS, Elishaev E, Podzielinski I, Yunokawa M,* et al. Significance of histologic pattern of carcinoma and sarcoma components on survival outcomes of uterine carcinosarcoma. Ann Oncol 2016; 27(7): 1257–66.
9. *Lopez-Garcia MA, Palacios J.* Pathologic and molecular features of uterine carcinosarcomas. Semin Diagn Pathol 2010; 27(4): 274–86.
10. *McCluggage WG.* Malignant biphasic uterine tumours: carcinosarcomas or metaplastic carcinomas? J Clin Pathol 2002; 55(5): 321–5.
11. *Emoto M, Iwasaki H, Ishiguro M, Kikuchi M, Horiuchi S, Saito T,* et al. Angiogenesis in carcinosarcomas of the uterus: Differences in the microvessel density and expression of vascular endothelial growth factor between the epithelial and mesenchymal elements. Hum Pathol 1999; 30(10): 1232–41.
12. *De Jong RA, Nijman HW, Wijbrandi TF, Reyners AK, Boezen HM, Hollema H.* Molecular markers and clinical behavior of uterine carcinosarcomas: focus of the epithelial tumour component. Mod Pathol 2011; 24(10): 1368–79.

13. *Şükür YE, Taşkın S, Varlı B, Ateş C, Güngör M, Ortaç F.* Prognostic factors for disease-free and overall survival of patients with uterine carcinosarcoma. Int J Clin Oncol 2018; 23(1): 114–20.
14. *Zwahlen DR, Schick U, Bolukbasi Y, Thariat J, Abdah-Bortnyak R, Kuten A*, et al. Outcome and Predictive Factors in Uterine Carcinosarcoma Using Postoperative Radiotherapy: A Rare Cancer Network Study. Rare Tumors 2016; 8(2): 6052.
15. *Rojas C, Tian C, Powell MA, Chan JK, Bateman NW, Conrads TP*, et al. Racial disparities in uterine and ovarian carcinosarcoma: A population-based analysis of treatment and survival. Gynecol Oncol 2020; 157(1): 67–77.
16. *Mbatani N, Olawaiye AB, Prat J.* Uterine sarcomas. Int J Gynaecol Obstet 2018; 143(Suppl 2): 51–8.
17. *Artioli G, Wabersich J, Ludwig K, Gardiman MP, Borgato L, Garbin F.* Rare uterine cancer: carcinosarcomas. Review from histology to treatment. Crit Rev Oncol Hematol 2015; 94(1): 98–104.
18. *Cory L, Brensinger C, Burger RA, Giuntoli RL, Morgan MA, Latif N,* et al. Patterns of adjuvant treatment and survival outcomes in stage I uterine carcinosarcoma, Gynecol Oncol Rep 2022; 39: 100930.
19. *Beckmann K, Selva-Nayagam S, Olver I, Miller C, Buckley ES, Powell K,* et al. Carcinosarcomas of the Uterus: Prognostic Factors and Impact of Adjuvant Treatment. Cancer Manag Res 2021; 13: 4633–45.
20. *Bodner-Adler B, Bodner K, Obermair A, Czerwenka K, Petru E, Leodolter S,* et al. Prognostic parameters in carcinosarcomas of the uterus: a clinico-pathologic study. Anticancer res 2001; 21(4B): 3069–74.
21. *Hosh M, Antar S, Nazzal A, Warda M, Gibreel A, Refky B.* Uterine Sarcoma: Analysis of 13,089 Cases Based on Surveillance, Epidemiology, and End Results Database. Int J Gynecol Cancer 2016; 26(6): 1098–104.
22. *McEachron J, Heyman T, Shanahan L, Tran V, Friedman M, Gorelick C*, et al. Multimodality adjuvant therapy and survival outcomes in stage I–IV uterine carcinosarcoma. Int J Gynecol Cancer 2020; 30(7): 1012–7.
23. *Terblanche L, Botha MH.* Uterine carcinosarcoma: A 10-year single institution experience. PLoS One 2022; 17(7): e0271526.
24. *Hapsari K, Bhugwandass C, Van Rijn GWJ, Van der Wurff AAM, Van 't Veer M, Boll D,* et al. Treatment and Outcome of Patients with Uterine Carcinosarcoma in a Comprehensive Cancer Network. Indian J Gynecol Oncol 2020; 18: 17.
25. *Kurnit KC, Previs RA, Soliman PT, Westin SN, Klopp AH, Fellman BM*, et al. Prognostic factors impacting survival in early stage uterine carcinosarcoma. Gynecol Oncol 2019; 152(1): 31–7.
26. *Nama N, Cason FD, Misra S, Hai S, Tucci V, Haq F,* et al. Carcinosarcoma of the Uterus: A Study From the Surveillance Epidemiology and End Result (SEER) Database. Cureus 2020; 12(9): e10283.
27. *Dave KS, Chauhan A, Bhansali R, Arora R, Purohit S.* Uterine carcinosarcomas: 8-year single center experience of 25 cases. Indian J Med Paediatr Oncol 2011; 32(3): 149–53.
28. *Pautier P, Genestie C, Rey A, Morice P, Roche B, Lhommé C,* et al. Analysis of clinicopathologic prognostic factors for 157 uterine sarcomas and evaluation of a grading score validated for soft tissue sarcoma. Cancer 2000; 88(6): 1425–31.
29. *Yilmaz U, Alanyali S, Aras AB, Ozsaran Z.* Adjuvant radiotherapy for uterine carcinosarcoma: A retrospective assessment of treatment outcomes. J Cancer Res Ther 2019; 15(6): 1377–82.
30. *Corrado G, Ciccarone F, Cosentino F, Legge F, Rosati A, Arcieri M,* et al. Role of minimally invasive surgery versus open approach in patients with early-stage uterine carcinosarcomas: a retrospective multicentric study. J Cancer Res Clin Oncol 2021; 147(3): 845–52.
31. *Toboni MD, Crane EK, Brown J, Shushkevich A, Chiang S, Slomovitz BM*, et al. Uterine carcinosarcomas: From pathology to practice. Gynecol Oncol 2021; 162(1): 235–41.
32. *Moatasim A, Hameed Z, Ahmad I.* Assessment of lymphovascular invasion in early stage endometrial carcinoma- a retrospective study. Surg Exp Pathol 2021; 4: 9.
33. *Gonzalez Bosquet J, Terstriep SA, Cliby WA, Brown-Jones M, Kaur JS, Podratz KC,* et al. The impact of multi-modal therapy on survival for uterine carcinosarcomas. Gynecol Oncol 2010; 116(3): 419–23.
34. *Gunther JR, Christensen EN, Allen PK, Ramondetta LM, Jhingran A, Fleming ND*, et al. Role of Radiation Therapy in the Multidisciplinary Management of Uterine Carcinosarcoma. Int J Gynecol Cancer 2018; 28(1): 114–21.
35. *Callister M, Ramondetta LM, Jhingran A, Burke TW, Eifel PJ.* Malignant mixed Müllerian tumors of the uterus: analysis of patterns of failure, prognostic factors, and treatment outcome. Int J Radiat Oncol Biol Phys 2004; 58(3): 786–96.
36. *Patel N, Hegarty SE, Cantrell LA, Mishra MV, Showalter TN.* Evaluation of brachytherapy and external beam radiation therapy for early stage, node-negative uterine carcinosarcoma. Brachytherapy 2015; 14(5): 606–12.
37. *Matsuzaki S, Klar M, Matsuzaki S, Roman LD, Sood AK, Matsuo K.* Uterine carcinosarcoma: Contemporary clinical summary, molecular updates, and future research opportunity. Gynecol Oncol 2020; 160(2): 586–601.

Received on September 1, 2023
Accepted on October 17, 2023
Online First October 2023

ORIGINAL ARTICLE

UDC: 616-053.2:616-006]:616.6-022.7
DOI: https://doi.org/10.2298/VSP230620057K

Urinary tract infections in children with cancer and febrile neutropenia – single center experience

Infekcije mokraćnih puteva kod dece obolele od malignih tumora sa febrilnom neutropenijom – iskustvo jednog centra

Nataša Kovač*†, Maja Samardžić Lukić*†, Nataša Kaćanski*, Aleksandra Kovač†‡, Tijana Latinović§, Jovanka Kolarović*†

***Institute for Child and Youth Health Care of Vojvodina, Novi Sad, Serbia; ‡Oncology Institute of Vojvodina, Sremska Kamenica, Serbia; §University of Belgrade, Faculty of Medicine, Belgrade, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia**

Abstract

Background/Aim. Urinary tract infection (UTI) in children with febrile neutropenia (FN) after receiving chemotherapy could be followed by atypical symptoms and signs. The absence of routine urine culture (UC) sampling for analysis could lead to undiagnosed disease and inadequate treatment of these patients. The aim of the study was to indicate the importance of sampling UC in children who have developed FN and to point out the most probable causative agents of UTI in children with FN and antibiotic sensitivity/resistance of the isolated strains. **Methods.** During a five-year observation period, 40 UTIs were registered in 30 patients with FN. In the study group of patients with FN, the number of UTIs, the number of recurrent UTIs, isolated pathogens, their sensitivity to antibiotics, characteristics of urine sediment, the presence of localized symptoms of UTI, and the presence of urosepsis were analyzed. The obtained results were compared with the control group which consisted of children who were healthy prior to hospitalization due to febrile urinary infection. **Results.** When compared to the control group, significant differences in the presence of symptoms of UTI and urine sediment findings in patients with FN were observed. A higher percentage of resistant strains of *Escherichia coli* isolated from UC of cancer patients with FN was noted when compared to the control group. Three UTI cases were followed by urosepsis. **Conclusion.** UC findings are important not only in establishing the diagnosis of UTI and detecting multi-resistant bacterial strains but also in choosing appropriate antibiotics and selecting a subgroup of patients with recurrent UTI who require further monitoring and detecting potential complications in a timely manner.

Key words:
bacteriological techniques; child; febrile neutropenia; medical oncology; urinary tract infection; urinalysis.

Apstrakt

Uvod/Cilj. Infekcije mokraćnih puteva (IMP) kod dece koja su razvila febrilnu neutropeniju (FN) nakon primene hemioterapije, mogu biti praćene atipičnim simptomima i znacima. Odsustvo rutinskog uzimanja uzorka urinokulture (UK) za analizu može dovesti do neprepoznavanja i neadekvatnog lečenja tih bolesnika. Cilj rada bio je da se ukaže na značaj uzimanja UK kod dece kod kojih se razvila FN, na najčešće uzročnike IMP kod ove dece, kao i da se ukaže na osetljivost/rezistenciju na antibiotike izolovanih sojeva uzročnika IMP. **Metode.** U posmatranom petogodišnjem periodu registrovano je 40 IMP kod 30 bolesnika sa FN. U grupi bolesnika sa FN analiziran je broj IMP, broj ponovljenih IMP, izolovani uzročnici infekcije, njihova osetljivost na antibiotike, karakteristike sedimenta urina, prisustvo lokalnih simptoma IMP i prisustvo urosepse. Dobijeni podaci su upoređivani sa kontrolnom grupom, koju su činila zdrava deca prethodno hospitalizovana zbog urinarne infekcije praćene febrilnošću. **Rezultati.** Kod bolesnika sa FN, u odnosu na kontrolnu grupu uočena je statistički značajna razlika povezana sa prisustvom simptoma IMP i nalazom sedimenta urina. Registrovan je viši procenat izolovanih rezistentnih sojeva *Escherichia coli* iz UK bolesnika sa FN, u odnosu na kontrolnu grupu. Tri bolesnika sa IMP imala su prateću urosepsu. **Zaključak.** Nalaz UK je značajan ne samo u postavljanju dijagnoze IMP i otkrivanju multirezistentnih bakterijskih sojeva, već i u pravilnom odabiru antibiotske terapije i selekciji onih bolesnika sa ponovljenim IMP, koji zahtevaju dalje praćenje i pravovremeno otkrivanje potencijalnih komplikacija.

Ključne reči:
bakteriološke tehnike; deca; neutropenija, febrilna; onkologija, medicinska; urinarni trakt, infekcije; mokraća, analiza.

Correspondence to: Nataša Kovač, University of Novi Sad, Faculty of Medicine, Institute for Child and Youth Health Care of Vojvodina, Hajduk Veljkova 10, 21 000 Novi Sad, Serbia. E-mail: natasa.kovac@mf.uns.ac.rs

Introduction

Urinary tract infections (UTIs) present a significant cause of morbidity in children. They are characterized by significant bacteriuria in urine culture, clinical signs of infection (fever, dysuria, pollakiuria, hematuria, abdominal pain), as well as the presence of pyuria. Pyuria is defined by the presence of more than five leukocytes per high-power field in centrifuged urine, which is consistent with positive leukocyte esterase on dipstick. The presence of nitrites, microscopical findings of bacteria, and the occurrence of microscopic hematuria in urine sediment can indicate the presence of infection. UTIs can be divided into two types: upper (acute pyelonephritis – renal parenchyma infected) and lower UTIs (cystitis) [1].

While the above-mentioned characteristics can be implemented in patients with normal blood leukocyte counts, in cancer patients with febrile neutropenia (FN), fever could often be the only sign of infection. Frequently, the presence of leukocytes in urine sediment could fail due to the existing leukopenia. In these patients, infection is often asymptomatic with normal urine findings, and diagnosis of UTI is established by the presence of significant bacteriuria [2–4].

Considering the fact that these infections could be caused by bacterial strains that are resistant to empiric antibiotic therapy, the omission of urine sampling in patient work-up could lead to undiagnosed UTIs and inadequate treatment. Consequently, this could cause the occurrence of renal scarring and may give rise to complications such as hypertension and chronic renal insufficiency [1].

At the moment, a small number of studies focused on UTI in children with FN have been conducted. The lack of studies might be attributed to the fact that according to Infectious Diseases Society of America recommendations from 2010, sampling urine in febrile oncology patients was recommended only when symptoms of UTI are present, a urinary catheter is placed, or if ultrasound of urinary tract shows evidence of urinary tract pathology [5]. According to current guidelines, urinalysis and urine culture (UC) should be obtained routinely as part of the diagnostic evaluation.

The aim of this paper was to present the importance of UC sampling in children who have developed FN after a cycle of chemotherapy. The absence of localized symptoms of UTI and normal urine sediment findings cannot safely exclude the presence of UTI in these patients. The presence of fever and significant bacteriuria can be the only signs of UTI.

Methods

This retrospective study was conducted in the Department of Oncology of the Institute for Health Care of Children and Youth of Vojvodina, Serbia. Ethical approval was obtained from the local Ethics Committee (No. 1402-1, from April 01, 2022). During a five-year observation period (from January 1, 2016, to January 1, 2021), 40 UTIs were registered in 30 patients with FN. The patients were children aged 3 to 18 years diagnosed with malignant diseases that established normal urinary continence and developed FN after a cycle of chemotherapy. Patients without normal urinary continence and patients with fever of another known origin were excluded from this study.

The obtained results were compared to the control group which consisted of 40 previously healthy children, in the same age range (3 to 18 years; average 6.9 years) hospitalized due to febrile urinary infection. Patients with congenital anomalies of the urinary system were excluded. Children from both groups were hospitalized during the same five-year study period.

Diagnosis of UTI was established due to the presence of significant bacteriuria in patients with an unknown origin of infection. In patients who had a urinary catheter placed, UC samples were taken from the catheter, while in other cases, a sample of mid-stream urine was obtained in a sterile container after cleaning the perineal/genital area. Standard bacterial, chemical, and microscopic techniques were used to analyze the urine samples, which included the analysis of urine appearance, pH, specific gravity, and presence of proteins, ketones, bilirubin, urobilinogen, nitrites, leukocytes, erythrocytes, and bacteria. After urine sampling, uricult was cultured, and if positive, UC was also tested. Significant bacteriuria was > 100,000 colony forming unit (CFU)/mL isolated from a voided specimen. Moreover, a sample for blood culture from the peripheral vein was obtained in all patients, and in patients who had central venous (CVC) catheters placed, a sample from CVC was also obtained. Urosepsis is defined by the isolation of the same causative agent from UC and blood culture.

During the study, we analyzed demographic characteristics (age, gender), the number of UTIs in patients with FN, the number of recurrent UTIs, isolated causative agents, their sensitivity to antibiotics, characteristics of urine sediment, the presence of localized symptoms of UTI (dysuria, pollakiuria, hematuria, and abdominal pain), as well as the presence of urosepsis. Available data on complete blood count (leukocytes, hemoglobin, platelets) and the inflammatory marker C-reactive protein (CRP) were collected.

Statistical package SPSS 23.0 was used for data processing and analysis. Besides descriptive statistics, the Chi-squared test was also implemented in order to compare the differences between the groups.

Results

The gender distribution in patients was equal (male/female ratio 50% : 50%). The majority (83%) of patients had hematologic malignancies (leukemia and lymphoma) while a smaller number (17%) had solid tumors (neuroblastoma, nephroblastoma, rhabdomyosarcoma, brain and testicular tumors). One UTI was present in 22 (73.3%) patients, while recurrent UTI was present in 8 (26.7%) patients. In half of the patients, recurrent UTIs occurred one month after the first infection. The largest number (70%) of examined patients previously received trimethoprim/sulfamethoxazole (TMP/SMZ) prophylaxis against *Pneumocystis jirovecii* in-

fection, out of whom 28% had recurrent UTI. The patients who did not receive prophylactic therapy had recurrent UTIs in 40% of the cases. Statistically significant differences were detected in the presence of symptoms of UTI and urine sediment findings in patients with FN when compared to the control group (Table 1). The symptoms of UTI (dysuria, pollakiuria, haematuria, and/or abdominal pain) were present in only 3 (7.7%) patients, while other (92.3%) patients, besides fever, did not have any other symptoms. By examining the differences in clinical manifestation, statistically significant differences between the groups with FN and the control group were detected ($\chi^2 = 25.813$, $p < 0.001$). All patients in the control group had pyuria, 55% of them had microscopic hematuria, and 25% had positive nitrites in urine sediment. In contrast, the patients with FN did not have pyuria in urine sediment in 90% of UTI episodes. This difference was also statistically significant ($\chi^2 = 65.455$, $p < 0.001$). Patients with pyuria (10%) most commonly manifested mild pyuria (5–10 leukocytes). Microscopic hematuria was recorded in two cancer patients with UTI compared to 22 patients in the control group; the difference was statistically significant ($\chi^2 = 23.8095$, $p < 0.001$). The presence of nitrite was recorded in one patient in the study group, while it was recorded in 10 UTI episodes in the control group. These differences were statistically significant ($\chi^2 = 8.5375$, $p < 0.05$). Three cancer patients with UTI subsequently developed urosepsis (7.5%). In all three patients, fever was the only sign of infection – all of them had prolonged neutropenia, and one of them had a central venous line. In the above-mentioned patients, one patient manifested mild pyuria and positive nitrites in urine sediment, another patient had microscopic hematuria, and the third patient had completely normal urine sediment. The only isolated causative agent in the UC of the control group was *Escherichia (E.) coli* in patients with FN. Besides *E. coli* (70% of all isolates), infections caused by other bacterial strains (*Klebsiella* spp 12.5%, *Enterococcus* and *Enterobacter* spp 5%, *Proteus*, *Pseudomonas* and *Morganella morganii* 2.5%) were also noticed. *E. coli* was a causative agent of urosepsis in two patients, while one patient developed urosepsis caused by *Klebsiella*. All of the *Klebsiella* strains

Table 1

Differences in UTI presence and in urine sediment findings in patients with FN and children without FN

Parameter	Group		Total	χ^2	*p*-level
	FN	control			
Urine leucocytes					
no	36 (90.0)	0 (0.0)	36 (45.0)		
yes	4 (10.0)	40 (100.0)	44 (55.0)	65.455	0.000
total	40 (100.0)	40 (100.0)	80 (100.0)		
Urine erythrocytes					
no	38 (95.0)	18 (45.0)	56 (70.0)		
yes	2 (5.0)	22 (55.0)	24 (30.0)	23.809	0.000
total	40 (100.0)	40 (100.0)	80 (100.0)		
Urine nitrites					
no	39 (97.5)	30 (75.0)	69 (86.3)		
yes	1 (2.5)	10 (25.0)	11 (13.8)	8.537	0.009
total	40 (100.0)	40 (100.0)	80 (100.0)		
UTI symptoms					
no	36 (90.0)	14 (35.0)	50 (62.5)		
yes	4 (10.0)	26 (65.0)	30 (37.5)	25.813	0.000
total	40 (100.0)	40 (100.0)	80 (100.0)		

UTI – urinary tract infection; FN – febrile neutropenia.
All values are expressed as numbers (percentages).

Table 2

Difference in the percentage of resistant strains of *Escherichia coli* in urine culture findings in patients with FN and children without FN

Antibiotics	Group	
	FN	control
Ampicillin	89.65	29.50
Amoxicillin/clavulanic acid	55.17	14.30
Amikacin	30.00	6.25
Piperacillin/tazobactam	57.15	11.76
Cephalexin	58.62	14.30
Cefixime	48.00	15.15
Ceftriaxone	50.00	15.15
Ciprofloxacin	48.14	6.66
Trimethoprim/sulphamethoxazole	79.31	20.00
Meropenem	17.86	0.00
Imipenem	10.72	0.00

FN – febrile neutropenia.
All values are expressed as percentages.

were resistant to ampicillin, amoxicillin, cephalexin, and cefixime; 83% of the strains showed resistance to ceftriaxone, amoxicillin/clavulanic acid and TMP/SMZ, 66% showed resistance to piperacillin/tazobactam, 22% to amikacin, and 16% to meropenem and imipenem. A higher percentage of resistant strains of *E. coli* was isolated from UC of patients with FN when compared to the control group (Table 2).

By observing the inflammatory markers, we found that the average value of CRP in patients with FN was 47.5 mg/L [minimum (min) 0.5 mg/L, maximum (max) 359 mg/L; reference range (RR) 0–5 mg/L], while the control group had a higher average value of 122 mg/L. In most episodes of FN (71.8%), the average value of CRP was below 50 mg/L. Mean values of leukocyte count was 1.2×10^9/L (min 0.3×10^9/L, max 3.2×10^9/L; RR 4.0–10.4 $\times 10^9$), hemoglobin levels were 93 g/L (min 72 g/L, max 131 g/L; RR 110–165 g/L), and platelet count 106 x 10^9/L (min 11 x 10^9/L, max 350×10^9/L; RR $150–450 \times 10^9$).

Discussion

FN is one of the most common complications of chemotherapy. Since it presents the leading cause of morbidity and mortality in children with malignant diseases, it should be suspected in every febrile cancer patient who received chemotherapy in the past 14 days [6–9]. Chemotherapy damages the skin and mucous membranes, disrupts the cellular and humoral immune response, and causes inadequate production of antibodies and depletion of immunoglobulin subclasses. After chemotherapy, it takes months for B and T lymphocyte counts to recover, while it takes years for the recovery of their subclasses. The mucocutaneous junction can also be damaged by the presence of intravascular and urinary catheters. After the pathogens enter the bloodstream, tissue damage, and systemic inflammatory response occur, potentially leading to organic dysfunction. Coagulopathy, as well as both quantitative and qualitative platelet disorders, can occur [10–14]. During the period of this study, the largest number of patients developed pancytopenia after a cycle of chemotherapy and became prone to infection. The majority (90%) of patients presented with fever on hospital admission. Three patients developed urosepsis, and fever was the only sign of systemic infection in all of them.

In children who have developed FN after the chemotherapy cycle, fever over 38 °C could be the only sign of systemic infection. Moreover, the inflammatory response can be absent, and thus, in children who have developed sepsis, instead of fever, signs of hypothermia, hypotension, confusion, poor general condition, or signs coming from the site of the primary infection may be present. Furthermore, children with normal absolute neutrophil count might have occult infection due to qualitative disorder of white blood cells. Neutropenic sepsis development and other life-threatening infections pose a high risk to these patients. In up to 50% of patients with FN, the primary infection site remains unidentified, while a causative agent is cultivated from only 20–30% of blood cultures [9, 11, 15, 16]. Therefore, a meticulous search for the primary infection site is mandatory.

The presence of UTI in adult cancer patients with FN is estimated at around 5–30% [2]. The exact prevalence of UTI in children with FN is unknown. In previously conducted studies, the estimated risk of UTI was around 8% [3, 4, 17]. In a study conducted by Klaassen et al. [18], only 4% of the UTI episodes in children with FN were associated with pyuria in urine sediment. The data about the presence of nitrites and microscopic hematuria are missing. In this study, 10% of the UTI episodes were associated with pyuria, 5% with microscopic hematuria, and 2.5% with the presence of nitrites in urine sediment. Both studies are retrospective, and the data about the presence of vulvitis/balanitis, which could possibly cause pyuria, are missing. Rahman et al. [17] have reached a similar conclusion, the largest number of patients with analyzed UTI episodes had normal urine sediment (the absence of pyuria, negative urinary nitrite test, and leukocyte esterase test), and none of the patients had symptoms of localized UTI. In the mentioned study, all patients, except one, received *Pneumocystis jirovecii* prophylaxis with TMP/SMZ three days weekly, and only one patient had a UTI with *E. coli* sensitive to this antibiotic. Prophylactic use of TMP/SMZ caused the most common UTI causative agents to develop resistance to the mentioned antibiotic. Our research showed similar results – 79.31% of *E. coli* isolates, and 83% of *Klebsiella spp* isolates were resistant to TMP/SMZ. On the other hand, in the control group, resistance to this antibiotic was present in 20% of the cases. The largest number of patients with FN was previously treated with prophylactic antibiotic therapy; out of them, 28% had recurrent UTI. In 40% of the cases, patients who were not treated with prophylactic therapy had recurrent UTIs. In our research, all three study patients with FN and urosepsis had additional risk factors for infection. All of them had prolonged neutropenia, and two of them had a central venous line. One of these patients presented with pyuria and positive nitrites, the second had microscopic hematuria, and the third had completely normal urine sediment.

Growing antimicrobial resistance is becoming a considerable problem worldwide, and thus, the selection of adequate antibiotics for treating episodes of FN is also becoming limited. Due to the high morbidity and mortality rates, empirical antibiotic therapy is introduced to every patient with FN. For the initial treatment of children with FN and high risk for developing complications, empirical antipseudomonal penicillin monotherapy (piperacillin/tazobactam, ticarcillin/clavulanic acid), fourth-generation antipseudomonal cephalosporins monotherapy (cefepime) or carbapenem monotherapy (meropenem, imipenem) is recommended. Usage of other antibiotics efficient in the treatment of Gram-negative bacteria (aminoglycosides) or glycopeptide antibiotics efficient in the treatment of Gram-positive bacteria (vancomycin, teicoplanin) is reserved only for clinically unstable patients suspected of developing infections with resistant microorganisms and centers with high-frequency rates of antimicrobial resistance. A Korean study in pediatric patients with FN showed evidence of a growing number of infections caused by Gram-negative bacteria since 2010, with a special focus on strains of *E. coli* and *Klebsiella spp*, which produce extended-spectrum beta-

lactamase (ESBL) [19]. Higher frequencies of ESBL-producing strains (30.6%) were noted by comparison with a previously conducted study in the same center [20]. The main risk factors in patients with FN are previous treatment with antibiotics and former FN episodes treated with broad-spectrum antibiotics. In the study of Hirmas et al. [21], the most isolated causative agents of UTI in pediatric cancer patients were Gram-negative bacteria (84%) with *E. coli* (51%), *Klebsiella pneumoniae* (9%), and *Pseudomonas aeruginosa* (8%) being the most frequent. ESBL-producing strains were present in 37% of the cases, and multidrug-resistant bacterial strains were present in 3%. Our study showed similar results – the most frequently isolated bacteria in patients with FN were *E. coli* and *Klebsiella spp*. Strains of *E. coli* isolated from UC were resistant to carbapenem in 17.86% of the cases, while in 57.15% of the cases, strains were resistant to piperacillin/tazobactam. Strains of *Klebsiella spp* were resistant to carbapenem in 16% of the cases and piperacillin/tazobactam in 66% of the cases. Sensitivity to cefepime was not routinely analyzed. Concerning the above-mentioned facts, recommended empirical antibiotic therapy for the treatment of pediatric UTI is likely to be ineffective in FN patients due to increased resistance of causative microorganisms. Moreover, failing to obtain UC in these patients may finally lead to an undiagnosed disease and inadequate treatment of UTI.

Regarding the fact that FN is an emergency that requires urgent empirical antibiotic treatment, it is necessary to start treatment regardless of the urine findings [22].

Conclusion

The urine sampling is extremely important, not only in establishing the diagnosis of UTI but also in detecting multiresistant bacterial strains. Possible isolation of a causative agent will improve appropriate antibiotic choice and selection of patients with recurrent UTIs who require further monitoring and early detection of potential complications in a timely manner.

R E F E R E N C E S

1. *Uwaezuoke S, Ayuk A, Muoneke U*. Urinary tract infection in children: A review of the established practice guidelines. EMJ Microbiol Infect Dis 2020; 1(1): 57–65.
2. *Hamzeh F, Kanj SS, Uwaydah M*. Febrile neutropenia in cancer patients in a tertiary care medical center in Lebanon: microbial spectrum and outcome. J med Liban 2000; 48(3): 136–42.
3. *Munyi ST, Macharia WM, Alwar AJ, Njeru EK*. Screening for urinary tract infection in children with cancer. East Afr Med J 1998; 75(5): 264–7.
4. *Sandoval C, Sinaki B, Weiss R, Munoz J, Ozkaynak MF, Tugal O*, et al. Urinary tract infections in pediatric oncology patients with fever and neutropenia. Pediatr Hematol Oncol 2012; 29(1): 68–72.
5. *Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA*, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis 2011; 52(4): e56–93.
6. *Barton CD, Waugh LK, Nielsen MJ, Paulus S*. Febrile neutropenia in children treated for malignancy. J Infect 2015; 71(Suppl 1): S27–35.
7. *Agrawal AK, Feusner J*. Supportive Care of Patient's with cancer. In: *Lanzkowsky P, Lippton JM, Fish JD,* editors. Lanzkowsky's Manual of Pediatric Hematology and Oncology. 6th ed. London: Elsevier Inc; 2016. p. 621–7.
8. *Haeusler GM, Phillips RS, Lehrenbecher T, Thursky KA, Sung L, Amman RA*. Core outcomes and definitions for pediatric fever and neutropenia research: A consensus statement from an international panel. Pediatr Blood Cancer 2015; 62(3): 483–9.
9. *Davis K, Wilson S*. Febrile neutropenia in paediatric oncology. Paediatr Child Health (Oxford) 2019; 30(3): 93–7.
10. *Kar YD, Özdemir ZC, Bör Ö*. Evaluation of febrile neutropenic attacks of pediatric hematology-oncology patients. Turk Pediatri Ars 2017; 52(4): 213–20.
11. *Meckler G, Lindemulder S*. Fever and neutropenia in pediatric patients with cancer. Emerg Med Clin North Am 2009; 27(3): 525–44.
12. *Kebudi R, Kizilocak H*. Febrile Neutropenia in children with cancer: Approach to diagnosis and treatment. Curr Pediatr Rev 2018; 14(3): 204–9.
13. *Van Tilburg CM, van Gent R, Bierings MB, Otto SA, Sanders EA, Nibbelke EE,* et al. Immune reconstitution in children following chemotherapy for haematological malignancies: a long-term follow-up. Br J Haematol 2010; 152(2): 201–10.
14. *Mongkolrattanothai K, Bard JD*. Sepsis in children with febrile neutropenia. J Appl Lab Med 2019; 3(4): 530–3.
15. *Penack O, Buchheidt D, Christopeit M, von Lilienfeld-Toal M, Massenkeil G, Hentrich M*, et al. Management of sepsis in neutropenic patients: guidelines from the infectious diseases working party of the German Society of Hematology and Oncology. Ann Oncol 2011; 22(5): 1019–29.
16. *Hakim H, Flynn PM, Knapp KM, Srivastava DK, Gaur AH*. Etiology and clinical course of febrile neutropenia in children with cancer. J Pediatr Hematol Oncol 2009; 31(9): 623–9.
17. *Rahman AA, Gupta SK, Ferdouse Z, Islam A*. Urinary tract infections in pediatric oncology patients with fever and neutropenia. Chattagram Maa-O-Shishu Hosp Med College J 2013; 12(2): 19–22.
18. *Klaassen IL, de Haas V, van Wijk JA, Kaspers GJ, Bijlsma M, Bökenkamp A*. Pyuria is absent during urinary tract infections in neutropenic patients. Pediatr Blood Cancer 2011; 56(5): 868–70.
19. *Lee J*. Antibiotic-resistant gram-negative bacteremia in febrile neutropenic children. Infect Chemother 2016; 48(3): 249–51.
20. *Kwon JC, Kim SH, Choi JK, Cho SY, Park YJ, Park SH,* et al. Epidemiology and clinical features of bloodstream infections in hematology wards: one year experience at the catholic blood and marrow transplantation center. Infect Chemother 2013; 45(1): 51–61.
21. *Hirmas N, Mubarak S, Sultan I*. Patterns of microbial growth in urine cultures in a pediatric hematology/oncology unit over a one-year period: a single institution study. Int J Pediatr Adolesc Med 2017; 4(3): 95–9.
22. *Fletcher M, Hodgkiss H, Zhang S, Browning R, Hadden C, Hoffman T,* et al. Prompt administration of antibiotics is associated with improved outcomes in febrile neutropenia in children with cancer. Pediatr Blood Cancer 2013; 60: 1299–306.

Received on June 20, 2022
Revised on June 27, 2023
Accepted on October 17, 2023
Online First October 2023

ORIGINAL ARTICLE

UDC: 616.61-008.6-08
DOI: https://doi.org/10.2298/VSP230805061K

Evaluating the renoprotective effectiveness of sodium-glucose co-transporter 2 inhibitor therapy in patients with chronic kidney disease: a prospective study

Procena renoprotektivne efikasnosti terapije inhibitorima natrijum-glukoznog kotransportera tipa 2 kod bolesnika sa hroničnom bolešću bubrega: prospektivna studija

Vidna Karadžić Ristanović*, Selena Gajić*, Ana Bontić*†, Jelena Pavlović*†, Aleksandra Kezić*†, Jovana Radovanović‡, Milan Radović*†

***University Clinical Center of Serbia, Clinic for Nephrology, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ‡University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia**

Abstract

Background/Aim. Chronic kidney disease (CKD) is a global health concern associated with increased cardiovascular risks and premature mortality. Proteinuria is a key prognostic indicator for CKD outcome. Sodium-glucose cotransporter 2 (SGLT2) inhibitors show potential for reducing proteinuria and slowing CKD progression. The aim of the study was to determine the impact of SGLT2 inhibitor therapy on CKD patients by evaluating the changes in the level of serum creatinine (sCr), 24-hour (24h) urine protein (UP), estimated glomerular filtration rate (GFR), and blood pressure (BP). **Methods.** This prospective study monitored 79 patients with CKD on therapy with SGLT2 inhibitors, who were followed up for one year. Patients received an SGLT2 inhibitor (dapagliflozin) once daily (10 mg), and assessment of specific parameters was conducted at baseline, 6 months, and 1 year later during the therapy. The study evaluated the levels of sCr, 24h UP, GFR, systolic BP (BPs), diastolic BP (BPd), uric acid (UA), total cholesterol (TC), triglycerides (Tg), low-density lipoprotein (LDL) cholesterol, sodium (Na^+), and potassium (K^+). **Results.** Over the one-year follow-up, significant changes were seen in UA levels (5.36, 4.99, 4.94 mg/dL, respectively; $p = 0.032$), 24h UP (662.60, 574.11, 417.09 mg/dL, respectively; $p = 0.028$), as well as BPs (128.44, 125.64, 126.12 mmHg, respectively; $p = 0.026$). No significant variations were observed in GFR, BPd, sCr, TC, Tg, LDL, and K^+ levels. Na^+ levels displayed a notable decrease (148.21, 147.57, 146.41 mmol/L, respectively; $p = 0.021$). **Conclusion.** The study suggests a potential benefit of SGLT2 inhibitors in managing CKD.

Key words:
drug therapy; proteinuria; renal insufficiency, chronic; sodium-glucose transporter 2 inhibitors; treatment outcome.

Apstrakt

Uvod/Cilj. Hronična bolest bubrega (HBB) predstavlja globalni zdravstveni problem, povezan sa povećanim kardiovaskularnim rizicima i preranom smrtnošću. Proteinurija je ključni prognostički pokazatelj ishoda HBB. Inhibitori natrijum-glukoznog kotransportera tipa 2 (NGKT2) pokazuju potencijal za smanjivanje proteinurije i usporavanje progresije HBB. Cilj rada bio je da se utvrdi uticaj terapije inhibitorima NGKT2 na bolesnike sa HBB procenom promene u nivou serumskog kreatinina (sKr), 24-časovne (24h) proteinurije (PU), brzine glomerularne filtracije (*glomerular filtration rate* – GFR) i krvnog pritiska (KP). **Metode.** U prospektivnu studiju bilo je uključeno 79 bolesnika sa HBB, praćenih tokom godinu dana. Bolesnici su primali inhibitor NGKT2 (dapagliflozin) jednom dnevno (10 mg), a merenja određenih parametara sprovedena su na početku terapije, šest meseci kasnije i godinu dana posle početka terapije. Studijom su procenjivane vrednosti sKr, 24h PU, GFR, sistolnog KP (KPs), dijastolnog KP (KPd), mokraćne kiseline (MK), ukupnog holesterola (UH), triglicerida (Tg), holesterola niske gustine (*low density lipoprotein* – LDL), natrijuma (Na^+) i kalijuma (K^+). **Rezultati.** Tokom godinu dana praćenja, pokazane su značajne promene u nivoima MK (5,36, 4,99, 4,94 mg/dL, redom; $p = 0,032$), 24h PU (662,60, 574,11, 417,09 mg/dL, redom; $p = 0,028$), kao i KPs (128,44, 125,64, 126,12 mmHg, redom; $p = 0,026$). Nisu zapažene značajne varijacije u vrednostima za GFR, KPd, sKr,

Correspondence to: Vidna Karadžić Ristanović, University Clinical Center of Serbia, Clinic for Nephrology, Pasterova 2, 11 000 Belgrade, Serbia. E-mail: vidnakaradzic@gmail.com

UH, Tg, LDL i K^+. Nivo Na^+ pokazao je primetno smanjenje (148,21, 147,57, 146,41 mmol/L, redom; p = 0,021). **Zaključak.** Studija ukazuje na postojanje potencijalne koristi od inhibitora NGKT2 u lečenju bolesnika sa HBB.

Ključne reči:
lečenje lekovima; proteinurija; bubreg, hronična insuficijencija; natrijum-glukozni transporter 2, inhibitori; lečenje, ishod.

Introduction

Chronic kidney disease (CKD) is a highly prevalent and serious global health condition, affecting a substantial population worldwide [1, 2]. This medical condition is associated with an escalated vulnerability to cardiovascular disease, end-stage renal disease, and premature mortality [3, 4]. A distinguishing hallmark of CKD is proteinuria, which serves as a robust prognostic indicator for adverse outcomes in those afflicted [5–8].

Sodium-glucose cotransporter 2 (SGLT2) inhibitors represent an innovative class of antidiabetic medications that exhibit encouraging potential in ameliorating proteinuria and decelerating the progression of CKD. Recent investigations have provided compelling evidence of the renoprotective benefits of SGLT2 inhibitor therapy in CKD patients. Heerspink et al. [9] noted that SGLT2 inhibitor therapy was associated with a slower rate of kidney function decline and lower risk of major kidney events compared to initiation of other glucose-lowering drugs. The meta-analysis conducted by Kalay et al. [10] provides compelling evidence supporting the likely beneficial role of SGLT2 inhibitors in patients with nephrotic-range proteinuria concerning the reduction of proteinuria and the deceleration of CKD progression. The findings contribute to a deeper understanding of the therapeutic potential of these inhibitors in managing CKD and proteinuria, shedding light on their promise as a treatment strategy for patients with nephrotic-range proteinuria.

In light of the aforementioned knowledge gaps, the primary aim of this study was to conduct an in-depth investigation into the impact of SGLT2 inhibitor therapy on patients suffering from CKD throughout a period of one year (yr). The study assessed the comprehensive changes in several critical parameters, encompassing serum creatinine (sCr), 24-hour (24h) urine protein (UP), estimated glomerular filtration rate (GFR), systolic blood pressure (BP) (BPs), diastolic BP (BPd), lipid profile, uric acid (UA). This research intends to analyze the variations in these parameters before and after the initiation of SGLT2 inhibitor therapy, shedding light on the potential renoprotective effectiveness of this treatment modality.

Methods

Study design and subjects

This prospective investigation involved a cohort of 79 patients who underwent continuous monitoring at the Clinic for Nephrology, University Clinical Center of Serbia, as part of the standard follow-up procedure from June 1, 2022, to August 21, 2023. The research was conducted in accordance with the Helsinki Declaration, and informed consent for participation was obtained from the patients involved. The research was approved by the Ethics Committee of the University Clinical Center of Serbia (No. 341/15, from September 14, 2023).

Inclusion criteria for the patients were the presence of CKD, with various grades (G) of the disease expressed as various levels of GFR (mL/min/1.73m^2) [the number 1.73 denotes the average body surface area (m^2) of an adult weighing 70 kg]: G2 (60–89 mL/min/1.73m^2), G3 (30–59 mL/min/1.73m^2), G4 (15–29 mL/min/1.73m^2), and G5 (< 15 mL/min/1.73m^2), with underlying diseases such as arterial hypertension (AH), glomerulopathies or type 2 diabetes mellitus (T2DM), age between 18 and 75 yrs, and the ability of the patient to follow the therapy. Individuals with contraindications for SGLT2 inhibitors such as allergic reactions to drugs, severe kidney diseases, acute infections, systemic lupus erythematosus, vasculitis, autosomal dominant polycystic kidney disease, hospitalization during the follow-up period, or discontinuation of SGLT2 inhibitor therapy for either medical or non-medical reasons, were excluded from the study.

Measurements

The study assessed mean values of sCr, 24h UP, GFR, BPs, and BPd at baseline (BL) and after 6 months (mos) and 1 yr of SGLT2 inhibitor therapy duration. Additionally, it examined UA, total cholesterol (TC), triglycerides (Tg), low-density lipoprotein (LDL) cholesterol, as well as sodium (Na^+) and potassium (K^+). sCr and 24h UP values were measured using spectrophotometry on an Alinity C device (Abbott, Ravenswood, Chicago), while GFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula: $186 \times [\text{serum creatinine (mg/dL)}]^{-1.154} \times (\text{age})^{-0.203} \times$ (0.742 if female) [11]. Serum values of TC, Tg, LDL, and UA levels were analyzed using the Olympus AU600 chemistry immuno analyzer (Olympus, Japan).

Study protocol

All patients were prescribed oral therapy with SGLT2 inhibitor (dapagliflozin) once daily at a dose of 10 mg according to the patient's condition. There were no changes in the dosage of the therapy during the follow-up period. Measurements were taken on three occasions: initial measurement, first follow-up measurement after 6 mos (± 15 days) of therapy duration, and second follow-up measurement after 1 yr (± 15 days) of therapy duration.

Statistical analysis

Statistical analysis was performed using IBM SPSS (version 26.0, Statistical Package for the Social Sciences, Chicago, Illinois, USA). The normality of the distribution for

the observed variables was assessed using the Kolmogorov-Smirnov test. Nonparametric tests were applied when deviations from the normal distribution were detected. The Chi-square test was used to examine significant differences in categorical variables. To determine whether there were statistically significant differences between BL and control measurements, a Friedman's test was performed. Statistical significance was set at a *p*-value of ≤ 0.05.

Results

The total number of participants was 79, of which 45 (57.0%) were males and 34 (43.0%) were females ($\chi^2 = 1.53$; $p = 0.216$). The mean age of the participants was 56 ± 19.3 yrs (minimum-maximum: 19–88 yrs), and the majority of the participants were over 65 yrs old (40.5%). Among the participants, 39.2% were categorized in CKD stage G2, closely followed by 36.8% of patients in stage G3. A smaller contingent of participants was allocated to the more advanced stages, with 21.5% of patients in stage G4 and a mere 2.5% of patients in the pinnacle stage G5. The patients in stage G5 also had heart failure, for which an SGLT2 inhibitor was introduced, so therapy was continued. Of all the participants, 26.6% had T2DM, 64.6% had AH, and 59.5% had glomerulonephritis (GN). The statistical analysis showed significant differences in age ($\chi^2 = 10.37$; $p = 0.016$), prevalence of T2DM ($\chi^2 = 17.3$; $p < 0.001$), AH ($\chi^2 = 6.70$; $p = 0.010$), and CKD stage ($\chi^2 = 27.076$; $p < 0.001$) among the participants. However, there were no significant differences in gender ($\chi^2 = 1.53$; $p = 0.216$) and prevalence of GN ($\chi^2 = 2.85$; $p = 0.091$). Participants' therapeutic regimens encompassed a range of pharmacological interventions. Notably, angiotensin-converting enzyme inhibitors were prescribed to 34.3% of individuals ($\chi^2 = 7.911$; $p = 0.005$) and angiotensin receptor blockers to 43.0% of individuals ($\chi^2 = 1.532$; $p = 0.216$). Loop diuretics were administered to 35.4% of participants ($\chi^2 = 6.696$; $p = 0.010$), and statins were prescribed to 41.8% of participants ($\chi^2 = 2.139$; $p = 0.144$) (Table 1).

Table 1

Demographic and clinical characteristics of patients

Variable	n (%)	χ^2	*p*-value
Demographic characteristics			
Gender			
male	45 (57.0)	1.53	0.216
female	34 (43.0)		
Age, (years)			
18–35	14 (17.7)	10.37	**0.016**
36–50	17 (21.5)		
51–65	16 (20.3)		
> 65	32 (40.5)		
mean ± SD (min-max)	56 ± 19.3 (19–88)		
CKD (mL/min/1.73m^2)			
G2 (60–89)	31 (39.2)	27.076	**< 0.001**
G3 (59–30)	29 (36.8)		
G4 (29–15)	17 (21.5)		
G5 (< 15)	2 (2.5)		
Comorbidities			
Type 2 diabetes mellitus			
no	58 (73.4)	17.3	**<0.001**
yes	21 (26.6)		
Arterial hypertension			
no	28 (35.4)	6.70	**0.010**
yes	51 (64.6)		
Glomerulonephritis			
no	32 (40.5)	2.85	0.091
yes	47 (59.5)		
Therapy			
ACEi			
no	52 (65.8)	7.911	**0.005**
yes	27 (34.3)		
ARB			
no	45 (57.0)	1.532	0.216
yes	34 (43.0)		
Loop diuretics			
no	51 (64.6)	6.696	**0.010**
yes	28 (35.4)		
Statins			
no	46 (58.2)	2.139	0.144
yes	33 (41.8)		

SD – standard deviation; CKD – chronic kidney disease; G – grade of disease; ACEi – angiotensin-converting enzyme inhibitors; ARB - angiotensin 2 receptor blockers; χ^2 – Friedman's Chi-square coefficient; n – number of patients. Bolded values indicate statistically significant results.

BL and control values of observed variables are shown in Table 2. The mean values of sCr were 136.78 ± 71.97 μmol/L [reference range (RR): 59–104 μmol/L] at BL and 136.21 ± 70.75 μmol/L after 6 mos. After 1 yr, the mean sCr level was 137.27 ± 73.31 μmol/L ($\chi^2 = 5.842$, $p = 0.054$). For 24h UP, the mean values were 662.60 ± 545.33 mg/dL (RR < 150 mg/dL) at BL, 574.11 ± 507.72 mg/dL after 6 mos and 417.09 ± 513.48 mg/dL after 1 yr ($\chi^2 = 7.117$, $p = 0.028$). The GFR showed minimal variation over time. At BL, the mean GFR was 45.01 ± 17.21 mL/min/1.73m² (RR > 90 mL/min/1.73m^2), and it remained relatively stable at 44.97 ± 16.46 mL/min/1.73m² after 6 mos and 45.80 ± 17.98 mL/min/1.73m² after 1 yr ($\chi^2 = 0.320$, $p = 0.572$). Regarding blood pressure, both BPs and BPd levels exhibited some changes. BPs decreased from 128.44 ± 15.54 mmHg at BL to 125.64 ± 10.35 mmHg after 6 mos and 126.12 ± 10.63 mmHg after 1 yr. This reduction was statistically significant (Friedman's $\chi^2 = 7.270$, $p = 0.026$). BPd showed no significant changes over time, with mean values of 80.45 ± 9.91 mmHg at BL, 80.93 ± 7.48 mmHg after 6 mos, and 80.99 ± 7.79 mmHg after 1 yr (Friedman's $\chi^2 = 359.0$, $p = 0.907$). UA levels exhibited a significant decrease, with mean values of 5.36 ± 1.21 mg/dL (RR: 2–7 mg/dL) at BL, 4.99 ± 1.37 mg/dL after 6 mos, and 4.94 ± 1.39 mg/dL after 1 yr follow-up ($\chi^2 = 7.421$, $p = 0.032$). TC remained stable, ranging from 5.34 ± 1.39 mmol/L (RR < 5.2 mmol/L) at BL, 5.38 ± 1.83 mmol/L after 6 mos, to 5.35 ± 1.91 mmol/L after 1 yr ($\chi^2 = 0.105$, $p = 0.974$). Tg showed a trend towards reduction, registering 2.12 ± 1.03 mmol/L (RR < 1.7 mmol/L) at BL, 1.97 ± 0.65 mmol/L after 6 mos, and 1.96 ± 0.67 mmol/L after 1 yr ($\chi^2 = 5.240$, $p = 0.052$). LDL levels remained steady, with mean values of 2.74 ± 1.21 mmol/L (RR < 3.4 mmol/L) at BL, 2.58 ± 1.29 mmol/L after 6 mos, and 2.54 ± 1.36 mmol/L after 1 yr ($\chi^2 = 0.311$, $p = 0.582$). Na^+ levels exhibited a statistically significant change, varying from 148.21 ± 10.24 mmol/L (RR: 135–148 mmol/L) at BL, 147.57 ± 9.44 mmol/L after 6 mos, to 146.41 ± 10.48 mmol/L after 1 yr ($\chi^2 = 7.870$, $p = 0.021$). K^+ levels remained consistent, showing mean values of 4.81 ± 0.47 mmol/L (RR: 3.5–5.0 mmol/L) at BL, 4.82 ± 0.48 mmol/L after 6 mos, and 4.81 ± 0.44 mmol/L after 1 yr ($\chi^2 = 0.059$, $p = 0.987$).

Discussion

The study revealed a statistically significant decrease in 24h UP, UA, BPs, and Na^+ levels, while other monitored parameters, including sCr, GFR, BPd, TC, Tg, LDL, and K^+ exhibited stability with no significant changes observed. The research findings provide compelling evidence in favor of a male predominance over females, diverging from the observations in the study by Kao et al. [12]. Furthermore, a recent investigation conducted by Lewandowski et al. [13] in Austria concluded that women exhibit a higher susceptibility to CKD compared to men. Concerning age distribution, most of our sample consisted of individuals over 65 yrs. Correspondingly, Liu et al. [14] presented data that aligns with our results, asserting that CKD is more prevalent among older individuals, with advancing age correlating to a higher incidence of complications arising from CKD. However, there is a noticeable shift in boundaries, as CKD is increasingly manifesting in younger individuals [15]. The accelerated disease progression observed in the younger cohort is akin to numerous other diseases, entailing a multitude of comorbidities, which, in most cases, share a causal relationship with CKD [16, 17].

The research outcomes underscore GN as the most prevalent comorbidity linked to CKD, signifying a significant association between these two states. This observation is corroborated by Wetmore et al. [18], whose study elucidated that approximately 10–15% of individuals with GN progress to the terminal stage of renal failure. Conversely, Meremo et al. [19] documented a notably lower GN comorbidity among CKD patients. AH emerges as the second most frequent comorbidity, which is to be expected given the established correlation between elevated blood pressure and the development of CKD [20, 21]. Additionally, T2DM constitutes a significant comorbidity within this study. The well-established link between T2DM and CKD stems from diabetes' propensity to inflict kidney damage over time [21, 22]. The three aforementioned factors are the most common comorbidities causally related to CKD, albeit the order of prevalence may fluctuate in other investigations [23, 24].

Table 2

Baseline and follow-up values of the specific observed variables

Variable	Baseline	Follow-up		χ^2	*p*-value
		6 months	1 year		
sCr (μmol/L)	136.78 ± 71.97 (51.0–443.0)	136.21 ± 70.75 (44.0–375.0)	137.27 ± 73.31 (44.21–376.0)	5.842	0.054
24 h UP (mg/dL)	662.60 ± 545.33 (0.02–44,970.0)	574.11 ± 507.72 (0.07–45,139.0)	417.09 ± 513.48 (0.05–44,970.0)	7.117	**0.028**
GFR (mL/min/1.73m^2)	45.01 ± 17.21 (9.0–108.0)	44.97 ± 16.46 (10.0–90.0)	45.80 ± 17.98 (10–91)	0.320	0.572
BPs (mmHg)	128.44 ± 15.54 (90.0–180.0)	125.64 ± 10.35 (100.0–150.0)	126.12 ± 10.63 (100.0–150.0)	7.270	**0.026**
BPd (mmHg)	80.45 ± 9.91 (60.0–100.0)	80.93 ± 7.48 (60.0–90.0)	80.99 ± 7.79 (60–90)	359.0	0.907
UA (mg/dL)	5.36 ± 1.21 (1.49–10.21)	4.99 ± 1.37 (1.58–7.81)	4.94 ± 1.39 (1.59–7.78)	7.421	**0.032**
TC (mmol/L)	5.34 ± 1.39 (3.55–7.49)	5.38 ± 1.83 (3.42–7.57)	5.35 ± 1.91 (3.24–7.44)	0.105	0.974
Tg (mmol/L)	2.12 ± 1.03 (0.35–4.52)	1.97 ± 0.65 (0.41–4.27)	1.96 ± 0.67 (0.54–4.51)	5.240	0.052
LDL (mmol/L)	2.74 ± 1.21 (0.81–3.12)	2.58 ± 1.29 (0.98–3.12)	2.54 ± 1.36 (1.1–3.37)	0.311	0.582
Na^+ (mmol/L)	148.21 ± 10.24 (145.24–154.01)	147.57 ± 9.44 (144.84–152.11)	146.41 ± 10.48 (145.28–154.05)	7.870	**0.021**
K^+ (mmol/L)	4.81 ± 0.47 (4.44–5.57)	4.82 ± 0.48 (4.51–5.68)	4.81 ± 0.44 (4.37–5.68)	0.059	0.987

sCr – serum creatinine; 24 h UP – 24-hour urine protein; GFR – estimated glomerular filtration rate; BPs – systolic blood pressure; BPd – diastolic blood pressure; UA – uric acid; TC – total cholesterol; Tg – triglycerides; LDL – low-density lipoprotein; χ^2 – Friedman's Chi-square coefficient. All results are shown as mean ± standard deviation (minimum-maximum). Bolded values indicate statistically significant results.

Regarding the impact of SGLT2 inhibitors, the study results demonstrate insignificant alterations in sCr and GFR levels and significant alterations when it comes to BPs between BL and control measurements. Notably, mean BPd values remained within the reference range, indicative of compensated hypertension. Additionally, after 1 yr of SGLT2 inhibitor therapy, a substantial reduction in the range and standard deviation of BP was observed, signifying hypertension stabilization. The results of our study align with the findings of Briasoulis et al. [25] and Baker et al. [26]. Regarding sCr values, prior investigations have shown that SGLT2 inhibitors may elevate sCr levels due to the tubuloglomerular feedback mechanism [27]. Although SGLT2 inhibitors hold renoprotective potential, their judicious use is of paramount importance, as they may entail risks, such as increased sCr levels in CKD patients [27, 28].

This study confirmed significantly decreased follow-up values in 24h UP excretion levels, indicative of the SGLT2 inhibitors' impact. As emphasized by Takashima et al. [29], the renoprotective effect encompasses diminished protein secretion, particularly in patients with T2DM. Furthermore, this renoprotective effect is linked to a reduced risk of cardiovascular incidents [30]. Reduction in protein excretion has also been documented in studies exploring nephrotic syndrome as a consequence of CKD, demonstrating marked improvements in hypoalbuminemia following SGLT2 inhibitor therapy [31, 32]. However, to achieve the complete renoprotective effect of SGLT2 inhibitors, a certain duration of action is required [28, 33].

Furthermore, it is noteworthy that there was a statistically significant reduction in UA levels during the follow-up period, which is an expected outcome. Earlier researchers, including Banerjee et al. [34], confirmed such findings in their meta-analysis of randomized controlled trials. The reduction in Na^+ levels has been confirmed, as observed in the study by Tang et al. [35]. The decrease in blood Na^+ concentration was expected due to the direct action of SGLT2 inhibitors on its reabsorption in the kidneys.

It is of great importance to mention the results of a randomized trial conducted by Perkovic et al. [36], which confirms the renoprotective effects of SGLT2 inhibitors in patients with T2DM. The study claims that the use of SGLT2 inhibitors extends the time of CKD onset by 2.62 yrs compared to patients who do not use SGLT2 inhibitors. Heerspink et al. [37] also achieved significant results, elucidating that dapagliflozin, belonging to the SGLT2 inhibitor class, substantially reduces the risk of CKD progression. In a recent study by the EMPA-KIDNEY Collaborative Group [38], it was found that empagliflozin, another SGLT2 inhibitor, reduces the risk of cardiovascular events and death in CKD patients by 27%.

The preceding three studies provide deeper insights into the role of SGLT2 inhibitors concerning CKD and emphasize their renoprotective potential. Our study's results support the existence of the protective effects of SGLT2 inhibitors, given the observed reduction in proteinuria. However, it is essential to consider the limitation of a short follow-up period when evaluating other variables in this study.

Conclusion

The study's conclusion indicates a correlation between CKD and comorbidities, with GN, AH, and T2DM being the most common accompanying conditions. SGLT2 inhibitors demonstrated a statistically significant reduction in proteinuria, suggesting potential renoprotective effects. However, longer-term monitoring is necessary to fully understand the impact on other measured parameters. These findings provide a basis for further research to better elucidate the role of SGLT2 inhibitors in managing CKD.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Borg R, Carlson N, Søndergaard J, Persson F.* The Growing Challenge of Chronic Kidney Disease: An Overview of Current Knowledge. Int J Nephrol 2023; 2023: 9609266.
2. *Georgianos PI, Agarwal R.* Hypertension in chronic kidney disease - treatment standard 2023. Nephrol Dial Transplant 2023; gfad118.
3. *Elendu C, Elendu RC, Enyong JM, Ibhiedu JO, Ishola IV, Egbunu EO,* et al. Comprehensive review of current management guidelines of chronic kidney disease. Medicine (Baltimore) 2023; 102(23): e33984.
4. *Liu W, Zhou L, Yin W, Wang J, Zuo X.* Global, regional, and national burden of chronic kidney disease attributable to high sodium intake from 1990 to 2019. Front Nutr 2023; 10: 1078371.
5. *Kataoka H, Mochizuki T, Ohara M, Tsuruta Y, Iwasa N, Yoshida R,* et al. Urate-lowering therapy for CKD patients with asymptomatic hyperuricemia without proteinuria elucidated by attribute-based research in the FEATHER Study. Sci Rep 2022; 12(1): 3784.
6. *Lafayette RA, Canetta PA, Rovin BH, Appel GB, Novak J, Nath KA,* et al. A Randomized, Controlled Trial of Rituximab in IgA Nephropathy with Proteinuria and Renal Dysfunction. J Am Soc Nephrol 2017; 28(4): 1306–13.
7. *Provenzano M, Puchades MJ, Garofalo C, Jongs N, D'Marco L, Andreucci M,* et al. Albuminuria-Lowering Effect of Dapagliflozin, Eplerenone, and Their Combination in Patients with Chronic Kidney Disease: A Randomized Crossover Clinical Trial. J Am Soc Nephrol 2022; 33(8): 1569–80.
8. *Swartling O, Rydell H, Stendahl M, Segelmark M, Trolle Lagerros Y, Evans M.* CKD Progression and Mortality Among Men and Women: A Nationwide Study in Sweden. Am J Kidney Dis 2021; 78(2): 190–9.e1.
9. *Heerspink HJL, Karasik A, Thuresson M, Melzer-Cohen C, Chodick G, Khunti K,* et al. Kidney outcomes associated with use of SGLT2 inhibitors in real-world clinical practice (CVD-REAL 3): a multinational observational cohort study. Lancet Diabetes Endocrinol 2020; 8(1): 27–35.

10. *Kalay Z, Sahin OE, Copur S, Danaci S, Ortiz A, Yau K,* et al. SGLT-2 inhibitors in nephrotic-range proteinuria: emerging clinical evidence. Clin Kidney J 2022; 16(1): 52–60.
11. *Lingli X, Qing Z, Wenfang X.* Diagnostic value of the modification of Diet in Renal Disease and Chronic Kidney Disease Epidemiology Collaboration equations in diabetic patients: a systematic review and meta-analysis. J Int Med Res 2020; 48(6): 300060520925950.
12. *Kao HY, Chang CC, Chang CF, Chen YC, Cheewakriangkrai C, Tu YL.* Associations between Sex and Risk Factors for Predicting Chronic Kidney Disease. Int J Environ Res Public Health 2022; 19(3): 1219.
13. *Lewandowski MJ, Krenn S, Kurnikowski A, Bretschneider P, Sattler M, Schwaiger E,* et al. Chronic kidney disease is more prevalent among women but more men than women are under nephrological care: Analysis from six outpatient clinics in Austria 2019. Wien Klin Wochenschr 2023; 135(3–4): 89–96.
14. *Liu P, Quinn RR, Lam NN, Al-Wahsh H, Sood MM, Tangri N,* et al. Progression and Regression of Chronic Kidney Disease by Age Among Adults in a Population-Based Cohort in Alberta, Canada. JAMA Netw Open 2021; 4(6): e2112828.
15. *Kerklaan J, Hannan E, Hanson C, Guha C, Cho Y, Christian M,* et al. Perspectives on life participation by young adults with Chronic kidney disease: an interview study. BMJ Open 2020; 10(10): e037840.
16. *Mallappallil M, Friedman EA, Delano BG, McFarlane SI, Salifu MO.* Chronic kidney disease in the elderly: evaluation and management. Clin Pract (Lond) 2014; 11(5): 525–35.
17. *Ferris ME, Miles JA, Seamon ML.* Adolescents and Young Adults with Chronic or End-Stage Kidney Disease. Blood Purif 2016; 41(1–3): 205–10.
18. *Wetmore JB, Guo H, Liu J, Collins AJ, Gilbertson DT.* The incidence, prevalence, and outcomes of glomerulonephritis derived from a large retrospective analysis. Kidney Int 2016; 90(4): 853–60.
19. *Meremo AJ, Masalu MB, Sabi I, Ngilangwa DP, Kapinga J, Tagalile R,* et al. Prevalence and Risk Factors Associated With Chronic Kidney Disease Among Patients Presenting at a Haemodialysis Unit in Dodoma, Tanzania. East Afr Health Res J 2018; 2(1): 53–7.
20. *Gupta A, Nagaraju SP, Bhojaraja MV, Swaminathan SM, Mohan PB.* Hypertension in Chronic Kidney Disease: An Update on Diagnosis and Management. South Med J 2023; 116(2): 237–44.
21. *Hamrahian SM, Falkner B.* Hypertension in Chronic Kidney Disease. Adv Exp Med Biol 2017; 956: 307–25.
22. *Żyłka A, Gala-Błądzińska A, Rybak K, Dumnicka P, Drożdż R, Kuśnierz-Cabala B.* Role of new biomarkers for the diagnosis of nephropathy associated with diabetes type 2. Folia Med Cracov 2015; 55(4): 21–33.
23. *Lee WC, Lee YT, Li LC, Ng HY, Kuo WH, Lin PT,* et al. The Number of Comorbidities Predicts Renal Outcomes in Patients with Stage 3¯5 Chronic Kidney Disease. J Clin Med 2018; 7(12): 493.
24. *MacRae C, Mercer SW, Guthrie B, Henderson D.* Comorbidity in chronic kidney disease: a large cross-sectional study of prevalence in Scottish primary care. Br J Gen Pract 2021; 71(704): e243–9.
25. *Briasoulis A, Al Dhaybi O, Bakris GL.* SGLT2 Inhibitors and Mechanisms of Hypertension. Curr Cardiol Rep 2018; 20(1): 1.
26. *Baker WL, Smyth LR, Riche DM, Bourret EM, Chamberlin KW, White WB.* Effects of sodium-glucose co-transporter 2 inhibitors on blood pressure: a systematic review and meta-analysis. J Am Soc Hypertens 2014; 8(4): 262–75.e9.
27. *Chu C, Lu YP, Yin L, Hocher B.* The SGLT2 Inhibitor Empagliflozin Might Be a New Approach for the Prevention of Acute Kidney Injury. Kidney Blood Press Res 2019; 44(2): 149–57.
28. *Yau K, Dharia A, Alrowiyti I, Cherney DZI.* Prescribing SGLT2 Inhibitors in Patients with CKD: Expanding Indications and Practical Considerations. Kidney Int Rep 2022; 7(7): 1463–76.
29. *Takashima H, Yoshida Y, Nagura C, Furukawa T, Tei R, Maruyama T,* et al. Renoprotective effects of canagliflozin, a sodium glucose cotransporter 2 inhibitor, in type 2 diabetes patients with chronic kidney disease: A randomized open-label prospective trial. Diab Vasc Dis Res 2018; 15(5): 469–72.
30. *Petrykiv S, Sjöström CD, Greasley PJ, Xu J, Persson F, Heerspink HJL.* Differential Effects of Dapagliflozin on Cardiovascular Risk Factors at Varying Degrees of Renal Function. Clin J Am Soc Nephrol 2017; 12(5): 751–9.
31. *Murashima R, Sai E, Tagawa Y, Yanagawa H, Ishiwata S, Kawaguchi Y,* et al. Usefulness of Dapagliflozin for Nephrotic Syndrome Secondary to Diabetic Kidney Disease. Intern Med 2022; 61(24): 3699–702.
32. *Tanaka A, Nakamura T, Sato E, Node K.* Therapeutic potential of tofogliflozin on nephrotic syndrome secondary to diabetic nephropathy. J Cardiol Cases 2017; 16(1): 30–3.
33. *Bailey CJ, Day C, Bellary S.* Renal Protection with SGLT2 Inhibitors: Effects in Acute and Chronic Kidney Disease. Curr Diab Rep 2022; 22(1): 39–52.
34. *Banerjee M, Pal R, Maisnam I, Chowdhury S, Mukhopadhyay S.* Serum uric acid lowering and effects of sodium-glucose cotransporter-2 inhibitors on gout: A meta-analysis and meta-regression of randomized controlled trials. Diabetes Obes Metab 2023; 25(9): 2697–703.
35. *Tang H, Cui W, Li D, Wang T, Zhang J, Zhai S,* et al. Sodium-glucose co-transporter 2 inhibitors in addition to insulin therapy for management of type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. Diabetes Obes Metab 2017; 19(1): 142–7.
36. *Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM,* et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. N Engl J Med 2019; 380(24): 2295–306.
37. *Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF,* et al. Dapagliflozin in Patients with Chronic Kidney Disease. N Engl J Med 2020; 383(15): 1436–46.
38. *The EMPA-KIDNEY Collaborative Group; Herrington WG, Staplin N, Wanner C, Green JB, Hauske SJ,* et al. Empagliflozin in Patients with Chronic Kidney Disease. N Engl J Med 2023; 388(2): 117–27.

Received on August 5, 2023
Revised on September 7, 2023
Revised on October 9, 2023
Accepted on October 17, 2023
Online First October 2023

ORIGINAL ARTICLE

UDC: 613.96:[613.2:613.71
DOI: https://doi.org/10.2298/VSP230131058D

Association between eating habits and low physical activity in adolescents

Povezanost između navika u ishrani i nedovoljne fizičke aktivnosti među adolescentima

Dragan Djurdjević*, Aleksandra Nikolić†, Sanja Mazić‡, Sandra Šipetić-Grujičić†

University of Belgrade, Faculty of Medicine, *Department of Sports Medicine, †Institute of Epidemiology, ‡Medical Physiology, Belgrade, Serbia

Abstract

Background/Aim. Insufficient levels of physical activity and poor nutrition, have led to an increase in the number of obese adolescents and cardiometabolic problems. The aim of this study was to analyze the association between eating habits and low physical activity levels among adolescents. **Methods.** The cross-sectional study, conducted in May 2017, included a total of 389 participants, aged 15–19 years. The study instrument was a questionnaire regarding demographic characteristics, physical activity (International Physical Activity Questionnaire-Short Form), as well as eating habits. The data were analyzed using univariate and multivariate logistic regression analysis. **Results.** A total of 131 participants (33.7%) had a low level of physical activity, and 258 (66.3%) had a moderate/high level of physical activity. Adolescents with moderate/high physical activity were significantly more likely to be male and medical school students. Moreover, they had breakfast significantly more often, consumed fish more than twice a week or at least twice a week, ate fruit once a day or more, and generally ate healthy, compared to the high school students with low physical activity. Among the factors associated with low physical activity were the following: the female gender, eating breakfast never/sometimes, consuming fish less than twice a week, fruit less than once a day, maintaining a healthy diet, and adolescents aged 18–19 years. **Conclusion.** This study identified several statistically significant independent factors associated with low physical activity in adolescents. Based on the obtained results, it is necessary to undertake certain interventions, promote physical activity, correct eating habits, and the overall lifestyle, with a special emphasis on the female population and teenagers aged 18–19 years. The education of both adolescents and their parents is essential.

Key words:
adolescent; attitude to health; exercise; feeding behavior; healthy lifestyle; serbia; sex factors; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Nedovoljan nivo fizičke aktivnosti i loša ishrana doveli su do povećanja broja gojaznih adolescenata i kardiometaboličkih problema. Cilj rada bio je da se analizira povezanost između navika u ishrani i niskog nivoa fizičke aktivnosti kod adolescenata. **Metode**. Studijom preseka, sprovedenom tokom maja 2017. godine, obuhvaćeno je ukupno 389 ispitanika, uzrasta 15–19 godina. Instrument istraživanja bio je upitnik o demografskim karakteristikama, fizičkoj aktivnosti (*International Physical Activity Questionnaire-Short Form*), kao i navikama u ishrani. Podaci su analizirani korišćenjem univarijantne i multivarijantne logističke regresione analize. **Rezultati.** Ukupno 131 (33,7%) ispitanik imao je nizak nivo fizičke aktivnosti, a 258 (66,3%) umeren/visok nivo fizičke aktivnosti. Adolescenti sa umerenim/visokim nivoom fizičke aktivnosti su značajno češće bili muškarci i učenici medicinske škole. Takođe, oni su značajno češće doručkovali, jeli ribu češće ili bar dva puta nedeljno, jeli voće jednom ili više puta dnevno i generalno su se hranili zdravo, u poređenju sa srednjoškolcima sa niskim nivoom fizičke aktivnosti. Među faktorima povezanim sa niskim nivoom fizičke aktivnosti bili su: ženski pol, doručkovanje nikada/ponekad, konzumiranje ribe ređe od dva puta nedeljno, voća ređe nego jednom dnevno, održavanje zdrave ishrane i adolescentni uzrast 18–19 godina. **Zaključak.** Ovom studijom identifikovano je nekoliko statistički značajnih nezavisnih faktora, koji su povezani sa niskim nivoom fizičke aktivnosti kod adolescenata. Na osnovu dobijenih rezultata, neophodno je preduzeti određene korektivne mere, promovisati fizičku aktivnost, korigovati navike u ishrani i način života, posebno u ženskoj populaciji i kod adolescenata uzrasta 18–19 godina. Veoma je važna edukacija adolescenata i njihovih roditelja.

Ključne reči:
adolescent; stav prema zdravlju; vežbanje; ishrana, navike; zdravlje, način života; srbija; pol, faktor; ankete i upitnici.

Correspondence to: Dragan Djurdjević, University of Belgrade, Faculty of Medicine, Department of Sports Medicine, Višegradska 26/2, 11 000 Belgrade, Serbia. E-mail: d_djurdjevic@yahoo.com

Introduction

A balanced diet and physical activity (PA) are essential for healthy growth and development of adolescents. Poor eating habits and insufficient PA are associated with cardiometabolic problems in this population [1]. Insufficient levels of PA and poor nutrition, according to the World Health Organization (WHO), have led to an increase in the number of obese and overweight children and adolescents, from 11 million to 124 million obese and 216 million overweight, from 1975–2016 [2]. Furthermore, the increase in type 2 diabetes mellitus in adolescents worldwide in the last 20 years is dramatic, where the prevalence is three times higher at the age of 15–18 compared to the age of 10–14, and girls have a 60% higher prevalence than boys [3].

Elevated blood pressure in childhood and adolescence can be manifested in adulthood through the development of hypertension and metabolic syndrome and can be associated with poor glucose metabolism and dyslipidemia [4].

PA helps develop and improve the musculoskeletal system and cardiorespiratory fitness, enables better control of energy balance and body weight, and reduces sedentary behaviors. Moreover, PA affects social interaction and has a positive impact on mental health through the reduction of anxiety and stress, better cognitive function, self-confidence, and achievement in school [5]. The recommendation for daily PA in adolescence is 60 min of moderate to vigorous PA [6, 7]. Exercises to strengthen the musculoskeletal system should be included at least three times a week. The WHO data show that in the category of adolescents, more than 80% of them do not meet the level of recommended PA [6]. The prevalence of insufficient PA worldwide in the period 2001–2016 was lower in boys by 2.5%, while in girls, it remained unchanged, with an increased gender gap of 7.1%. In Serbia in 2015, 65.5% of adolescents (aged 15–18) did not meet the level of recommended PA, where the girls were less active by 19% [8].

In order to make the strategic and necessary interventions to promote PA and prevent the negative trend of the development of chronic non-communicable diseases, it is very important to understand the eating habits that affect insufficient PA. A study conducted among adolescents in the United States of America shows a strong association between PA and nutritional behavior and that higher levels of PA are associated with higher intake of fruits and vegetables [9]. On the other hand, a lower intake of fruits and vegetables correlates with a lower PA level [10]. Some studies have shown that older adolescents are less physically active compared to the younger ones, that the male gender is associated with higher PA [10, 11], and that adolescents who skip breakfast have lower PA levels [12, 13]. In contrast, Sila et al. [14] have shown that breakfast consumption does not affect PA.

The aim of this study was to analyze the association between eating habits and low PA levels among adolescents in Valjevo, Serbia.

Methods

The cross-sectional study was conducted in May 2017; 389 adolescents aged 15–19 years were randomly selected for this study from all five Valjevo high schools (Medical School, Valjevo High School, Technical School, School of Economics, and School of Agriculture). Valjevo is a city and the administrative center of the Kolubara District in western Serbia. The coverage rate was 14.4%.

The sample size was calculated using Epi Info 7 (population 2,700, expected prevalence 81%, acceptable margin of error: 5%, design effect 1). The required sample size was 291. Due to potential losses, the number of participants included in the study was increased by 20%.

Information about the study and the study protocol were submitted to the School Board and then to the Parents' Council, after which the parents gave their consent for the children's participation in the study. The number of respondents who refused to participate in this study was 14.4%. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Serbia (No. 29/XII-18, from December 28, 2015).

Data collection

All data were collected by a questionnaire. It contained questions related to the demographic characteristics of adolescents, their PA, and their eating habits.

Sociodemographic characteristics

Sociodemographic characteristics that were collected *via* a questionnaire were gender (male/female), age (15–17/18–19), type of school (Medical School, Valjevo High School, Technical School, School of Economics, and School of Agriculture), and residence (with parents, with one parent, tenant).

Physical activity level

The International Physical Activity Questionnaire-Short Form (IPAQ-SF) was used for assessing PA [15]. This questionnaire consists of seven questions related to the assessment of the level of PA for the last seven days. Obtained time in min is converted to metabolic equivalent (MET)-min *per* week:

MET/week = high-intensity PA (MET) + moderate-intensity PA (MET) + low-intensity PA (MET) [16]; 8.0 MET is used for high-intensity PA, 4.0 MET for medium-intensity PA, and 3.3 MET for low-intensity PA. Energy expenditure was calculated in the following way:

MET/week = number of days with PA x duration in min x k*; *k is intensity (8/4/3.3 MET)

All participants were classified into three groups according to the degree of their PA: insufficient/low level of PA (< 600 MET-min/week), sufficient/moderate level of PA (601–3,000 MET-min *per* week), and sufficient/high level of PA (> 3,000 MET-min *per* week) [16]. Since the aim of this study was to determine the factors that affect low PA, two categories of participants were made – those with low PA and those with moderate/high PA, based on the recommendation for minimal PA [7].

Anthropometric measurements

The height was measured on the altimeter SECA 213 (Seca GmbH & Co.KG.). Weight was measured with the InBody 230 (Biospace, Seoul, Korea). Body mass index (BMI) percentiles were calculated according to the WHO standards [17].

Body composition analysis

The percentage of body fat (% BF) of the participants was obtained by body composition analysis that was performed on the device InBody 230 (Biospace, Seoul, Korea). Measurements were made according to the manufacturer's instructions – in the morning, after at least 2 hrs of not consuming any food or fluids, and after emptying the bladder. Participants did not engage in any PA prior to performing the analysis. They were classified into four categories based on their % BF and age (underweight, with normal body weight, overweight, and obese) [18].

Eating habits

In our study, we used adapted items from the questionnaire applied in the Serbian National Health Survey 2013 [19]. Our questionnaire contained nine questions that related to breakfast (every day/sometimes/never), consumption of milk and dairy products (2 or more cups every day/one cup every day/sometimes, not every day/never), type of bread most often used (white/semi-white/integral/combined-all types/do not eat bread), type of fat most often used for food preparation (lard, butter/vegetable fat, margarine/oil/do not use fat), salting food (yes, almost always before tasting food/yes, when food is not salty enough/never), fish consumption (more than twice a week/twice a week/less than twice a week, never), fruit consumption (once and several times a day/4 to 6 times a week/1 to 3 times a week/less than once a week/never), vegetable consumption (once and several times a day/4 to 6 times a week/1 to 3 times a week/less than once a week/never), and thinking about health when choosing a diet (always/often/sometimes/never). The variables were analyzed individually, but we also formed a cumulative variable "healthy diet", based on the Healthy Eating Index, as a measure of diet quality [20].

The cumulative variable was formed as the sum of the answers to eight questions related to nutrition. The total score of "healthy diet" had values from 0 to 8. The variable was divided into two categories (unhealthy diet < 4 and healthy diet 4+). Answers are marked with 1 in the following categories: breakfast (every day), consumption of milk and dairy products (2 or more cups/one cup every day), the type of bread most often used (integral/combined-all types), the type of fat most often used for food preparation (oil/do not use fat), salting food (never), fish consumption (more than twice a week/twice a week), fruit consumption (once and several times a day), and vegetable consumption (once and several times a day).

Data analysis

A total of 16 variables were analyzed: gender, age, school, housing, BMI, % BF, breakfast, consumption of milk and dairy products, type of bread most often used, type of fat most often used for food preparation, salting food, fish consumption, fruit consumption, vegetable consumption, thinking about health when choosing a diet, and a healthy diet.

Data analysis was performed using the statistical analysis software IBM SPSS Statistics 24.0 for Mac (Chicago, IL, USA). Methods of descriptive statistics (mean, standard deviation, and median) were used in statistical data analysis. Univariate logistic regression analysis (ULRA) and multivariate logistic regression analysis (MLRA) were used to examine significant independent factors for low PA. Variables with a *p*-value of < 0.1 according to ULRA were included in the MLRA model. The difference was marked as significant if $p < 0.05$.

Results

High school students (389 participants) had an average value of % BF 23.30 ± 8.30, average value of BMI 21.80 ±

Table 1

Distribution of moderate/high physical activity (PA) and low PA of high school students in relation to their demographic characteristics

Demographic characteristics	Moderate and high PA n = 258	Low PA n = 131	OR (95% CI)	*p*-value*
Gender				
male	116 (45.0)	24 (18.3)	3.64 (2.19–6.04)	< 0.001
female	142 (55.0)	107 (81.7)		
Age (years)				
15–17	206 (79.8)	87 (66.4)	2.00 (1.25–3.22)	0.004
18–19	52 (20.2)	44 (33.6)		
School				
medical	56 (21.7)	17 (13.0)	1.86 (1.03–3.35)	0.039
other**	202 (78.3)	114 (87.0)		
Residence				
with parents	218 (84.5)	104 (79.4)	1 (Ref.)	
with one parent	33 (12.8)	19 (14.5)	1.20 (0.65–2.21)	0.237
tenant	7 (2.7)	8 (6.1)	2.38 (0.84–6.75)	

Moderate and high PA – more than 600 metabolic equivalent (MET)-min *per* week; Low PA – less than 600 MET-min *per* week; OR – odds ratio; CI – confidence interval; Ref. – reference category; **p*-value according to univariate logistic regression analysis. **other – Valjevo High School, Technical School, School of Economics, School of Agriculture.
Data are expressed as numbers (percentages).

3.17 kg/m^2, average total score of healthy diet 3.59 ± 1.49, and in MET-min 1,633.82 ± 1,302.31.

Of the 389 participants who participated in the study, 258 (66.3%) had moderate/high PA, and 131 (33.7%) had low PA. Students with moderate/high PA were significantly more likely to be male, 15–17 years old, and enrolled in medical school. There was no significant difference between the examined groups in terms of the place of residence during studies (Table 1).

No statistical differences among high school students were observed between BMI and % BF and PA levels (Table 2).

Students with moderate/high PA had breakfast significantly more often, consumed fish at least twice a week, ate fruit at least once a day, and generally ate healthy compared to high school students with low PA. There was no significant difference between the two groups concerning the frequency of consumption of vegetables and milk and/or dairy products, as well as the type of bread and fats consumed, or salting food (Table 3).

Table 2

Distribution of moderate/high physical activity (PA) and low PA of high school students in relation to their anthropometric characteristics

Level of nutrition	Moderate and high PA n = 258	Low PA n = 131	OR (95% CI)	*p*-value*
Body mass index				
malnutrition/normal body weight	210 (81.4)	113 (86.3)	1 (Ref.)	
overweight	36 (14.0)	14 (10.7)	0.72 (0.37–1.39)	0.456
obesity	12 (4.7)	4 (3.1)	0.61 (0.19–1.95)	
Body fat percentage				
malnutrition/normal body weight	198 (76.7)	88 (67.2)	1 (Ref.)	
overweight	36 (14.0)	24 (18.3)	1.50 (0.84–2.66)	0.120
obesity	24 (9.3)	19 (14.5)	1.78 (0.92–3.42)	

Data are expressed as numbers (percentages). *p-value according to univariate logistic regression analysis. For other abbreviations, see Table 1.

Table 3

Distribution of moderate/high physical activity (PA) and low PA of high school students concerning eating habits

Eating habits	Moderate and high PA n = 258	Low PA n = 131	OR (95% CI)	*p*-value*
How many times during the week do you have breakfast?				
every day	235 (91.1)	103 (78.6)		0.001
sometimes/never	23 (8.9)	28 (21.4)	2.78 (1.53–5.05)	
How often do you drink milk and/or dairy products?				
2 or more cups every day	62 (24.0)	26 (19.8)	1 (Ref.)	
one cup every day	80 (31.0)	35 (26.7)	1.04 (0.56–1.91)	0.285
sometimes, not every day/never	116 (45.0)	70 (53.4)	1.44 (0.83–2.48)	
What type of bread do you use most often in your diet?				
integral	20 (7.8)	8 (6.1)	1 (Ref.)	
semi–white	11 (4.3)	8 (6.1)	1.82 (0.53–6.19)	
combined (all types)	78 (30.2)	39 (29.8)	1.25 (0.50–3.09)	0.768
white	122 (47.3)	66 (50.4)	1.35 (0.56–3.29)	
do not eat bread	27 (10.5)	10 (7.6)	0.93 (0.31–2.77)	
What type of fat is most often used for food preparation (cooking, baking, making a cake, etc.) in your household?				
oil/do not use fat	179 (69.4)	93 (71.0)	1 (Ref.)	
lard, butter	65 (25.2)	31 (23.7)	0.92 (0.55–1.51)	0.944
vegetable fat, margarine	14 (5.4)	7 (5.3)	0.96 (0.37–2.47)	
Do you add salt to the food you eat?				
never	71 (27.5)	36 (27.5)	1 (Ref.)	0.44
yes, when the food is not salty enough	159 (61.6)	75 (57.3)	0.93 (0.57–1.51)	
yes, almost always before I try food	28 (10.9)	20 (15.3)	1.41 (0.70–2.84)	
How often do you eat fish?				
more than twice a week	10 (3.9)	1 (0.8)	1 (Ref.)	
twice a week	53 (20.5)	4 (3.1)	0.75 (0.07–7.48)	< 0.001
less than twice a week/never	195 (75.6)	126 (96.2)	6.46 (0.81–51.10)	
How often do you eat fruit other than juice made from fruit concentrate?				
once and several times a day	106 (41.1)	28 (21.4)	2.56 (1.57–4.17)	< 0.001
less than once a day	152 (58.9)	103 (78.6)		
How often do you eat vegetables and salads, except potatoes and juice from vegetable concentrates?				
once and several times a day	93 (36.0)	36 (27.5)	1.49 (0.93–2.36)	0.091
less than once a day	165 (64.0)	95 (72.5)		
Healthy diet** 4+	140 (54.5)	45 (34.6)	0.44 (0.28–0.69)	< 0.001

Data are expressed as numbers (percentages). *p-value according to univariate logistic regression analysis. **Healthy diet minimum value 0, maximum value 8. For other abbreviations, see Table 1.

Table 4

Factors of low physical activity according to the results of multivariate logistic regression analysis

Parameter	B	OR (95% CI)	*p*-value
Model 1			
gender: female	1.42	4.12 (2.36–7.18)	< 0.001
age: 18–19 years	0.83	2.29 (1.33–3.92)	0.003
breakfast: never/sometimes	0.98	2.67 (1.36–5.22)	0.004
fish: less than twice a week	1.92	6.82 (2.54–18.27)	< 0.001
fruit: less than once a day	0.73	2.08 (1.22–3.55)	0.007
Model 2			
gender: female	1.59	4.90 (2.82–8.51)	< 0.001
age: 18–19 years	0.879	2.41 (1.42–4.07)	0.001
healthy diet 4+	-0.402	0.67 (0.56–0.79)	< 0.001

For abbreviations, see Tables 1 and 3.

The first MLRA model included the variables gender, age, school, breakfast, and consumption of fish, fruits, and vegetables, which were converted into variables with two categories each for the needs of MLRA. Significant independent factors of low PA were the female gender, age 18–19 years, breakfast never/sometimes, consuming fish less than twice a week, and fruit less than once a day (Table 4).

A healthy diet significantly positively correlates with all individual variables related to nutrition, which served to form the given variable.

The second MLRA model included gender, age, and healthy diet (overall assessment of healthy eating in high school students), and it was found that all three variables represent significant independent factors of low PA (Table 4). Female gender and age of 18–19 years were risk factors, while a healthy diet was a protective factor for low PA.

Discussion

The results of this study showed that two-thirds of adolescents had moderate/high PA, and one-third had low PA, in contrast to the data of WHO, which show that in the category of adolescents, more than 80% of them do not meet the level of recommended PA [6]. This study shows that adolescents in Valjevo are more physically active than adolescents in Belgrade (low PA 65.5%), the capital of Serbia, according to data from urban areas [8]. On the one hand, that is probably due to the availability and proximity to sports facilities in smaller communities and participation in sports and recreational activities, and on the other hand, it could be due to the use of IPAQ-SF as an indirect method of measuring PA.

According to MLRA results, significant independent factors of low PA among adolescents are female gender, breakfast never/sometimes, consuming fish less than twice a week, and fruit less than once a day. These factors are significantly independent even after age control, and being in the 18–19 age group represents a significant independent risk factor, but so does a healthy diet.

Despite the high prevalence of PA in Valjevo, we found that females have about four times higher likelihood to have low PA, as shown by some other studies [10, 11]. That indicates that women have lower levels of PA compared to men in all age groups, which directly impacts their health [21], while the study of Miljanovic-Damjanovic et al. [22] shows that in this period of adolescence, there is a decrease in PA in both genders. In addition to the aforementioned benefits, PA is important for women from a psychological point of view due to the reduction of stress, anxiety, and depression. In addition, PA is vital because of self-esteem, social integration, and equality in society [23]. Furthermore, PA is essential for expectant mothers and a healthy pregnancy. During pregnancy, PA is primarily important in the prevention of preeclampsia and gestational diabetes mellitus, the most frequent metabolic disorder during pregnancy.

As shown by the results of other studies, adolescents who skip breakfast or have breakfast sometimes are about 2.5 times more likely to have low PA, i.e., those who eat breakfast more regularly (2–3 days/3 days, frequent breakfast consumption) are more physically active [24] when it comes to moderate/high PA [25]. Skipping breakfast is associated with female gender and low PA [12]. On the other hand, the study of Sila et al. [14] shows that skipping breakfast is not related to PA, and there is no consensus. In addition to the influence on PA, breakfast as the most important meal of the day is positively associated with academic achievement, mental performance, and quality of life and negatively associated with obesity [26]. Consuming fish less than twice a week and consuming fruit less than once a day fits into a poor diet and lifestyle in general. It increases the likelihood of low PA by almost 6.5 times when it comes to consuming fish and more than 2.5 times when it comes to consuming fruit. Manz et al. [11] found that adolescents with high PA levels were twice as likely to consume large amounts of fruit. When it comes to consuming vegetables, we did not find any significant association with PA, while some other studies did show that [11]. According to MLRA, older adolescents aged 18–19 have a 2.3 times higher likelihood of having low PA than the younger ones. This indicates that this is the period when high school students are approaching the period of going to college and that their obligations are increasing, which affects their PA. Moreover, the study of Farooq et al. [21] shows that PA decreases in late adolescence. In addition to the above, despite factors associated with low PA, our results show that a healthy diet is negatively associated with low PA, while some studies show that a healthy balanced diet is positively associated with PA [11]. This can be explained by the adoles-

cents' way of thinking that the impact on health is sufficient if they only take care of a healthy diet and that it is not necessary to practice any kind of PA. In addition, girls think more about their body shape, tend to control or lose weight, and have increased awareness about their diet.

It is known that insufficient levels of PA and poor nutrition are associated with overweight and obesity. Adolescents with obesity are at a high risk of being obese in adulthood and, thus, at a higher risk of developing cardiometabolic diseases. In Serbia, the number of obese children and adolescents in 2018 was 5.7%, and 18.2% were overweight [27]. In addition to medical comorbidities, social and psychological comorbidities are present, mostly associated with depression, negative moods, destructive behavior, and physical and verbal abuse, often as a result of peer stigmatization. These problems lead to a worse social life, poor school performance [28], and, as a result, poorer quality of life [29].

On the one hand, reduced PA and poor nutrition are elements for the development of obesity, and on the other hand, overweight and obesity affect reduced volume or absence of PA. However, it is interesting that in our study, there was no statistical difference between students of different PA levels and their BMI, but we got that low PA is associated with skipping breakfast, insufficient consumption of dairy products and whole grains, insufficient daily intake of vegetables, and salting of food, which on the other hand affects overweight and obesity.

Limitations of the study

The study has certain limitations, hence the recommendations for future research are as follows: this study included a random sample of adolescents aged 15–19 but did not include the category of younger adolescents. Instead of IPAQ-SF, it would be better to use more accurate measuring instruments, for instance, an accelerometer, to obtain more reliable PA data and avoid bias. In addition, longitudinal dietary monitoring should be used in the following research on eating habits. The strength of the study is in identifying significant independent factors affecting low PA, and the use of IPAQ-SF makes our results comparable to numerous studies that use the same questionnaire.

Conclusion

This study identified several statistically significant independent factors that are associated with low PA in adolescents. They refer to the female gender, age 18–19, breakfast never/sometimes, consuming fish less than twice a week and fruit less than once a day, and an unhealthy diet. According to the obtained results, it is necessary to undertake certain interventions, such as education and promotion of PA, aimed at correcting current eating habits, as well as the overall lifestyle. The education of both adolescents and parents is essential. This would be of great importance for the health of adolescents and future generations, with a special emphasis on the female population. It is necessary to continuously promote PA and healthy eating habits in schools while introducing new programs aimed at increasing the number of physically active adolescents.

Conflict of interest

The authors declare no conflict of interest.

This research received no specific grant from funding agencies in the public, commercial, or not-profit sectors.

REFERENCES

1. *Cunha CM, Costa PRF, de Oliveira LPM, Queiroz VAO, Pitangueira JCD, Oliveira AM.* Dietary patterns and cardiometabolic risk factors among adolescents: Systematic review and meta-analysis. Br J Nutr 2018; 119(8): 859–79.
2. *World Health Organization.* Tenfold increase in childhood and adolescent obesity in four decades: new study by Imperial College London and WHO [Internet]. London; 2017 [cited 2022 Nov 13; accessed on 2023 Oct 20]. Available from: https://www.who.int/news-room/detail/11-10-2017-tenfold-increase-in-childhood-and-adolescent-obesity-in-four-decades-new-study-by-imperial-college-london-and-who
3. *Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J,* et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA 2014; 311(17): 1778–86.
4. *Tasy A, Fisher M.* The medical evaluation and management of children and adolescents with obesity. Curr Probl Pediatr Adolesc Health Care 2020; 50(9): 100874.
5. *2018 Physical Activity Guidelines Advisory Committee.* 2018 Physical Activity Guidelines Advisory Committee Scientific Report [Internet]. Washington (DC): U.S. Department of Health and Human Services; 2018 [accessed on 2023 Oct 20]. Available from: https://health.gov/sites/default/files/2019-09/PAG_Advisory_Committee_Report.pdf
6. *World Health Organization.* Physical activity [Internet]. Switzerland; 2020 [cited 2022 Nov 15; accessed on 2023 Oct 20]. Available from: https://www.who.int/news-room/fact-sheets/detail/physical-activity
7. *NHS.* Physical activity guidelines for children and young people [Internet]. 2019 [cited 2022 Nov 15; accessed on 2023 Oct 23]. Available from: https://www.nhs.uk/live-well/exercise/physical-activity-guidelines-children-and-young-people/
8. *Božić P, Ostojić S, Berjan-Bačvarević B, Radisavljević-Janić S, Pažin N, Milanović I,* et al. Youth Physical Activity and Nutrition Guidelines [Internet]. Belgrade: Association for Sports Medicine of Serbia; 2016 [accessed on 2023 November 8]. Available from: https://rzsport.gov.rs/wp-content/uploads/2020/12/Vodic-za-fizicku-aktivnost-i-ishranu-mladih.pdf
9. *Lowry R, Michael S, Demissie Z, Kann L, Galuska DA.* Associations of Physical Activity and Sedentary Behaviors with Dietary Behaviors among US High School Students. J Obes 2015; 2015: 876524.
10. *Darfour-Oduro SA, Buchner DM, Andrade JE, Grigsby-Toussaint DS.* A comparative study of fruit and vegetable consumption and physical activity among adolescents in 49 Low-and-Middle-Income Countries. Sci Rep 2018; 8(1): 1623.
11. *Manz K, Mensink GBM, Finger JD, Haftenberger M, Brettschneider AK, Barbosa CL,* et al. Associations between physical activity and food intake among children and adolescents: Results of KIGGS wave 2. Nutrients 2019; 11(5): 1060.

12. *Smith KJ, Breslin MC, McNaughton SA, Gall SL, Blizzard L, Venn AJ*. Skipping breakfast among Australian children and adolescents; findings from the 2011–12 National Nutrition and Physical Activity Survey. Aust N Z J Public Health 2017; 41(6): 572–8.
13. *Harris JA, Carins JE, Rundle-Thiele S*. A systematic review of interventions to increase breakfast consumption: A socio-cognitive perspective. Public Health Nutr 2021; 24(11): 3253–68.
14. *Sila S, Ilić A, Mišigoj-Duraković M, Sorić M, Radman I, Šatalić Z*. Obesity in adolescents who skip breakfast is not associated with physical activity. Nutrients 2019; 11(10): 2511.
15. *Ministry of Health of the Republic of Serbia.* Research on the health of the inhabitants of the Republic of Serbia 2006 – final report [Internet]. Serbia: Ministry of Health; 2007 [accessed 2023 November 17]. Available from: https://www.batut.org.rs/download/publikacije/Finalni%20izvestaj%202006.pdf
16. *Todorovic J, Terzic-Supic Z, Djikanovic B, Nesic D*. Can social media intervention improve physical activity of medical students? Public Health 2019; 174: 69–73.
17. *World Health Organization.* BMI-for-age (5-19 years) [Internet]. Switzerland: WHO; 2020 [cited 2022 Oct 22; accessed on 2023 Oct 20]. Available from: https://www.who.int/toolkits/growth-reference-data-for-5to19-years/indicators/bmi-for-age
18. *Tanita*. Body Fat Percentage Chart [Internet] [cited 2022 Oct 15; accessed on 2023 Oct 20]. Available from: https://www.tanita.com/data/BodyFatPercentageChart.pdf?rev=CE2E
19. *Boričić K, Vasić M, Grozdanov J, Gudelj Rakić J, Živković Šulović M, Jaćović Knežević N*, et al. Research results on the health of the population of Serbia, 2013 [Internet]. Belgrade: Institute of Public Health of Serbia "Dr. Milan Jovanović Batut"; 2014 [accessed on 2023 November 8]. Available from: https://batut.org.rs/download/publikacije/IstrazivanjeZdravljaStanovnistvaRS2013.pdf
20. *Hiza HA, Casavale KO, Guenther PM, Davis CA*. Diet quality of Americans differs by age, sex, race/ethnicity, income, and education level. J Acad Nutr Diet 2013; 113(2): 297–306.
21. *Farooq MA, Parkinson KN, Adamson AJ, Pearce MS, Reilly JK, Hughes AR,* et al. Timing of the decline in physical activity in childhood and adolescence: Gateshead Millennium Cohort Study. Br J Sports Med 2018; 52(15): 1002–6.
22. *Miljanovic Damjanovic V, Obradovic Salcin L, Zenic N, Foretic N, Liposek S*. Identifying Predictors of Changes in Physical Activity Level in Adolescence: A Prospective Analysis in Bosnia and Herzegovina. Int J Environ Res Public Health 2019; 16(14): 2573.
23. *McMahon EM, Corcoran P, O'Regan G, Keeley H, Cannon M, Carli V*, et al. Physical activity in European adolescents and associations with anxiety, depression and well-being. Eur Child Adolesc Psychiatry 2017; 26(1): 111–22.
24. *Garcia AS, Takahashi S, Anderson-Knott M, Dev D*. Determinants of Physical Activity for Latino and White Middle School-Aged Children. J Sch Health 2019; 89(1): 3–10.
25. *Zakrzewski-Fruer JK, Gillison FB, Katzmarzyk PT, Mire EF, Broyles ST, Champagne CM,* et al. Association between breakfast frequency and physical activity and sedentary time: A cross-sectional study in children from 12 countries. BMC Public Health 2019; 19(1): 1–11.
26. *Lundqvist M, Vogel NE, Levin LÅ*. Effects of eating breakfast on children and adolescents: A systematic review of potentially relevant outcomes in economic evaluations. Food Nutr Res 2019; 63.
27. *Gudelj Rakić J, Jovanović V, Kilibarda B, Vesić M, Tošić M, Kisić Tepavčević D*. Health Behaviour Research in School-aged Children in the Republic of Serbia in 2018. Belgrade: Institute of Public Health of Serbia "Dr. Milan Jovanović Batut"; 2019 [accessed on 2023 November 8]. Available from: https://www.batut.org.rs/download/novosti/RezultatiIstrazivanjaPonasanjaDeceSkolskogUzrasta.pdf
28. *Asigbee FM, Whitney SD, Peterson CE*. The Link Between Nutrition and Physical Activity in Increasing Academic Achievement. J Sch Health 2018; 88(6): 407–15.
29. *Reinehr T*. Long-term effects of adolescent obesity: Time to act. Nat Rev Endocrinol 2018; 14(3): 183–8.

Received on January 31, 2023
Revised on August 29, 2023
Accepted on October 17, 2023
Online First October 2023

CASE REPORTS

UDC: 616.342-007.64:616.37]:616.599
DOI: https://doi.org/10.2298/VSP230626043T

Pancreatic panniculitis associated with periampullary duodenal diverticulum

Panikulitis pankreasa udružen sa periampularnim divertikulumom duodenuma

Tanja Tirnanić*†, Tatjana Radević*†, Andrea Djordjević*†, Nenad Petrov†‡, Željko Mijušković*†

Military Medical Academy, *Clinic for Dermatovenerology, ‡Institute of Pathology and Forensic Medicine, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Pancreatic panniculitis (PP) is a rare type of lobular panniculitis that manifests as painful erythematous nodules on the skin of the lower extremities. Subcutaneous fat necrosis caused by the release of pancreatic enzymes is the underlying cause of the disease, affecting around 2–3% of patients with pancreatic diseases. **Case report.** We present a case of a 58-year-old male patient who was admitted to our clinic due to the appearance of painful erythematous nodules on the lower extremities and trunk. Laboratory results revealed increased levels of pancreatic enzymes, amylase, and lipase, as well as heightened levels of glucose and inflammation markers. The histological analysis of the skin lesion biopsy revealed the presence of predominantly lobular panniculitis in the hypodermis, with areas of fatty tissue necrosis/saponification and remnants of adipocytes ("ghost cells"). Abdominal computed tomography scan demonstrated periampullary diverticulum (PD) of the duodenum, with no signs of pancreatitis or other pancreatic abnormalities. Esophagogastroduodenoscopy showed a wide opening of PD in the D2 segment of the duodenum. The patient was successfully treated with pancreatin therapy, resulting in a significant reduction of skin lesions and decreased levels of pancreatic enzymes. **Conclusion.** Duodenal PDs can be the cause of PP, most likely due to the pressure they exert on the pancreatic duct, which can lead to elevated values of pancreatic enzymes. Depending on the individual characteristics of the patient, symptomatic duodenal PD may be treated with operative or non-operative measures. Treatment of PP primarily involves addressing any underlying medical condition.

Key words:
diagnosis; diverticulum; endoscopy, gastrointestinal; histological techniques; pancreatin; pancreatitis; panniculitis; tomography, x-ray computed.

Apstrakt

Uvod. Panikulitis pankreasa (PP) je retka forma lobularnog panikulitisa koja se manifestuje pojavom eritematoznih bolnih nodusa na koži predominantno donjih ekstremiteta. U osnovi ovog oboljenja je supkutana masna nekroza uzrokovana oslobađanjem pankreasnih enzima koja se viđa kod 2–3% bolesnika sa bolestima pankreasa. **Prikaz bolesnika.** Prikazujemo 58-godišnjeg bolesnika koji je primljen u našu kliniku zbog pojave bolnih, eritematoznih nodusa na koži donjih ekstremiteta i trupa. Laboratorijske analize pokazale su povišene vrednosti pankreasnih enzima, amilaze i lipaze, povišene vrednosti glukoze i markera zapaljenja. Histopatološkom analizom bioptirane lezije kože pokazano je prisustvo pretežno lobularnog panikulitisa u hipodermu, sa poljima nekroze/saponifikacije masnog tkiva i ostacima adipocita („ćelijama-duhovima"). Kompjuterizovanom tomografijom abdomena utvrđeno je postojanje periampularnog divertikuluma (PD) duodenuma, bez znakova pankreatitisa ili drugih abnormalnosti pankreasa. Ezofagogastroduodenoskopijom viđen je širok otvor PD u D2 segmentu duodenuma. Bolesnik je uspešno lečen pankreatinom i došlo je do početne regresije promena na koži, uz sniženje nivoa pankreasnih enzima. **Zaključak.** Duodenalni PD mogu biti uzročnici PP, najverovatnije zbog pritiska koji vrše na izvodni kanal pankreasa, što može dovesti do povišenih vrednosti pankreasnih enzima. U zavisnosti od individualnih karakteristika bolesnika, terapija PD može biti hirurška i nehirurška. Lečenje PP podrazumeva lečenje oboljenja i stanja koja se nalaze u njegovoj osnovi.

Ključne reči:
dijagnoza; divertikulum; endoskopija, gastrointestinalna; histološke tehnike; pankreatin; pankreatitis; panikulitis; tomografija, kompjuterizovana, rendgenska.

Correspondence to: Tanja Tirnanić, Military Medical Academy, Clinic for Dermatovenerology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: storebarce@gmail.com

Introduction

Pancreatic panniculitis (PP) is a skin condition that affects a small percentage (2–3%) of individuals with pancreatic diseases [1]. While the exact cause is unknown, some studies suggest that pancreatic enzymes may trigger the condition by releasing fatty acids from the fat beneath the skin, leading to fat necrosis [1, 2].

PP can be linked to several pancreatic conditions, such as acute and chronic pancreatitis, pancreatic tumors, and cysts [1–4]. The skin symptoms can occur before, during, or after the development of pancreatic disease [2, 4].

The condition typically presents as painful, red nodules beneath the skin, mostly observed on the lower legs, although other body areas can also be affected. In more severe cases, the symptoms can lead to skin ulcers and other complications [3].

Case report

A male 58-year-old patient was hospitalized in our clinic due to the presence of painful erythematous nodules on his lower extremities and trunk, that persisted for two months. The patient did not exhibit any constitutional symptoms. Initial treatment with systemic antibiotics on an outpatient basis did not yield a therapeutic response.

In his personal medical history, the patient reported that he suffered from acute pancreatitis in 2011. The patient also reported cigarette smoking with no previous history of alcohol consumption.

During the physical examination, numerous subcutaneous nodules, measuring 1.5–4.0 cm in diameter, displaying erythematous coloration and fluctuation, were observed primarily on the lower legs, thighs, and trunk (Figure 1). Additionally, mild edema of both feet and ankles was present, and the patient's abdomen was found to be soft and non-tender. He did not report abdominal pain, nausea, vomiting, or any other gastrointestinal symptoms.

Abnormal laboratory findings showed increased sedimentation rate [106 mm/h, reference range (RR) < 20 mm/h], increased leucocyte count (14.47 × 10^9/L, RR 4–10 × 10^9/L), increased levels of C-reactive protein (280.57 mg/L, RR 0–5 mg/L), glucose (10.5 mmol/L, RR 4.1–5.9 mmol/L), urea (13.1 mmol/L, RR 2.5–7.5 mmol/L), creatinine (139 mmol/L, RR 62–115 mmol/L), amylase (133 U/L, RR 30–115 U/L), lipase (1,041 U/L, RR 73–393 U/L), alpha-1 antitrypsin (2.88 g/L, RR 0.78–2 g/L) and decreased levels of iron (3 μmol/L, RR 8–30 μmol/L), erythrocytes (3.68 × 10^{12}/L, RR 4.5–6.5 × 10^{12}/L), and hemoglobin (101 g/L, RR 130–180 g/L). Serum tumor marker levels of carcinoembryonic antigen, CA 19.9, and alpha-fetoprotein were within the RR. Immunologic analyses: anti-nuclear antibodies - ANA, C3, C4, extractable nuclear antigen - ENA screening, antineutrophil cytoplasmic antibodies - ANCA with a cytoplasmic staining pattern (cANCA), perinuclear ANCA - pANCA, cryoglobulins, and circulating immune complexes were undetectable.

The histological analysis of the skin lesion biopsy revealed the presence of predominantly lobular panniculitis in the hypodermis, characterized by a mixed inflammatory infiltrate, areas of fatty tissue necrosis/saponification, and remnants of adipocytes ("ghost cells") (Figure 2). Granuloma formation or atypical cells were not observed, and the pathological findings were consistent with subcutaneous nodular fat necrosis.

The culture of the skin tissue sample showed no presence of deep fungal infection or acid-resistant bacteria.

Additional diagnostic procedures were done. Abdominal ultrasound showed signs of liver steatosis. Computed tomography (CT) of the chest, abdomen, and pelvis demonstrated the periampullary diverticulum (PD) of the duodenum adjacent to the pancreatic head. CT scans showed no signs of pancreatitis, pancreatic neoplasm, or any other pancreatic disease. Esophagogastroduodenoscopy showed a wide opening of PD in the D2 segment of the duodenum.

Initially, before obtaining the results of histologic analysis for skin lesions, systemic antibiotic therapy (intravenous metronidazole and meropenem for seven days) and topical corticosteroids (betamethasone dipropionate 0.05%) were started twice a day for skin lesions along with bed rest and leg elevation, with insufficient therapeutic effect. Since the diagnoses of PD and PP were established and pancreatic enzymes were elevated, a gastroenterologist was consulted, and pancreatin 10,000 IU was administered three times a day.

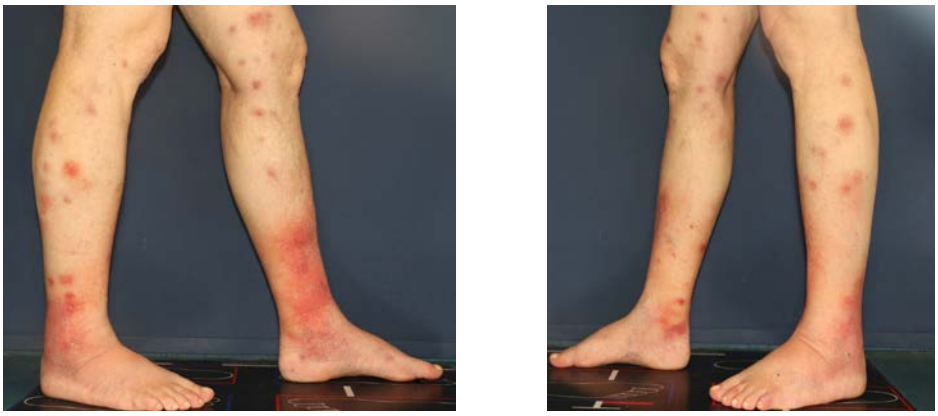


Fig. 1 – Multiple fluctuant erythematous subcutaneous nodules measuring 1.5–4 cm in diameter on both lower legs and thighs.

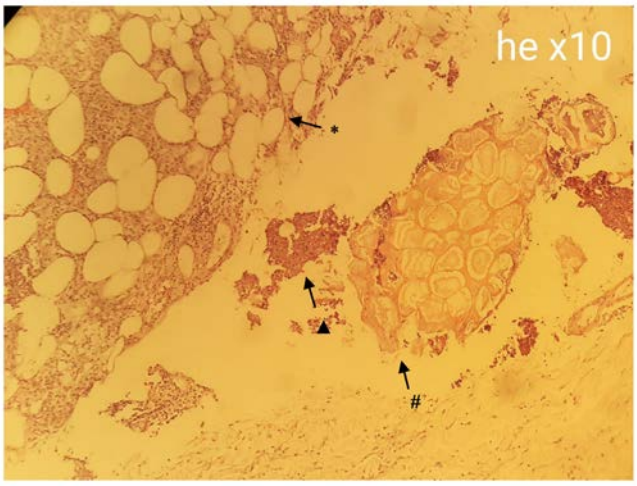


Fig. 2 – Predominantly lobular panniculitis (*) with "ghost-cells" (#), necrotic adipocytes with amorphous granular debris (▲) (hematoxylin-eosin, ×10).

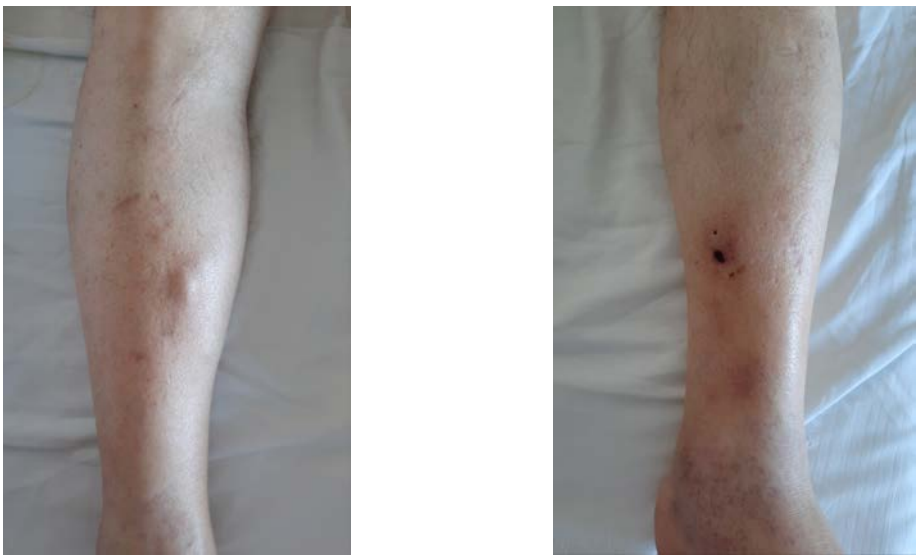


Fig. 3 – Initial regression of skin lesions.

Ten days after starting pancreatin therapy, there was a significant reduction in skin lesions along with decreasing levels of pancreatic enzymes (amylase, 129 U/L; lipase, 120 U/L) (Figure 3).

Discussion

PP is an infrequent disorder that occurs when pancreatic enzymes are released in large quantities into the bloodstream, leading to the development of subcutaneous nodules and necrosis of the fatty tissue [1, 2]. This condition is an uncommon complication of pancreatic diseases, often observed in cases of acute or chronic pancreatitis, pancreatic carcinoma (specifically the acinar cell variant), and less frequently in conjunction with pancreatic pseudocysts and pancreas divisum [1–4]. Nevertheless, there have been documented cases of its occurrence linked to various other diseases and conditions like HIV infection, haemophagocytic syndrome, diabetic ketoacidosis, sepsis, liver carcinoma, and systemic lupus erythematosus [5–9]. To the best of our knowledge, this case report presents the first documented instance of PP associated with a duodenal PD.

Due to its clinical presentation, PP may have similarities with other types of septal and lobular panniculitis (erythema nodosum, erythema induratum, lupus panniculitis, etc.) [2, 4]. Therefore, it is crucial to conduct various diagnostic procedures, placing particular emphasis on performing a biopsy of skin lesions and submitting the sample for thorough histological and microbiological analysis. In the initial stages, early skin lesions may exhibit septal panniculitis without fat necrosis. On the other hand, fully developed lesions display distinct saponification and "ghost cells", which represent remnants of adipocytes with amorphous granular debris [2–4].

The presumed mechanism behind the development of skin lesions involves trypsin increasing the permeability of blood vessels in the tissue, which allows lipase to enter the subcutaneous tissue and break down fatty acids [2, 4].

Elevated serum levels of amylase, lipase, and trypsin are often observed in PP, even though the presence of elevat-

ed levels of a single enzyme without concurrent elevation in others is not uncommon. The development and severity of panniculitis do not necessarily correlate with enzyme levels [2]. In some cases, the patient may display elevated levels of serum lipase without any clinical or radiologic evidence of pancreatitis or other pancreatic diseases [8].

In this particular case, laboratory analyses revealed a 2.6-fold increase in serum lipase levels (1,043 U/L, normal < 393 U/L) with slightly increased levels of amylase (133 U/L, normal < 115 U/L). Abdominal CT indicated the presence of duodenal PD with no signs of pancreatitis or other pancreatic diseases. The patient did not report any constitutional or gastrointestinal symptoms at presentation.

PDs refer to sac-like expansions in the mucosal lining situated near the ampulla of Vater. It is worth noting that approximately 70–75% of duodenal diverticula are classified as periampullary [10]. There have been reports linking PDs with acute and chronic pancreatitis. Complications arising from these diverticula can occur when they exert pressure on the duodenal wall, common bile duct, or pancreatic duct, potentially leading to pancreatitis [10–12].

In the case presented, it is possible that distension of a diverticulum caused compression of the pancreatic duct, resulting in an asymptomatic increase in pancreatic enzymes and subsequent PP. Furthermore, elevated pancreatic enzymes were observed despite the absence of clinical examination or CT scan findings indicative of pancreatitis (CT scans have a sensitivity of approximately 92% for detecting pancreatitis) [13]. Notably, the patient had experienced an episode of acute pancreatitis ten years prior, which may have been related to the same underlying cause. In 40% of cases, lesions of PP can appear before the onset of pancreatic disease, with a time interval ranging from one to seven months, thereby serving as a significant diagnostic indicator [14].

The primary approach to treating PP involves addressing the underlying pancreatic disease [1, 2]. In cases associated with acute pancreatitis, resolution of skin lesions is typically seen with the normalization of pancreatic enzymes. Nevertheless, in cases associated with neoplasia, PP tends to exhibit a chronic and persistent nature [2]. The use of octreotide, a somatostatin analog, could potentially offer advantages in the treatment of individuals diagnosed with PP [15].

Symptomatic duodenal diverticula can be managed through operative or non-operative measures, with surgical intervention reserved for complicated cases [16]. In the presented case, symptomatic treatment led to initial regression of skin changes and a decrease in pancreatic enzyme levels.

Conclusion

This report emphasizes the uncommon occurrence of PP and its association with multiple diseases. Despite the absence of pancreatitis or other pancreatic abnormalities on imaging, the patient's history of acute pancreatitis and the presence of PD suggest a possible connection to the development of PP. The successful treatment with pancreatin further supports this hypothesis.

In conclusion, diagnosing PP involves assessing pancreatic enzyme levels and performing a skin biopsy. PP can be associated with various pancreatic disorders and other conditions, necessitating the exclusion of underlying diseases in order to manage effectively both the primary disease and cutaneous manifestations.

REFERENCES

1. *Sánchez RB, Bustos BD, Bel PH, Aguilar AT, De Miquel VA*. Pancreatic panniculitis. A review of 7 cases. Piel 2012; 27(7): 367–71. (Spanish)
2. *García-Romero D, Vanaclocha F*. Pancreatic panniculitis. Dermatol Clin 2008; 26(4): 465–70.
3. *Requena L, Sánchez Yus E*. Panniculitis. Part II. Mostly lobular panniculitis. J Am Acad Dermatol 2001; 45(3): 325–61; quiz 362–4.
4. *Naeyaert C, de Clerck F, De Wilde V*. Pancreatic panniculitis as a paraneoplastic phenomenon of a pancreatic acinar cell carcinoma. Acta Clin Belg 2016; 71(6): 448–50.
5. *Martínez-Escribano JA, Pedro F, Sabater V, Quacedo E, Navarro V, Aliaga A*. Acute exanthem and pancreatic panniculitis in a patient with primary HIV infection and haemophagocytic syndrome. Br J Dermatol 1996; 134(4): 804–7.
6. *Koh WL, Tay YK, Ng VW*. Pancreatic Panniculitis Sans Pancreatitis in a Patient with Diabetic Ketoacidosis. Ann Acad Med Singap 2017; 46(6): 252–3.
7. *Milani-Nejad N, Johnson AG, Chung CG*. Nonpancreatic Pancreatic Panniculitis: An Incidental Finding in Individuals without Pancreatic Disease? A Case Series and Review of the Literature. J Clin Aesthet Dermatol 2021; 14(4): 28–30.
8. *Corazza M, Salmi R, Strumia R*. Pancreatic panniculitis as a first sign of liver carcinoma. Acta Derm Venereol 2003; 83(3): 230–1.
9. *Goyal A, Jain M, Rehberg K, Goodman W, Gertner E*. Pancreatic panniculitis in active systemic lupus erythematosus. J Cutan Pathol 2019; 46(9): 688–90.
10. *Rizwan MM, Singh H, Chandar V, Zulfiqar M, Singh V*. Duodenal diverticulum and associated pancreatitis: case report with brief review of literature. World J Gastrointest Endosc 2011; 3(3): 62–3.
11. *Uomo G, Manes G, Ragozzino A, Cavallera A, Rabitti PG*. Periampullary extraluminal duodenal diverticula and acute pancreatitis: an underestimated etiological association. Am J Gastroenterol 1996; 91(6): 1186–8.
12. *Chen Q, Zhang Y, Tang Z, Yu M, Liu Z, Zhou X*, et al. The role of periampullary diverticulum on the incidence of pancreaticobiliary diseases and the outcome of endoscopic retrograde cholangiopancreatography. Arch Med Sci 2020; 17(4): 905–14.
13. *Clavien PA, Hauser H, Meyer P, Rohner A*. Value of contrast-enhanced computerized tomography in the early diagnosis and prognosis of acute pancreatitis. Am J Surg 1988; 155(3): 457–66.
14. *Rongioletti F, Caputo V*. Pancreatic panniculitis. G Ital Dermatol Venereol 2013; 148(4): 419–25.
15. *Hudson-Peacock MJ, Regnard CF, Farr PM*. Liquefying panniculitis associated with acinous carcinoma of the pancreas responding to octreotide. J R Soc Med 1994; 87(6): 361–2.
16. *Chait JS, Galli LD, Clark CJ*. Indications for Operative Management of Complicated Duodenal Diverticula: A Review. Am Surg 2023; 89(7): 3043–6.

Received on Jun 26, 2023
Accepted on August 22, 2023
Online First August 2023

CASE REPORT
(CC BY-SA)

UDC: 616.438:616.15
DOI: https://doi.org/10.2298/VSP230803045A

Thymic hyperplasia as a rare etiology of pure red cell aplasia

Hiperplazija timusa kao retka etiologija čiste aplazije eritroidne loze

Isidora Arsenović*, Danijela Leković*†, Dijana Šefer*, Jelena Ivanović*, Mihajlo Smiljanić*, Andrija Bogdanović*†

***University Clinical Center of Serbia, Clinic of Hematology, Belgrade, Serbia;**
†University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Abstract

Introduction. Thymic hyperplasia is a rare condition caused by an increase in cellular thymic mass and, in some cases, is associated with autoimmune diseases, such as pure red cell aplasia (PRCA). Thymectomy is considered the most effective therapy for PRCA associated with thymoma, with a 31.5% complete remission rate. Other treatments may induce partial remissions, but complete remission remains elusive. A case of PRCA attributed to thymic hyperplasia is presented, highlighting the effectiveness of thymectomy. **Case report.** A previously healthy 18-year-old woman presented with severe anemia and after hematological evaluation, including bone marrow biopsy confirmation, a diagnosis of PRCA was made. Immunological and virological analyses were unremarkable. Given the history of thymoma in the family and the known association between thymoma and PRCA, a chest magnetic resonance imaging was performed, which proved the existence of thymic hyperplasia. The patient underwent the least invasive surgical procedure – total thymectomy using video-assisted thoracic surgery approach. Pathohistological examination of the operative material confirmed the presence of thymic hyperplasia with a simple intrathymic cyst. Following thymectomy, the patient's hematological values significantly improved. **Conclusion.** The course and outcome of the patient's treatment support the role of thymectomy in PRCA associated with thymic hyperplasia. However, further research and follow-up are needed to optimize management strategies for this rare condition.

Key words:
anemia; diagnosis; histological techniques; red-cell aplasia, pure; thoracic surgery, video-assisted; thymoma; thymus hyperplasia.

Apstrakt

Uvod. Hiperplazija timusa je retko stanje koje nastaje kao posledica povećanog broja ćelija timusa, koje se u nekim slučajevima može dovesti u vezu sa autoimunskim bolestima kao što je čista aplazija eritroidne loze (*pure red cell aplasia* – PRCA). Timektomija se smatra najefikasnijim terapijskim modalitetom za lečenje PRCA povezane sa timomom, sa stopom potpune remisije od 31,5%. Ostali načini lečenja mogu dovesti do parcijalne ali ne i kompletne remisije. Prikazana je bolesnica kod koje je PRCA bila povezana sa hiperplazijom timusa, sa naglaskom na efikasnosti timektomije kao incijalnog terapijskog pristupa. **Prikaz bolesnika.** Prikazana je, prethodno zdrava, žena stara 18 godina, sa teškom anemijom kod koje je nakon hematoloških ispitivanja, uključujući i potvrdu biopsijom koštane srži, postavljena dijagnoza PRCA. Vrednosti imunoloških i virusoloških analiza bile su u granicama referentnih vrednosti. Zbog pojave timoma u porodičnoj anamnezi i poznate veze između timoma i PRCA, urađena je magnetna rezonanca grudnog koša kojom je dokazano postojanje hiperplazije timusa. Kod bolesnice je sprovedena najmanje invazivna hirurška procedura – totalna timektomija primenom video-asistirane torakoskopske hirurgije. Patohistološki pregled operativnog materijala potvrdio je prisustvo hiperplazije tkiva timusa, sa prisutnom „jednostavnom" cistom unutar timusa. Nakon timektomije, hematološki parametri bolesnice su se značajno poboljšali. **Zaključak.** Tok i ishod lečenja bolesnice podržavaju primenu timektomije u lečenju PRCA koja je povezana sa hiperplazijom timusa. Ipak, potrebna su dalja istraživanja i praćenja kako bi se optimizovao terapijski pristup za to retko stanje.

Ključne reči:
anemija; dijagnoza; histološke tehnike; aplazija crvene loze, čista; hirurgija, torakalna, video-asistirana; timom; timus, hiperplazija.

Introduction

Pure red cell aplasia (PRCA) is an exceptionally rare disorder characterized by the failure of erythropoiesis, resulting in anemia. This condition is characterized by normocytic, normochromic anemia, associated with severe reticulocytopenia and notable absence or severe decrease in erythroblasts from an otherwise normal bone marrow [1]. PRCA can mani-

Correspondence to: Isidora Arsenović, University Clinical Center of Serbia, Clinic of Hematology, Koste Todorovića 2, 11 000 Belgrade, Serbia. E-mail: isidoraarsenovic@yahoo.com

fest either as a congenital disorder, known as Diamond-Blackfan syndrome, or as an acquired disease resulting from various causes, including autoimmune disorders, some leukemias, lymphoproliferative disorders, ABO incompatible stem cell transplant, viral infections, medications, and notably, solid tumors. Among the last, thymoma is a prominent example strongly linked to PRCA [2].

Thymic hyperplasia (TH) refers to the enlargement of the thymus gland caused by an increase in the number of cells. During puberty, TH hyperplasia is not always a pathological condition – when the thymus grows beyond the expected size for a person's age, it warrants further investigation. In the pediatric population, TH is the most prevalent benign tumor found in the anterior mediastinum [3]. We present a case in which PRCA was attributed to TH, an exceptionally rare association. Notably, PRCA is presented as the primary feature of thymic enlargement in particular cases.

Case report

In July 2018, a previously healthy 18-year-old female was referred to hematology due to progressively worsening fatigue and malaise over the past two months. Moreover, she was also suffering from heavy menstrual bleeding. Physical examination revealed pallor of the skin but no enlarged lymph nodes or hepatosplenomegaly. Laboratory evaluation showed severe normocytic anemia [hemoglobin 56 g/L, reference range (RR) for female: 120–140 g/L; MCV 82 fL, RR: 80–100 fL] with normal white blood cell count (4×10^9/L, RR: 4–10×10^9/L) and normal platelet count (482×10^9/L, RR: 150–450×10^9/L).

Ferritin, total iron binding capacity, and iron levels were within the RR, even with a history of heavy menstrual bleeding. Due to the severity of the anemia, the patient received a packed red cell transfusion and was advised to take iron supplements for the following month. Regardless of that, her hemoglobin level declined further. The bone marrow aspirate was mildly hypocellular with a marked decrease in erythroid lineage, without signs of dysplasia. Cytogenetic analysis revealed a normal female karyotype. A bone marrow trephine biopsy demonstrated sparse erythroid lineage, with erythroblasts in various maturation stages lacking well-formed erythroid islets. With the present morphological features of PRCA, an immunology workup for connective tissue disease was performed and showed a complete absence of any immunological marker abnormalities. Antinuclear antibodies – ANA (Hep-2), antineutrophilic cytoplasmic antibody - ANCA, anti-transglutaminase antibodies - ATA, and anti-cardiolipin antibodies - ACA were negative; levels of complement (C) component 3 (C3), C4, and rheumatoid factor - RF were also within the RR. Virological analyses excluded cytomegalovirus, Epstein-Barr virus, parvovirus B19, human immunodeficiency virus, hepatitis C virus, and hepatitis B virus infections as possible causes. To evaluate further the mechanism responsible for the presence of PRCA, an in vitro hematopoietic progenitor culture assay was performed, showing preserved normal in vitro erythropoietic growth with an even more increased response of colony-forming unit (CFU)-E on erythropoietin stimulation (finding not consistent with typical PRCA). Erythropoietin level was 95.9 IU/mL (RR: 3.3–16.6 IU/mL), which is five times elevated.

Afterward, the co-cultivation cross-match assay was performed with the healthy bone marrow. This testing revealed that the patient's serum caused a dose-dependent humoral inhibition of erythroid colony growth at the level of CFU-E precursors in culture. Considering the patient's family history, especially the presence of thymoma in her aunt having myasthenia gravis (MG), it was decided to do a complete chest magnetic resonance imaging examination with a focus on the thymus due to the established association between thymoma and PRCA. The magnetic resonance imaging revealed an abnormal formation in the anterior mediastinum, measuring 41 × 11 mm, suggesting TH (Figure 1). After referral to the Thoracic Surgery Board, a decision was made to proceed with a total thymectomy, which was successfully performed by the video-assisted thoracoscopic sur-

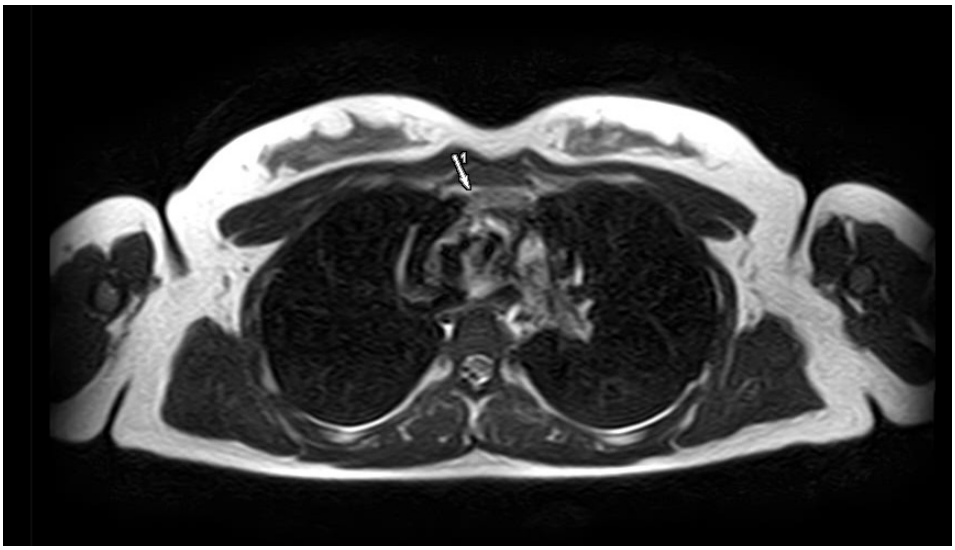


Fig. 1 – Chest magnetic resonance imaging demonstrating abnormal formation in the anterior mediastinum (arrow).

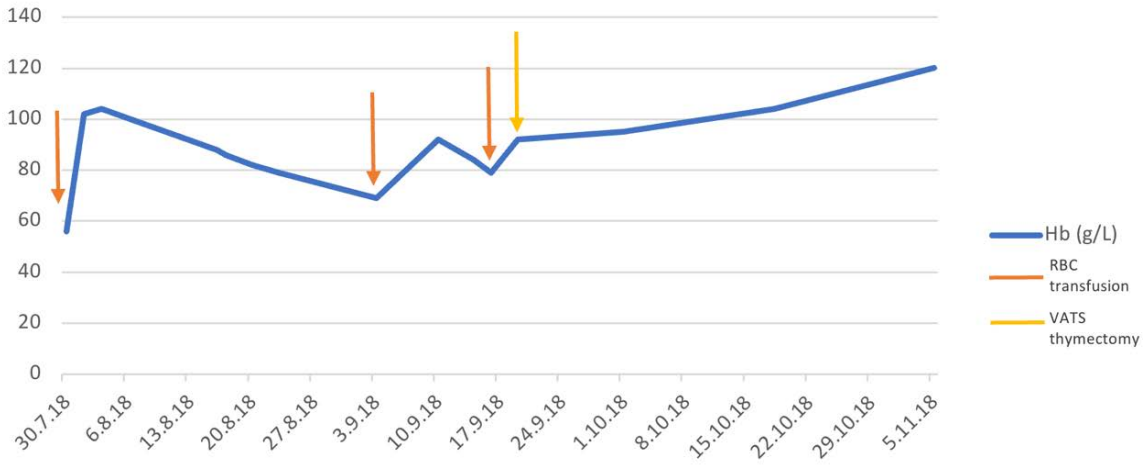


Fig. 2 – Effect of different treatment modalities on the patient's hemoglobin levels. Hb – hemoglobin; RBC – red blood cells; VATS – video-assisted thoracoscopic surgery.

gery (VATS) technique. The pathohistological examination of the excised thymus revealed morphological features consistent with thymus tissue containing an intrathymic simple cyst. Following the surgical intervention, the hematological parameters were stabilized with a subsequent rise toward normal values, indicating a positive outcome (Figure 2). During the five-year follow-up, her blood counts remained normal.

Discussion

The thymus is a crucial organ for the development of T-cells and for forming the adaptive immune system. It consists of various types of stromal cells and maturing T lymphocytes working together to build a strong cellular immune response [4].

Distinguishing between a normal and hyperplastic thymus can be challenging, but certain guidelines can help in this distinction, including the absence of rounded soft-tissue masses larger than 7 mm, the absence of a convex contour of the thymus in individuals above 19 years of age, the absence of soft-tissue lobulation, and absence of excessive thymic thickness (should be ≤ 1.3 cm in individuals above 20 years of age). Additionally, the absence of conditions associated with TH, like MG, is also important to consider [5]. In our patient, the thymus measured 41 × 11 mm, and it was presented as a convex formation in the anterior mediastinum. Moreover, the association with PRCA provided evidence that we were indeed dealing with TH.

TH itself is a benign condition characterized by an increase in the number of cells within the thymus gland. While TH and thymoma are separate entities, they can sometimes have similar clinical and radiological features, making it challenging to differentiate them based on imaging alone [6]. A definitive diagnosis usually requires a combination of clinical evaluation, imaging studies, and histopathological examination of a tissue sample obtained through biopsy or surgery. TH is associated with various autoimmune diseases, including MG, Graves' disease, systemic lupus erythematosus, ulcerative colitis, rheumatoid arthritis, and others [7]. The most common diseases associated with thymic function are MG and Grave's disease. MG is a neurological autoimmune disease characterized by autoantibodies targeting components of the neuromuscular junction, leading to disabling fatigability. Most MG patients have anti-acetylcholine receptor (AChR) antibodies. Histological abnormalities in MG are frequently found in the thymus, which can exhibit either TH or thymoma. TH is particularly associated with elevated levels of the anti-AChR antibody titer, which decreases after thymectomy. The hyperplastic thymus includes all components involved in the anti-AChR response: AChR, B-cells producing anti-AChR antibodies, and anti-AChR auto-reactive T-cells [8]. Graves' disease is an autoimmune disease characterized by the development of antibodies directed against the thyrotropin receptor. The relationship between TH and Graves' disease was first described in 1912, highlighting the importance of thorough thymus evaluation in patients with Graves' disease. The exact pathogenesis involves complex hormonal and immunological mechanisms, which remain to be fully elucidated [7]. In addition to these autoimmune diseases, there are certain hematological autoimmune conditions associated with thymic diseases, including aplastic anemia (AA) and PRCA. AA, as the worst manifestation of bone marrow failure, is characterized by low levels of blood cells in circulation and severely reduced marrow cellularity [9]. AA is a rare complication of thymoma and is even less common after surgical removal of a thymic tumor. The pathogenesis of AA associated with thymoma seems to be explained by bone marrow suppression related to unbalanced T-cell regulation and inverted $CD4^+/CD8^+$ T-cells ratio due to an increase in cytotoxic T-cells [9].

TH causing PRCA is very rare, with a limited number of published cases so far confirming this relationship [10–13] (Table 1).

The precise pathophysiology mechanism underlying PRCA associated with TH is yet to be determined. However,

Table 1

Other cases of pure red cell aplasia (PRCA) associated with thymic hyperplasia (TH)

Author (year)	Age at diagnosis (years)	Time of TH diagnosis	Time of PRCA diagnosis	Treatment	Follow-up (months)
Mohammad et al. (2021) [10]	25	2019	2019	thymectomy + prednisolone	21
Wong et al. (1995) [13]	28	1992	1992	thymectomy	24
Konstantopoulos et al. (1995) [11]	35	N/A	N/A	thymectomy	1
Suto et al. (2004) [12]	31	1975	2001	thymectomy + cyclosporine A	312

N/A – not available.

there is some understanding of the mechanisms contributing to PRCA in patients with thymoma. The published data suggests that multiple pathways may be involved in this clinical syndrome, including the presence of a humoral factor that suppresses the erythroid lineage, antibodies targeting erythropoietin, but also cell-mediated suppression involving T-cells, large granular lymphocytes, and natural killer cells [14]. In our case, we have demonstrated that TH induced an unbalanced immune response with humoral erythroid suppression even with high levels of endogenous erythropoietin. While TH is commonly associated with one autoimmune disease, there was a case in which TH was connected to both MG and PRCA [12]. Investigation of the pathogenesis in this case revealed increased $CD8^+$ T-cells and a decrease in $CD19^+$ B-cells. Interestingly, the $CD8^+$ T-cells were found to impede the maturation of early erythroid cells despite elevated erythropoietin levels. These results suggest that PRCA might be induced by a T-cell clonal disorder [15]. Similarly, in other reports in complex patients with polyglandular autoimmune syndrome, PRCA was associated with rearranged T-cell receptors (TCR) in response to calcineurin inhibitors. Due to the good response to VATS thymectomy, we have decided not to perform a TCR rearrangement study, as this might be a diagnostic step towards a decision for immunomodulatory treatment [16].

Moreover, some research has suggested that a thymoma or the thymus itself might, in some cases, share antigens with erythroblastic cells [17]. In a unique case, a patient with end-stage renal failure developed PRCA. The investigation revealed that the chronic antigenic stimulation of the thymus, caused by repeated blood transfusions and hemodialysis, resulted in lymphoid hyperplasia, ultimately leading to the development of PRCA [13].

Thymectomy stands out as the most potent anti-tumor therapy for PRCA associated with thymoma, yielding a 31.5% complete remission (CR) rate when performed alone or in conjunction with other treatment modalities [17]. While alternative anti-tumor interventions may induce partial remissions, the attainment of CR remains elusive based on available reports. It is worth noting that in some cases, PRCA can still occur after thymectomy. This finding suggests that immunological alterations of T- and B-cells may persist and continue to impact the maturation of erythroid precursors even after the surgical removal of the thymus [12]. Therefore, in instances where PRCA persists post-thymectomy, considering immuno-modulatory therapies, particularly cyclosporine, becomes crucial, as it has shown the most promising CR rates [18]. When it comes to treating PRCA associated with TH, there is a lack of official guidelines. However, there have been cases indicating that CR was achieved through thymectomy either alone or in combination with immunomodulatory therapy [10–13]. In one of these cases, thoracotomy was utilized to access the anterior mediastinum, while in the other case, a median sternotomy was chosen to allow for optimal exposure and complete clearance of mediastinal tissue [10, 11]. In our specific case, we opted for the VATS method, which has evolved over time to become a time-efficient, effective, and well-tolerated procedure with a low conversion rate and minimal complications [19]. As a result, the significant improvement in erythropoiesis and the subsequent normalization of laboratory values strongly support the compelling evidence that TH, operating through one of the previously discussed mechanisms, was indeed a causative factor behind PRCA.

Conclusion

Considering the previously discussed case, where PRCA was attributed to TH, our findings further support the effectiveness of thymectomy as a basic therapeutic approach for PRCA associated with this particular etiology. However, it is important to note that this is a rare condition that accounts for only a small subset of thymic masses associated with PRCA. Further research and exploration are warranted to deepen our understanding of this complex relationship and optimize management strategies for PRCA related to TH.

REFERENCES

1. *Sawada K, Fujishima N, Hirokawa M.* Acquired pure red cell aplasia: updated review of treatment. Br J Haematol 2008; 142(4): 505–14.
2. *Mangla A, Hamad H.* Pure Red Cell Aplasia [updated 2022 Nov 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 July 13]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549833/
3. *Khan MA, Anjum F.* Thymic Hyperplasia [updated 2023 Apr 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 July 15]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560558/
4. *Shichkin VP, Antica M.* Key Factors for Thymic Function and Development. Front Immunol 2022; 13: 926516.

5. *Ackman JB, Wu CC.* MRI of the thymus. AJR Am J Roentgenol. 2011; 197(1): W15–20.
6. *Den Bakker MA, Oosterhuis JW.* Tumours and tumour-like conditions of the thymus other than thymoma; a practical approach. Histopathology 2009; 54(1): 69–89.
7. *Le Panse R, Bismuth J, Cizeron-Clairac G, Weiss JM, Cufi P, Dartevelle P,* et al. Thymic remodeling associated with hyperplasia in myasthenia gravis. Autoimmunity 2010; 43(5–6): 401–12.
8. *Haider U, Richards P, Gianoukakis AG.* Thymic Hyperplasia Associated with Graves' Disease: Pathophysiology and Proposed Management Algorithm. Thyroid 2017; 27(8): 994–1000.
9. *de Castro MA, de Castro MA, Arantes Ade M, Roberti Mdo R.* Thymoma followed by aplastic anemia – two different responses to immunosuppressive therapy. Rev Bras Hematol Hemoter 2011; 33(6): 476–7.
10. *Mohammad A, Dawson AG, Bajaj A, Rathinam S.* True thymic hyperplasia causing pure red cell aplasia: a case report. Interact Cardiovasc Thorac Surg 2022; 34(4): 697–9. Erratum in: Interact Cardiovasc Thorac Surg 2022.
11. *Konstantopoulos K, Androulaki A, Aessopos A, Patsouris E, Dosios TH, Psychogios A,* et al. Pure red cell aplasia associated with true thymic hyperplasia. Hum Pathol 1995; 26(10): 1160–2.
12. *Suto Y, Araga S, Sakuma K, Nakano T, Ishiga K, Tajima F,* et al. Myasthenia gravis with thymus hyperplasia and pure red cell aplasia. J Neurol Sci 2004; 224(1–2): 93–5.
13. *Wong KF, Chau KF, Chan JK, Chu YC, Li CS.* Pure red cell aplasia associated with thymic lymphoid hyperplasia and secondary erythropoietin resistance. Am J Clin Pathol 1995; 103(3): 346–7.
14. *Thompson CA, Steensma DP.* Pure red cell aplasia associated with thymoma: clinical insights from a 50-year single-institution experience. Br J Haematol 2006; 135(3): 405–7.
15. *Masuda M, Arai Y, Okamura T, Mizoguchi H.* Pure red cell aplasia with thymona: evidence of T-cell clonal disorder. Am J Hematol 1997; 54(4): 324–8.
16. *Bakrac M, Jurisic V, Kostic T, Popovic V, Pekic S, Kraguljac N,* et al. Pure red cell aplasia associated with type I autoimmune polyglandular syndrome-successful response to treatment with mycophenolate mofetil: case report and review of literature. J Clin Pathol 2007; 60(6): 717–20.
17. *Masaoka A, Hashimoto T, Shibata K, Yamakawa Y, Nakamae K, Iizuka M.* Thymomas associated with pure red cell aplasia. Histologic and follow-up studies. Cancer 1989; 64(9): 1872–8.
18. *Lesire B, Durieux V, Grigoriu B, Girard N, Berghmans T.* Management of thymoma associated autoimmune pure red cell aplasia: Case report and systematic review of the literature. Lung Cancer 2021; 157: 131–46.
19. *Infante M, Benato C, Giovannetti R, Bonadiman C, Canneto B, Falezza,* et al. VATS thymectomy for early stage thymoma and myasthenia gravis: combined right-sided uniportal and left-sided three-portal approach. J Vis Surg 2017; 3: 144.

Received on August 3, 2023
Accepted on August 22, 2023
Online First August 2023

BOOK REVIEW/PRIKAZ KNJIGE

Imunosupresivna terapija kod pacijenata sa transplantiranim bubregom

Autori: Nemanja Rančić, Neven Vavić, Viktorija Dragojević Simić
Izdavač: Medija centar „Odbrana“, Beograd
Godina izdanja: 2023
ISBN: 978-86-335-0820-9

Monografiju „Imunosupresivna terapija kod pacijenata sa transplantiranim bubregom“ napisali su viši naučni saradnik doc. dr Nemanja Rančić, farmakolog i radiolog, prodekan Medicinskog fakulteta Vojnomedicinske akademije Univerziteta odbrane u Beogradu, pukovnik doc. dr Neven Vavić, specijalista interne medicine, načelnik Klinike za nefrologiju Vojnomedicinske akademije i prof. dr Viktorija Dragojević Simić, specijalista kliničke farmakologije, načelnik Centra za kliničku farmakologiju Vojnomedicinske akademije.

Glavna tema rukopisa, jedno od najznačajnijih područja savremene medicine, jeste imunosupresivna terapija kod pacijenata sa transplantiranim bubregom. Ovo je jedno od područja današnje medicine koje omogućava značajno produženje ljudskih života ali i poboljšanje kvaliteta života pacijenata u završnom stadijumu hroničnog oboljenja bubrega. Ako sagledamo rezultate koje je Hrvatska napravila na ovom planu i uspela da se pozicionira među najboljim organizacijama službe u Evropi, ali i šire, značaj ovakvog rukopisa je veliki, jer bi on pomogao lekarima iz Srbije da što pre uspešno savladaju težak put kojim je prošla i Hrvatska, kako bi se postigli odlični rezultati na polju transplantacije. Rukopis ima 282 strane i 629 citiranih referenci a tekstualni deo dopunjuje 9 slika i 21 tabela. Nakon *Uvoda*, rukopis ima dva velika poglavlja – *Farmakološki pristup u transplantaciji bubrega* i *Najznačajniji ishodi transplantacije bubrega*. U okviru prvog poglavlja nalazi se pet podpoglavlja: *Transplantacija bubrega*, *Imunosupresivna terapija*, *Najčešća komedikacija koja može uticati na imunosupresiju*, *Terapijski monitoring lekova* i *Parametri koji mogu uticati na nivo takrolimusa u krvi*. U okviru drugog poglavlja nalaze se sledeća podpoglavlja: *Pacijenti sa transplantiranim bubregom*, *Protokol lečenja u Centru za transplantaciju solidnih organa VMA*, *Uticaj KOVID infekcije na imunosupresivnu terapiju* i *Završna razmatranja*. Na kraju se nalazi *Zaključak* i citirana *Literatura*. U tekstu su citirane savremene i značajne reference, kako starijeg, tako i novijeg datuma. Među citatima uočavam da postoje i 22 autocitata, čime se dokazuje kompetentnost autora u ovom području.

Autori rukopisa izneli su najznačajnije rezultate u trenutno dostupnoj medicinskoj literaturi vezano za imunosupresivnu terapiju kod pacijenata sa transplantiranim bubregom. Oni su izneli i svoje dosadašnje rezultate prikazane u mnogobrojnim radovima, od kojih su mnogi objavljeni u časopisima na SCI listi, sa visokim faktorom uticaja. Na osnovu veličine rukopisa, broja autocitata i broja recenzenata, a u skladu sa okvirnim zakonodavstvom na području nauke, ovaj tekst se kategoriše kao istaknuta nacionalna monografija.

Nakon uvida u ceo tekst, može se zaključiti da je ovaj tekst namenjem ne samo kao dopunska literatura na osnovnim i postdiplomskim studijama, već i specijalističkim i subspecijalističkim studijama, kao i svim transplantolozima

koji se svakodnevno bave transplantacionom medicinom. Na kraju, još jedan razlog za preporuku ove knjige je i to što ovakav obim i sadržaj knjige iz oblasti imunosupresivne terapije u transplantaciji nije dostupan na srpskom jeziku. Sadržaj ove knjige može značajno olakšati lekarima u transplantacionim timovima rad sa pacijentima, kojima je imunosupresivna terapija osnovna terapija, kako bi se sprečilo odbacivanje presađenog organa i omogućilo dugogodišnje preživljavanje i bolji kvalitet života.

Prof. dr Nikolina Bašić Jukić
Kliničko bolnički centar Zagreb
Klinika za unutarnje bolesti
Zavod za nefrologiju, arterijsku hipertenziju i dijalizu
E-mail: nikolina.basic.jukic@kbc-zagreb.hr

INSTRUCTIONS TO THE AUTHORS

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

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

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

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

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

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

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

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

Preparation of manuscript

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

1. ***Title page***

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

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

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

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

e) Data on the corresponding author.

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

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

3. ***Text***

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

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

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

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

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

References

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

Examples of references:

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

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

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

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

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

Tables

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

Illustrations

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

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

Abbreviations and acronyms

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

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

Detailed Instructions are available at the web site:

www.vma.mod.gov.rs/vsp

UPUTSTVO AUTORIMA

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

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

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

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

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

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

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

Priprema rada

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

1. ***Naslovna strana***

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

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

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

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

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

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

3. ***Tekst članka***

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

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

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

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

Literatura

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

Primeri referenci:

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

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

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

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

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

Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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