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EDITORIAL
UVODNIK

ANTIMICROBIAL RESISTANCE – A CROSSROADS OF HEALTH, EQUITY, AND SUSTAINABILITY

ANTIMIKROBNA REZISTENCIJA – RASKRSNICA ZDRAVLJA, JEDNAKOSTI I ODRŽIVOSTI

Milka SOKOLOVIĆ

ORCID NUMBER

European Public Health Alliance, Brussels, Belgium

Milka Sokolović - 0000-0002-3957-4656

Editorial
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Abstract
Antimicrobial resistance is one of the most complex global health challenges, posing substantial threats to public health, economies, and sustainable development. This editorial explores the multifaceted nature of antimicrobial resistance, which intersects human, animal, and environmental health. Misuse of antibiotics in clinical settings, agricultural practices, and environmental contamination has accelerated the proliferation of resistant pathogens. The One Health approach, which underscores the interconnectedness of these sectors, is critical for effectively addressing antimicrobial resistance. International organizations, such as Food and Agriculture Organization, World Health Organization, World Organisation for Animal Health, and United Nations Environment Programme, advocate for coordinated, cross-sectoral effort to combat antimicrobial resistance, highlighting its connections to neglected tropical diseases, food systems, and climate change. Although the recent United Nations General Assembly Declaration on antimicrobial resistance marks progress, substantial gaps persist, particularly in the context of antimicrobial overuse in agriculture and veterinary medicine. Civil society groups and public health advocates have raised concerns regarding the absence of concrete global targets and regulatory frameworks. In the absence of stronger international commitments, antimicrobial resistance will continue to disproportionately affect low- and middle-income countries, further exacerbating global health inequalities Ultimately, this editorial emphasises the need for sustainable funding, stronger policies, and global cooperation to tackle antimicrobial resistance and promote health equity. The One Health approach offers a pathway to mitigate the burden of antimicrobial resistance, protect human rights, and promote universal access to effective treatments.
Key words: Drug Resistance, Microbial; One Health; Health Inequities; Public Health; Environmental Health; Human Rights

Sažetak
Antimikrobna rezistencija predstavlja jedan od najsloženijih globalnih zdravstvenih izazova, koji preti javnom zdravlju, ekonomiji i globalnom razvoju. Ovaj uvodnik istražuje višestruku prirodu antimikrobne rezistencije, koja povezuje ljudsko, životinjsko i zdravlje životne sredine. Neadekvatna upotreba antibiotika u kliničkom okruženju i poljoprivredi, "ruku pod ruku" sa zagađenjem životne sredine, dodatno je pogoršala rast otpornosti patogena. Pristup „jedno zdravlje" (*One Health*), koji naglašava međusobnu povezanost ovih sektora, ključan je za efikasno rešavanje antimikrobne rezistencije. Međunarodne organizacije, različite agencije Ujedinjenih nacija, zalažu se za koordinisanu, međusektorsku akciju u borbi protiv antimikrobne rezistencije, prepoznajući njene veze sa zanemarenim tropskim bolestima, prehrambenim sistemima i klimatskim promenama. Iako nedavna Deklaracija Generalne skupštine Ujedinjenih nacija o antimikrobnoj rezistenciji predstavlja pozitivan korak, i dalje postoje značajni nedostaci, posebno u vezi sa prekomernom upotrebom antimikrobnih sredstava u poljoprivredi i veterini. Predstavnici civilnog društva i stručnjaci za javno zdravlje izrazili su zabrinutost zbog manjka konkretnih globalnih ciljeva i regulatornih okvira. Bez izričitijih međunarodnih obaveza, antimikrobna rezistencija će nastaviti da nesrazmerno pogađa zemlje sa niskim i srednjim prihodima, dodatno pogoršavajući svetske zdravstvene neravnopravnosti. Konačno, ovaj tekst naglašava neophodnost održivog finansiranja, izričitijih politika i globalne saradnje kako bi se našlo rešenje za antimikrobnu rezistenciju i unapredila zdravstvena ravnopravnost. Pristup „Jedno zdravlje" nudi put za smanjenje tereta antimikrobne rezistencije, uz očuvanje ljudskih prava i promovisanje univerzalnog pristupa efikasnim tretmanima.
Ključne reči: Amikrobna rezistencija na lekove; jedno zdravlje; zdravstvene nejednakosti; jabno zdravlje; ekološko zdravlje; ljudska prava

An extraordinary nexus of challenges

According to the United Nations, antimicrobial resistance (AMR) ranks among the top 10 global health threats facing humanity. The emergence of "superbugs", including bacteria no longer treatable by antibiotics, poses one of the most significant and complex global health challenges of our time. AMR's complexity stems from its multifaceted nature, intersecting human, animal, and environmental health, and its potential to erode decades of medical progress. AMR emerges from a nexus of factors, making it a challenge that transcends borders and sectors.

Human health lies at the core of the AMR crisis. The misuse and overuse of antibiotics in clinical settings continue to drive the proliferation of resistant

✉ Corresponding author: Milka Sokolović, E-mail: milka.sokolovic@epha.org

Abbreviations

UN – United Nations
AMR – antimicrobial resistance
NCDs – non-communicable diseases
UNGA – United Nations General Assembly
FAO – Food and Agriculture Organization
WHO – World Health Organization
WOAH – World Organisation for Animal Health
UNEP – United Nations Environment Programme
NTDs – neglected tropical diseases
LMICs – low- and middle-income countries
EPHA – European Public Health Alliance
HCWH – Health Care Without Harm

pathogens, particularly in countries with weaker healthcare infrastructures [1, 2]. However, the problem extends beyond hospitals and clinics. In animal husbandry, antibiotics are often overused to promote growth and prevent disease in livestock, facilitating the transfer of resistant bacteria passing from animals to humans through food systems, direct contact, or environmental pathways [3, 4].

Environmental factors also significantly contribute to AMR. Antibiotic residues from pharmaceuticals and agricultural runoff enter soil and water systems, creating reservoirs for resistant bacteria. These environmental reservoirs further complicate efforts to control AMR, as they can spread resistant genes across microbial communities worldwide [5]. This issue is intricately linked to other global health crises, including climate change [6] and the transformation of food systems [7]. With climate change affecting both the spread of infections and food production practices, the misuse of antimicrobials increases, thereby accelerating resistance. By altering ecosystems, climate change influences the spread of infectious diseases and impacts the effectiveness of antimicrobial treatments. Moreover, our food systems keep contributing to the rise of non-communicable diseases (NCDs), and AMR further burdens this landscape by limiting treatment options for infections that arise as secondary complications in patients with chronic conditions [8].

Recent publications, including the March 2024 issue of The Lancet [9] and reports released ahead of the United Nations General Assembly (UNGA) High-Level Meeting on AMR, underscore the urgency of addressing AMR. They emphasize that AMR is not only a public health crisis but also an economic, environmental, and social threat, with the potential to cause millions of deaths annually and severely undermine global development goals.

One Health to address it all

Addressing the AMR challenge requires a holistic One Health approach, which recognizes the interconnectedness of human, animal, and environmental health. In recent years, the One Health concept has gained prominence, with organisations such as the Food and Agriculture Organization (FAO), World Health Organization (WHO), World Organisation for Animal Health (WOAH), and United Nations Environment Programme (UNEP) collaborating through Quadripartite Secretariat for One Health to drive policies and actions that integrate all these dimensions [10, 11].

This approach highlights the interdependency of sectors such as animal husbandry, food safety, and environmental health with combating AMR [10, 12]. For example, controlling zoonotic diseases, which can be transmitted from animals to humans, is essential for preventing the spread of resistant bacteria. Equally important are the strengthening of laboratory services and improvement of surveillance of resistant pathogens across species, as well as strict regulation of antimicrobials in veterinary settings to prevent resistance proliferation.

The One Health approach also extends to tackling neglected tropical diseases (NTDs) [13], which are prevalent in many low- and middle-income countries (LMICs). These diseases often require antimicrobial treatment, and without proper stewardship, they risk accelerating resistance in regions already struggling with high rates of infectious diseases. Integrating AMR strategies into broader efforts to control NTDs and improve environmental health is therefore vital to the global AMR response [14].

Recent collaborative efforts by the FAO, UNEP, WHO, and WOAH, leading up to the UNGA High-Level Meeting on AMR, have emphasised the importance of coordinated, cross-sectoral action [15]. Such collaboration is critical to equipping the world to address the AMR crisis in a comprehensively, balancing public health needs with animal health and environmental sustainability.

Much left to desire

The recent UNGA Declaration on Antimicrobial Resistance [16, 17], with its meaningful global commitments and targets to advance the fight against AMR, marked an important step forward, received broad support from member states, international organisations, the commercial sector, and civil society. While the declaration acknowledges the growing threat of AMR and calls for coordinated action across human, animal, and environmental health sectors, significant gaps remain that must be addressed to ensure a comprehensive global response to AMR.

Despite the strengths, the final version of the declaration falls short of many ambitious goals, especially in regulating over-the-counter sales and antibiotic use in food systems - areas strongly advocated by civil society organisations and public health groups [18]. Although the declaration highlights the need for a global framework to tackle AMR, it lacks specific commitments to curb the overuse of antimicrobials in agriculture and food production. For instance, a proposal to reduce antimicrobial use in agriculture by 30% over the next six years was replaced by "strive to meaningfully reduce", leaving countries without a clear target. This dilution resulted from pushback by the agriculture and veterinary drug industry, along with meat-producing nations [19]. Civil society groups have expressed concerns that, without firm regulations and a binding framework, progress against AMR will remain slow and uneven [20].

Antimicrobial resistance respects no borders, and a collective response is still urgently needed. More concerted efforts from governments, civil society, and international organisations are necessary to fill the gaps left by the UNGA declaration. This includes establishing stronger regulatory frameworks, allocating more resources to LMIC, and setting more ambitious targets for reducing antimicrobial use in agriculture.

The win-wins and other happy endings

It is no surprise that AMR crisis bears a huge economic impact. According to the most extensive modelling to date, drug-resistant pathogens could threaten the food supply for over two billion people and increase healthcare costs by $159 billion annually by 2050. The EcoAMR series (Health and Economic Impacts of AMR in Human and Food-Producing Animals), led by WOAH, projects a return of $28 for every $1 invested in drug innovation and healthcare improvements [21].

The One Health approach not only protects public health and the economy but also helps preserve ecosystems from the effects of unconstrained antimicrobial use. By implementing strategies such as better waste management and regulation of antibiotic use in agriculture, the contamination of ecosystems can be reduced, which is crucial for maintaining environmental health [22].

Most importantly, AMR endangers basic human rights by limiting access to effective treatments, with resistant infections disproportionately affecting LMIC where healthcare access is limited. Addressing AMR through a One Health approach directly supports reduction of health inequalities by expanding healthcare access and promoting equity in health outcomes globally [23]. It prioritises universal access to essential medicines, reduces the disparities between high- and low-income countries, and aligns with the principle of health as a human right.

The role of global civil society organisations cannot be overstated, among others in ensuring that the health equity and human rights aspects of AMR crisis are properly addressed. In Europe, organisations such as the European Public Health Alliance (EPHA), Health Care Without Harm (HCWH), ReAct, and others have been at the forefront of advocacy, ensuring that AMR remains a priority even when other public health issues dominate the headlines. However, for civil society to sustain this critical role, they need sustainable funding and support from governments and international bodies [24]. Through the recent approval of the resolution on Social participation for universal health coverage, health and well-being, Members States have committed to implementing, strengthening and sustaining regular and meaningful social participation in health-related decision-making processes. It is now their responsibility to ensure that civil society organisations are adequately resourced, enabling them to continue their vital work in raising awareness, improving antimicrobial stewardship, and pushing for stronger policy measures [25].

About EPHA

European Public Health Alliance is the leading public health civil society organisation in Europe, advocating for better public health policies. During the last decade, EPHA has been promoting an integrated approach to AMR by addressing the interconnections between human, animal, and environmental health, ensuring that policies reflect this interconnectedness [26]. Through coordinating its AMR Stakeholder Network [27], supporting the Members of the European Parliament Interest Group on AMR, and collaborating in the European Commission-funded One Health AMR European partnerships, EPHA fosters cross–sectoral cooperation among stakeholders and policymakers, helping build more resilient systems to address both climate-related health impacts and AMR.

European Public Health Alliance's work focuses on raising awareness among, healthcare professionals, patient organisations, and policymakers, enhancing their understanding on the prudent use of antimicrobials. This includes topics of infection prevention, surveillance and control, health literacy, and proper waste disposal. Together with its members and partners, EPHA advocates for better antimicrobial stewardship by sharing best practices across healthcare and related sectors, applying an interdisciplinary, multistakeholder

approach [28]. It has consistently advocated for AMR prioritisation on the global agenda and promoted transparent, evidence-based policymaking by translating research findings into policy recommendations.

References

1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. London: Government of the United Kingdom; 2016.

2. World Health Organization. Antimicrobial resistance: global report on surveillance [Internet]. Geneva: World Health Organization; 2014 [cited 2024 Aug 15]. Available from: https://www.who.int/publications/i/item/9789241564748

3. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A. 2015;112(18):5649-54.

4. European Medicines Agency. Sales of veterinary antimicrobial agents in 31 European countries in 2019 and 2020 [Inter-net]. Luxembourg: Publications Office of the European Union; 2021 [cited 2024 Aug 15]. Available from: https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-31-european-countries-2019-2020-trends-2010-2020-eleventh_en.pdf

5. Food and Agriculture Organization of the United Nations. The FAO action plan on antimicrobial resistance 2021-2025 [Internet]. Rome: FAO; 2021 [cited 2024 Aug 15]. Available from: http://www.fao.org/3/cb5545en/cb5545en.pdf

6. Jevtic M. The voice of health: finding a cure for the climate change malady. Med Pregl. 2016;69(11-12):339-44.

7. Interagency Coordination Group on Antimicrobial Resistance. No time to wait: securing the future from drug-resistant infections [Internet]. Geneva: WHO; 2019 [cited 2024 Aug 15]. Available from: https://cdn.who.int/media/docs/default-source/documents/no-time-to-wait-securing-the-future-from-drug-resistant-infections-en.pdf?sfvrsn=5b424d7_6&download=true

8. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-55.

9. The Lancet. The Lancet Series on Antimicrobial Resistance: the need for sustainable access to effective antibiotics [Internet]. 2024 [cited 2024 Aug 15]. Available from: https://www.thelancet.com/series/antibiotic-resistance

10. World Health Organization (WHO); Food and Agriculture Organization (FAO); World Organisation for Animal Health; UN Environment Programme. Strategic framework for collaboration on antimicrobial resistance – together for One Health [Internet]. Geneva: WHO; 2022 [cited 2024 Aug 15]. Available from: https://iris.who.int/bitstream/handle/10665/352625/9789240045408-eng.pdf?sequence=1

11. World Health Organization. Antimicrobial resistance [Internet]. 2023 [cited 2024 Aug 15]. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

12. European Commission. A European One Health action plan against antimicrobial resistance (AMR) [Internet]. [cited 2024 Aug 15]. Available from: https://health.ec.europa.eu/system/files/2020-01/amr_2017_action-plan_0.pdf

13. Laing G, Vigilato MAN, Cleaveland S, Thumbi SM, Blumberg L, Salahuddin N, et al. One Health for neglected tropi-cal diseases. Trans R Soc Trop Med Hyg. 2021;115(2):182-4.

14. World Health Organization. Global action plan on antimicrobial resistance [Internet]. Geneva: WHO; 2015 [cited 2024 Aug 15]. Available from: https://www.amcra.be/swfiles/files/WHO%20actieplan_90.pdf

15. World Health Organization; Food and Agriculture Organization of the United Nations; United Nations Environment Programme; World Organisation for Animal Health. A one health priority research agenda for antimicrobial resistance [In-ternet]. Geneva: WHO; 2023 [cited 2024 Aug 15]. Available from: https://nora.nerc.ac.uk/id/eprint/536179/1/N536179CR.pdf

16. Political declaration of the high-level meeting on antimicrobial resistance [Internet]. 2024 [cited 2024 Sep 15]. Available from: https://www.un.org/pga/wp-content/uploads/sites/108/2024/09/FINAL-Text-AMR-to-PGA.pdf

17. World Health Organization. Joint News Release. World leaders commit to decisive action on antimicrobial resistance [Internet]. 2024 [cited 2024 Sep 27]. Available from: https://hq_who_departmentofcommunications.cmail20.com/t/d-e-euyithd-tujhurkjju-g/

18. ReAct. ReAct's statement at the UN High level meeting on AMR in New York [Internet]. 2024 [cited 2024 Sep 27]. Available from: https://www.reactgroup.org/news-and-views/news-and-opinions/year-2024/statement-by-otto-cars-on-behalf-of-react-un-high-level-meeting-on-amr-panel/

19. US Right to Know. US pressure weakens global commitments on antimicrobial resistance [Internet]. 2024 [cited 2024 Sep 27]. Available from: https://usrtk.org/factory-farming/us-pressure-weakens-global-commitments-on-antimicrobial-resistance/

20. CIDRAP. UN calls for action, accountability on antimicrobial resistance [Internet]. 2024 [cited 2024 Sep 27]. Available from: https://www.cidrap.umn.edu/antimicrobial-stewardship/un-calls-action-accountability-antimicrobial-resistance

21. World Organisation for Animal Health. Superbugs could jeopardise food security for over two billion people and in-crease annual health care costs by US$ 159 billion annually by 2050, finds most extensive modelling to date [Internet]. 2024 [cited 2024 Sep 27]. Available from: https://www.woah.org/en/superbugs-could-jeopardise-food-security-for-over-two-billion-people-and-increase-annual-health-care-costs-by-us-159-billion-annually-by-2050-finds-most-extensive-modelling-to-date/

22. Marvasi M, Purchase D, editors. Antimicrobial resistance and the environment: one health approach. 2nd ed. Antibiot-ics. 2024;(Special issue).

23. World Health Organization; Food and Agriculture Organization of the United Nations; United Nations Environment Programme; World Organisation for Animal Health. A one health priority research agenda for antimicrobial resistance. Geneva: WHO; 2023.

24. Nambiar D, Boldosser-Bosch A, Costongs C, Sokolovič M, Kozlovič M, Frieder K, et al. Standing for, with and be-hind each other: how to foster civil society capacities for social participation. EuroHealth. 2024;30(1):44-9.

25. World Health Organization. Seventy-Seventh World Health Assembly. Social participation for universal health cover-age, health and well-being [Internet]. Geneva: WHO; 2024 [cited 2024 Sep 27]. Available from: https://apps.who.int/gb/ebwha/pdf_files/WHA77/A77_ACONF3-en.pdf

26. European Public Health Alliance. Stepping up the fight against antimicrobial resistance (AMR): EPHA position paper (2020 update) [Internet]. 2020 [cited 2024 Sep 27]. Available from: https://epha.org/wp-content/uploads/2020/12/epha-amr-position-paper-updated-2020.pdf

27. European Public Health Alliance. AMR Stakeholder network: Roadmap for action on antimicrobial resistance: 5 key strategies to tackle this global health threat [Internet]. 2022 [cited 2024 Sep 27]. Available from: https://epha.org/wp-content/uploads/2022/01/amr-roadmap-22.pdf

28. EPHA. Civil Society Joint Statement on the Roadmap on antimicrobial resistance for the WHO European Region 2023–2030 [Internet]. 2023 [cited 2024 Sep 27]. Available from: https://cdn.who.int/media/docs/librariesprovider2/regional-committee-meeting-reports/rc73-meeting-reports/rc73-nsa-statements/written---item-05--epha-1.pdf?sfvrsn=5d3b50bd_1&download=true

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ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

CONGENITAL ANOMALIES OF THE AORTIC ARCH – AUTOPSY STUDY

UROĐENE ANOMALIJE AORTNOG LUKA – AUTOPSIJSKA STUDIJA

Jovan RADOJEVIĆ[1], Stevan STOJANOVIĆ[2,3], Sofija GLUMAC[4], Nebojša PRIJOVIĆ[5] and Danica STANIĆ[6]

ORCID NUMBER
Jovan Radojević - 0009-0009-3826-0813
Stevan Stojanović - 0000-0002-9067-7933
Sofija Glumac - 0000-0002-2720-4148
Nebojša Prijović - 0000-0002-5268-5801
Danica Stanić - 0009-0001-3576-2887

General Hospital "Dr Radivoj Simonović", Sombor[1]
University Clinical Center of Vojvodina, Urology Clinic, Novi Sad[2]
University of Novi Sad, Faculty of Medicine Novi Sad[3]
University of Belgrade, Faculty of Medicine, Pathology Institute "Dr Djordje Joanović", Belgrade[4]
University Clinical Center of Serbia, Urology Clinic, Belgrade[5]
Clinic of Emergency Surgery[6]

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Abstract

Introduction. Congenital anomalies of the aortic arch encompass a diverse range of cardiovascular malformations that are less well-known and infrequently diagnosed in clinical settings. The study aims to assess the prevalence of aortic arch anomalies, their correlation with other heart defects, and their distribution by gender. **Material and Methods.** Data were collected through a retrospective analysis of autopsy records from the Archive for Congenital Heart Defects at the Institute of Pathology, Faculty of Medicine, University of Belgrade, spanning from 1961 to 2013. The archive also included autopsies conducted at the Institute for Mother and Child Health Care "Dr. Vukan Čupić" and the Gynecology and Obstetrics Clinic at the Clinical Center of Serbia. The study sample consisted of 245 autopsies. Both descriptive and analytical statistical methods were employed for data analysis. **Results.** The most common anomaly identified was the right aortic arch, observed in 26.94% of cases. A vascular ring was detected in 3.67% of cases, while tubular hypoplasia was found in 20.41% of cases. Aortic coarctation was present in 21.63% of cases, and interrupted aortic arch was noted in 12.65%. Aortic arch atresia was identified in 6.94% of the cases. **Conclusion.** Congenital anomalies of the aortic arch are relatively obscure and seldom clinically diagnosed cardiovascular anomalies. The findings of this study indicate that the right aortic arch is the most prevalent congenital anomaly of the aortic arch, occurring in 26.94% of cases. Among the anomalies incompatible with life, vascular ring, interruption of the aortic arch, and aortic arch atresia were observed.

Key words: Aorta, Thoracic; Congenital Abnormalities; Heart Defects, Congenital; Autopsy

Sažetak

Uvod. Urođene anomalije aortnog luka predstavljaju širok spektar manje poznatih i ređe klinički dijagnostikovanih kardiovaskularnih anomalija. Cilj rada je ispitivanje učestalosti anomalija aortnog luka, njihove udruženosti sa drugim srčanim manama i učestalost prema polu. **Materijal i metode.** Podaci su dobijeni retrospektivnom analizom obdukcionih protokola Instituta za patologiju Medicinskog fakulteta Univerziteta u Beogradu (Arhiva za urođene srčane mane) iz vremenskog perioda od 1961. do 2013. godine. Arhiva je obuhvatala i autopsije rađene na Institutu za zdravstvenu zaštitu majke i deteta "Dr Vukan Čupić" i na Ginekološko-akušerskoj klinici Kliničkog centra Srbije. Uzorak obuhvata 245 autopsija. Za analizu podataka korišćene su deskriptivne i analitičke statističke metode. **Rezultati.** Desni aortni luk se javio kod 26,94% slučajeva. Vaskularni prsten je uočen kod 3,67% slučajeva. Tubularna hipoplazija je zabeležena kod 20,41% slučajeva. Koarktacija aorte je uočena kod 21,63% slučajeva. Prekid aortnog luka je zabeležen kod 12,65% slučajeva, a atrezija aortnog luka se javila u 6,94% slučajeva. **Zaključak.** Urođene anomalije aortnog luka su manje poznate i retko klinički dijagnostikovane kardiovaskularne anomalije. Rezultati istraživanju su pokazali da je najčešća urođena anomalija aortnog luka desni aortni luk, koji je zabeležen kod 26,94% slučajeva. Od anomalija koje su inkompatibilne sa životom uočeni su vaskularni prsten, prekid aortnog luka i atrezija aortnog luka.

Ključne reči: grudna aorta; urođene anomalije; kongenitalne srčane mane; autopsija

Introduction

Congenital heart anomalies refer to structural abnormalities of the heart or major blood vessels that are present at birth. Most of these anomalies originate during embryogenesis, typically between the third and eighth week of gestation, while the primary structures of the cardiovascular system are formed. Although the reported prevalence can vary across different studies, congenital heart defects generally occur in 1% of the general population, making them the most common type heart disease in children [1].

Congenital anomalies of the aortic arch are among the lesser-known and less frequently diagnosed cardiovascular malformations. The spectrum of these anomalies is broad, ranging from anatomical variations that may be an incidental finding during autopsy to severe malformations incompatible with life,

✉ Corresponding author: Jovan Radojević, E-mail: jovan91so@hotmail.com, stevan.stojanovic@mf.uns.ac.rs

Abbreviations

ASD – atrial septal defect
VSD – ventricular septal defect
PDA – patent ductus arteriosus
TGA – transposition of great arteries
DAL – right aortic arch
LAL – left aortic arch
VP – vascular ring
TH – tubular hipoplasia
CoA – coartaction of aorta
PAL – interrupted aortic arch
AtrAo – aortic arch atresia

such as vascular ring and interrupted aortic arches. Early detection and clinical diagnosis of these anomalies are essential, as timely surgical intervention can often lead to favorable outcome for the patient [1].

To classify and understand the morphology of different subtypes of aortic arch anomalies, it is crucial to consider the developmental model proposed by Edwards. He introduced a hypothetical model that consists of paired aortic arches on either side, paired bilateral ductus arteriosus, and a single dorsal aorta. In this model, the carotid and subclavian vessels originate from the aortic arch, with the carotid arteries positioned anteriorly and the subclavian arteries posteriorly.

The normal left aortic arch is formed by the regression of the right aortic arch, right-sided ductus arteriosus, and right dorsal aorta. The proximal segment of the right dorsal aorta contributes to the formation of the right subclavian artery, while the left dorsal aorta forms the distal aortic arch and the descending thoracic aorta.

Various aortic arch anomalies can be explained by abnormal persistence or regression of different segments in the Edwards' double aortic arch model, resulting in a range of malformations [2, 3].

The objective of this study is to investigate the prevalence of aortic arch anomalies, their association with other heart defects, and their distribution according to gender.

Material and Methods

This study is based on a retrospective analysis of autopsy records from the Archive for Congenital Heart Defects at the Institute of Pathology, Faculty of Medicine, University of Belgrade, covering the period from 1961 to 2013. The dataset also includes autopsy reports from the Institute for Mother and Child Health Care "Dr. Vukan Čupić" and at the Gynecology and Obstetrics Clinic of the Clinical Center of Serbia. The total sample comprises 245 autopsies.

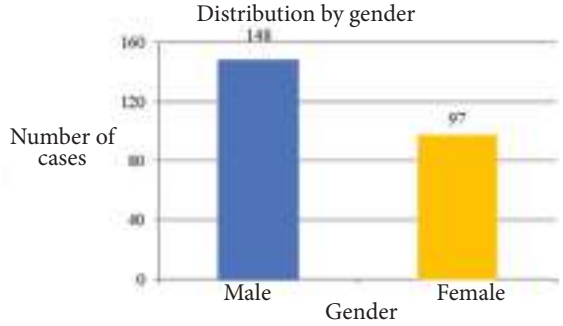
A pathological and morphological autopsy analysis of cases involving congenital heart defects was conducted using the modified Rokitansky technique.

For investigating the genesis of aortic arch anomalies, the study applied Edward's model of the primary double aortic arch in embryonic development. Data were analyzed using appropriate statistical methods, including measures of central tendency (mean and standard deviation), the chi-squared (χ^2) test. All statistical analyses were performed using SPSS software, version 17.0.

Results

From 1961 to 2013, a total of 1460 patients who underwent autopsies were diagnosed with congenital heart defects. Among these, congenital anomalies of the aortic arch were identified in 245 cases, representing 16.78% of the total.

These anomalies were found in 148 (60.41%) males and 97 (39.59%) females **(Graph 1).** This difference was statistically significant, with a higher prevalence in males (χ^2=10.616, p=0.0011).

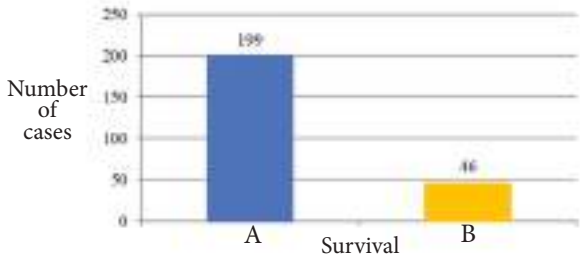


Graph 1. Prevalence of congenital aortic arch defects by gender

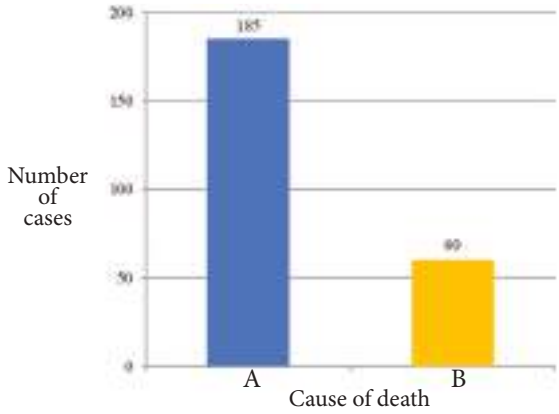
A survival rate beyond the first year of life was observed in 46 cases (18.78%), while 199 cases (81.22%) did not survive beyond one year **(Graph 2)**, showing a statistically significant difference (χ^2=95.547, p<0.001).

Cardiopulmonary diseases were recorded as the immediate cause of death in 185 cases (75.51%), whereas the remaining 60 cases (24.49%) died due to other etiologies, such as brain edema, cerebral hemorrhage, or peritonitis **(Graph 3).** The predominance of cardiopulmonary diseases as the cause of death was statistically significant (χ^2=63.776, p<0.001).

Among the 245 cases with aortic arch anomalies, the right aortic arch was noted in 66 cases (26.94%) **(Graph 4)**. The right aortic arch was observed in 36 males (54.55%) and 30 females (45.45%), with no statistically significant difference by gender (χ^2=0.545, p=0.4602). A survival rate greater than one year was noted in 16 cases (24.24%), while 50 cases (75.76%) did not survive beyond the first year, showing a statistically significant difference (χ^2=17.515, p<0.001). In 42 cases (63.64%), the immediate cause of death was a congenital heart defect,



Graph 2. Survival in relation to the first year of life
A – cardiopulmonary etiology; B – other causes (brain edema, cerebral hemorrhage, peritonitis, pericarditis)

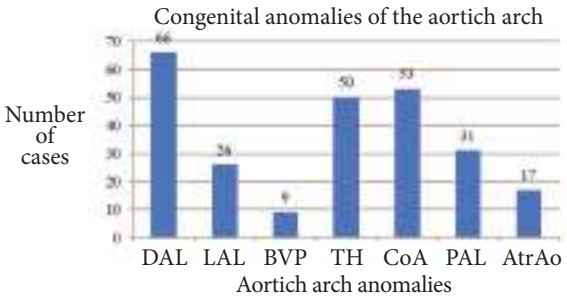


Graph 3. Cause of death in congenital aortic arch anomalies
A – cardiopulmonary etiology; B – other causes (brain edema, cerebral hemorrhage, peritonitis, pericarditis)

while in 24 cases (36.36%), it was due to another etiology, with a statistically significant difference (χ^2=4.909, p=0.0267). The right aortic arch was associated with atrial septal defect (ASD) and ventricular septal defect (VSD) in 26 cases (39.39%).

A left aortic arch with an aberrant subclavian artery was present in 26 cases (10.61%) in the analyzed cohort of aortic arch anomalies **(Graph 4)**.

A vascular ring was diagnosed in 9 cases (3.67%) (**Graph 4)**, with a male predominance: 7 cases (77.77%) in males and 2 cases (22.23%) in females. All 9 cases did not survive beyond the first year of life. Of these, 6 cases (66.67%) were associated with other congenital heart defects, while 3 cases (33.33%) presented as isolated aortic arch anomalies. In 8 cases (88.89%), the



Graph 4. Distribution of congenital aortic arch anomalies

immediate cause of death was cardiopulmonary in nature.

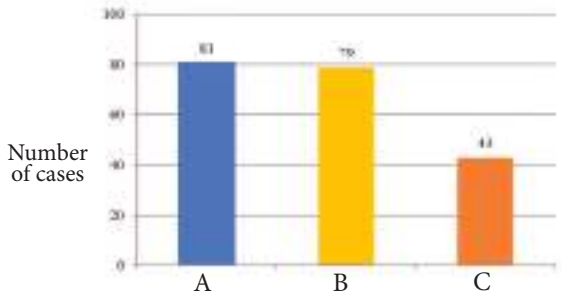
Tubular hypoplasia was observed in 50 cases (20.41%) **(Graph 4)**, with 33 cases (66%) in males and 17 cases (34%) in females, indicating a statistically significant higher occurrence in males (χ^2=5.120, p=0.0237). Only 2 cases (4%) survived beyond the first year of life, whereas 48 cases (96%) did not (χ^2=42.320, p<0.001). Tubular hypoplasia was associated with aortic coarctation in 26 cases (52%) and with Patent ductus arteriosus (PDA) in 21 cases (42%), while in only 3 cases it occurred as an isolated defect. Cardiopulmonary insufficiency was the immediate cause of death in 40 cases (80%), which was statistically significant (χ^2=18.000, p<0.001).

Aortic coarctation was recorded in 53 cases (21.63%), with 33 cases (62.26%) in males and 20 cases (37.74%) in females, without a statistically significant gender difference (χ^2=3.189, p = 0.0741). A total of 25 cases (47.16%) survived beyond the first year of life, while 28 cases (52.84%) did not (χ^2= 0.170, p= 0.6803). An association with other congenital heart defects was noted in 26 cases (49.06%) with tubular hypoplasia, in 11 cases (20.75%) with other congenital heart defects, and in 16 cases (30.19%) as an isolated defect, with a distribution nearing statistical significance (χ^2=5.895, df=2, p=0.0525). Cardiorespiratory failure was the immediate cause of death in 40 cases (75.47%) (χ^2=13.755, p<0.001).

Interrupted aortic arch was found in 31 cases (12.65%), with no significant difference in distribution between males (15 cases) and females (16 cases) (χ^2=0.032, p=0.8575). Of these, 26 cases (83.87%) were associated with other congenital heart defects (χ^2=14.226, p=0.0002), including 12 cases (38.71%) associated with VSD, and 5 cases (16.13%) as isolated defects. None of these cases survived beyond the first year of life. In 22 cases (71%), the cause of death was of cardiopulmonary etiology, while 9 cases died of other causes (χ^2=5.452, p=0.0196).

Aortic arch atresia was present in 17 cases (6.93%), with 12 cases (70.59%) in males and 5 cases (29.41%) in females. None of these cases survived the first year of life. In 14 cases (82.35%), aortic arch atresia was associated with other congenital heart defect, while in 3 cases (17.65%) it occurred in isolation. Cardiopulmonary insufficiency was the cause of death in 13 cases (76.47%), and in 4 cases (23.53%) the cause of death was of other etiology.

Among the 245 autopsied cases with congenital anomalies of the aortic arch, 202 cases (82.45%) were associated with other congenital heart defects **(Graph 5)**, while 43 cases (17.55%) presented as isolated forms, showing statistical significance (χ^2 = 103.188, p<0.001). Among the associated congenital heart defects **(Graph**



Graph 5. Association of aortic arch defects with other congenital heart defects
A – anomalies associated with TGA, PDA; B – anomalies associated with ASD or VSD; C – aortic arch anomalies associated with each other

5), 30 cases (14.85%) were found with transposition of the great arteries (TGA), 12 cases (5.94%) with PDA, 79 cases (39.11%) with ASD or VSD, and 81 cases (40.1%) with other aortic arch anomalies such as tubular hypoplasia and aortic coarctation.

Discussion

Congenital anomalies of the aortic arch result from aberrant development of one or more embryonic components of the pharyngeal arches. During early embryogenesis, six symmetrical pairs of pharyngeal arches merge with the branchial arches to form a primitive vascular structure. Each segment of the embryological arch system may abnormally regress or persist, resulting in aortic arch anomalies [4].

Anomalies of the aortic arch represent a rare subset of cardiovascular anomalies, accounting for 16.78% of all congenital heart defects.

The results of our study demonstrate a higher frequency of these anomalies in the male population, comprising 60.41% of cases. The severity of these defects is underscored by the high mortality rate when surgical correction is not performed, often due to cardiac or renal failure or intracranial bleeding [5, 6]. Our findings showed that 199 (81.23%) did not survive beyond the first year if life, with a statistically significant difference (p=0.01), highlighting the crucial nature of these defects when left untreated. Furthermore, 185 cases (75.51%) died from cardiopulmonary causes, consistent with the findings of other studies [5, 6].

The most common anomaly of the aortic arch identified in our study was the right aortic arch, found in 66 cases (26.94%). According to the literature, this anomaly occurs in approximately 1 in 2,500 autopsied cases [7]. In our data, 26 cases (39.39%) presented with concurrent ASD or VSD, aligning with previously reported results [7, 8]. Of those with a right aortic arch, 24.24% survived beyond one year, while 75.76% did not, demonstrating a statistically significant difference (p=0.016). The immediate cause of death was congenital heart anomalies in 63.64% of cases, with other etiologies accounting for the remaining 36.36%, thought this difference was not statistically significant (p>0.05).

Vascular ring is an exceedingly rare defect in our dataset, with only 9 cases (3.67%) of all congenital aortic arch anomalies. Among these, 8 cases (89%) died from cardiopulmonary causes, which is consistent with literature suggesting that these patients most commonly succumb to aspiration bronchopneumonia [9].

Tubular hypoplasia, another aortic arch anomaly, rarely appears in isolation; in our study, it was isolated in only 3 cases (6%). It was most commonly associated with aortic coarctation in 26 cases (52%) and with other congenital heart defects in 21 cases (42%), which is in agreement with literature data [9, 10].

Aortic coarctation was identified in 53 cases (21.63%). Literature suggests that aortic coarctation ranks fifth in frequency, accounting for 6-8% of all congenital heart defects, and occurs in approximately 1 in 2,500 cases [11, 12]. Our results revealed a male-to-female ratio of 1.62:1, closely matching the literature-reported ratio of 1.74:1 [13]. Among those affected, coarctation of the aorta had the highest percentage of cases surviving beyond the first year, with 47.16% of cases achieving this milestone. Aortic coarctation was most frequently associated with tubular hypoplasia, observed in 49.05% of cases, while 20.75% were associated with other anomalies of the aortic arch. The literature commonly cites association with hypoplastic left heart syndrome or VSD [14].

Interrupted aortic arch was present in 31 cases (12.65%), which constitute 2.12% of all congenital heart defects in our study. The literature reports that this anomaly accounts for about 1% of all congenital heart defects [15]. In our findings, 38.71% of cases of interrupted aortic arch were associated with VSD, aligning with literature that indicates this association is most common [16]. None of the cases with an interrupted aortic arch survived beyond the first year of life, indicating a high statistical significance (p<0.0001). The literature also states that without surgical intervention, the mortality rate exceeds 90% [17].

Aortic arch atresia was identifies in 17 cases (6.93%). None of these cases survived the first year of life, demonstrating a high statistical significance (p<0.0001), underscoring the severity of this defect if not diagnosed and managed promptly.

Conclusion

The study examined the frequency of congenital aortic arch anomalies, their distribution between

sexes, survival rates, and association with other defects. We analyzed 245 cases of congenital aortic arch anomalies and found that these are indeed rare defects. Among these, interrupted aortic arch, vascular ring, and aortic arch atresia are particularly uncommon, accounting for only a small percentage of cases. However, without surgical intervention, these anomalies are invariably fatal. Our findings also indicate that aortic arch anomalies are more prevalent in males, and the majority of deaths were due to cardiopulmonary insufficiency. Early diagnosis and prompt surgical management of these anomalies are crucial for improving survival and extending life expectancy.

References

1. Priya S, Thomas R, Nagpal P, Sharma A, Steigner M. Congenital anomalies of the aortic arch. Cardiovasc Diagn Ther. 2018;8(Suppl 1):S26-44.

2. Hanneman K, Newman B, Chan F. Congenital variants and anomalies of the aortic arch. Radiographics. 2017;37(1):32-51.

3. Türkvatan A, Büyükbayraktar FG, Ölçer T, Cumhur T. Congenital anomalies of the aortic arch: evaluation with the use of multidetector computed tomography. Korean J Radiol. 2009;10 (2):176-84.

4. Jakanani GC, Adair W. Frequency of variations in aortic arch anatomy depicted on multidetector CT. Clin Radiol. 2010; 65(6):481-7.

5. Tawfik AM, Sobh DM, Ashamallah GA, Batouty NM. Prevalence and types of aortic arch variants and anomalies in congenital heart diseases. Acad Radiol. 2019;26(7):930-6.

6. Lodeweges JE, Dikkers FG, Mulder BJM, Roos-Hesselink JW, Vliegen HW, van Dijk APJ, et al. The natural and unnatural history of congenital aortic arch abnormalities evaluated in an adult survival cohort. Can J Cardiol. 2019;35(4):438-45.

7. Zidere V, Tsapakis EG, Huggon IC, Allan LD. Right aortic arch in the fetus. Ultrasound Obstet Gynecol. 2006;28(7):876-81.

8. Tsai IC, Tzeng WS, Lee T, Jan SL, Fu YC, Chen MC, et al. Vertebral and carotid artery anomalies in patients with aberrant right subclavian arteries. Pediatr Radiol. 2007;37(10):1007-12.

9. Goo HW, Park IS, Ko JK, Kim YH, Seo DM, Yun TJ, et al. CT of congenital heart disease: normal anatomy and typical pathologic conditions. Radiographics. 2003;23(Suppl 1):S147-65.

10. Dillman JR, Yarram SG, D'Amico AR, Hernandez RJ. Interrupted aortic arch: spectrum of MRI findings. AJR Am J Roentgenol. 2008;190(6):1467-74.

11. Bravo-Valenzuela NJ, Peixoto AB, Arauji Júnior E. Prenatal diagnosis of congenital heart disease: a review of current knowledge. Indian Heart J. 2018;70(1):150-64.

12. Dijkema EJ, Leiner T, Grotenhuis HB. Diagnosis, imaging and clinical management of aortic coarctation. Heart. 2017;103(15):1148-55.

13. Singh S, Hakim FA, Sharma A, Roy RR, Panse PM, Chandrasekaran K, et al. Hypoplasia, pseudocoarctation and coarctation of the aorta – a systematic review. Heart Lung Circ. 2015;24 (2):110-8.

14. Teo LL, Cannell T, Babu-Narayan SV, Hughes M, Mohiaddin RH. Prevalence of associated cardiovascular abnormalities in 500 patients with aortic coarctation referred for cardiovascular magnetic resonance imaging to a tertiary center. Pediatr Cardiol. 2011;32(8):1120-7.

15. Zhao Q, Wang J, Yang ZG, Shi K, Diao KY, Huang S, et al. Assessment of intracardiac and extracardiac anomalies associated with coarctation of aorta and interrupted aortic arch using dual-source computed tomography. Sci Rep. 2019;9(1):11656.

16. Vogel M, Vernon MM, McElhinney DB, Brown DW, Colan SD, Tworetzky W. Fetal diagnosis of interrupted aortic arch. Am J Cardiol. 2010;105(5):727-34.

17. Marek J, Fenton M, Khambadkone S. Aortic arch anomalies: coarctation of the aorta and interrupted aortic arch. In: Lai WW, Martens L, Cohen M, Geva T, editors. Echocardiography in pediatric and congenital heart disease: from fetus to adult. Hoboken: Wiley-Blackwell; 2022. p. 430-62.

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THE IMPORTANCE OF TRAINING IN DELIVERING HIGH-QUALITY CHEST COMPRESSIONS

ZNAČAJ EDUKACIJE U POSTIZANJU VISOKOKVALITETNIH KOMPRESIJA GRUDNOG KOŠA

Aleksandar ĐURIČIN[1,2], Nikolina KONSTANTINOVIĆ[1], Radojka JOKŠIĆ MAZINJANIN[1,2], Nikolina MARIĆ[1,2], Dragan TURANJANIN[1,3] and Vanja TATALOVIĆ[1,4]

ORCID NUMBER
Aleksandar Đuričin – 0000-0002-8473-4022
Nikolina Konstantinović – 0009-0003-8878-8110
Radojka Jokšić Mazinjanin – 0000-0002-4436-336X
Nikolina Marić – 0009-0004-3029-385X
Dragan Turanjanin – 0009-0002-9638-0935
Vanja Tatalović – 0009-0008-1942-706X

University of Novi Sad, Faculty of Medicine Novi Sad[1]
Institute for Emergency Medical Services Novi Sad[2]
Children and Youth Health Care Institute of Vojvodina, Clinic of Pediatric Surgery, Department for Pediatric Anesthesia, Intensive Care and Pain Therapy, Novi Sad[3]
University Clinical Center of Vojvodina, Clinic for Plastic and Reconstructive Surgery, Novi Sad[4]

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Abstract

Introduction. Sudden cardiac arrest is an abrupt cessation of the heartbeat, leading to a critical interruption in blood circulation and oxygen delivery to vital organs. Immediate initiation of cardiopulmonary resuscitation upon recognizing cardiac arrest significantly enhances the chances of survival. Basic Life Support helps to mitigate organ damage while awaiting more advanced medical interventions. The objective of this study is to evaluate the effectiveness of chest compressions delivered by students trained in Basic Life Support compared to those without such training. **Material and Methods.** A total of 120 first-year medical students participated in the study. Sixty students had not undergone Basic Life Support training, while the remaining 60 had successfully completed the course. Data about chest compression quality were gathered using SimPad PLUS Laerdal software and the Little Anne QCPR manikin. **Results.** Students who had received Basic Life Support training demonstrated significantly better performance across key parameters, including compression rate, depth, and the proportion of compressions meeting adequate depth criteria. Additionally, there was a statistically significant improvement in overall compression success rate among the students trained in Basic Life Support. **Conclusion.** The findings of this study highlighted the positive impact of Basic Life Support on the quality of chest compressions.

Key words: Heart Arrest; Cardiopulmonary Resuscitation; Education; Students, Medical; Heart Massage; Quality Improvement; Manikins; Life Support Care

Sažetak

Uvod. Iznenadni srčani zastoj predstavlja neočekivani prestanak rada srca koji dovodi do prekida krvotoka i snabdevanja organa kiseonikom. Započinjanje mera kardiopulmonalne reanimacije bez odlaganja, odmah nakon registrovanja znaka srčanog zastoja, povećava šanse za preživljavanjem. Osnovne mere oživljavanja usporiće razvoj oštećenja, dok se složenijim postupcima stručne medicinske pomoći ne obezbedi dalje zbrinjavanje. Cilj rada je analiza uspešnosti izvođenja visokokvalitetnih kompresija grudnog koša kod studenata nakon edukacije o osnovnoj životnoj podršci u odnosu na grupu studenata koji nisu prošli edukaciju. **Materijal i metode.** U istraživanju je učestvovalo 120 studenata prve godine medicine, od toga 60 studenata je činilo prvu grupu koja nije prošla edukaciju o osnovnoj životnoj podršci, dok se druga grupa sastojala od 60 studenata koji su prethodno prošli kurs. Za rad je upotrebljena lutka *Little Anne QCPR* uz korišćenje softvera (*SimPad PLUS Laerdal*) koji meri informacije o kvalitetu kompresija grudnog koša. **Rezultati.** Kada se razmatraju parametri kompresije grudnog koša, studenti grupe koja je prošla edukaciju o osnovnoj životnoj podršci pokazali su značajno bolje rezultate u brzini, dubini i procentu dovoljno dubokih kompresija. Studenti koji su prošli edukaciju pokazali su i statistički značajnu veću ukupnu uspešnost kompresija grudnog koša. **Zaključak.** Rezultati našeg istraživanja pokazali su da edukacija utiče na kvalitet kompresija grudnog koša.

Ključne reči: srčani zastoj; kardiopulmonalna reanimacija; edukacija; studenti medicine; masaža srca; unapređenje kvaliteta; lutke; osnovna životna podrška

Introduction

Sudden cardiac arrest (SCA) is an abrupt cessation of the heartbeat, resulting in a loss of blood flow and oxygen to vital organs. Immediate resuscitation can potentially reverse the effects of SCA, but delays in starting resuscitation often lead to irreversible damage to the brain and other critical organs, ultimately causing death. Out-of-hospital survival rates for SCA are just below 10%, making it the third leading cause of death in developed countries [1–3]. Whether occurring in or out of the hospital, the treatment of cardiac arrest requires the rapid initiation of bystander cardiopulmonary resuscitation (CPR), with the primary goal of maintaining blood flow to essential organs, including the brain and heart. Survival chances increase two to threefold if CPR is started immediately following the onset of cardiac arrest [4, 5].

External chest compressions and artificial respiration, the key components of Basic Life Support (BLS),

✉ Corresponding author: Aleksandar Đuričin, E-mail: aleksandar.djuricin@mf.uns.ac.rs

Abbreviations

SCA – sudden cardiac arrest
CPR – cardiopulmonary resuscitation
BLS – Basic Life Support
ERC – European Resuscitation Council
ILCOR – International Liaison Committee on Resuscitation
AHA – American Heart Association
ROSC – return of spontaneous circulation

play a critical role in delaying heart and brain damage in individuals experiencing SCA. These interventions are designed to sustain vital organ functions until more advanced medical care can be provided by trained professionals. The effectiveness of CPR is largely determined by the quality of chest compressions, which depend on proper hand placement, depth, and compression rate. Full chest recoil after each compression is also crucial to maximize blood flow. Furthermore, maintaining continuous and uninterrupted compressions is essential for preserving coronary perfusion pressure, creating optimal conditions for successful resuscitation [6, 7].

Continuous education and training are necessary to ensure high-quality CPR. Advances in technology have led to the development of tools such as software applications, smartphones, and portable devices, which are integrated with training manikins to provide real-time feedback and quantitative assessment of CPR quality. These innovations aim to enhance the training process and improve CPR performance by offering immediate, accurate feedback on technique and effectiveness [8–10].

The aim of this study is to evaluate the effectiveness of delivering high-quality chest compressions in students trained in BLS compared to those without training.

Material and Methods

This research was conducted at the Educational Center of the Institute for Emergency Medical Assistance in Novi Sad during November and December 2021. The study was approved by the Director of the Institute. Participants were first-year medical students from the Faculty of Medicine Novi Sad, who regularly attended first aid exercises. A total of 120 students, of both sexes, were included and divided into two groups of 60 each. Prior to the study, all participants were informed in detail about the study's objectives, methodologies, and the measurements involved. Students who declined to participate for any reason were excluded from the research.

The study included two different groups: the first group comprised students who had not received any BLS training, while the second group consisted of students who had completed a standard BLS course based on the 2021 European Resuscitation Council (ERC) guidelines as part of their first aid curriculum at the Faculty of Medicine. The training lasted four hours, focusing on practical application. Following the training, all participants underwent a formal skills assessment using the Laerdal Resusci Anne QCPR manikin and the ZOLL AED PRO defibrillator. The interval between the BLS training and the assessment ranged from 7 to 10 days.

After a brief introduction to the study equipment, each participant performed 2 minutes of chest compressions on the Laerdal Resusci Anne QCPR manikin. This manikin, designed specifically for BLS training, is integrated with the Laerdal SimPad PLUS software, which monitors and provides real-time feedback on the quality of chest compressions. Although the software can evaluate both ventilations and compressions, our study focused solely on chest compressions.

The data extracted from the software included: the total number of compressions performed in 2 minutes, the average compression rate per minute, the percentage of compressions delivered at the correct rate, the average depth of compression, the percentage of compressions that reached sufficient depth, the percentage of compressions with complete chest recoil, and the percentage of correct hand placements. The software then synthesized these parameters into an overall performance score, ranging from 0% to 100%, which reflected the quality of the chest compressions. The evaluation was based on the criteria outlined in the 2021 European Resuscitation Council Guidelines [6].

Data analysis was performed using IBM SPSS Statistics 23. Descriptive statistics, including mean and standard deviation, minimum and maximum values, or median and interquartile range (P25-P75), were calculated based on the normality of the data distribution, as assessed by the Shapiro-Wilk test.

Comparisons between the two groups were conducted using either the independent samples Student´s t-test or the Mann-Whitney U test, depending on the data's distribution. Gender differences were evaluated using the chi-squared (χ^2) test. Results were presented in both tabular and graphical formats. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 120 participants were included in the study. Statistical analysis revealed a significant gender disparity, with a higher proportion of female participants (89; 74.2%) compared to male (31; 25.8%) ($\chi^2 = 28.033$, df = 1, p = 0.000). However, no statisti-

Table 1. ‡T-test with repeated measurements; *Mann-Whitney U* test; SD-Standard deviation; *IQR*- interquartile range: P25-25. percentile; P75-75. percentile; Bolded values are statistically significant.

	Group 1	Group 2	Overall	p
Number of compressions				
Mean±SD	234.9±32.0	206.5±26.33	220.7±32.5	<0.0001
Min-Max	163-307	150-262	150-307	
Mean depth of all compressions (mm)				
Median	39.00	44.5	42.5	0.005
IKR (P25-P75)	22 (28-49.8)	13 (37.2-50)	16 (34-51)	
Mean speed of all compressions per minute				
Mean±*SD*	118.0±16.2	103.8±12.9	110.9±16.3	<0.0001
Min-Max	82-154	78-131	78-154	
Compressions at correct speed (%)				
Median	38.5	34	35.5	0.398
IQR (P25-P75)	82 (2-84)	77 (9.2-86.5)	80 (5.2-85)	
Correct hand position (%)				
Median	100	100	100	0.077
IQR (P25-P75)	0 (100-100)	91 (9.5-100)	23 (77.2-100)	
Compressions with complete chest recoil (%)				
Median	37.5	60	55.5	0.084
IQR (P25-P75)	88 (3-91)	76 (17.2-93)	84 (8.5-92.5)	
Sufficiently deep compressions (%)				
Median	2	11.5	5.5	0.003
IQR (P25-P75)	41 (0-41.2)	49 (3-51.8)	50 (0-49.5)	
Overall success (%)				
Median	9.5	25.5	17	0.003
IQR (P25-P75)	54 (0-53.8)	75 (6.5-81.5)	62 (3-64.8)	

cally significant difference in gender distribution was observed between the two groups ($\chi^2 = 0.174$, df = 1, p = 0.677).

Table 1 presents a descriptive statistics for the parameters examined. Only the variables "number of compressions" and "mean compression depth (mm)" followed a normal distribution. In the group of students without Basic Life Support (BLS) training (Group 1), the number of compressions ranged from 163 to 307, with a mean of 234.9 ± 32.0. In contrast, among the students who received BLS training (Group 2), the number of compressions was significantly lower (t = 5.308; p = 0.000), ranging from 150 to 262, with a mean of 206.5 ± 26.33. Additionally, the mean compression rate per minute was significantly lower in Group 2 (103.8 ± 12.9) compared to Group 1 (118.0 ± 16.2) (t = 5.302; p = 0.000), as shown in **Figure 1.**

The mean compression depth among students in Group 1 was 39 mm, whereas in Group 2, it was significantly higher at 44.5 mm (Z = -2.812, p = 0.005), as depicted in **Figure 2.**

The percentage of compressions performed at correct speed did not how a statistically significant difference between the two groups (Z = -0.846, p = 0.398), although the median value in Group 1 (38.5%) was slightly higher than in Group 2 (34%). Similarly, the percentage of compressions with complete chest recoil did not differ significantly between the groups (Z = -1.726, p = 0.084). In Group 1, half of the participants achieved 37.5% chest recoil, whereas in Group 2, half achieved 60%, as illustrated in **Figure 3.**

There was a statistically significant difference in achieving adequate compression depth (%) between students who had not undergone BLS training and those who had (Z = -2.995, p = 0.003). However, no

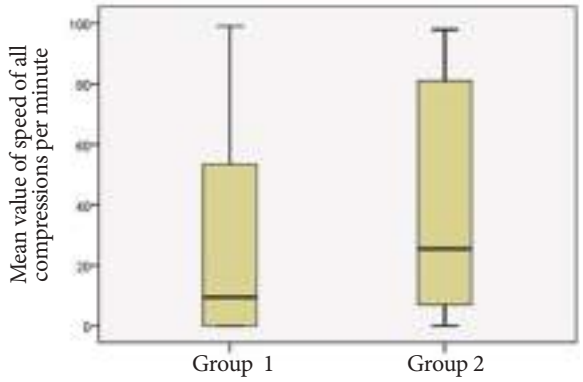

Figure 1. Mean value of the speed of all compressions per minute

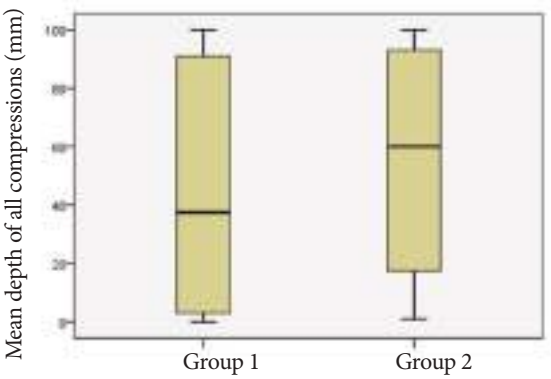

Figure 2. Mean depth of all compressions in mm

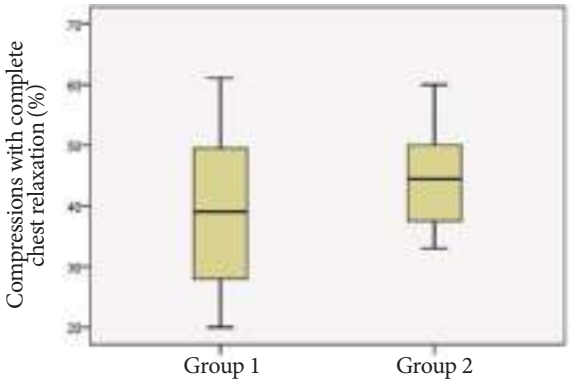

Figure 3. Compressions with complete chest relaxation (%) per group

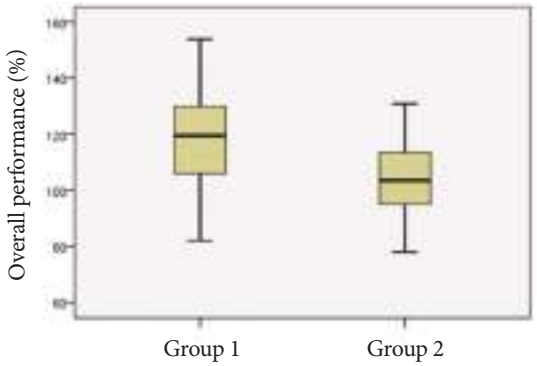

Figure 4. Mean value of speed of all compressions per minute

statistically significant difference was observed between the groups regarding hand positioning (%) during compressions (Z = -1.771, p = 0.077). Overall, participants who had completed the BLS course demonstrated a significantly higher overall success rate (%) compared to those without prior BLS training (Z = -2.934, p = 0.003), as illustrated in **Figure 4.**

Discussion

CPR is a structured set of interventions aimed at restoring cardiac and respiratory function in individuals who have experienced cardiac arrest or respiratory failure. Immediate initiation of CPR at the scene is vital, following a predefined sequence known as the "chain of survival", which has been endorsed by the European Resuscitation Council (ERC) since 2005 [10]. The first link in the chain involves rapid recognition of the emergency and immediate activation of emergency medical services to prevent cardiac arrest. Subsequent links focus on early CPR and prompt defibrillation, followed by post-resuscitation care strategies to preserve vital organ function [11]. The 2020 Guidelines for Cardiopulmonary Resuscitation, based on research from the International Liaison Committee on Resuscitation (ILCOR), provide a widely accepted framework for effective and safe resuscitation practices [12].

Effective chest compressions are crucial for the survival of cardiac arrest victims [13–17]. The guidelines emphasize that high-quality compressions involve maintaining an appropriate rate and depth, ensuring full chest recoil, and positioning the hands correctly. Adherence to these parameters correlates with improved survival rates for both in-hospital and out-of-hospital cardiac arrest cases [13, 18]. Maintaining the correct rate and depth optimizes blood flow and oxygen delivery to the heart and brain, enhancing the likelihood of a return of spontaneous circulation (ROSC) and improving neurological outcomes at hospital discharge. In contrast, inadequate chest recoil impedes venous return, reducing mean arterial pressure and compromising perfusion in the coronary and cerebral circulations [11, 19, 20].

Despite widespread training in CPR, sudden cardiac arrest remains one of the leading causes of death in Europe, with resuscitation success rates below 10% [21]. Continuing education is essential to maintain and improve CPR skills, which can be enhanced through regular practice [22]. Technological advancements have transformed medical training with the introduction of tools such as mobile applications, smartwatches, and software devices designed to improve CPR training quality [8–10]. Studies consistently show that real-time feedback during CPR improves the depth and rate of compressions in both novice and experienced individuals [23–26].

This study aimed to evaluate the differences in the efficiency and quality of chest compressions between students who had completed a BLS course and those who had not. Participants performed compressions on a Laerdal Resusci Anne QCPR manikin connected to Laerdal's SimPad PLUS software, which measures and provides feedback on compression quality. The evaluation was based on ERC-recommended parameters such as compression rate, depth, full chest relaxation, and hand positioning. BLS-trained students demonstrated a statistically lower compression rate

per minute compared to their untrained peers. However, their rates remained within the recommended range of 100 to 120 compressions per minute [13, 18]. The mean compression depth and the percentage of adequately deep compressions were also statistically significantly higher among BLS-trained students. Although participants did not achieve the guideline-recommended minimum compression depth of 50 mm, these results align with other studies conducted in training settings [27–30]. Vadeboncoeur et al. found that deeper compressions were associated with better survival outcomes in out-of-hospital cardiac arrest patients, with a mean compression depth of 49.8 ± 11.0 mm, and the mean rate of 113.9 ± 18.1 in min. Survivors had a significantly deeper mean compression depth (53.6 mm) compared to non-survivors (48.8 mm) [31]. In our study, no significant differences were observed between the groups concerning chest compression with full recoil and hand position. However, BLS-trained students had a statistically significantly higher overall compression success rate, as indicated by the SimPad PLUS software, which integrates all performance parameters into a comprehensive score ranging from 0 to 100%.

This study has several limitations. First, it was conducted using a manikin simulation, which do not replicate the real-world cardiac arrest situations, where panic and confusion can influence outcomes. Second, the study focused solely on chest compressions and did not consider ventilation, which is a critical aspect of CPR. Finally, some students in the non-BLS group may have had prior exposure to CPR techniques through secondary medical school or first aid courses, potentially influencing their performance.

Conclusion

Our study highlights the vital role of training in enhancing the quality of chest compressions during cardiopulmonary resuscitation. Participants trained in Basic Life Support demonstrated superior performance in terms of compression rate, depth, and proportion of compressions reaching adequate depth. Additionally, those who had completed Basic Life Support training achieved a significantly higher overall success rate in delivering effective chest compressions.

Ongoing education and training are crucial for maintaining proficiency in cardiopulmonary resuscitation. Regularly updating and assessing acquired skills is essential for ensuring competence. Moreover, training in managing sudden cardiac arrest should extend beyond health care professionals to include the general population, fostering a broader level of competence and readiness for emergency situations.

References

1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics – 2020 update: a report from the American Heart Association. Circulation. 2020;141(9):e139-596.

2. Kiguchi T, Okubo M, Nishiyama C, Maconochie I, Ong MEH, Kern KB, et al. Out-of-hospital cardiac arrest across the World: first report from the International Liaison Committee on Resuscitation (ILCOR). Resuscitation. 2020;152:39-49.

3. Grasner JT, Wnent J, Herlitz J, Perkins GD, Lefering R, Tjelmeland I, et al. Survival after out-of-hospital cardiac arrest in Europe – results of the EuReCa TWO study. Resuscitation. 2020;148:218-26.

4. Valenzuela TD, Roe DJ, Cretin S, Spaite DW, Larsen MP. Estimating effectiveness of cardiac arrest interventions: a logistic regression survival model. Circulation. 1997;96(10):3308-13.

5. Kragholm K, Wissenberg M, Mortensen RN, Hansen SM, Malta Hansen C, Thorsteinsson K, et al. Bystander efforts and 1-year outcomes in out-of-hospital cardiac arrest. N Engl J Med. 2017;376(18):1737-47.

6. Olasveengen TM, Semeraro F, Ristagno G, Castren M, Handley A, Kuzovlev A, et al. European Resuscitation Council guidelines 2021: basic life support. Resuscitation. 2021;161:98-114.

7. Popović V, Gvozdenović L, Ivanov I, Milić S. Out-of-hospital treatment in case of drowning. Med Pregl. 2011;64(1-2):64-7.

8. Abella BS. High-quality cardiopulmonary resuscitation: current and future directions. Curr Opin Crit Care. 2016;22(3):218-24.

9. Couper K, Kimani PK, Abella BS, Chilwan M, Cooke MW, Davies RP, et al. The system-wide effect of real-time audiovisual feedback and postevent debriefing for in-hospital cardiac arrest: the cardiopulmonary resuscitation quality improvement initiative. Crit Care Med. 2015;43(11):2321-31.

10. Vahedian-Azimi A, Hajiesmaeili M, Amirsavadkouhi A, Jamaati H, Izadi M, Madani SJ, et al. Effect of the Cardio First Angel™ device on CPR indices: a randomized controlled clinical trial. Crit Care. 2016;20(1):147.

11. Nolan J, Soar J, Eikeland H. The chain of survival. Resuscitation. 2006;71(3):270-1.

12. Kiguchi T, Okubo M, Nishiyama C, Maconochie I, Ong MEH, Kern KB, et al. Out-of-hospital cardiac arrest across the World: first report from the International Liaison Committee on Resuscitation (ILCOR). Resuscitation. 2020;152:39-49.

13. Kleinman ME, Brennan EE, Goldberger ZD, Swor RA, Terry M, Bobrow BJ, et al. Part 5: adult basic life support and cardiopulmonary resuscitation quality 2015 American Heart Association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation. 2015;132(18 Suppl 2):S414-35.

14. Bohn A, Weber TP, Wecker S, Harding U, Osada N, Van Aken H, et al. The addition of voice prompts to audiovisual feedback and debriefing does not modify CPR quality or outcomes in out of hospital cardiac arrest - a prospective, randomized trial. Resuscitation. 2011;82(3):257-62.

15. Meaney PA, Bobrow BJ, Mancini ME, Christenson J, de Caen AR, Bhanji F, et al. Cardiopulmonary resuscitation quality: improving cardiac resuscitation outcomes both inside and outside the hospital a consensus statement from the American Heart Association. Circulation. 2013;128(4):417-35.

16. Nolan JP. High-quality cardiopulmonary resuscitation. Curr Opin Crit Care. 2014;20(3):227-33.

17. Stiell IG, Brown SP, Christenson J, Cheskes S, Nichol G, Powell J, et al. What is the role of chest compression depth during out-of-hospital cardiac arrest resuscitation? Crit Care Med. 2012;40(4):1192-8.

18. Perkins GD, Handley AJ, Koster RW, Castren M, Smyth MA, Olasveengen T, et al. European Resuscitation Council guidelines for resuscitation 2015: section 2. Adult basic life support and automated external defibrillation. Resuscitation. 2015; 95:81-99.

19. Wallace SK, Abella BS, Becker LB. Quantifying the effect of cardiopulmonary resuscitation quality on cardiac arrest outcome: a systematic review and meta-analysis. Circ Cardiovasc Qual Outcomes. 2013;6(2):148-56.

20. Lin S, Scales DC. Cardiopulmonary resuscitation quality and beyond: the need to improve real-time feedback and physiologic monitoring. Crit Care. 2016;20(1):182.

21. Perkins GD, Travers AH, Berg RA, Castren M, Considine J, Escalante R, et al. Part 3: adult basic life support and automated external defibrillation: 2015 International Consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. Resuscitation. 2015;95:e43-69.

22. van Tulder R, Laggner R, Kienbacher C, Schmid B, Zajicek A, Haidvogel J, et al. The capability of professional and lay-rescuers to estimate the chest compression-depth target: a short, randomized experiment. Resuscitation. 2015;89:137-41.

23. Buléon C, Parienti JJ, Halbout L, Arrot X, De Facq Régent H, Chelarescu D, et al. Improvement in chest compression quality using a feedback device (CPRmeter): a simulation randomized crossover study. Am J Emerg Med. 2013;31(10):1457-61.

24. Krasteva V, Jekova I, Didon JP. An audiovisual feedback device for compression depth, rate and complete chest recoil can improve the CPR performance of lay persons during self-training on a manikin. Physiol Meas. 2011;32(6):687-99.

25. Skorning M, Beckers SK, Brokmann JCh, Rörtgen D, Bergrath S, Veiser T, et al. New visual feedback device improves performance of chest compressions by professionals in simulated cardiac arrest. Resuscitation. 2010;81(1):53-8.

26. Li Q, Ma EL, Liu J, Fang LQ, Xia T. Pre-training evaluation and feedback improve medical students' skills in basic life support. Med Teach. 2011;33(10):e549-55.

27. Kaminska H, Wieczorek W, Matusik P, Czyzewski L, Landy JR, Smereka J, et al. Factors influencing high-quality chest compressions during cardiopulmonary resuscitation scenario, according to 2015 American Heart Association guidelines. Kardiol Pol. 2018;76(3):642-7.

28. Abelairas-Gómez C, Barcala-Furelos R, Szarpak L, García-García Ó, Paz-Domínguez Á, López-García S, et al. The effect of strength training on quality of prolonged basic cardiopulmonary resuscitation. Kardiol Pol. 2017;75(1):21-7.

29. Shinchi M, Kobayashi M, Soma K, Maeda A. Comparison of chest compression quality in walking versus straddling cardiopulmonary resuscitation during stretcher transportation: a prospective randomised crossover study using manikins. PLoS One. 2019;14(5):e0216739.

30. Sanri E, Karacabey S. The impact of backboard placement on chest compression quality: a Mannequin study. Prehosp Disaster Med. 2019;34(2):182-7.

31. Vadeboncoeur T, Stolz U, Panchal A, Silver A, Venuti M, Tobin J, et al. Chest compression depth and survival in out-of-hospital cardiac arrest. Resuscitation. 2014;85(2):182-8.

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ASSOCIATION BETWEEN INHERITED THROMBOPHILIA AND ISCHEMIC BRAIN DISEASE IN VOJVODINA

POVEZANOST NASLEDNE TROMBOFILIJE I ISHEMIJSKE BOLESTI MOZGA U VOJVODINI

Tamara IVKOVIĆ[1], Iva BARJAKTAROVIĆ[2,3], Miljen MALETIN[1], Stanislava NIKOLIĆ[3,4] and Marko SUBAŠIĆ[1]

ORCID NUMBER
Tamara Ivković – 0009-0000-3995-2744
Iva Barjaktarović – 0000-0003-2460-3458
Miljen Maletin – 0000-0002-4135-0758
Stanislava Nikolić – 0000-0002-7859-0923
Marko Subašić – 0009-0004-3881-4752

University Clinical Center of Vojvodina, Novi Sad,
Center for Forensic Medicine, Toxicology and Molecular Genetics[1]
University of Novi Sad, Faculty of Medicine Novi Sad,
Department of General Education Subjects[2]
University Clinical Center of Vojvodina, Novi Sad, Laboratory Medicine Center[3]
University of Novi Sad, Faculty of Medicine Novi Sad, Department of Pathophysiology and Laboratory Medicine[4]

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Abstract

Introduction. The most common genetic causes of thrombophilia include deficiency of histidine-rich glycoprotein, proteins C and S, antithrombin III, as well as mutations in the factor V and factor II genes. Thrombophilia is primarily associated with an elevated risk of deep vein thrombosis and/or thromboembolism; however, thrombosis can also occur in atypical locations such as the retinal, cerebral, and splanchnic veins. Ischemic stroke can result from restricted blood flow to the brain. Some studies suggest a potential link between ischemic brain disease and inherited thrombophilia. **Material and Methods.** Venous blood samples were used to extract genomic DNA, and detection of allele types in patients and controls was performed using real-time polymerase chain reaction on Gentier 96R Real-Time PCR System (TianLong, China) with an allelic discrimination assay. **Results.** Among the risk factors evaluated, the most common one linked to the development of cerebral ischemia was resistance to activated protein C due to the presence of FV Leiden mutation. **Conclusion.** Further studies involving larger cohorts of patients with reported cases of cerebral ischemia are necessary to determine whether a significant association exists between inherited thrombophilia and cerebral ischemia.

Key words: Blood Coagulation Disorders, Inherited; Brain Ischemia; Thrombophilia; Risk Factors; Activated Protein C Resistance; Polymerase Chain Reaction; Mutation

Sažetak

Uvod. Najčešći genetički faktori koji uzrokuju trombofiliju su mutacije u genima za faktor V i faktor II, kao i deficit glikoproteina bogatog histidinom, antitrombina III, proteina C i proteina S. Trombofilija se najčešće dovodi u vezu sa trombozom dubokih vena i/ili tromboembolijom, ali se tromboza može dogoditi i na mestima kao što su splanhične, cerebralne i retinalna vena. Prekid protoka krvi do mozga može dovesti do ishemijskog moždanog udara. Istraživanja su ukazala na moguću povezanost između ishemijske bolesti mozga i nasledne trombofilije. **Materijal i metode.** Nakon ekstrakcije DNK iz uzoraka venske krvi, određen je tip alela metodom lančane reakcije polimeraze u realnom vremenu na instrumentu *Gentier 96R Real-Time PCR System (TianLong, China)*, metodom alelske diskriminacije. **Rezultati.** Među ispitivanim faktorima rizika za razvoj cerebralne ishemije najzastupljeniji je bio rezistencija na aktivirani protein C usled potvrđene *FV Leiden* mutacije. **Zaključak.** Potrebno je sprovesti ovakvo istraživanje na većem broju pacijenata sa dokumentovanom cerebralnom ishemijom kako bi se sa sigurnošću utvrdilo da li postoji značajna povezanost između nasledne trombofilije i ishemijske bolesti mozga.

Ključne reči: nasledni poremećaji koagulacije; ishemija mozga; trombofilija; faktori rizika; rezistencija na aktivirani protein C; PCR; mutacije

Introduction

Hypercoagulability results from either an excessive activity of pro-coagulant factors or a deficiency in anti-coagulant factors [1]. Thrombophilia, characterized by an increased tendency for blood to clot, can be either inherited or acquired [2, 3], although thrombotic events typically result from the interaction of both genetic and environmental factors [4]. Genetic causes of thrombophilia include mutations in the factor V (FV) gene, antithrombin III deficiency, protein C or S deficiencies, histidine-rich glycoprotein deficiency, and prothrombin-related thrombophilia [2].

The most common form of inherited thrombophilia is the FV Leiden mutation, caused by a guanine-to-adenine substitution at nucleotide 1691 in the FV (proaccelerin) gene. This substitution leads to the replacement of arginine with glutamine at position 506 of the heavy chain, resulting in partial resistance to activated protein C (APC)-mediated proteolysis, thereby sustaining factor V's pro-coagulant activity [2, 5].

The second most common hereditary thrombophilia is prothrombin-related thrombophilia [2]. Factor II (FII), or prothrombin, is a vitamin K-dependent proenzyme produced by the liver produces. It plays a crucial role in converting fibrinogen into fibrin. Elevated FII levels are associated with adenine-to-gua-

✉ Corresponding author: Tamara Ivković, E-mail: ivkovictamara28@gmail.com, stanislava.nikolic@mf.uns.ac.rs

Abbreviations

FV – factor V
APC – activated protein C
FII – factor II
AT – antithrombin
CI – cerebral ischemia
EDTA – ethylenediamine tetraacetic acid
DNA – deoxyribonucleic acid
PCR – polymerase chain reaction
qPCR – real-time polymerase chain reaction
TIA – transient ischemic attack
RFU – relative fluorescence unit

nine replacement at position 20210. Mutations in the factor II gene are correlated with an increased risk of thrombosis in specific venous regions, such as the portal vein and intracranial veins [6].

The three major inhibitors of blood coagulation are antithrombin, protein C, and protein S [7]. Antithrombin (AT), a serine proteinase inhibitor, enhances the inhibitory process in the presence of the co-factor heparin. Antithrombin directly inactivates thrombin as well as factors IX, X, and XI through the formation of a covalent complex. Thrombin, in turn, activates protein C, which proteolytically inactivates factors Va and VIIIa, inhibiting thrombin production and subsequent coagulation. Protein S, a vitamin K-dependent glycoprotein synthesized in the liver, megakaryocytes, endothelial cells, and Leydig cells acts as a co-factor for protein C [3, 8].

Non-genetic factors also influence coagulation, including age, tissue damage, malignancy, pregnancy, oral contraception and hormone replacement therapy, physical inactivity, and obesity [9].

While deep venous thrombosis and/or thromboembolism are the most frequent manifestations of thrombophilia, thrombosis can also occur in uncommon locations, such as the retinal, cerebral, and splanchnic veins [2].

Given that approximately 25% of ischemic strokes are cryptogenic [10], genetic testing for thrombophilia is often sought to uncover possible stroke causes. Ischemic strokes, which account for about 87% of all stroke cases, are a leading cause of death or severe disability [11]. Ischemic strokes can be caused by a thrombosis, embolism, or systemic hypoperfusion, all of which restrict blood flow to the brain, depriving it of sufficient oxygen and glucose, and leading to cell death [12].

A 2019 study by Chiasakul et al. found that patients with arterial ischemic stroke were significantly more likely to have inherited thrombophilia, such as FV Leiden or the prothrombin G20210 mutation, or deficiencies in protein C and protein S [13].

Conversely, other studies have found limited evidence to support strong association between thrombophilia and cerebral ischemia (CI). Some argue that FV Leiden and prothrombin gene mutations are not significantly related to ischemic stroke, questioning the cost-effectiveness and utility of thrombophilia testing in various stroke scenarios [14–16].

The aim of this study is to evaluate the potential correlation between hereditary thrombophilia and the development of CI in a population from Vojvodina.

Material and Methods

Genetic testing to detect point mutations in the factor II and factor V genes was conducted at the Center for Forensic Medicine and Laboratory Medicine Center of the University Clinical Center of Vojvodina from January 2022 to July 2024. This case-control study included 83 patients from the Neurology Clinic, University Clinical Center of Vojvodina, diagnosed with ischemic brain disease, and 112 healthy controls (volunteers with no known health conditions), matched by sex and age. Informed consent was obtained from all participants, and the study was approved by the Institutional Ethics Committee, in accordance with the Declaration of Helsinki. Venous blood samples were collected from each participant into ethylenediamine tetraacetic acid (EDTA) tubes, and dried blood spots were prepared from each sample. Genomic DNA was extracted from these dried blood spots using the Chelex100® Molecular Grade Resin reagent (Bio-Rad, Hercules, CA, USA), following the manufacturer's protocol. Diluted DNA samples were added to a reaction mix consisting of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), along with the primers and specific probes (TaqMan SNP Genotyping Assay, Applied Biosystems, Foster City, CA, USA) to differentiate between wild-type and mutant alleles. Allele detection in patients and controls was performed using real-time polymerase chain reaction (qPCR) on the Gentier 96R Real-Time PCR System (TianLong, China), employing the allelic discrimination assay. Data on antithrombin III, protein C, protein S, lupus anticoagulant, and beta-2 glycoprotein 1 antibody levels were retrieved from patient's medical records. Statistical analysis was conducted using SPSS software, version 23.0 (Chicago, IL, USA). A p-value of ≤ 0.05 was considered statistically significant. The data were analyzed using the χ^2 test to examine individual predictors and binomial logistic regression analysis to assess the combined effect of all potential predictors on ischemic brain disease.

Results

Of the 83 patients, 43 (51.81%) were male and 40 (48.19%) were female, resulting in a male-to-female

Table 1. Frequency of analyzed parameters in patients and controls

Parameter	Patients N (%)	Controls N (%)	p
APC resistance with FV Leiden mutation	17 (20.48%)	1 (0.89%)	< 0.001
FII G20210A	9 (10.84%)	1 (0.89%)	0.05
Antithrombin III	1 (1.20%)	0 (0%)	0.16
Protein S	3 (3.61%)	0 (0%)	0.02
Protein C	1 (1.20%)	0 (0%)	0.17
Lupus anticoagulant	3 (3.61%)	0 (0%)	0.02
Anti-beta-2-glycoprotein-1 antibodies	1 (1.20%)	0 (0%)	0.16

ratio of 1.08:1. In the control group, 58 (51.79%) were male and 54 (48.21%) were female, with a similar male-to-female ratio of 1.07:1. The average age of patients was 47.91 years (95% CI: 43.58 – 48.74), while the average age of the controls was 46.16 (95% CI: 46.29 – 49.53). A small percentage of participants were in the older age group, with 5 patients (6.02%) and 6 controls (5.36%) classified as such, while the remaining participants fell into the middle-aged category (98.92%).

Among the inherited thrombophilias studied, the most common risk factor for developing CI in the patient group was APC resistance due to the presence of FV Leiden mutation (20.48%). This was followed by the prothrombin (FII) G20210A gene mutation (10.84%), protein S deficiency (3.61%), positive lupus anticoagulant test results (3.61%), protein C deficiency (1.20%), and a positive beta-2 glycoprotein-1 antibody test (1.20%) **(Table 1).** Combined thrombophilia was observed in 2.41% of patients, with one patient exhibiting APC resistance with protein C deficiency and another showing APC resistance with protein S deficiency. Both the FII G20210A and FV Leiden mutations were found in 0.89% of healthy controls. All patients with confirmed mutation had heterozygous genotype **(Figures 1 and 2),** and the remaining participants did not carry either factor II **(Figure 3)** or factor V **(Figure 4)** gene mutations.

The prevalence of the FII G20210A mutation, protein S deficiency, lupus anticoagulant, FV Leiden mutation, and APC resistance was significantly higher in patients with CI (p = 0.05, 0.02, 0.02, and < 0.001, respectively). However, the Phi and Cramer's V values

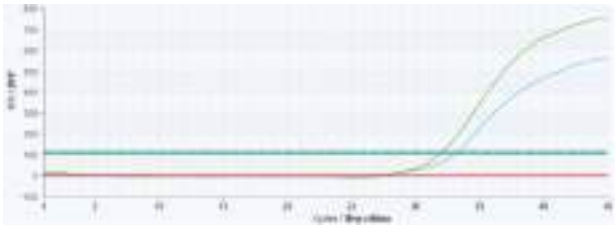

Figure 2. Sample amplification curve with heterozygous mutation in gene for factor II (prothrombin)
RFU – relative fluorescence unit

for the FII G20210A, protein S deficiency, and lupus anticoagulant were 0.14, 0.18, and 0.18, respectively, indicating weak associations. In contrast, the FV Leiden mutation and APC resistance showed moderate associations with CI, as reflected in the Phi and Cramer's V values of 0.31 and 0.32, respectively. No statistically significant associations were found between CI and antithrombin III, protein C deficiency, or the presence of anti-beta-2-glycoprotein antibodies (p = 0.16, 0.17, and 0.16, respectively). To account for potential confounding factors, binomial logistic regression was applied. The analysis confirmed that both the FII G20210A and the FV Leiden mutations were significantly associated with CI (p = 0.02 for both).

Discussion

This study demonstrates a significant association between certain inherited thrombophilias and ischemic brain disease. These findings contradict much of the previous literature, which has largely failed to establish a clear causal link between inherited

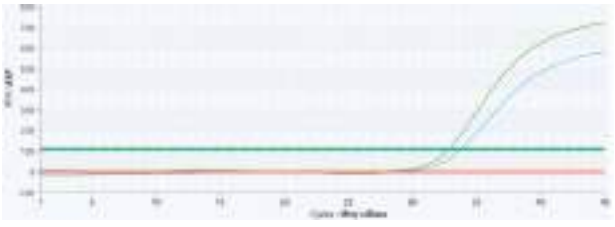

Figure 1. Sample amplification curve with heterozygous mutation in gene for Factor V
RFU – relative fluorescence unit

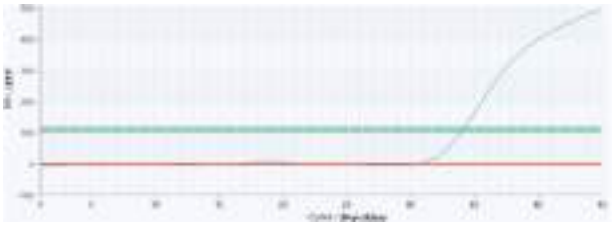

Figure 3. Sample amplification curve without mutation in gene for factor II (prothrombin)
RFU – relative fluorescence unit

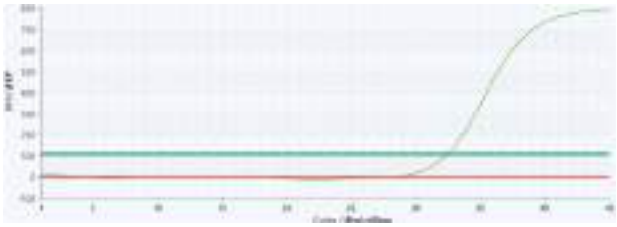

Figure 4. Sample amplification curve without mutation in gene for factor V
RFU – relative fluorescence unit

thrombophilia and stroke [14–16]. The most prominent thrombophilic risk factor identified in our study was APC resistance due to the FV Leiden mutation. Other thrombophilias, including the FII G20210A mutation, antithrombin III, protein C and protein S deficiencies, and the presence of a lupus anticoagulant or anti-beta-2-glycoprotein-1 antibodies, were only weakly associated with CI. Our results align with those reported by Chiasakul et al. [13], where 10% of 1,900 acute ischemic stroke patients were tested for thrombophilia markers, including APC resistance, FII G20210A mutation, homocysteine levels, factor VIII, antithrombin, protein S, protein C, anti-beta-2-glycoprotein-1 antibodies, anticardiolipin antibodies, and lupus anticoagulant. Of these patients, 72.1% had at least one abnormal test result [17]. Similarly, a 2018 retrospective analysis of 628 patients with transient ischemic attacks (TIAs) and stroke found that 57% underwent thrombophilia testing, with 14% yielding positive results [18]. The findings of our study may provide preliminary evidence to support the consideration of long-term anticoagulant therapy in patients with CI who also have hereditary thrombophilia. Such an approach could reduce the risk of recurrent cerebral ischemia and other thrombophilia-related complications. Furthermore, it is advisable that family members of patients with confirmed inherited thrombophilia undergo testing, ensuring that those diagnosed with a coagulation disorder receive appropriate prophylactic treatment.

Conclusion

Based on the findings of this study, we recommend conducting similar research on a larger cohort of patients with reported cerebral ischemia to determine whether a significant association between inherited thrombophilia and cerebral ischemia exists. Some of the results from our study deviate from the majority of comparable research, emphasizing the need for more extensive studies. Such research will provide more definitive results, allowing for the validation or refinement of current clinical recommendations.

References

1. Senst B, Tadi P, Basit H, Jan A. Hypercoagulability. In: StatPearls [Internet]. Treasure Island: StatPearls Publishing; 2024 [updated 2023 Aug 22, cited 2024 Jul 24]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538251/

2. Dautaj A, Krasi G, Bushati V, Precone V, Gheza M, Fioretti F, et al. Hereditary thrombophilia. Acta Biomed. 2019;90(10-S):44-6.

3. Khan S, Dickerman JD. Hereditary thrombophilia. Thromb J. 2006;4:15.

4. Zöller B, García de Frutos P, Hillarp A, Dahlbäck B. Thrombophilia as a multigenic disease. Haematologica. 1999; 84(1):59-70.

5. Kujovich JL. Factor V Leiden thrombophilia. Genet Med. 2011;13(1):1-16.

6. Szpecht D, Gadzinowski J, Seremak-Mrozikiewicz A, Kurzawińska G, Drews K, Szymankiewicz M. The role of FV 1691G>A, FII 20210G>A mutations and MTHFR 677C>T; 1298A>C and 103G>T FXIII gene polymorphisms in pathogenesis of intraventricular hemorrhage in infants born before 32 weeks of gestation. Childs Nerv Syst. 2017;33(7):1201-8.

7. Esmon CT. The regulation of natural anticoagulant pathways. Science. 1987;235(4794):1348-52.

8. Obradović D. Thrombophilia and thrombosis. Med Pregl. 2005;58(7-8):368-74.

9. März W, Nauck M, Wieland H. The molecular mechanisms of inherited thrombophilia. Z Kardiol. 2000;89(7):575-86.

10. Ornello R, Degan D, Tiseo C, Di Carmine C, Perciballi L, Pistoia F, et al. Distribution and temporal trends from 1993 to 2015 of ischemic stroke subtypes: a systematic review and meta-analysis. Stroke. 2018;49(4):814-9.

11. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, et al. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2007;115 (5):e69-171.

12. Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. Neuropharmacology. 2008;55(3):310-8.

13. Chiasakul T, De Jesus E, Tong J, Chen Y, Crowther M, Garcia D, et al. Inherited thrombophilia and the risk of arterial ischemic stroke: a systematic review and meta-analysis. J Am Heart Assoc. 2019;8(19):e012877.

14. Salehi Omran S, Hartman A, Zakai NA, Navi BB. Thrombophilia testing after ischemic stroke: why, when, and what? Stroke. 2021;52(5):1874-84.

15. Jasaraj RB, Proskuriakova E, Gaire S, Chaudhary A, Khosla P. Thrombophilia testing in stroke: a case report and review of evidence. Cureus. 2023;15(12):e50348.

16. Majmundar S, Thapa S, Miller ES, Bell R, Dharia R, Tzeng D, et al. Low value of inherited thrombophilia testing among patients with stroke or transient ischemic attack: a three-year retrospective study. J Stroke Cerebrovasc Dis. 2023;32(10): 107308.

17. May J, Lin C, Martin K, Taylor LJ, Gangaraju R. Thrombophilia testing in hospitalized patients with acute ischemic stroke: an opportunity for hematology input. Blood. 2019;134(Suppl 1):2105.

18. Alakbarzade V, Taylor A, Scully M, Simister R, Chandratheva A. Utility of current thrombophilia screening in young patients with stroke and TIA. Stroke Vasc Neurol. 2018;3(4):231-6.

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TREATMENT DYNAMICS OF TIBIAL SHAFT FRACTURES IN CHILDREN – THE ROLE OF GENDER, AGE AND TREATMENT METHOD

DINAMIKA LEČENJA PRELOMA POTKOLENICE KOD DECE – ULOGA POLA, UZRASTA I METODA LEČENJA

Božo TOPALOVIĆ[1], Ivan MRATINKOVIĆ[2], Boris RADULOVIĆ[3], Mile BJELOBRK[1,4] and Vukadin MILANKOV[1,2]

ORCID NUMBER
Božo Topalović - 0009-0007-6890-286X
Ivan Mratinković - 0009-0006-8789-3367
Boris Radulović - 0009-0006-4631-4656
Mile Bjelobrk - 0000-0003-3169-2989
Vukadin Milankov - 0000-0001-5009-0372

University of Novi Sad, Faculty of Medicine Novi Sad[1]
Institute for the Health Care of Children and Youth of Vojvodina, Novi Sad[2]
Atlas General Hospital, Belgrade[3]
University Clinical Center of Vojvodina, Clinic for Orthopedics and Traumatology, Novi Sad[4]

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Abstract

Introduction. Tibial shaft fractures in pediatric patients pose challenges due to bone growth. This study examined the influence of gender, age, and treatment methods on the duration of hospitalization, total treatment time, and timing of osteosynthetic material removal. **Material and Methods.** We conducted an analysis of 50 pediatric patients treated for tibial shaft fractures at the Institute for Children and Youth Healthcare of Vojvodina between 2016 and 2022. Patients with systemic diseases, neuromuscular disorders, polytrauma, or incomplete medical records were excluded. The cohort comprised 36 boys (72%) and 14 girls (28%), with a mean age of 11.76 years (SD=3.93). The primary mechanisms of injury included falls (38%), traffic accidents (30%), and sports activities (26%). An isolated tibial fracture occurred in 30% of the cases, while 70% of the patients sustained an associated fibular fracture. Statistical analysis were performed using Wilcoxon Rank Sum, Kruskal-Wallis, and Fisher's exact tests in RStudio. **Results.** Boys had a longer hospital stay than girls (11.28 vs. 6.14 days), whereas girls experienced a longer overall treatment duration (330.23 vs. 211.58 days) and a delay in the removal of osteosynthetic material (312 vs. 218.9 days). Adolescents aged 13-17 years exhibited longer hospitalization periods and delayed surgical intervention compared to children under 13 years of age ($p<0.05$). Age did not impact the time to weight-bearing or the total treatment duration. Surgical treatment was associated with a longer overall treatment course compared to conservative management ($p<0.05$). **Conclusion.** Gender and age play a significant role in the management of pediatric tibial shaft fractures, with girls and older adolescents experiencing extended treatment durations. While surgical intervention remains essential in severe cases, it is linked to prolonged treatment duration. These findings highlight the need for personalized treatment plans and call for further research into gender-specific approaches and long-term outcomes.

Key words: Tibial Fractures; Child; Adolescent; Treatment Outcome; Hospitalization; Orthopedic Fixation Devices; Age Factors; Sex Factors

Sažetak

Uvod. Prelomi dijafize tibije kod dece predstavljaju poseban izazov zbog rasta kostiju. Ova studija je ispitivala efekte pola, starosti i metoda lečenja na trajanje hospitalizacije, dužinu lečenja i vreme uklanjanja osteosintetskog materijala. **Materijal i metode.** U Institutu za zdravstvenu zaštitu dece i omladine Vojvodine (2016–2022) ispitano je 50 pedijatrijskih pacijenata sa prelomom tibije. Isključeni su oni sa sistemskim oboljenjima, neuromuskularnim poremećajima, politraumom ili nekompletnom evidencijom. Uzorak je obuhvatio 36 dečaka (72%) i 14 devojčica (28%), prosečne starosti 11,76 godina (SD = 3,93). Najčešći uzroci povreda bili su padovi (38%), saobraćajne nesreće (30%) i sportske aktivnosti (26%). Izolovana fraktura tibije bila je prisutna kod 30% pacijenata, dok je 70% imalo udruženi prelom fibule. U analizi je korišten Vilkoksonov test (*Wilcoxon Rank Sum*), Kruskal-Volisov test i Fišerovi egzaktni testovi u RStudio. **Rezultati.** Dečaci su duže boravili u bolnici od devojčica (11,28 > 6,14 dana), dok je kod devojčica ukupno trajanje lečenja bilo duže (330,23 > 211,58 dana) i odloženo uklanjanje osteosintetskog materijala (312 > 218,9 dana). Adolescenti (13–17 godina) su imali dužu hospitalizaciju i odlaganje operacije u poređenju sa decom mlađom od 13 godina ($p < 0,05$). Starost nije uticala na vreme do oslonca ili ukupnu dužinu lečenja. Hirurško lečenje je dovelo do dužeg ukupnog vremena lečenja u poređenju sa konzervativnim ($p < 0,05$). **Zaključak.** Pol i starost utiču na lečenje preloma dijafize tibije kod dece, pri čemu devojčice i stariji adolescenti imaju duže lečenje. Posle hirurške intervencije, koja je neophodna u teškim slučajevima, lečenje duže traje. Ovi nalazi naglašavaju potrebu za personalizovanim planiranjem lečenja i predlažu dalje istraživanje rodno specifičnih strategija lečenja.

Ključne reči: prelomi tibije; dete; adolescent; ishod lečenja; hospitalizacija; ortopedski fiksatori; starost; pol

Introduction

Tibial fractures account for about 15% of long-bone fractures in children and adolescents [1]. Among lower extremity fractures, 47% involve the tibia and fibula, with 26% occurring in the distal segment, 16% in the shaft, and 5% in the proximal segment of the tibia [2]. Tibial diaphysis fractures, commonly referred to as tibial shaft fractures in children, pose a significant clinical challenge due to various injury mechanisms and manifestations that differ according to the patient's age and circumstances. Notably, tibial shaft fractures

✉ Corresponding author: Božo Topalović, E-mail: bozotopalovic98@hotmail.com, vukadin.milankov@mf.uns.ac.rs

Abbreviations
FIN – flexible intramedullary nailing
ESIN – elastic stable intramedullary nailing
CRIM – Closed Reduction Immobilization

are the second most frequent type of fracture among children affected by abuse, with 26% of these young patients presenting with such injuries. This statistic underscores the importance of considering non-accidental trauma in the differential diagnosis when evaluating pediatric fractures [3].

Treating tibial shaft fractures in children requires a careful approached to avoid potential serious complications, the most severe of which is impaired bone healing. For instance, in type III open tibial fractures, as classified by Gustilo-Anderson, the incidence of healing complications is as high as 60% [4]. Therefore, individualized treatment strategies are essential to achieve optimal outcomes. The treatment of tibial shaft fractures in pediatric patients differs from adults primarily due to the presence of growth plates and the potential for bone remodeling. In children, conservative management with casting or bracing is often favored for minimally displaced fractures, given that their bones have a greater capacity for healing and correcting deformities over time. In contrast, adults typically require surgical intervention, such as intramedullary nailing or plating, due to a lower potential for remodeling and a higher risk of complications like nonunion or malalignment. Additionally, pain management, rehabilitation, and monitoring strategies must be tailored to the child's growth and developmental needs [5].

Most pediatric tibial shaft fractures are managed non-operatively, typically involving closed reduction and immobilization in a plaster cast, leading to favorable long-term outcomes [6]. However, more severe fractures may necessitate surgical intervention. Indications for operative treatment include the inability to maintain proper fracture alignment with non-operative methods, open fractures requiring frequent wound care, fractures complicated by compartment syndrome, and fractures with significant swelling, such as those involving ipsilateral femoral fractures ("floating knee"). Other indications include tibial fractures with an intact fibula, particularly when the fracture line extends from the distal anteromedial to the proximal posterolateral aspect of the tibia, as these tend to drift into varus even in well-molded casts, then comminuted and unstable fractures, and fractures where prolonged immobilization is impractical due to social or activity-related factors [7].

Surgical options include flexible intramedullary nailing (FIN), K-wiring, rigid intramedullary nailing, open reduction with internal (plate and screw) fixation, and external fixation. Studies have consistently demonstrated that titanium elastic nails provide the most effective treatment management with the lowest complication rates, making this technique the preferred option whenever applicable [8, 9]. However, the use of this method is limited by the child's size and the severity of the fracture. In cases of open, complex, or unstable fractures, or when non-operative or other treatments modalities fail, the Ilizarov method is considered a favorable option [10].

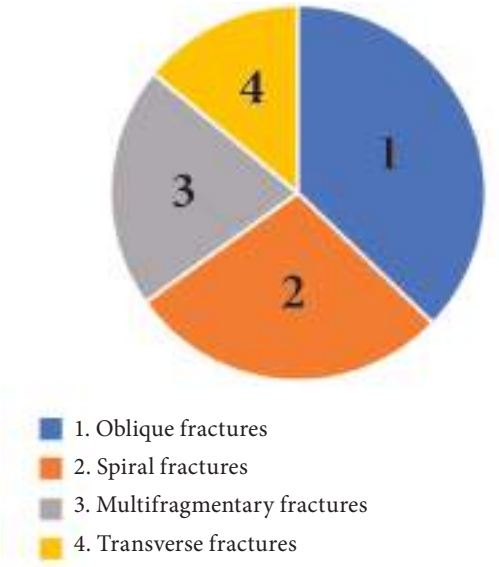
Over the past two decades, flexible intramedullary nailing has become increasingly popular for treating shaft fractures in children aged 4 to 15 years. Known as elastic stable intramedullary nailing (ESIN), this technique evolved from flexible rodding methods developed in Nancy, France. ESIN offers numerous benefits, including minimal invasiveness, shorter hospital stays, early mobilization, and fewer complications. ESINs differ from other flexible nail systems, such as Ender nails, which lack the necessary elasticity for pediatric fractures and can alter the natural bone curvature. ESIN is widely accepted for length-stable femoral fractures, such as transverse and short oblique patterns, particularly in children weighing under 60 kilograms, reducing the risk of complications like femoral head osteonecrosis and premature greater trochanteric epiphysiodesis with rigid intramedullary techniques. Nonetheless, the application of ESIN in unstable fractures remains a topic of debate due to concerns over a higher incidence of angular and rotational deformities [11–13].

Once bone healing and remodeling are complete, the implants are removed in a secondary surgery. This procedure is crucial to prevent complications such as chronic infections, interference with surrounding soft tissues, and long-term issues with fracture healing [14]. The procedure, however, carries risks [15] including tendon rupture, infections, neurovascular injury, and granuloma formation, highlighting the need for careful evaluation the optimal timing for osteosynthetic implant removal.

This study aimed to evaluate the influence of factors such as gender, age, and treatment methods on hospitalization duration and overall treatment length in pediatric patients treated over a five–year period in a single institution. It also sought to compare the length of therapy between operated and non-operated patients to identify any significant differences in outcomes based on the chosen treatment approach.

Material and Methods

The study received approval from the Ethics Committee of the Institute for the Health Care of Children and Youth of Vojvodina. It was conducted



Graph 1. Prevalence of different fractures based on the type of fracture line

retrospectively from 2016 to 2022, involving fifty participants of both sexes aged 0 to 17 years with tibial shaft fractures who consented to participate in the research. Exclusion criteria included the presence of systemic diseases, neuromuscular disorders, polytrauma, and incomplete medical documentation. Among the 50 subjects, 36 were male (72%) and 14 were female (28%). The average age of the patients was 11.76 years (SD=3.93), with 7 (14%) in the 0-6 years age group, 18 (36%) in the 7-12 years group, and 25 (50%) in the 13-17 years group. Injuries to the left leg were recorded in 46% of cases, while the right leg was affected in 54% of cases. The most frequent mechanism of injury was a fall, reported by 19 patients (38%), followed by traffic accidents in 15 patients (30%), sports-related activities in 13 (26%), and other causes in 3 patients (6%).

A total of 30% of patients presented with an isolated tibial fracture, whereas 70% had an associated fibular fracture. Among the 35 patients with an accompanying fibula fracture, 28 underwent surgical treatment. In contrast, out of the 15 patients with an isolated tibial fracture, 14 required surgical intervention. Although there was a seemingly higher rate of surgical treatment in the group with isolated tibial fractures, this difference was not statistically significant based on the data provided.

Based on the fracture line orientation, the distribution of fractures was classified as follows: oblique fractures 37.2%, spiral fractures 27.9%, multifragmentary fractures 20.95%, and transverse fractures 13.95% (as illustrated in **Graph 1**).

The average time from injury to treatment was 4.28 days, and the average duration of hospitalization was 9.84 days. Forty (80%) patients were treated surgically, while the remaining 10 patients (20%) underwent closed reduction and immobilization with a plaster cast (Closed Reduction Immobilization – CRIM). The average period of cast immobilization was 62.54 days.

All surgical procedures and follow-up treatments were carried out by the same surgeon, with detailed documentation maintained during each follow-up visit. Statistical analysis was performed using RStudio software, employing the Wilcoxon Rank Sum Test, Kruskal-Wallis test, and Fisher's exact test to analyze the data.

Results

For the 36 male patients, the average treatment duration was 211.58 days, with osteosynthetic material removal occurring after an average of 218.91 days. For the 14 female patients, the average treatment duration was 330.23 days, with the removal of osteosynthetic material taking place after an average of 312 days. There is a statistically significant difference in the treatment duration based on the patient's ($p<0.01$), as well as in the time to osteosynthetic material removal ($p<0.05$). On average, male children spent 11.28 days in the hospital, compared to female children, who spent an average of 6.14 days there, as detailed in **Table 1.**

There was a statistically significant difference in the hospitalization duration between sexes ($p<0.05$). However, gender did not have a statistically significant impact on the average time to begin weight-bearing in these patients.

Table 1. Treatment and hospitalization duration depending on gender

	Boys	Girls
Duration of hospitalization	11.3 days	6.1 days
Duration of presence OS material	219 days	312 days
Duration of treatment	212 days	330 days

Table 2. Hospitalization duration depending on age

Age (years)	0-6	7-12	13-17
Average duration of hospitalization in days	3.4	4.6	13.2

Graph 2. Period from hospitalization to surgery in days

The overall average hospitalization duration for the patient cohort was 9.8 days. Patients aged 0-6 years had an average hospitalization period of 3.4 days, those aged 7-12 years averaged 4.6 days; and patients aged 13-17 years had an average stay of 13.2 days **(Table 2)**. There was a statistically significant difference in the length of hospitalization depending on age group ($p<0.05$), with post-hoc tests indicating that patients aged 13-17 years differed significantly from the other two age groups. The average time from hospitalization to intervention for the entire sample was 5.1 days. For the age group 0-6 years, it was 1 day; for the 7-12 years group, it was 3 days; and for those aged 13-17 years, it was 7.8 days (as illustrated in **Graph 2**). This age-based difference in the period from hospitalization to surgery was statistically significant ($p<0.05$), with post-hoc tests again confirming that the 13-17 years age group differed significantly from the other two groups.

The average time from injury to initiation of weight-bearing on the injured leg was 64.72 days, and the total treatment duration for both surgically and conservatively treated patients averaged 229.22 days. For surgically treated patients, osteosynthetic material was removed after an average of 249.54 days. Statistical analysis revealed no statistically significant difference in the time to begin weight-bearing, the duration of osteosynthetic material presence, or the overall treatment length when stratified by age.

The mean treatment duration for surgically treated patients was 258 days, while non-surgically treated patients had shorter average treatment period of 137 days, demonstrating a statistically significant difference ($p<0.05$) based on whether the patient underwent surgery.

Discussion

In adults, tibial shaft fractures account for about 1.9% of all fractures [16], whereas in pediatric population, they represent approximately 2.5% of all pediatric fractures [17]. Similarly to adult cases, these fractures are observed to be two to three times more common in males [16, 17]. Our study's findings align with this sex ratio.

Among the 35 patients with a combined fibula fracture, 28 (80%) underwent surgery, while 14 (93.3%) of the 15 patients with an isolated tibial fracture also received surgical treatment. This data indicates a higher proportion of patients receiving surgical intervention in the group with isolated tibial fractures, corroborating the assertion that isolated tibial fractures often warrant surgical intervention [7].

Regarding the fracture line orientation, oblique fractures were the most frequent type in this study, occurring in over 37% of cases. This observation differs from the studies conducted by Stenroos et al. [18] and Ho et al. [19], where spiral fractures were the predominant type (57-62%). Nonetheless, both studies ranked oblique fractures as the second most common type after spiral fractures.

The average period from hospitalization to surgery in our study was 5.1 days, which is slightly longer than 3.9 days reported in the study by Gamal et al. [20]. According to our data, we notice that both hospitalization period and time to surgery in the 13-17 age group were significantly longer compared to younger age groups. One plausible explanation is that our pediatric surgery clinic typically has ESIN nails available for patients up to 16 years of age. For those older than 16 years, rigid intramedullary nails are used, which may take additional time to acquire. This factor likely contributed to the slightly longer average period from hospitalization to surgery compared to Gamal et al. study [20].

Our study revealed that male patients had a significantly longer hospital stays than female patients. This finding contrasts with the results reported by Pogorelić et al. [21], where no gender-based differences in hospital stay duration were noted. This discrepancy might be due to age distribution in our study, where 19 out of 36 male patients (52.8%) were over 12 years of age, compared to only 6 out of 14 female patients (42.8%) falling into this older age group. Given our findings that older patients tend to have longer hospital stays, the higher proportion of older males in our sample might account for the observed difference in hospitalization duration between genders. Future research should aim for a more balanced age and gender distribution to better understand these dynamics.

Although nonoperative treatment is by far the most common method of treatment in most studies [3, 5, 18, 19, 22], only 20% of our patients were treated conservatively. This can be explained by the fact that the data were collected from a tertiary care facility that primarily manages severe cases, which usually require surgical treatment.

In our study, osteosynthetic material was extracted after an average of 249.54 days, considerably sooner than in the studies by Stenroos et al. [18] and

Goodwin et al. [23], where extraction was performed after an average of one year. It should be noted that their studies mainly included open fractures, which are usually the most severe and require later extraction of osteosynthetic material.

Statistical analysis of this study indicates longer treatment duration for surgically treated patients compared to conservatively treated patients, aligning with the findings by Kinney et al. [24]. This difference can be explained by the fact that surgical intervention is usually reserved for the most severe cases that are expected to have a poor outcome with nonoperative treatment, thus necessitating extended postoperative follow-up.

No instances of refracture, remanipulation, or repeat surgery were recorded in our study, which is consistent to the findings of O'Brien et al. [25].

While this study provides valuable insights into management of pediatric tibial shaft fractures, several limitations must be acknowledged. The retrospective design limits control over confounding variables and may introduce biases due to its reliance on previously recorded data. The small sample size of 50 patients restricts the generalizability of the findings and may reduce the statistical power, particularly in subgroup analyses. As the study was conducted at a single institution, the results may not be applicable to other populations. Additionally, the absence of long-term follow-up data leaves gaps in understanding the full scope of functional recovery and complications. Given that 80% of patients underwent surgery, insights into non-operative management remain limited. Furthermore, the study does not thoroughly explore the relationship between injury mechanisms and outcomes, and the lack of functional outcome measures restricts the assessment of the injury's overall impact on quality of life. These limitations highlight the need for cautious interpretation of the results and further research.

Conclusion

Based on the analysis of the results from this study and a review of the literature, several conclusions can be drawn regarding diaphyseal tibial fractures in children. Firstly, these fractures are observed to be two to three times more prevalent in males than in females. In terms of treatment outcomes, girls demonstrated a statistically significant increase in both treatment duration and the time until the extraction of osteosynthetic material ($p<0.05$), while boys experienced significantly longer hospitalization periods.

Additionally, adolescents aged 13 to 17 years exhibited a statistically significantly longer hospitalization duration and an extended period from admission to surgery when compared to patients younger than 13 years ($p<0.05$). However, no significant differences were noted regarding the time to onset of weight-bearing, the duration of osteosynthetic material presence, or the overall length of treatment based on age.

It is also notable that patients who underwent surgical intervention had significantly longer treatment duration than those treated nonoperatively. This finding can be attributed to the fact that surgical intervention is typically reserved for the most severe cases, whereas milder fractures are managed without surgery.

Future research can build on these findings to further enhance management strategies for pediatric tibial fractures, aiming to improve outcomes and reduce the treatment burden on young patients.

References

1. Gothefors M, Wolf O, Hailer YD. Epidemiology and treatment of pediatric tibial fractures in Sweden: a nationwide population-based study on 5828 fractures from the Swedish Fracture Register. Eur J Trauma Emerg Surg. 2023;49(2):911-9.

2. Chaibi E, Zambelli PY, Merckaert S. Epidemiology of paediatric lower extremity fractures in a tertiary care center in Switzerland. Eur J Trauma Emerg Surg. 2022;48(5):3449-59.

3. Santili C, Gomes CMO, Akkari M, Waisberg G, Braga SR, Lino W Jr, et al. Fraturas da diáfise da tíbia em crianças. Acta Ortop Bras. 2010;18(1):44-8.

4. Gajdobranski Đ, Micić I, Mitković MB, Mladenović D, Milankov M. Management of impaired fracture healing: historical aspects. Med Pregl. 2005;58(9-10):507-12.

5. Murphy D, Raza M, Monsell F, Gelfer Y. Modern management of paediatric tibial shaft fractures: an evidence-based update. Eur J Orthop Surg Traumatol. 2021;31(5):901-9.

6. Setter KJ, Palomino KE. Pediatric tibia fractures: current concepts. Curr Opin Pediatr. 2006;18(1):30-5.

7. Gordon JE, O'Donnell JC. Tibia fractures: what should be fixed. J Pediatr Orthop. 2012;32(Suppl 1):S52-61.

8. Nandra RS, Wu F, Gaffey A, Bache CE. The management of open tibial fractures in children: a retrospective case series of eight years' experience of 61 cases at a paediatric specialist centre. Bone Joint J. 2017;99-B(4):544-53.

9. Palmu SA, Auro S, Lohman M, Paukku RT, Peltonen JI, Nietosvaara Y. Tibial fractures in children: a retrospective 27-year follow-up study. Acta Orthop. 2014;85(5):513-7.

10. Messner J, Johnson L, Taylor DM, Harwood P, Britten S, Foster P. Treatment and functional outcomes of complex tibial fractures in children and adolescents using the Ilizarov method. Bone Joint J. 2018;100-B(3):396-403.

11. Till H, Huttl B, Knorr P. Elastics stable intramedullary nailing (ESIN) provides good long-term results in pediatric long-bone fractures. Eur J Pediatr Surg. 2000;10(3):319-22.

12. Patil C, Amar J. Study of the outcome of titanium elastic nail system (TENS) in diaphyseal fractures of femur and tibia

in children. International Journal of Orthopaedics Sciences. 2019;5(3):617-20.

13. Luo Y, Wang L, Zhao LH, Wang YC, Chen MJ, Wang S, et al. Elastic stable titanium flexible intramedullary nails versus plates in treating low grade comminuted femur shaft fractures in children. Orthop Surg. 2019;11(4):664-70.

14. Peterson HA. Metallic implant removal in children. J Pediatr Orthop. 2005;25(1):107-15.

15. Lieber J, Dietzel M, Scherer S, Schäfer JF, Kirschner HJ, Fuchs J. Implant removal associated complications after ESIN osteosynthesis in pediatric fractures. Eur J Trauma Emerg Surg. 2022;48(5):3471-8.

16. Court-Brown CM, Caesar B. Epidemiology of adult fractures: a review. Injury. 2006;37(8):691-7.

17. Rennie L, Court-Brown CM, Mok JY, Beattie TF. The epidemiology of fractures in children. Injury. 2007;38(8):913-22.

18. Stenroos A, Puhakka J, Nietosvaara Y, Kosola J. Treatment of closed tibia shaft fractures in children: a systematic review and meta-analysis. Eur J Pediatr Surg. 2020;30(6):483-9.

19. Ho CA, Dammann G, Podeszwa DA, Levy J. Tibial shaft fractures in adolescents: analysis of cast treatment successes and failures. J Pediatr Orthop B. 2015;24(2):114-7.

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Prihvaćen za štampu 8. X 2024.
BIBLID.0025-8105:(2024):LXXVII:5-6:165-170.

20. El-Adl G, Mostafa MF, Khalil MA, Enan A. Titanium elastic nail fixation for paediatric femoral and tibial fractures. Acta Orthop Belg. 2009;75(4):512-20.

21. Pogorelić Z, Vegan V, Jukić M, Llorente Muñoz CM, Furlan D. Elastic stable intramedullary nailing for treatment of pediatric tibial fractures: a 20-year single center experience of 132 cases. Children (Basel). 2022;9(6):845.

22. Patel NK, Horstman J, Kuester V, Sambandam S, Mounasamy V. Pediatric tibial shaft fractures. Indian J Orthop. 2018;52(5):522-8.

23. Goodwin RC, Gaynor T, Mahar A, Oka R, Lalonde FD. Intramedullary flexible nail fixation of unstable pediatric tibial diaphyseal fractures. J Pediatr Orthop. 2005;25(5):570-6.

24. Kinney MC, Nagle D, Bastrom T, Linn MS, Schwartz AK, Pennock AT. Operative versus conservative management of displaced tibial shaft fracture in adolescents. J Pediatr Orthop. 2016; 36(7):661-6.

25. O'Brien TJ, Weisman DS, Ronchetti P, Piller CP, Maloney M. Flexible titanium nailing for the treatment of the unstable pediatric tibial fracture. J Pediatr Orthop. 2004;24(6):601-9.

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REVIEW ARTICLES
PREGLEDNI ČLANCI

REPRODUCTIVE HEALTH SCREENING FOR ENDOMETRIOSIS, REDUCED OVARIAN RESERVE, POLYCYSTIC OVARY SYNDROME AND THE MOST COMMON SEXUALLY TRANSMITTED DISEASES

SKRINING REPRODUKTIVNOG ZDRAVLJA ZA ENDOMETRIOZU, SMANJENU OVARIJALNU REZERVU, SINDROM POLICISTIČNIH JAJNIKA I NAJČEŠĆE SEKSUALNO PRENOSIVE BOLESTI

Artur BJELICA[1,2], Jelena ĆURČIĆ[1,2], Dragan STAJIĆ[1,2] and Marko ILINČIĆ[1,2]

ORCID NUMBER
Artur Bjelica – 0000-0002-1219-4936
Jelena Ćurčić – 0009-0002-1984-7263
Dragan Stajić – 0009-0002-1387-6338
Marko Ilinčić – 0000-0002-3404-7865

University of Novi Sad, Faculty of Medicine Novi Sad[1]
University Clinical Center of Vojvodina, Novi Sad, Clinic of Gynecology and Obstetrics[2]

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Abstract

Introduction. This review explores screening options for the most common disorders that significantly impact reproductive health, based on a review of recent literature. **Endometriosis.** Diagnosis of endometriosis typically involves evaluating symptoms such as pelvic pain, alongside gynecological examination, imaging, and surgical exploration of the abdomen. Currently, there is no reliable biomarker for detecting asymptomatic endometriosis. Early detection, therefore, relies on elevated serum levels of the cancer antigen 125 and symptom-based questionnaires. **Reduced ovarian reserve.** Anti-Müllerian hormone levels are a highly effective screening tool for assessing diminished ovarian reserve, providing critical guidance for infertility treatment. **Polycystic ovary syndrome.** While diagnosing this complex disorder is relatively straightforward, a reliable screening method remains elusive. Gene expression analysis in blood, alongside the identification of genes associated with the condition, may serve as potential biomarkers for future screening approaches. **Sexually transmitted diseases.** Early identification of causative agents in asymptomatic phases has been instrumental in reducing the spread of these diseases and preventing pelvic inflammatory disease – one of the leading causes of infertility and ectopic pregnancy. Standard screenings target Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, and Neisseria gonorrhoeae. Current efforts aim to develop more reliable, accessible screening methods for broader populations and to identify women at higher risk. **Conclusion.** In addition to routine reproductive health screenings, such as anti-Müllerian hormone testing for ovarian reserve and pathogen detection in sexually transmitted infections, further research is needed to identify biomarkers for effective screening of endometriosis and polycystic ovary syndrome.

Key words: Reproductive Health; Mass Screening; Polycystic Ovary Syndrome; Sexually Transmitted Diseases; Ovarian Reserve; Endometriosis

Sažetak

Uvod. Mogućnost skrininga najznačajnijih poremećaja koji utiču na reproduktivno zdravlje sagledane su uvidom u noviju stručnu literaturu. **Endometrioza.** Dijagnoza endometrioze postavlja se uvidom u simptome bolesti, odnosno različito ispoljen bol u predelu karlice, ginekološkim pregledom, imidžingom i hirurškom eksploracijom abdomena. Ne postoji zadovoljavajući biomarker za otkrivanje endometrioze u asimptomatskoj fazi pa se primenjuje koncept ranog otkrivanja bolesti za šta se koriste povišene serumske koncentracije antigena 125 za kancer i različiti upitnici zasnovani na simptomima i znacima bolesti. **Smanjena ovarijalna rezerva.** Određivanje serumskog nivoa anti-Milerovog hormona suverena je metoda skrininga koja može značajno da usmeri dalje lečenje infertiliteta. **Sindrom policističnih jajnika.** Dijagnoza ovog složenog poremećaja postavlja se relativno lako, ali za njegov skrining za sada nemamo zadovoljavajuću metodu. Obećavajući rezultati postižu se identifikacijom gena povezanih sa ovom bolešću u krvi. **Seksualno prenosive bolesti.** Identifikacija uzročnika seksualno prenosivih bolesti u asimptomatskoj fazi sprovodi se već duže vreme različitim metodama što doprinosi smanjenoj stopi širenja tih bolesti u populaciji i prevenciji upalne bolesti karlice kao značajnom etiološkom faktoru steriliteta i vanmaterične trudnoće. Najčešće se sprovodi skrining na hlamidiju trahomatis, mikoplazmu hominis, ureoplazmu urealitikum i naiseriju gonoreje. Naglasak je na pronalasku pouzdanijih i dostupnijih metoda koje bi mogle da se primene na široj populaciji, kao i na identifikaciji grupa žena sa povišenim rizikom za obolevanje. **Zaključak.** Pored metoda koje se rutinski primenjuju u reproduktivnoj medicini kao skrining za smanjenu ovarijalnu rezervu i identifikaciju seksualno prenosivih bolesti potrebno je učiniti dodatni napor da se nađu biomarkeri podesni za skrining endometrioze i sindroma policističnih jajnika.

Ključne reči: reproduktivno zdravlje; skrining; sindrom policističnih jajnika; seksualno prenosive bolesti; ovarijalna rezerva; endometrioza

Introduction

Screening, which focuses on detecting diseases and conditions during their asymptomatic phase or shortly after the onset of initial symptoms, has driven signifi-

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✉ Corresponding author: Jelena Ćurčić, E-mail: jelenacurcic.98@gmail.com, artur.bjelica@mf.uns.ac.rs

Abbreviations

PCOS – polycystic ovary syndrome
STDs – sexually transmitted diseases
CA-125 – cancer antigen-125
FSH – follicle stimulating hormone
E2 – estradiol
AMH – anti-Müllerian hormone
AFC – antral follicle count
ART – assisted reproductive techniques
DHEA – dehydroepiandrosterone
DHEA-S – dehydroepiandrosterone-sulfate
HOMA – homeostatic model assessment
OGTT – oral glucose tolerance test
HDL – high density lipoprotein
LDL – low density lipoprotein
CRP – C-reactive protein
ZAG – Zinc-alpha2-glucoprotein
PCR – polymerase chain reaction

cant progress in modern medicine. This proactive approach not only enhances therapeutic outcomes but also considerably reduces healthcare costs. Screening has achieved its most notable successes in oncology, targeting diseases with high mortality rates. Nevertheless, it also holds great potential for conditions that, while not fatal, profoundly affect quality of life and are prevalent in the general population.

Infertility is one such condition, defined as the inability to conceive after 12 months of regular, unprotected intercourse. Screening for reproductive health disorders - such as endometriosis, polycystic ovary syndrome (PCOS), reduced ovarian reserve, and sexually transmitted diseases (STDs) - can greatly influence family planning. By providing patients with early insights into their likelihood of conceiving naturally and outlining the available options for assisted reproduction, these screenings can facilitate earlier interventions and significantly improve treatment outcomes.

Endometriosis

Endometriosis is a chronic condition defined by the presence of endometrium-like tissue outside the uterus, commonly located on the peritoneum, ovaries, bladder, intestines, and, in some cases, outside the pelvic region. It is estimated to affect approximately 10% of women of reproductive age and 30-40% of infertile women [1]. In addition to fertility challenges, endometriosis is linked to chronic pelvic pain, dysmenorrhea (painful menstruation), dyspareunia (painful intercourse), dysuria (painful urination), and dyschezia (painful defecation). Diagnosis is often delayed by several years following the onset of symptoms. The gold standard for diagnosis is surgical exploration, typically via laparoscopy with histopathological confirmation [2]. However, many experts advocate for avoiding surgery, especially in the early stages of the disease and in younger patients. Instead, a non-invasive, clinical diagnosis based on symptomatology, imaging, and laboratory findings is recommended [3]. Traditionally, it is widely believed among gynecologists that effective surgical treatment of confirmed endometriosis would improve fertility outcomes, including pregnancy rates. However, recent debates question the extent of surgery's impact on fertility, suggesting its benefits may be overstated [4].

Detecting endometriosis during its asymptomatic phase would represent a major breakthrough; however, current predictive models rely on early symptom-based diagnosis using patient questionnaires and laboratory assessments. A review of 23 different studies highlighted a variety of questionnaires designed to capture data on symptoms, lifestyle, family history, and other factors potentially associated with endometriosis. Despite these efforts, existing predictive models have failed to meet expectations. They often struggle to strike a balance between sensitivity and specificity, and are generally perceived as complex, time-consuming, and difficult for patients to navigate. Developing a predictive model that minimizes subjectivity, simplifies complexity, and remains patient-friendly while being validated through large-scale testing continues to be a significant challenge for researchers [5, 6].

At present, there are no widely accepted biochemical screening markers for endometriosis. The association between cancer antigen 125 (CA-125) and endometriosis was first identified in the mid-1980s. While CA-125 was originally linked to ovarian cancer, it has since been shown to correlate with the severity of endometriosis, although its diagnostic accuracy is diminished in the early stages. The threshold for CA-125 is set at 30 U/mL. Meta-analyses suggest that CA-125 has a sensitivity of 52% and a specificity of 93% in diagnosing endometriosis, regardless of the disease stage [4].

Reduced ovarian reserve

Ovarian reserve is the functional capacity of the ovaries, which is determined by both the number and quality of oocytes available at any given point in a woman's life, specifically the population of immature primordial ovarian follicles. Reduced ovarian reserve is a significant factor in infertility, contributing independently to infertility in 30% of women [7]. The assessment of ovarian reserve involves a combination of biochemical tests that measure follicle-stimulating hormone (FSH), estradiol (E2), inhibin B, and anti-Müllerian hormone (AMH) levels, as well as a provocative test with clomiphene citrate. Ultrasono-

graphic evaluations of antral follicle count (AFC) and ovarian volume are also utilized.

Among these methods, measuring serum AMH levels has proven to be the most reliable test for evaluating ovarian reserve. AMH testing is objective, unaffected by intra- or inter-cycle fluctuations in gonadotropin, and levels below 0.7 ng/mL are indicative of reduced ovarian reserve [8, 9]. AMH, a glycoprotein secreted by granulosa cells in preantral and small antral follicles, plays a crucial role in regulating follicle maturation. In males, AMH is vital for the regression of Müllerian ducts during fetal development, supporting sexual differentiation towards the male phenotype. In women, the total number of oocytes is determined genetically after fertilization and steadily declines throughout life until menopause. Women are born with approximately 1 to 2 million oocytes, which decrease to around 25,000 by age 40, and to fewer than 1,000 by the time of postmenopause. AMH is extensively used to assess ovarian reserve in the context of assisted reproductive techniques (ART), to predict the risk of premature ovarian failure, and in the field of oncofertility, both pre- and post-oncological therapy [10]. Additionally, it serves as a tumor marker for granulosa-cell ovarian tumors and is elevated in PCOS, reflecting the increased number of small antral follicles characteristic of this disorder [11].

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrinopathies in women of reproductive age, with an estimated prevalence of 5-10%. It presents with a variety of symptoms, the most common being menstrual irregularities due to chronic anovulation, hyperandrogenism, and polycystic ovarian morphology [12, 13]. Diagnosis is primarily based on the "Rotterdam" criteria, established by the European Society for Human Reproduction and Embryology in 2003. It is essential to exclude other disorders that also lead to chronic anovulation and hyperandrogenism [14].

Hyperandrogenism, characterized by elevated levels of ovarian androgens such as testosterone, androstenedione, dehydroepiandrosterone (DHEA), and DHEA-sulfate (DHEA-S), manifests clinically as hirsutism, acne, and androgenic alopecia. While clinical signs are often sufficient for diagnosis, severe hyperandrogenism, especially if sudden or progressive, may warrant investigation for androgen-producing tumors. Menstrual abnormalities, such as oligomenorrhea and amenorrhea (cycle intervals exceeding 35 days or absence of menstruation), are also common in PCOS. Polycystic ovaries, identified via ultrasound, typically exhibit 12 or more small follicles (2-9 mm) or an ovarian volume exceeding 10 cubic milliliters. Notably, this morphology may also appear in healthy women without PCOS symptoms [14, 15].

PCOS is associated with several conditions beyond the diagnostic criteria, including gonadotropin secretion abnormalities, insulin resistance, metabolic disorders, inflammation, and subfertility or sterility [16]. Atypical gonadotropin secretion, characterized by elevated luteinizing hormone (LH) levels with normal follicle-stimulating hormone (FSH) levels, leads to an increased LH/FSH ratio. Insulin resistance and hyperinsulinemia affect around 35% of PCOS patients, especially those who are obese. Many women with PCOS and insulin resistance are younger and possess a good reserve of B cells in the pancreas, enabling compensatory hyperinsulinemia to maintain glucose homeostasis. Insulin resistance is confirmed by measuring fasting insulinemia (above 20-30 mU/mL) or calculating the HOMA index (homeostatic model assessment) - fasting glucose level in mmol/l times fasting insulinemia in mU/ml divided by 22.5, where values above 3.2-3.9 indicate insulin resistance [12]. In addition to determining routine glycemia and insulinemia in women with PCOS, an oral glucose tolerance test (OGTT) is also recommended as diabetes appears in 10% of cases. Lipid metabolism disorder - particularly reduced HDL cholesterol and elevated LDL cholesterol and triglycerides are present in 70% of PCOS patients. Chronic inflammation, indicated by elevated inflammatory cytokines and C-reactive protein (CRP), and unopposed estrogenic stimulation from chronic anovulation increase the risk of endometrial cancer, even in the premenopausal period [14, 15].

The Rotterdam criteria facilitate PCOS diagnosis through the detection of two of the three main diagnostic features. The evaluation can be performed by general practitioner and gynecologist, with clinical signs such as hirsutism, acne, and androgenic alopecia being significant indicators of hyperandrogenism. A comprehensive menstrual history and ovarian ultrasound are relatively simple diagnostic tools, yet access to healthcare and patients' reluctance to seek medical attention for mild symptoms pose challenges. Given that PCOS is not associated with significant mortality, encouraging preventive screenings among reproductive-age women is difficult. However, selective screening could be integrated into routine gynecological exams, particularly for malignant cervical diseases, using medical history and ultrasound.

Many experts advocate screening via well-designed questionnaires that could identify women with potential PCOS. These surveys gather data on socioeconomic factors, lifestyle, anthropometric measurements (e.g., BMI, waist-to-hip ratio), menstrual his-

tory, skin changes, metabolic conditions, psychological symptoms, and family history of PCOS [17, 18]. Some questionnaires incorporate biochemical analyses [19, 20], while others emphasize emotional health and quality of life, as suggested by Boivin, who compared 12 different questionnaires and recommended that they be concise and simple [21].

To date, no reliable biomarker has been established for screening PCOS, particularly in younger, asymptomatic women. Emerging research suggests that cytokines such as Zinc-alpha2-glycoprotein (ZAG), irisin, and betatrophin may serve as potential biomarkers [22]. Additionally, gene expression analysis could help identify genes associated with PCOS, offering promise for future screening tools. PCOS is a complex, multifactorial, polygenic disorder influenced by genetic and environmental factors. Unhealthy lifestyle habits, poor diet, and physical inactivity, along with various infectious agents, are believed to trigger disease onset. Modifying lifestyle habits can mitigate immune system dysregulation and reduce the risk of PCOS. Identifying key genes involved in PCOS development and using gene expression analysis could provide valuable tools for early screening and diagnosis [23–25].

Sexually transmitted diseases

Sexually transmitted diseases pose a major global public health issue, particularly as a leading cause of pelvic inflammatory disease and its associated reproductive complications, including infertility, ectopic pregnancies, and premature births, all of which adversely affect neonatal health. The causative agents of STDs span a wide range of pathogens, including bacteria, viruses, fungi, and parasites [26–28]. Among bacterial STDs, common pathogens include Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, and Neisseria gonorrhoeae. These organisms often colonize the cervix, with many infections remaining asymptomatic for extended periods, making them ideal candidates for screening. Effective screening can not only reduce the incidence of pelvic inflammatory disease and its subsequent reproductive health implications but also limit disease transmission to sexual partners, thereby curbing the broader spread within populations. Various screening strategies have been developed to address these concerns. General screening, while theoretically appealing, is not recommended due to the lower prevalence of STDs in the general population, which could lead to high rate of false positives. Instead, targeted screening focuses on specific high-risk groups, including adolescents, infertile women, and individuals with risky sexual behaviors. Opportunistic screening, conducted during unrelated medical examinations, is another strategy often employed. For example, in Canada, routine screening for Chlamydia trachomatis is conducted among all sexually active women and men under 25, pregnant women, and other high-risk groups, though defining these groups can be challenging [29].

Adolescents and young adults (up to age 24) represent the demographic at the highest risk for STDs. In the United States, this age group comprises only 25% of the sexually active population but accounts for 50% of newly diagnosed STD cases. This disparity arises from both biological and social factors, including higher numbers of sexual partners, inconsistent condom use, limited access to healthcare services, and subjective factors such as immaturity, inadequate education, and psychological or financial barriers [23]. Many young individuals avoid seeking healthcare due to embarrassment, fear of judgment, or concerns over confidentiality. Pediatricians, typically focused on other health concerns, often lack time for thorough sexual health counseling or screening and frequently refer patients to gynecologists, adding another barrier to effective care [30].

Testing for common bacterial STDs such as chlamydia, mycoplasma, ureaplasma, and gonococci typically involves taking swabs from the cervix and cervical canal for laboratory analysis. Traditional diagnostic methods often can lack accuracy, but advancement in molecular diagnostics, particularly polymerase chain reaction (PCR) testing, have greatly improved sensitivity. However, molecular testing remains relatively expensive. To mitigate costs, group testing strategies have been proposed, particularly in populations with varying disease prevalence, which requires prior prevalence studies [31]. Recently, multiplex PCR tests capable of detecting multiple STD pathogens in a single sample have been developed. These tests are simpler, more cost-effective, and applicable to a broader patient population [32].

Conclusion

Recent advancement in reproductive health literature emphasize the potential of screening as a simple, non-invasive, and accessible method for identifying conditions that negatively impact female fertility. Effective screening tools, with high sensitivity and specificity, can detect certain disorders at early, often asymptomatic stages. Notably, measuring serum anti-Müllerian hormone levels in serum has become a reliable indicator of ovarian reserve, while advanced diagnostic methods now facilitate the detection of common sexually transmitted infections – such as chlamydia, mycoplasma, ureaplasma, and gonococci

- preventing complications arising from untreated infections. However, screening techniques for more complex disorders, such as endometriosis and polycystic ovary syndrome, remain under development. These conditions require a multifaceted diagnostic approach, combining clinical symptoms, laboratory findings, and imaging. Ongoing research is exploring potential biomarkers, including gene identification for PCOS, which could enhance early detection. A major challenge in effective screening lies in ensuring widespread participation among the target population. This necessitates a well-coordinated strategy involving both the healthcare system and broader societal engagement to maximize reach and impact.

References

1. Bjelica A. Endometrioza. In: Đurđević S, editor. Ginekologija. Novi Sad: Medicinski fakultet; 2019. p. 88-93.
2. Horne AW, Missmer SA. Pathophysiology, diagnosis, and management of endometriosis. BMJ. 2022;379:e070750.
3. Allaire C, Bedaiwy MA, Yong PJ. Diagnosis and management of endometriosis. CMAJ. 2023;195(10):E363-71.
4. Mear L, Pineau C, Vialard F, Velez de la Calle JF. Endometriosis screening in patients attending an IVF clinic: a proof-of-concept retrospective cohort study. Hum Fertil (Camb). 2022;25 (2):313-22.
5. Gkrozou F, Tsonis O, Sorrentino F, Nappi L, Vatopoulou A, Skentou C, et al. Endometriosis predictive models based on self-assessment questionnaire, evidence from clinical examination or imaging findings: a narrative review. J Clin Med. 2024; 13(2):356.
6. DiBenedetti DB, Soliman AM, Ervin C, Evans E, Coddington CC, Agarwal SK, et al. Development of the Painful Periods Screening Tool for endometriosis. Postgrad Med. 2018;130 (8):694-702.
7. Xavier P, Dantas S, Almeida-Santos T, Soares SR, Imhoff RJ, Genovez V, et al. Screening for diminished ovarian reserve in Portugal: a cost-saving answer to shorten the fertility journey. J Comp Eff Res. 2023;12(7):e230003.
8. Sinha S, Sharan A, Sinha S. Anti-mullerian hormone as a marker of ovarian reserve and function. Cureus. 2022;14(9): e29214.
9. Harris BS, Jukic AM, Truong T, Nagle CT, Erkanli A, Steiner AZ. Markers of ovarian reserve as predictors of future fertility. Fertil Steril. 2023;119(1):99-106.
10. Bjelica A, Trninić Pjević A, Jagodić I, Vukosavljević J. Fertility preservation in cancer patients. Med Pregl. 2018;71(1-2):53-9.
11. Shrikhande L, Shrikhande B, Shrikhande A. AMH and its clinical implications. J Obstet Gynecol India. 2020;70(5):337-41.
12. Pavičić Baldani D. Sindrom policističnih jajnika. In: Šimunić V. Reprodukcijska endokrinologija i neplodnost. Zagreb: Školska knjiga; 2012. p. 275-96.
13. Bjelica A, Trninić-Pjević A, Mladenović-Segedi Lj, Ćetković N, Petrović Đ. Comparison of the efficiency of clomiphene citrate and letrozole in combination with metformin in moderately obese clomiphene citrate-resistant polycystic ovarian syndrome patients. Srp Arh Celok Lek. 2016;144(3-4):146-50.
14. Taylor HS, Fritz MA, Pal L, Seli E. Speroff's clinical gynecologic endocrinology and infertility. 9th ed. Philadelphia: Wolters Kluwer; 2020. p. 978-98.
15. Bjelica A. Sindrom policističnih jajnika. In: Đurđević S, editor. Ginekologija. Novi Sad: Medicinski fakultet; 2019. p. 85-7.
16. Eledath Kolasseri A, Eledath Kolasseri A, Sivaraman J, Ramasamy T. Assessment of factors related to poly cystic ovarian syndrome – a comparative and correlational study. J Psychosom Obstet Gynaecol. 2024;45(1):2297166.
17. Bjelica A, Bjelanović J, Milić N, Mladenović-Segedi Lj, Ilić Đ, Dimitrijević J. Algorithm of ovulation induction in patients with polycistic ovary syndrome. Med Pregl. 2016;69(1-2): 25-30.
18. Bohsas H, Alibrahim H, Swed S, Abouainain Y, Aljabali A, Kazan L. Prevalence and knowledge of polycystic ovary syndrome (PCOS) and health-related practices among women of Syria: a cross-sectional study. J Psychosom Obstet Gynaecol. 2024;45(1):2318194.
19. Gourgari E, Spanakis E, Dobs AS. Pathophysiology, risk factors, and screening methods for prediabetes in women with polycystic ovary syndrome. Int J Womens Health. 2016;8:381-7.
20. Deswal R, Nanda S, Ghalaut VS, Roy PS, Dang AS. Cross-sectional study of the prevalence of polycystic ovary syndrome in rural and urban populations. Int J Gynaecol Obstet. 2019;146 (3):370-9.
21. Boivin MJ, Fatehi F, Phillips-Chan AE, Richardson JR, Summers AN, Foley SA. Exploratory study of a screening measure for polycytic ovarian syndrome, quality of life assessment, and neuropsychological evaluation. BMC Womens Health. 2020;20(1):132.
22. Luo P, Zhang C, He Y, Yang G, Liu H, Li L. Several circulating biomarkers for PCOS diagnosis. Exp Clin Endocrinol Diabetes. 2021;129(10):705-12.
23. Branavan U, Muneeswaran K, Wijesundera S, Chandrasekharan NV, Wijeyaratne C. Genotyping Sri Lankan women with polycystic ovary syndrome (PCOS) towards a novel screening tool. Ceylon Med J. 2021;66(3):129-37.
24. Liu S, Zhao X, Meng Q, Li B. Screening of potential biomarkers for polycystic ovary syndrome and identification of expression and immune characteristic. PloS One. 2023;18(10): e0293447.
25. Li X, Wang C, Yang H, Pei D, Liu Y, Yan S, et al. Screening and verification of genes related to polycystic ovary syndrome. J Int Med Res. 2023;51(1):3000605221147444.
26. Van Gerwen OT, Muzny CA, Marrazzo J. Sexually transmitted infections and female reproductive health. Nat Microbiol. 2022;7(8):1116-26.
27. Tantengco OAG, de Castro Silva M, Velayo CL. The role of genital mycoplasma infection in female infertility: a systematic review and meta-analysis. Am J Reprod Immunol. 2021;85 (6):e13390.
28. Sieving RE, Gewirtz O'Brien JR, Saftner MA, Argo TA. Sexually transmitted diseases among US adolescents and young adults: patterns, clinical considerations, and prevention. Nurs Clin North Am. 2019;54(2):207-25.
29. Pillay J, Wingert A, MacGregor T, Gates M, Vandermeer B, Hartling L. Screening for chlamydia and/or gonorrhea in primary health care: systematic reviews on effectiveness and patient preferences. Syst Rev. 2021;10(1):118.
30. Shafii T, Levine D. Office-based screening for sexually transmitted infections in adolescents. Pediatrics. 2020;145(2): S219-24.
31. Connolly S, Kilembe W, Inambao M, Visoiu AM, Sharkey T, Parker R, et al. A population-specific optimized GeneXpert pooling algorithm for Clamydia trachomatis and Neisseria gon-

orrhoeae to reduce cost of molecular sexually transmitted infection screening in resource-limited settings. J Clin Microbiol. 2020;58(9):e00176-20.

32. Bui HTV, Bui HT, Chu SV, Nguyen HT, Nguyen ATV, Truong PT, et al. Simultaneous real-time PCR detection of nine prevalent sexually transmitted infections using a predesigned double-quenched TaqMan probe panel. PLoS One. 2023;18(3): e0282439.

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PROFESSIONAL ARTICLES
STRUČNI ČLANCI

DETERMINING LEUKOCYTE ALKALINE PHOSPHATASE SCORE WITH CYTOCHEMICAL STAINING

ODREĐIVANJE INDEKSA ALKALNE FOSFATAZE LEUKOCITA PRIMENOM CITOHEMIJSKOG BOJENJA

Dušan SEDLAREVIĆ[1], Stanislava NIKOLIĆ[1,2], Maša SLADOJEVIĆ[1,2], Dragana ŽUVIĆ[1,2], Ivan SIVČEV[4] and Borivoj SEKULIĆ[2,3]

ORCID NUMBER
Dušan Sedlarević 0009-0008-2075-4754
Stanislava Nikolić 0000-0002-7859-0923
Maša Sladojević 0000-0001-7461-4345
Dragana Žuvić 0000-0002-0114-2989
Ivan Sivčev 0009-0006-3162-1581
Borivoj Sekulić 0000-0001-7136-8572

University Clinical Center of Vojvodina, Novi Sad, Center of Laboratory Medicine[1]
University of Novi Sad, Faculty of Medicine Novi Sad[2]
University Clinical Center of Vojvodina, Novi Sad, Clinic of Hematology[3]
Clinic of Otorhinolaryngology and Head and Neck Surgery[4]

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Abstract
Introduction. Leukocyte alkaline phosphatase is an enzyme found in white blood cells, with its activity measured by the leukocyte alkaline phosphatase score. The aim of this study was to determine the optimal performing time and the primary biological sample in order to optimize the above laboratory analysis. **Material and Methods.** A prospective study was conducted from December 2023 to July 2024 at the Center of Laboratory Medicine, University Clinical Center of Vojvodina, involving 80 patients. Among them, 50 had confirmed leukocytosis with absolute neutrophilia, while 30 had normal white blood cell count. Leukocyte alkaline phosphatase was measured from capillary and venous blood samples immediately after collection, three hours post-collection, and seven days post-collection. All samples were prepared using the modified Kaplow method. The leukocyte alkaline phosphatase score is graded from 0 to 4 based on the ability of granulocytes to absorb certain amount of reagent according to the degree of enzyme activity. **Results.** The analysis revealed statistically higher leukocyte alkaline phosphatase scores in capillary blood samples compared to venous blood (62 (2-305) vs. 52 (5-292) vs. 50 (6-275) vs. 42 (2-280), p= 0.000). A significant decline of the score was observed in venous blood samples if the analysis is performed immediately after venipuncture, at 3 hours (52 (5-292) vs. 50 (6-275), p=0.021), and at 7 days post-sampling (52 (5-292) vs. 42 (2-280), p=0.000). **Conclusion.** Both the type of blood sample and the kinetics of the determination time affect the reliability of leukocyte alkaline phosphatase score results.
Key words: Alkaline Phosphatase; Leukocytes; Histocytochemistry; Blood Specimen Collection; Capillaries; Veins

Sažetak
Uvod. Alkalna fosfataza leukocita je enzim zrelih formi ćelija bele krvne loze čija se aktivnost meri indeksom alkalne fosfataze. Cilj ovog rada bio je da se utvrdi optimalno vreme izvođenja i primarni biološki uzorak u svrhu optimizacije prethodno navedene laboratorijske analize. **Materijal i metode.** Od decembra 2023. do jula 2024. godine u Centru za laboratorijsku medicinu Univerzitetskog kliničkog centra Vojvodine sprovedena je prospektivna studija koja je obuhvatila ukupno 80 pacijenata, od toga 50 ispitanika sa potvrđenom leukocitozom i apsolutnom neutrofilijom i 30 ispitanika sa fiziološkim vrednostima parametara kompletne krvne slike. Svim ispitanicima je određena vrednost indeksa alkalne fosfataze leukocita iz uzoraka kapilarne kao i venske krvi pripremljene neposredno, zatim nakon tri sata i sedam dana od momenta venepunkcije. Svi uzorci krvi su pripremljeni korišćenjem modifikovane *Kaplov* metode. Određivanje skora alkalne fosfataze leukocita zasniva se na sposobnosti granulocita da apsorbuju određenu količinu reagensa prema stepenu aktivnosti enzima i skorira se 0–4. **Rezultati.** Statistički više vrednosti indeksa alkalne fosfataze leukocita utvrđene su analizom iz uzoraka kapilarne krvi u odnosu na vrednosti dobijene iz venske krvi (62 (2–305) vs. 52 (5–292) vs. 50 (6–275) vs. 42 (2–280), p = 0,000). Postoji statistički značajan trend smanjenja vrednosti indeksa alkalne fosfataze leukocita dobijenih iz uzoraka venske krvi ako se analiza uradi odmah nakon venepunkcije i nakon tri sata (52 (5–292) vs. 50 (6–275), p = 0,021) kao i nakon sedam dana od trenutka uzimanja (52 (5–292) vs. 42 (2–280), p = 0,000). **Zaključak.** Vrsta uzorka krvi, kao i kinetika vremena određivanja imaju značaja na pouzdanost rezultata indeksa alkalne fosfataze leukocita.
Ključne reči: alkalna fosfataza; leukociti; histocitohemija; uzimanje uzoraka krvi; kapilari; vene

Introduction

Alkaline phosphatase (AP) is an enzyme that removes phosphate groups from molecules within different types of body cells [1]. As a basic biochemical test, elevated AP activity is detected in a range of pathophysiological conditions, including liver, kidney, and bone tissue disorders, as well as during physio-

✉ Corresponding author: Dušan Sedlarević, E-mail: dusansedlarevic01@gmail.com, stanislava.nikolic@mf.uns.ac.rs

Abbreviations

AP	– alkaline phosphatase
LAP	– leukocyte alkaline phosphatase
CML	– chronic myeloid leukemia
UCCV	– University Clinical Center of Vojvodina
EDTA	– ethylendiaminetetraacid
CB	– capillary blood
VB0	– venous blood, zero hour
VB3	– venous blood, third hour
VB7	– venous blood, seventh day
CBC	– complete blood count
CG	– control group

logical growth and development [1, 2]. When detected in white blood cells, alkaline phosphatase is referred to as leukocyte alkaline phosphatase (LAP) [3]. While serum alkaline phosphatase measures total enzyme activity in the bloodstream, the LAP score specifically detects enzyme activity within white blood cells.

White blood cells are classified in two main lineages: myeloid and lymphoid, which are further divided into five major subtypes: neutrophils, eosinophils, basophils, monocytes, and lymphocytes. Neutrophils, a subtype of leukocytes containing AP, are the most abundant circulating leukocytes, comprising 50-70% of the total number of white blood cells. Neutrophil granulocytes play a crucial role in the innate immune system [4]. The alkaline phosphatase activity in neutrophil granules reflects LAP score fluctuations, providing insight into the state and degree of maturity of the observed cells [4–7]. As such, LAP activity varies significantly across different physiological and pathological conditions and is not associated with variations in serum alkaline phosphatase [5].

Although measurement of LAP scores is a highly subjective method, it remains a good starting point for the diagnosis and monitoring of chronic myeloid leukemia (CML) [8]. Moreover, LAP scores can be indicative in other conditions affecting the bone marrow or immune system disorders, such as infections, phases of blast crisis, or CML remission [9, 10]. Low LAP values are not due to altered structure or accelerated maturation of specific myeloid cell lineage but result from decreased enzyme content within leukocyte granules. Previous studies have ruled out catalytically defective enzyme synthesis as a cause of low LAP levels, confirming instead that these low values stem from insufficient synthesis of the functional enzyme [3, 5, 11].

The determination of LAP scores is based on the fact that leukocytes absorb certain amount of reagent responsible for specific staining inside the leukocyte cytoplasm. Microscopic examination then allows for subjective classification of staining intensity into five groups, scored from 0 to 4. If the sum of observed granulocytes falls within the range between 20 and 80, the result is considered to be within the reference range.

Despite its subjectivity, the LAP score remains a routine laboratory hematological analysis due to its quick turnaround time and low cost. Depending on the initial diagnosis, LAP score values can vary widely, from very low to very high. The aim of this study was to determine the optimal timing and type of primary biological sample for LAP score determination in order to optimize and standardize this laboratory analysis.

Material and Methods

A prospective study was conducted from December 2023 to July 2024. Blood samples from 80 patients were analyzed at the laboratory of the University Clinical Center of Vojvodina (UCCV) to determine their LAP values. We included 50 patients with confirmed leukocytosis with absolute neutrophilia in the complete blood count. Additional 30 participants with normal complete blood count were included as a control group. Capillary and venous blood samples were collected from all participants in the same laboratory in a single procedure. Venous blood was drawn from the cubital vein into EDTA tubes, while capillary blood was obtained from the palmar side of the distal phalanx of the third or fourth finger. For each participant, a drop of capillary blood was immediately applied to a glass slide to determine the LAP score. A second blood smear was prepared three hours after venous blood collection, as this timeframe was arbitrarily defined to represent the average delay between sample collection and the initial processing in the laboratory. At the same time, the second peripheral blood smear was prepared, fixed, and stored for seven days, after which it was stained, observed, and compared with previously obtained LAP results.

Blood samples were prepared using a modified Kaplow method [12]. After drying at room temperature, the preparations were fixed within 60 minutes of collection using a combination of methanol and formalin in a 10:1 ration, with the fixing process lasting for about 1 minute. The fixed slides were then rinsed with distilled water and dried at room temperature.

Next, the slides were placed in a Petri dish with a freshly prepared substrate solution consisting of 70 ml of Tris-hydroxymethyl-aminomethane, 60 mg of naphthyl phosphate, and 90 mg of Fast Red salt (pH= 9.2-9.4) for 30 minutes. was After incubation, the slides were rinsed under running water, dried at room temperature, and covered with Mayer hematoxylin solution for 15 minutes. Following the washing and drying steps, the preparations were observed and analyzed on the same day under a Nikon Eclipse E

400 electron microscope by two trained laboratory technicians.

Under the microscope, active granulocytes displaying a certain degree of discoloration were observed. A total of 100 neutrophil granulocytes were assessed, and their sum determined the final LAP score, which ranged from 0 to 400 depending on the number of stained leukocytes. Scores from 20 to 80 were considered within the reference range. LAP score was determined by the method of classifying leukocytes according to their enzyme activity. Activated neutrophil granulocytes release the enzyme alkaline phosphatase into the cytoplasm. LAP score determination was based on the ability of granulocytes to absorb the reagent, reflecting enzyme activity. This allowed subjective classification into five scoring groups. Absence of alkaline phosphatase activity in neutrophils was scored 0, while significant cytoplasmic staining due to a large amount of free enzyme and bound reagents received a score of 4. Scores 1, 2, and 3 were assigned based on shades in the discoloration of the cytoplasm of neutrophils. The normal distribution of continuous variables was assessed using the Shapiro-Wilk test. Data were presented as mean ± standard deviation for normally distributed continuous variables and median (min-max) for non-parametric continuous variables. Parametric (t-test) and non-parametric (Mann-Whitney) statistical tests were used. The obtained results were analyzed using the Data Analysis tool in Excel (Microsoft Corp, Redmond, WA). All data are presented in tables and graphs, accompanied by detailed legends for each analyzed parameter.

Results

The study included 34 male and 46 female patients. The average age of the female participants was 42 years, ranging from 19 to 73 years, while the average age of the male participants was 46 years old, with an age range of 18 to 74 years.

Analysis of complete blood count parameters revealed a statistically significant difference in the absolute number of leukocytes between the tested and control groups (14.48 (10.15-36.71) vs. 6.33 (4.20-8.46); p=0.002) as well as number of neutrophils (9.72 (5.57-25.8) vs. 2.70 (1.26-6.37); p=0.002). No statistically significant differences were observed in other measured parameters between the two groups **(Table 1).**

Further analysis showed that the leukocytosis group exhibited higher LAP score values across all sample modalities, but without statistical significance **(Table 2).**

A statistically significant difference was identified when comparing LAP scores obtained from capillary blood samples analyzed immediately after collection with those from venous blood samples analyzed immediately (Capillary blood (CB): 62 (2-305) vs. venous blood, zero hour (VB0): 52 (5-292), p=0.01), after 3 hours (CB: 62 (2-305) vs. venous blood, third hour (VB3): 50 (6-275), p=0.000), and after 7 days (CB: 62 (2-305) vs. venous blood, seventh day (VB7): 42 (2-280), p= 0.000). The highest values were observed in capillary blood samples analyzed immediately after sampling **(Table 3).**

Additionally, a statistically significant downward trend in LAP score was noted in venous blood sam-

Table 1. Results of descriptive statistics of complete blood count parameters

CBC parameters	Tested group (50)	CG (30)	p
Erythrocytes (x 10^9/L)	5.04 ± 1.09	5.65 ± 0.79	0.61
Thrombocytes (x 10^9/L)	352.5 (44-863)	212 (178-485)	0.250
Leucocytes (x 10^9/L)	14.48 (10.15-36.71)	6.33 (4.20-8.46)	0.002
Neutrophils (x 10^9/L)	9.72 (5.57-25.80)	2.7 (1.26-6.37)	0.002
Neutrophils (%)	64.69 ± 6.41	44.2 ± 14.52	0.090
Monocytes (%)	7.1 (3.60-21.80)	8.6 (6.8-10.32)	0.640
Lymphocytes (%)	25.55 ± 9.64	40.3 ± 12.12	0.090

Legend: tested group – group of participants with leukocytosis; CG – control group of participants with normal complete blood count; p – statistical significance

Table 2. Values of LAP results in the tested group and the control group of subjects

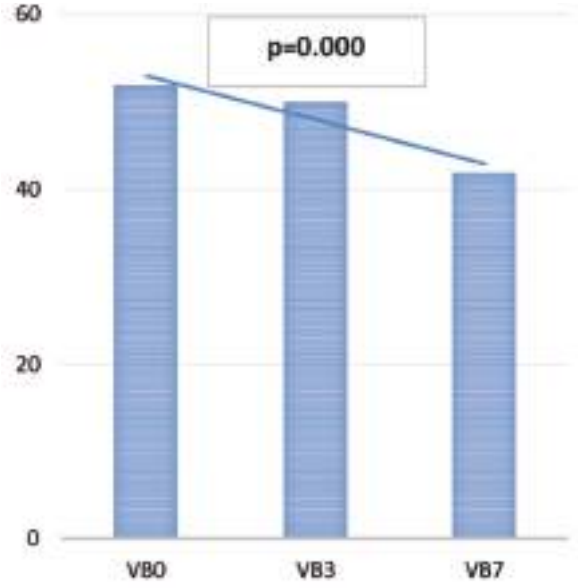
	$LAP_{tested\ group}$ (50)	LAP_{CG} (30)	p
CB	73 (2-305)	53 (30-129)	0.133
VB0	66 (5-292)	42 (26-111)	0.100
VB3	50.5 (2-280)	40 (15-108)	0.154
VB7	56.5 (6-275)	58 (22-95)	0.465

Legend: $LAP_{tested\ group}$ – leucocyte alkaline phosphatase index in the tested group LAP_{CG}–leucocyte alkaline phosphatase index in the healthy group; CB – capillary blood; VB0 – venous blood, zero hour; VB3 – venous blood, third hour; VB7 – venous blood, seventh day; p – statistical significance

Table 3. LAP score values in relation to the time of analysis and the type of sample from which the analysis was performed

CB (80)	VB0 (80)	VB3 (80)	VB7 (80)	p
62 (2-305)	52 (5-292)*	50 (6-275)**	42 (2-280)***	0.01* 0.000**, 0.000***

Legend: CB – capillary blood; VB0 – venous blood, zero hour; VB3 – venous blood, third hour; VB7 – venous blood, seventh day; *- p value between LAP values obtained from samples CB and VB0; ** - p value between the LAP values obtained from samples CB and VB3; *** - p value between the LAP values obtained from samples CB and VB7



Graph 1. Trend line of LAP score values obtained from venous blood samples in relation to the time of analysis
Legend: VB0 – venous blood, zero hour; VB3 – venous blood, third hour; VB7 – venous blood, seventh day; p – statistical significance

ples, with valued decreasing when analyzed immediately after venipuncture, after 3 hours (52 (5-292) vs. 50 (6-275), p=0.021), and 7 days after blood collection (52 (5-292) vs. 42 (2-280), p=0.000) **(Graph 1).**

Discussion

Leukocyte alkaline phosphatase activity plays an important role in differentiating leukemoid reaction from CML. This is achieved through cytochemical staining, which detects alkaline phosphatase activity in the neutrophils and bands [13]. Normal neutrophils and bands exhibit cytoplasmic alkaline phosphatase activity in the form of dark staining. Patients with leukemoid leukocytosis may exhibit very high LAP score. On the other hand, in patients with CML, the neutrophils show a very weak reaction to alkaline phosphatase staining, resulting in LAP score below the reference range. The simplicity and low cost of this laboratory test make it a useful tool for initial diagnosis of these conditions.

The reliability of this test is limited due to its subjectivity and experience of a technician performing the test. Also, the original method calls for completely fresh blood smears, but laboratory workflow constraints impose an alternative version, and routine analysis is performed from venous blood samples once a week. These facts were the starting point for re-examining the LAP score and its reliability in daily laboratory practice. The study included patients in whom varying LAP scores were expected. We compared LAP scores obtained from capillary and venous blood samples for all subjects, and found statistically higher LAP score values in capillary blood samples compared to venous blood samples, both immediately after collection and after seven days from the venous sampling **(Table 3).** Since this test is used to diagnose conditions where both slightly elevated and very low values are expected, standardized pre-analytical conditions is essential for accurate. Depending on the moment of processing, LAP score obtained from the venous blood samples continuously decreased **(Graph 1)**. As the method determined the reference range from 20 to 80, LAP score results can be interpreted incorrectly, and initially elevated values may be classified as normal, or referential ones pathologically lowered.

When analyzing LAP scores, peripheral blood samples are collected in vacutainers containing Ethylenediaminetetraacetic acid (EDTA), the most commonly used anticoagulant. However, literature suggests that EDTA may not be the optimal choice for alkaline phosphatase activity assays, even in routine biochemical testing [14]. Potassium EDTA, as an anticoagulant and chelating agent, interferes with calcium assays and clot formation, though it remains preferred for hematology testing. In most cases, relatively elevated EDTA levels due to insufficient sample volumes can intensify the chelation, which may interfere with reagent enzymes used for signal generation, such as alkaline phosphatase [15]. Based on this fact, we could assume that EDTA can also interfere with cytochemical staining, which may cause significant decline in the absolute values of the LAP scores in both patients with elevated and normal leukocyte counts.

Conclusion

Based on the study results, the highest leukocyte alkaline phosphatase score values were obtained from fresh capillary blood samples. At the same time, we proved that leukocyte alkaline phosphatase score results obtained from venous blood samples progressively decreased depending on the time of analysis - whether performed immediately or seven days after sampling.

However, the study has several basic limitations, including the small sample size, the subjectivity involved in microscopy and scoring, and the challenges in selecting non-hematological subjects. Future research would benefit from examining alternative modalities for determining and quantifying leukocyte alkaline phosphatase, such as flow cytometry or immunochemical chemiluminescence. By analyzing and validating these methods, one more key factor – the subjectivity of individuals performing the scoring during microscopic examination would be avoided.

References

1. Lowe D, Sanvictores T, Zubair M, John S. Alkaline phosphatase. Treasure Island: StatPearls Publishing; 2024.

2. Savić Lj, Savić D. Serum calcium and phosphorus concentration and alkaline phosphatase activity in healthy children during growth and development. Med Pregl. 2008;61(7-8):393-9.

3. Lipshitz J, Limaye S, Patel D. Leukocyte alkaline phosphatase score as a marker of severity and progression of myelodys-plastic syndrome. Blood. 2007;110(11):4625.

4. Tigner A, Ibrahim SA, Murray IV. Histology, white blood cell. Treasure Island: StatPearls Publishing; 2024.

5. Williams DM. Leucocyte alkaline phosphatase as a marker of cell maturity: a quantitative cytochemical and autoradiographic study. Br J Haematol. 1975;31(3):371-9.

6. Wiltshaw E, Moloney WC. Histochemical and biochemical studies on leukocyte alkaline phosphatase activity. Blood. 1955;10(11):1120-31.

7. Ullyot JL, Bainton DF. Azurophil and specific granules of blood neutrophils in chronic myelogenous leukemia: an ultrastructural and cytochemical analysis. Blood. 1975;45(4):469-82.

8. Kanegae MP, Ximenes VF, Falcão RP, Colturato VA, de Mattos ER, Brunetti IL, et al. Chemiluminescent determination of leukocyte alkaline phosphatase: an advantageous alternative to the cytochemical assay. J Clin Lab Anal. 2007;21(2):91-6.

9. Gantt S, Gervassi A, Jaspan H, Horton H. The role of myeloid-derived suppressor cells in immune ontogeny. Front Immunol. 2014;5:387.

10. Hughes A, Yong ASM. Immune effector recovery in chronic myeloid leukemia and treatment-free remission. Front Immu-nol. 2017;8:469.

11. Rosenblum D, Petzold SJ. Neutrophil alkaline phosphatase: comparison of enzymes from normal subjects and patients with polycythemia vera and chronic myelogenous leukemia. Blood. 1975;45(3):335-43.

12. Kaplow LS. Leukocyte alkaline phosphatase cytochemistry: apllications and methods. Ann N Y Acad Sci. 1968;155(2): 911-47.

13. Awale R, Maji R, Chandra D, Sharma S, Saxena R, Mukhopadhyay AK. Marked leucocytosis masquerading chronic leuke-mia: case series. IOSR Journal of Dental and Medical Sciences. 2016;15(11):65-9.

14. Bowen RA, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med (Zagreb). 2014;24(1):31-44.

15. Tate J, Ward G. Interferences in immunoassay. Clin Biochem Rev. 2004;25(2):105-20.

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Med Pregl 2024; LXXVII (5-6): 183-186. Novi Sad: maj-juni.

CASE REPORTS
PRIKAZI SLUČAJEVA

SURGICAL TREATMENT OF A DENTIGEROUS CYST AND ECTOPIC MAXILLARY MOLARS INVOLVING THE MAXILLARY SINUS – A CASE REPORT

HIRURŠKO LEČENJE ODONTOGENE CISTE MAKSILARNOG SINUSA SA EKTOPIČNIM GORNJIM MOLARIMA – PRIKAZ SLUČAJA

Denis BRAJKOVIĆ[1,2], Aleksandar KIRALJ[1,2], Miroslav P. ILIĆ[1,2], Ivana MIJATOV[1,2] and Saša MIJATOV[2]

ORCID NUMBER
Denis Brajković - 0000-0001-8484-9770
Aleksandar Kiralj - 0000-0003-4605-6408
Miroslav P. Ilić - 0000-0001-5088-4957
Ivana Mijatov - 0000-0002-4771-4102
Saša Mijatov - 0000-0003-2074-0527

University of Novi Sad, Faculty of Medicine Novi Sad,
Department of Stomatology with Maxillofacial Surgery, Novi Sad[1]
University Clinical Center of Vojvodina, Clinic for Maxillofacial Surgery, Novi Sad[2]

Case report
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Abstract

Introduction. This article aims to report a rare case of dentigerous cyst involving the left maxillary sinus and two unerupted ectopic teeth located in the lateral nasal cavity wall and pterygopalatine fossa. **Case Report.** A 13-year-old male patient was referred to our clinic due to a dentigerous maxillary sinus cyst with two unerupted ectopic teeth located in the lateral nasal cavity wall and pterygopalatine fossa. Radiographic investigation using cone-beam computed tomography was performed to determine the precise location of the ectopic teeth and the extension of the lesion. A differential diagnosis of dentigerous cyst, odontogenic keratocystic tumor, or odontogenic tumor involving the left maxillary sinus was considered. Surgical treatment comprised modified Caldwell-Luc approach, enucleation of the cyst, and extraction of ectopic teeth. Histopathological analysis confirmed the diagnosis of a dentigerous cyst. The postoperative period was uneventful. **Conclusion.** Caldwell-Luc procedure remains the preferred method for removing ectopic teeth in the maxillary sinus, whether or not associated with dentigerous cysts. Further refinement of intraoral endoscopy linked with the Caldwell-Luc technique should be particularly considered as it may lead to its establishment as the gold standard for such interventions.

Key words: Molar, Third; Tooth Eruption, Ectopic; Maxillary Sinus; Endoscopy; Dentigerous Cyst; Treatment Outcome

Sažetak

Uvod. Cilj ovog rada bio je da se predstavi redak slučaj odontogene ciste maksilarnog sinusa sa dva neizrasla ektopična zuba u bočnom zidu nosne šupljine i pterogopalatinskoj pterigopalatinskoj jami. **Prikaz slučaja**. U radu je prikazan pacijent muškog pola, starosti 13 godina, sa odontogenom cistom koja u potpunosti zahvata levi maksilarni sinus i dva ektopična zuba u bočnom zidu nosne duplje i pterigopalatinskoj jami. Preoperativno je učinjena kompjuterizovana tomografija konusnog zraka gornje vilice radi preciznog određivanja odnosa promene sa okolnim anatomskim strukturama. Na osnovu kliničkog i radiološkog pregleda postavljena je radna dijagnoza promene koja je ukazivala na odontogenu razvojnu cistu, odontogenu keratocistu ili odontogeni tumor. Operativni zahvat obuhvatao je modifikovan *Caldwell-Luc* pristup, enukleaciju ciste i ekstrakciju ektopičnih zuba. Histopatološki nalaz išao je ukazivao na folikularnu odontogenu cistu. Postoperativni tok je protekao bez komplikacija. **Zaključak.** *Caldwell-Luc* tehnika je i dalje tretman izbora za evakuaciju ektopičnih zuba u maksilarnom sinusu sa odontogenim cistama ili bez njih. Posebna pažnja se može posvetiti daljim modifikacijama *Caldwell-Luc* tehnike, posebno primenom intraoralne endoskopije, kako bi se smanjile postoperativne komplikacije.

Ključne reči: treći molar; ektopični umnjak; maksilarni sinus; endoskopija; odontogena cista; ishod lečenja

Introduction

Ectopic teeth most commonly involve mandibular third molars and maxillary canines [1]. However, ectopic maxillary third molars within the maxillary sinus are a rare clinical presentation. When located in the sinus cavity, these teeth may manifest with classical sinusitis symptoms such as facial pain, swelling, headache, and nasal obstruction or they may remain asymptomatic [1]. Symptoms usually occur when a dentigerous cyst is present in the sinus cavity. The development of dentigerous cysts in the maxillary sinus related to an ectopic tooth is rare. These cystic lesions are characterized by constant growth, leading to bone resorption and the displacement of surrounding structures and teeth [1].

The diagnosis of ectopic maxillary third molars and dentigerous cysts in the maxillary sinus is made through clinical and radiological examinations. Preoperative planning for the treatment of maxillary sinus pathologies is important due to the close proximity of vital anatomical structures [2]. Surgical extraction of ectopic third molars in the maxillary sinus is indicated for symptomatic patients as well as asymptomatic patients with dentigerous cysts [1, 2]. The Caldwell-Luc procedure (CL), first described in

✉ Corresponding author: Denis Brajković, E-mail: denis.brajkovic@mf.uns.ac.rs

Abbreviations

CL – Caldwell-Luc procedure
ESS – endoscopic sinus surgery
PPF – pterygopalatine fossa
CBCT – cone-beam computed tomography
CT – computed tomography
MRI – magnetic resonance imaging

1893, was the treatment of choice for approaching maxillary sinus lesions until the introduction of endoscopic sinus surgery [2]. The CL approach is associated with frequent complications such as postoperative swelling, infraorbital nerve paresthesia, damage to the roots of adjacent teeth, oroantral fistula formation, and postoperative anterior maxillary wall defects [3, 4]. Since the introduction of endoscopic sinus surgery (ESS), many authors have reported successful endoscopic management of ectopic teeth in the maxillary sinus, highlighting the reduced risk of injury to adjacent anatomical structures and less invasive nature of the procedure [3, 4].

This article aims to report a rare case of dentigerous maxillary sinus cyst with two unerupted ectopic teeth located in the lateral nasal cavity wall and pterygopalatine fossa (PPF).

Case Report

A 13-year-old male patient was referred to Clinic for Maxillofacial Surgery at University Clinical Center of Vojvodina with a primary complaint of intraoral swelling on the left side of the upper jaw persisting for two months. His past medical and dental history was unremarkable. Extraoral examination revealed no facial swelling. Intraoral examination showed expansion of the buccal cortical plate in the posterior region, along with the absence of teeth #27 and #28. A panoramic radiograph revealed ectopic eruption of teeth #27 and #28 in the maxillary sinus, accompanied by a hyperdense lesion obliterating the maxillary sinus cavity.

Further radiographic investigation using cone-beam computed tomography (CBCT) was performed to determine the precise location of the ectopic teeth and the extension of the lesion. The CBCT scan showed that ectopic tooth #28, with incompletely developed roots, was located in the antero-inferior aspect of the left PPF, while ectopic tooth #27 had been displaced into the lateral wall of the nose, in close proximity to the left orbital floor and nasolacrimal duct. The left maxillary sinus was completely obliterated by a well-defined, corticated hyperdense lesion measuring 40×30×30 mm, causing mediolateral expansion of the maxilla and slight elevation of the orbital floor **(Figure 1).** A differential diagnosis of dentigerous cyst, odontogenic keratocystic tumor, or odontogenic tumor involving the left maxillary sinus was considered. After analyzing the CBCT images, we were able to plan sur-

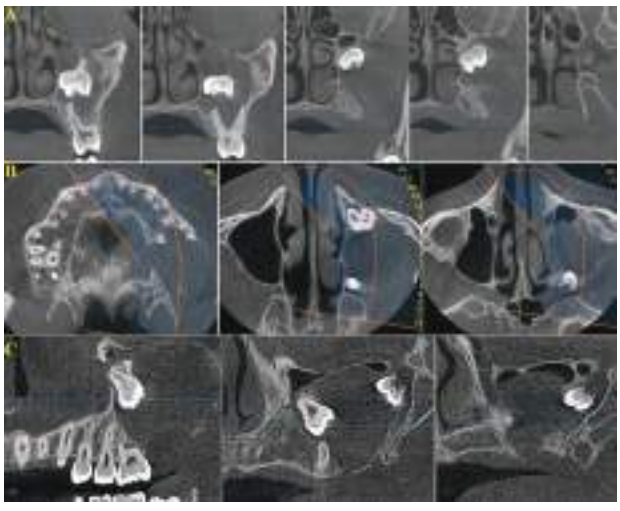

Figure 1. Preoperative cone-beam computed tomography of a dentigerous cyst in left maxillary sinus with two ectopic teeth. Axial (A), coronal (B) and sagittal (C) images of radiolucent cystic lesion filling the entire maxillary sinus and ectopic teeth #27 in the lateral nasal wall and #28 in pterygopalatine fossa

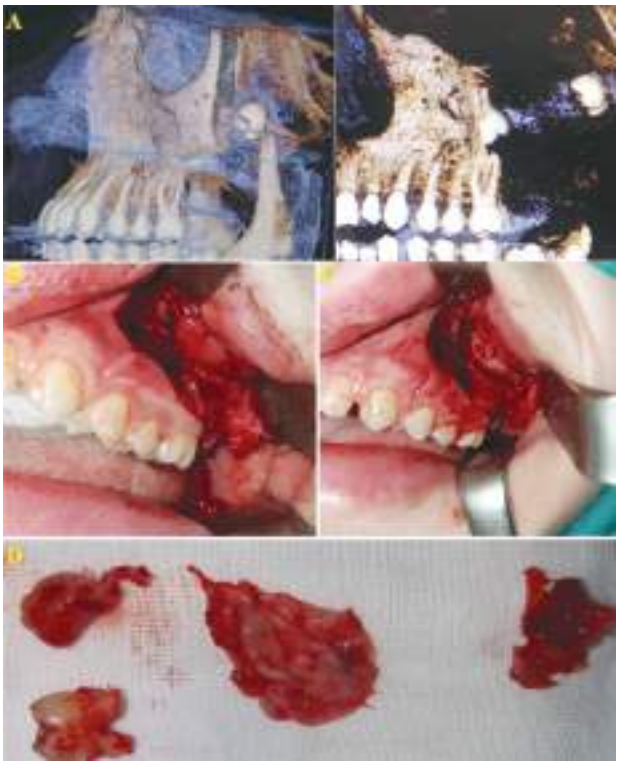

Figure 2. A) 3D reconstruction of the left maxillary sinus lesion; B) Surgical procedure – elevated mucosal-submucosal flap with the periost left attached to the anterior wall of the maxilla; C) bony flap with the periost left attached was marked and raised to enucleate the sinus cyst; D) enucleated dentigerous cyst and the enveloped teeth #27 and #28

gical treatment through a modified Caldwell-Luc approach. A vestibular incision was made from the canine to the first molar, and instead of a full-thickness mucoperiosteal flap, a mucosal-submucosal flap was raised, leaving the periosteum attached to the maxilla. Instead of creating a bony window, we elevated a 2.0 x 1.5 cm bony-periosteal flap with a superiorly placed base. The infraorbital nerve was carefully preserved. The bony-periosteal flap was carefully moved superiorly, and the ectopic teeth were extracted along with the enucleation of the cyst. The sinus cavity was irrigated, the bony-periosteal flap was repositioned, the periosteum was sutured, and the vestibular incision was then closed **(Figure 2).** Histopathological analysis confirmed the diagnosis of a dentigerous cyst. The postoperative period was uneventful.

Discussion

The most commonly reported surgical procedures for the extraction of ectopic teeth and maxillary sinus cysts were the Caldwell-Luc procedure, endoscopic sinus surgery, and endoscopic sinus surgery combined with the Caldwell-Luc approach [4]. The CL procedure has long been considered the classic surgical approach to the maxillary sinus due to its easy intraoral access to the sinus cavity through canine fossa, which can be performed under local anesthesia. The traditional CL operation involves accessing the sinus cavity through the canine fossa, removing the diseased sinus membrane, and drainage through an artificial aperture on the inferior nasal meatus. However, in the era of endoscopic surgery, the CL approach is considered aggressive due to common complications, including postoperative swelling, infraorbital nerve paresthesia, trauma to adjacent teeth, oroantral fistula, and postoperative anterior maxillary wall defects [3]. Also the removal of the entire sinus mucosa during the CL procedure leads to its replacement with nonfunctional mucosa, permanently altering physiological functions of the sinus [5]. Further complications may arise when an inferior meatal antrostomy is performed to sinus drainage, such as extended surgery time, nasolacrimal duct injury, and epistaxis [6]. In the present case, we presented a modification of the classic CL approach to enucleate large dentigerous cyst in the maxillary sinus and remove two ectopic teeth: #27 located in the lateral nasal wall and #28 located in the PPF. Three-dimensional CBCT image reconstruction was used for preoperative planning. The imaging revealed that despite the cyst's significant size, it was well demarcated from surrounding bone structures. Ectopic tooth #28 in the PPF was not in contact with neurovascular elements, and tooth #27 was not in contact with the nasolacrimal canal. Therefore, instead of creating a bony defect on the anterior maxillary wall (around 2x1 cm in diameter), we elevated a bony-periosteal flap and sutured it back into place, avoiding thus postoperative defect in the anterior wall of the maxilla.

To reduce the complications associated with the two standard techniques, several authors have reported modifications to these approaches. Li et al. [7] used an endoscopy-assisted CL operation to treat dentigerous cysts and ectopic third molars involving the maxillary sinus, demonstrating that this approach provides clear visibility during operation, less bleeding and nerve injury, minimal incisions, and minimal bone loss. The combination of ESS and CL operation has also been shown to be effective for the removal of foreign bodies in the sinus and even for the enucleation of maxillary sinus tumors such as nasal inverted papilloma. Seo et al. [6] reported a modified ESS technique that integrates elements of both the conventional Caldwell-Luc procedure and functional endoscopic sinus surgery. This approach includes repositioning the buccal bony window and enlarging the maxillary ostium via an endonasal approach, which enables postoperative reduction of the maxillary sinus resulting from postoperative cyst formation or scar tissues.

Radiological examinations are essential in the preoperative diagnosis and treatment planning for enucleation of ectopic teeth and cysts in maxillary sinus. Conventional panoramic radiographs are useful due to their low level of radiation and diagnostic accuracy for assessing lesions and location of the ectopic tooth [8]. However, 2D panoramic imaging has limitations in interpreting the exact location of the ectopic tooth, especially when displaced in the posterosuperior direction, due to superimposition of different bony structures [8]. Specialized imaging modalities like CT, MRI, and CBCT play a vital role in managing and definitely diagnosing dentigerous cysts involving the maxillary sinus. CBCT offers several advantages over CT and MRI, including multiplanar volume reconstructions, 3D volumetric planning, a significantly lower radiation dose, lower costs, shorter scan times, and fewer imaging artifacts [8]. CBCT is a useful diagnostic tool for evaluating maxillary sinus pathologies due to high sensitivity and specificity for detecting jaw bone pathologies compared with panoramic, CT and MRI. Given that dentigerous cyst are found in about 80% of cases involving ectopic maxillary third molars, differential diagnosis with other cystic radiolucent lesions in the maxillary sinus, such as ameloblastoma and odontogenic keratocyst, is essential due to varying treatment approaches for each lesion [9, 10].

Conclusion

We can conclude that the Caldwell-Luc procedure remains the preferred treatment for the removal of ectopic teeth associated with the maxillary sinus, with or without the presence of dentigerous cysts. However, recent reports on the use of endoscopic sinus surgery for the removal of ectopic teeth favor this less invasive approach. Special consideration should be given to further modifications of intraoral endoscopy in combination with the Caldwell-Luc technique, which may become the gold standard for this intervention.

References

1. Zhang LL, Yang R, Zhang L, Li W, MacDonald-Jankowski D, Poh CF. Dentigerous cyst: a retrospective clinicopathological analysis of 2082 dentigerous cysts in British Columbia, Canada. Int J Oral Maxillofac Surg. 2010;39(9):878-82.

2. Courtot R, Devoize L, Louvrier A, Pereira B, Caillet J, Meyer C, et al. Surgical approach of ectopic maxillary third molar avulsion: systematic review and meta-analysis. J Stomatol Oral Maxillofac Surg. 2021;122(1):77-82.

3. Ikeda K, Hirano K, Oshima T, Shimomura A, Suzuki H, Sunose H, et al. Comparison of complications between endoscopic sinus surgery and Caldwell-Luc operation. Tohoku J Exp Med. 1996;180(1):27-31.

4. Buyukkurt MC, Omezli MM, Miloglu O. Dentigerous cyst associated with an ectopic tooth in the maxillary sinus: a report of 3 cases and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(1):67-71.

5. Allen DZ, Sethia R, Hamersley E, Elmaraghy CA. Presentation of an iatrogenically displaced third molar into the maxillary sinus in a 14-year-old patient successfully removed with an endoscopic approach: a case report and a review of the literature. J Surg Case Rep. 2020;2020(10):rjaa290.

6. Seo MH, Lee JY, Frimpong P, Eo MY, Kim SM. Modified endoscopic-assisted approach for removal of ectopic third molar in the maxillary sinus. Appl Sci (Basel). 2021;11(18):8446.

7. Li SH, Wang Y, Huang ZX, Jin TT, Huang ZQ. Endoscope-assisted surgery in the treatment of dentigerous cyst involving the maxillary sinus - report of two cases. Chin J Dent Res. 2020;23(1):71-6.

8. Ngoc VTN, Anh LQ, Duc NM, Dinh TC, Dinh TC. Cone beam computed tomography application in finding ectopic tooth: a systemic analysis and a case report. Open Access Maced J Med Sci. 2019;7(24):4333-6.

9. Mijatov I, Kiralj A, Vučković N, Nikolić J, Tadić A, Mijatov S. Preoperative determination of tumor thickness in oral squamous cell carcinoma by computed tomography. Med Pregl. 2022;75(11-12):338-43.

10. Meng Y, Zhao YN, Zhang YQ, Liu DG, Gao Y. Three-dimensional radiographic features of ameloblastoma and cystic lesions in the maxilla. Dentomaxillofac Radiol. 2019;48(6):20190066.

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COVID-19 AND ACUTE HEPATITIS B CO-INFECTION – A CASE SERIES AND SINGLE CENTER EXPERIENCE

COVID-19 I AKUTNA HEPATITIS B VIRUSNA KOINFEKCIJA – SERIJA SLUČAJA I ISKUSTVO JEDNOG CENTRA

Natalija RAJIĆ[1], Maja RUŽIĆ[1], Maja DRLJAČA[1], Ivana MILOŠEVIĆ[2], Maria PETE[1] and Jelena ĐURICA[1]

ORCID NUMBER
Natalija Rajić – 0000-0002-2382-573X
Maja Ružić – 0000-0003-1820-9067
Maja Drljača – 0000-0003-0881-436X
Ivana Milošević – 0000-0002-5014-8949
Maria Pete – 0000-0002-1772-5564
Jelena Đurica – 0000-0001-7962-1413

University of Novi Sad, Faculty of Medicine Novi Sad,
University Clinical Center of Vojvodina, Novi Sad, Clinic for Infectious Diseases[1]
University of Belgrade, Faculty of Medicine, Belgrade
University Clinical Center of Serbia, Belgrade,
Clinic for Infectious and Tropical Diseases[2]

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Abstract

Introduction. The COVID-19 pandemic has demonstrated that COVID-19 is a systemic disease capable of causing various degrees of liver injury. However, the co-infection of COVID-19 and acute hepatitis B viruses has not been extensively studied in the literature. This study focuses on four cases of COVID-19 and acute HBV co-infections admitted to our institution. **Case Report.** This case series includes four patients - three male and one female - aged 35-44. Three patients were initially hospitalized and treated for acute hepatitis B infection, with COVID-19 co-infection diagnosed at least three weeks into their hospital stay. One patient tested positive for both COVID-19 and hepatitis B viruses upon admission. All patients received antiviral therapy for acute hepatitis B. Only one patient presented with respiratory symptoms and early-stage COVID-19 pneumonia, for which glucocorticoid therapy was administered. All four patients achieved full recovery from COVID-19 and complete resolution of hepatitis B, confirmed by virological tests during follow-up examinations. **Conclusion.** Although literature on this subject is limited, our observations suggest that co-infection with COVID-19 and acute hepatitis B virus generally results in mild clinical manifestations, without complications related to COVID-19. During hospitalization, sudden fever or worsening of serum transaminase levels may indicate a potential COVID-19 infection, particularly during epidemic periods. Further research is warranted to explore antiviral therapy of acute hepatitis and the clinical course of mild COVID-19 infection.

Key words: Hepatitis B; COVID-19; SARS-CoV-2; Coinfection; Pandemics; Treatment Outcome; Antiviral Agents

Sažetak

Uvod. Tokom pandemija izazvane virusom korone 19 (COVID-19) pandemije, dokazano je da je COVID-19 sistemska bolest koja na više načina može uzrokovati različit stepen oštećenja jetre. Koinfekcija virusima COVID-19 i virusom akutnog hepatitisa B u literaturi nije često istraživana, te se u ovoj studiji fokusiramo na jedina četiri slučaja koinfekcije kovida i akutnog hepatitisa B koja su hospitalizovana u našoj ustanovi. **Prikaz slučaja**. U ovom prikazu slučajeva, tri pacijenta su muškarci a jedna žena, od 35 do 44 godine. Od njih, tri pacijenta su inicijalno lečena pod dijagnozom akutne infekcije hepatitisom B, a kovid koinfekcija je nastala nakon najmanje tri nedelje hospitalizacije, dok je jedan pacijent od početka bolesti bio pozitivan na oba virusa. Svi pacijenti su lečeni antivirusnom terapijom za akutnu infekciju hepatitisom B, a samo jedan pacijent je imao respiratorne tegobe i incipijentnu COVID-19 pneumoniju i lečen je prema protokolu kortikosteroidnom terapijom. Svi pacijenti su imali potpun oporavak i od COVID-19 infekcije, kao i kompletnu rezoluciju infekcije hepatitisom B, što je nakon hospitalizacije na kontrolnim pregledima potvrđeno virusološkim ispitivanjima. **Zaključak**. Iako u svetu postoji vrlo mali broj studija sa ovom temom, možemo da zaključimo da koinfekcija ova dva virusa ima blagu kliničku sliku, bez komplikacija COVID-19 virusne infekcije. U toku hospitalizacije, iznenadni porast telesne temperature ili pogoršanje laboratorijskih vrednosti transaminaza bi moglo da ukaže na moguću infekciju COVID-19 u doba epidemije. Potrebno je još istraživanja o povezanosti antivirusne terapije akutnog hepatitisa B i blage kliničke slike COVID-19 infekcije.

Ključne reči: hepatitis B; COVID-19; SARS-CoV-2; koinfekcija; pandemija; ishod lečenja; antivirusni lekovi

Introduction

Since December 2019, the coronavirus disease 2019 (COVID-19) pandemic has rapidly spread worldwide. By January 2023, over 600 million cases of COVID-19 and more than 6 million deaths have been reported globally [1, 2]. In Serbia, more than 2 million cases and over 17,000 deaths were estimated by the same period [2]. While diffuse alveolar damage and acute respiratory failure are the primary features of COVID-19, the disease has been recognized as systemic, affecting multiple organs [3]. The mechanisms through which SARS-CoV-2 affects hepatocytes are well documented, with evidence suggesting that COVID-19 can lead to liver damage. Elevated serum levels of alanine aminotransferase (ALT) were observed in 28% of patients, and aspartate aminotransferase (AST) levels increased in 35% in previous studies [4].

✉ Corresponding author: Natalija Rajić, E-mail: 1423d20@mf.uns.ac.rs, maja.ruzic@mf.uns.ac.rs

Abbreviations

COVID-19 – coronavirus disease 2019
ALT – aminotransferase
AST – aminotransferase
ACE2 – angiotensin-converting enzyme 2
HBV – hepatitis B virus
Ag – antigen
PCR – Polymerase Chain Reaction
GGT – gamma-glutamil transferase
ALP – alkaline phosphatase

There are four proposed mechanisms for COVID-19-induced liver injury. The first is direct cytopathic damage, where SARS-CoV-2 directly affects hepatocytes and cholangiocytes via angiotensin-converting enzyme 2 (ACE2) receptors [5, 6]. The second is drug-induced liver injury from medications such as lopinavir/ritonavir, rececivir, chloroquine, tocilizumab, and mitifovir) [7]. The third mechanism involves a cytokine storm – a severe inflammatory reaction to the SARS-CoV-2 that can lead to multi-organ failure and secondary liver injury [6]. Lastly, underlying liver diseases – such as fatty liver disease, alcohol-related liver disease, and obesity may exacerbate liver damage. There have also been reports of chronic liver disease reactivation, such as hepatitis B virus (HBV), in COVID-19 patients treated with biological drugs such as tocilizumab, leading to liver function deterioration [6, 7].

The incidence rate of acute hepatitis B in Vojvodina in recent years is estimated at 1.2 per 100,000 and shows a declining trend, likely due to mandatory vaccination programs. Conversely, the incidence rates of chronic HBV infection are higher, ranging from 0.1 to 5.3 per 100,000 [8]. Hepatitis B infection (HBV) alone is associated with significant liver-related morbidity and mortality; however, co-infection with HBV and other viruses can accelerate liver disease progression [9].

Currently, it remains uncertain whether HBV infection predisposes patients to more severe COVID-19 or if COVID-19 exacerbates outcomes in HIV co-infected patients. While the impact of COVID-19 on chronic HBV infection has been well-documented, several studies have reported the risk of HBV reactivation during COVID-19 infection, irrespective of glucocorticoid use [4,10]. However, to our knowledge, few studies have documented cases of acute hepatitis B and COVID-19 co-infection [9]. This study presents a case series of patients co-infected with confirmed COVID-19 and acute HBV.

Material and Methods

During the COVID-19 pandemic, four patients at our institution were diagnosed with both acute HBV infection and COVID-19. These patients were selected for inclusion in this case series.

This study is a retrospective, single-center case series involving four patients who presented with acute hepatitis B and were infected with SARS-CoV-2. The patients were treated the University Clinical Center of Vojvodina, Clinic for Infectious Diseases, from March 2020 to May 2023. All patients were initially admitted to the Cohort Zone of the respective department, following the time standard protocol at the time. Upon admission to individual clinics within the University Clinical Center of Vojvodina, all patients underwent Real-Time SARS-CoV-2 PCR testing via nasopharyngeal swab and were confirmed negative prior to their transfer [11]. We reviewed medical records of these patients and collected demographic information, epidemiologic data, clinical characteristics, laboratory findings, treatment details, and outcomes of both acute HBV and COVID-19 infections. The study protocol was approved by the Medical Ethics Committee of University Clinical Center of Vojvodina. Statistical analysis was conducted using SPSS software, version 23.g.

Results

The age range of patient cohort was 35 to 44 years, with an average age of 39.7 years. The study included three male patients and one female patient. None of the patients had a prior history of chronic HBV infection, or any previous recorded underlying chronic liver diseases. All patients tested negative for secondary hepatotropic viruses. The average duration of hospitalization was 40.7 days. **Table 1** summarizes the clinical and laboratory characteristics observed during hospitalization.

Patient 1

A 35-year-old male presented with jaundice and abdominal pain lasting three days prior to admission to the Department of Abdominal Surgery with a diagnosis of acalculous cholecystitis. Laboratory results indicated acute liver injury, with transaminases significantly elevated (ALT 90x, AST 45x the normal range) and mixed hyperbilirubinemia, along with mild leucopenia. Further serologial testing confirmed acute hepatitis B with positive anti-HBc IgM, HBsAg and HbeAg. The patient was treated with Lamivudine, resulting in clinical and laboratory improvement. On the 22nd day of hospitalization, he developed a low-grade fever and showed an unexpected rise in transaminase levels. Per institutional protocols to prevent intra-hospital spread of COVID-19, an RT-PCR test and was performed, which confirmed COVID-19.

However, the patient did not develop respiratory symptoms, and there was no need for antiviral, antibiotic, or corticosteroid treatment. The chest radiograph showed no signs of viral pneumonia. The transaminase levels gradually decreased, and there were no signs of liver failure. The patient was discharged after 32 days with significantly improved liver enzyme levels. At the follow-up examination, the patient tested negative for HBsAg and positive for anti-HBs antibodies, indicating complete resolution of acute HBV infection.

Patient 2

A 41-year-old female was admitted to the Clinic for Infectious Diseases in the second week of symptoms, which included jaundice, nausea, vomiting, malaise, and pruritus. Initial lab results showed elevated transaminase levels (ALT 25x, AST 16x) and mixed hyperbilirubinemia. Acute HBV infection was confirmed by positive IgM anti-HBc. The patient was treated with Lamivudine and supportive therapy. In the fourth week, she developed a fever with a subsequent rise in previously improved transaminase levels. A PCR test confirmed COVID-19. The patient did not develop any respiratory symptoms, and the chest radiograph was normal; therefore, there was no need for oxygen support or specific COVID-19 treatment. She was discharged after 39 days with significantly improved transaminase levels. At the follow-up examination, she tested negative for HBsAg and positive for anti-HBs antibodies, indicating complete resolution of the acute HBV infection.

Patient 3

A 39-year-old male was admitted in the second week of symptoms, presenting with jaundice, nausea, vomiting, malaise, and pruritus. Initial laboratory results indicated elevated transaminase levels and mixed hyperbilirubinemia, suggesting acute hepatitis. Further testing confirmed positive HBsAg and anti-HBc IgM. The patient was started on Tenofovir but showed minimal clinical improvement. After 30 days of persisting high transaminase levels and jaundice, he was tested for COVID-19 due to possible exposure, following institutional protocols for preventing intra-hospital COVID-19 spread. The RT PCR test was positive for SARS-CoV-2. The patient remained asymptomatic for respiratory symptoms, and the chest radiograph showed no signs of pneumonia, so no specific treatment for SARS-CoV-2 was administered. After 44 days, he was discharged with improved laboratory findings and without any symptoms of hepatobiliary disease. At the follow-up, he tested negative for HBsAg and positive for anti-HBs antibodies, indicating complete resolution of the acute HBV infection.

Patient 4

A 44-year-old male was admitted with symptoms of high fever, jaundice, nausea, and abdominal pain lasting five days prior to admission. He tested positive for COVID-19 and had positive HBsAg and later positive anti-HBc IgM. Initial laboratory results revealed elevated transaminase levels, mixed hyperbilirubinemia, and elevated infection markers. The

Table 1. Demographic information, clinical presentation, laboratory, and radiology findings of the examined patients

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Age	35 years	41 years	39 years	44 years
Sex	Male	Female	Male	Male
Clinical presentation				
Jaundice	Yes	Yes	Yes	Yes
Fever	No	No	No	Yes
Abdominal pain	Yes	Yes	No	Yes
Nausea and vomiting	No	Yes	Yes	No
Laboratory tests				
WBC	5.6	6.6	5.0	9.2
Lymph	29%	26.1%	17.9%	21.3%
PLT	210	203	334	230
CRP	3.8	4.0	8.9	4.3
ALT	3428	921	2092	1756
AST	1622	577	820	604
Tbil	142.5	246.4	319.5	68.6
PT	1.06	1.30	1.16	1.06
Chest radiograph	Normal	Normal	Normal	Incipient pneumonia
USG of the abdomen	Perihepatic free fluid, periportal edema, cholecystitis	Focal changes in the liver parenchima, follow up is needed	Normal	Normal

Legend: WBC - white blood cells; Lymph - lymphocytes; PLT - platelets; CRP - C-reactive protein; ALT - alanine aminotransferase; AST - aspartate aminotransferase; GGT - gamma-glutamyl transferase; TBIL - total bilirubin; PT - prothrombin time

initial chest radiograph indicated suspected incipient viral pneumonia. The patient was started on Tenofovir, which showed a favorable initial response in treating acute hepatitis B. However, due to worsening respiratory symptoms and a chest radiograph confirming viral pneumonia, corticosteroid treatment (Dexamethasone) was administered according to the National COVID-19 treatment protocol. There was no indication for oxygen support, and follow-up chest radiographs showed significant improvement. The patient's clinical signs and laboratory results continued to improve, and he was discharged after 48 days. At the follow-up, he tested negative for HBsAg and positive for anti-HBs antibodies, indicating complete resolution of the infection, similar to the other patients.

Discussion

Hepatitis B virus (HBV) infection is a significant global health concern, capable of causing acute, fulminant, or chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [12]. In Vojvodina, the incidence of acute HBV is currently 1.2 per 100,000, with a declining trend attributed to compulsory vaccination [8]. In non-endemic countries, where the prevalence is below 1%, HBV infection primarily affects adults and in high-risk groups, such as men who have sex with men, intravenous drug users, and an increasing number of immigrants from hyperendemic regions [13].

As mentioned previously, COVID-19 can cause liver injury even in individuals without pre-existing liver disease [14]. Several patophysiological mechanisms have been proposed to explain liver damage in COVID-19. Patients with COVID-19 often exhibit elevated markers associated with liver injury, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamil transferase (GGT), and alkaline phosphatase (ALP). While the prognostic significance of elevated liver injury markers in COVID-19 patients remains debated, some studies have shown that increased AST and ALT levels are associated with a worse prognosis [15].

Few studies have examined the co-infection of viral hepatitis and COVID-19, making it challenging to determine the impact of HBV on COVID-19 severity. A Chinese study reported HBV in 12.2% of COVID-19 patients, and it remains the only study to suggest an association between HBV and increased COVID-19 severity and mortality rates [16]. Other studies, however, have found no evidence of worsening liver injury or prolonged hospitalization in HBV/COVID-19 co-infected patients [15].

Our findings are consistent with previous studies. The patients presented in our study experienced mild COVID-19 symptoms and a generally benign disease course. Only one patient (Patient 4) required corticosteroid treatment. This patient was also the only one who appeared to be co-infected with COVID-19 before the onset acute hepatitis B symptoms. Although fever in acute HBV could indicate a high risk of acute liver failure, in this case, it may have pointed out to an infection of a different etiology, possibly SARS-CoV-2 co-infection [17, 18]. In Patients 1 and 3, persistent lymphopenia was among the clinical signs that raised suspicion of a COVID-19 co-infection. Studies have shown that SARS-CoV-2-induced lymphopenia may pose a risk for patients with active HBV infection and may be associated with increased COVID-19 severity and mortality [19]. However, in our study, these patients did not experience COVID-19 progression and did not require immunosuppressive therapy or oxygen therapy.

In Patient 2, clinical suspicion of a possible COVID-19 infection arose after a sudden increase in serum transaminase levels and the development of fever. Previously, this patient had an uncomplicated clinical course and steady resolution of acute hepatitis B. Despite the co-infection, the patient did not develop any COVID-19 symptoms or viral pneumonia, and there was no need for specific COVID-19 treatment. This case highlights the need for clinicians to consider COVID-19 co-infection in the differential diagnosis of unexpected serum transaminase elevation or fever during the management of acute HBV infection.

It is noteworthy that Patients 1, 2, and 3 had already begun antiviral treatment for acute HBV infection and the time of their COVID-19 diagnosis. These patients had normal chest radiographs, required no specific COVID-19 therapies, and did not experience any clinical exacerbation of COVID-19. During the study period, antiviral drugs for COVID were not available to us, and these patients did not exhibit severe COVID-19 symptoms.

Our study suggests that the timing of HBV superinfection may determine the severity of COVID-19. In Patient 4, there appeared to be a superinfection of SARS-CoV-2 during the prodromal period of the HBV infection. This patient had the most severe clinical presentation compared to other patients, who acquired COVID-19 as a nosocomial infection with a mild course. Unlike the other patients, Patient 4 developed viral pneumonia and required corticosteroid therapy, and had the longest hospitalization. To our knowledge, this finding has not been reported in the literature, and it raises intriguing questions for further research.

Conclusion

In conclusion, cases of co-infection with acute hepatitis B and COVID-19 appear to have mild clinical manifestations. In this case series, three out of four exhibited no COVID-19 symptoms, had normal chest radiographs throughout hospitalization, and did not require specific SARS-CoV-2 treatment regimens. Further studies are needed to determine whether the use of antiviral agents, such as Lamivudine or Tenofovir, may be associated with a milder clinical course of COVID-19. Additionally, a sudden elevation in serum transaminase levels or an unexpected onset of fever in hospitalized patients could indicate a nosocomial COVID-19 infection during a SARS-CoV-2 epidemic. Physicians should be vigilant in monitoring serum transaminase levels in COVID-19 patients, as these could provide early indicators of disease progression or secondary infections.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.

2. Worldometer. COVID-19 coronavirus pandemic [Internet]. 2020 [cited 2020 Dec 25]. Available from: https://www.worldometers.info/coronavirus/

3. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. Nat Med. 2020;26(7):1017-32.

4. Li Y, Li C, Wang J, Zhu C, Zhu L, Ji F, et al. A case series of COVID-19 patients with chronic hepatitis B virus infection. J Med Virol. 2020;92(11):2785-91.

5. Baroiu L, Dumitru C, Iancu A, Leşe AC, Drăgănescu M, Baroiu N, et al. COVID-19 impact on the liver. World J Clin Cases. 2021;9(16):3814-25.

6. Yu D, Du Q, Yan S, Guo XG, He Y, Zhu G, et al. Liver injury in COVID-19: clinical features and treatment management. Virol J. 2021;18(1):121.

7. Sun J, Aghemo A, Forner A, Valenti L. COVID-19 and liver disease. Liver Int. 2020;40(6):1278-81.

8. Rajčević S, Medić S, Patić A, Dragnić N, Ristić M, Vuković V, et al. Seroprevalence study of anti-HBs antibodies in the general population of Vojvodina, Serbia. Medicina (Kaunas). 2024; 60(3):436.

9. Bekçibaşı M, Arslan E. Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2)/hepatitis B virus (HBV) co-infected patients: a case series and review of the literature. Int J Clin Pract. 2021;75(9):e14412.

10. Liu J, Wang T, Cai Q, Sun L, Huang D, Zhou G, et al. Longitudinal changes of liver function and hepatitis B reactivation in COVID-19 patients with pre-existing chronic hepatitis B virus infection. Hepatol Res. 2020;50(11):1211-21.

11. Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut". Stručno-metodološko uputstvo za kontrolu unošenja i sprečavanje širenja novog korona virusa SARS-CoV-2 u Republici Srbiji. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut"; 2020. p. 23.

12. Rosić I, Malićević S, Medić S. Immune response to hepatitis B vaccine in elite athletes. Med Pregl. 2008;61(1-2):55-9.

13. Chang MH. Hepatitis B virus infection. Semin Fetal Neonatal Med. 2007;12(3):160-7.

14. Wu J, Song S, Cao HC, Li LJ. Liver diseases in COVID-19: etiology, treatment and prognosis. World J Gastroenterol. 2020; 26(19):2286-93.

15. Martinez MA, Franco S. Impact of COVID-19 in liver disease progression. Hepatol Commun. 2021;5(7):1138-50.

16. Chen X, Jiang Q, Ma Z, Ling J, Hu W, Cao Q, et al. Clinical characteristics of hospitalized patients with SARS-CoV-2 and hepatitis B virus co-infection. Virol Sin. 2020;35(6):842-5.

17. Liang TJ. Hepatitis B: the virus and disease. Hepatology. 2009;49(5 Suppl):S13-21.

18. Du WJ, Liu L, Sun C, Yu JH, Xiao D, Li Q. Prodromal fever indicates a high risk of liver failure in acute hepatitis B. Int J Infect Dis. 2017;57:98-103.

19. Liu R, Zhao L, Cheng X, Han H, Li C, Li D, et al. Clinical characteristics of COVID-19 patients with hepatitis B virus infection - a retrospective study. Liver Int. 2021;41(4):720-30.

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BLOOD TRANSFUSION MANAGEMENT FOR PATIENTS WITH MULTIPLE MYELOMA TREATED WITH ANTI-CD38 MONOCLONAL ANTIBODIES – CASE REPORTS

TRANSFUZIOLOŠKO ZBRINJAVANJE PACIJENATA SA MULTIPLIM MIJELOMOM LEČENIH ANTI-CD38 MONOKLONSKIM ANTITELIMA – PRIKAZI SLUČAJEVA

Radovan DINIĆ[1], Nevenka BUJANDRIĆ[2,3] and Jasmina GRUJIĆ[2,3]

ORCID NUMBER
Radovan Dinić – 0009-0004-6712-8460
Nevenka Bujandrić – 0000-0001-7331-5562
Jasmina Grujić – 0000-0003-3092-4768

University Clinical Center of Serbia, Belgrade, Emergency Center, Department of Pretransfusion Testing, Hospital Blood Bank[1]
Blood Transfusion Institute of Vojvodina, Novi Sad[2]
University of Novi Sad, Faculty of Medicine Novi Sad, Department of Transfusiology[3]

Case report
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Abstract

Introduction. Daratumumab is a monoclonal antibody targeting CD38, commonly used in the treatment of newly diagnosed and relapsed multiple myeloma. The expression of CD38 on red blood cells poses a challenge as anti-CD38 can interact with red blood cells during the indirect antiglobulin test, complicating pre-transfusion testing and delaying the availability of antigen-negative blood units. **Case Report.** The first case involved a 49-year-old woman with anemia secondary to multiple myeloma, who had received treatment with Darzalex® three months prior. The second case was a 66-year-old woman with relapsed multiple myeloma, undergoing treatment with Darzalex®, who required a blood transfusion as part of her therapeutic protocol. Both patients' blood samples demonstrated complications during pre-transfusion testing, including panagglutination of red blood cells during the indirect antiglobulin test, affecting both antibody screening and cross-matching procedures. Following treatment of the red blood cells with Dithiothreitol, the indirect antiglobulin test results were rendered negative. However, since dithiothreitol destroys the Kell blood group antigen, both patients received Rh- and Kell-matched blood units to ensure compatibility. **Conclusion.** Dithioerythritol remains the most commonly available and effective method for resolving pre-transfusion testing issued in patients undergoing anti-CD38 monoclonal antibody therapy. Ensuring antigen matching patient for clinically significant blood group antigens between patient and donor red blood cells is crucial. Future efforts should focus on enhancing coordination between clinical teams and blood transfusion laboratories, developing unified guidelines, and investing in advanced testing modalities to overcome these transfusion challenges.

Key words: Blood Transfusion; Multiple Myeloma; Antibodies, Monoclonal; Antineoplastic Agents; Coombs Test; Genotyping Techniques

Sažetak

Uvod. Daratumumab je monoklonsko antitelo usmereno na CD38 i koristi se za lečenje novodijagnostikovanog i relapsirajućeg multiplog mijeloma. Zbog ekspresije CD38 na eritrocitima, ova antitela raguju sa eritrocitima koji se koriste za indirektni antiglobulinski test ometajući pretransfuzijsko testiranje i otežavajući pravovremeno obezbeđivanje antigen-negativnih jedinica krvi. **Prikaz slučaja.** Prvi pacijent je ženska osoba stara 49 godina, sa kliničkom manifestacijom anemije kao komplikacije multiplog mijeloma. Prošlo je tri meseca od kako je završila terapiju *Darzalex®*-om. Drugi pacijent je 66 godina stara žena sa relapsom multiplog mijeloma, kojoj je utvrđena potreba za transfuzijom krvi tokom terapije *Darzalex®*-om u sklopu odgovarajućeg protokola. Kod obe pacijentkinje su uzorci krvi pokazali smetnje u pretransfuzijskom testiranju koje je karakterisala panaglutinacija sa test-eritrocitima kod indirektnog antiglobulinskog testa, kako u skriningu antitela tako i kod krosmeča. Nakon što su eritrociti tretirani ditiotreitolom, andirektni antiglobulinski test je postao negativan. Međutim, s obzirom da ditioeritritol uništava antigen *Kell* krvne grupe, oba pacijenta su primila jedinice krvi kompatibilne sa njihovim *Rh* i *Kell* fenotipom. **Zaključak.** Primena ditioeritritola je najčešći, širokodostupan i efikasan metod za rešavanje problema tokom pretransfuzijskog testiranja kod pacijenata na terapiji anti-CD38 monoklonskim antitelima. Značajno je za pacijente obezbediti jedinice eritrocita koje su podudarne na klinički značajne antigene iz krvnogrupnih sistema. Koordinacija između klinika i laboratorija za transfuziju krvi, uspostavljanje jedinstvenih smernica i ulaganje u nove modalitete testiranja biće ključni za rešavanje ovih izazova u budućnosti.

Ključne reči: transfuzija krvi; multipli mijelom; monoklonska antitela; antineoplastični agensi; antiglobulinski test; genotipizacija

Introduction

Multiple myeloma (MM) is a type of hematologic cancer that originates in plasma cells, which are essential component of the immune system. These malignant plasma cells accumulate in the bone marrow, leading to a variety of health complications, including bone lesions, anemia, kidney dysfunction, and compromised immunity [1]. Despite advances in treatment, MM remains an incurable disease, highlighting the need for continuous research into novel therapeutic approaches. One of the most promising strategies

✉ Corresponding author: Radovan Dinić, E-mail: dinicradovan@yahoo.com, jasmina.grujic@mf.uns.ac.rs

Abbreviations

MM – multiple myeloma
mAbs – monoclonal antibodies
IAT – indirect antiglobulin test
RBCs – red blood cells
BTIV – Blood Transfusion Institute of Vojvodina
DAT – direct antiglobulin test
DTT – dithiothreitol
UCSS – University Clinical Center of Serbia

in recent years has been the use of monoclonal antibodies (mAbs), which have transformed the treatment landscape for MM, significantly improving patient outcomes [2, 3]. These therapies target specific antigens on myeloma cells, inducing their destruction through mechanisms like immune activation and direct cytotoxic effects. While the introduction of mAbs, particularly those targeting CD38, has marked a significant advancement in MM therapy, it has also presented new challenges in the management of blood transfusions – a critical component of supportive care for many MM patients. Anti-CD38 monoclonal antibodies, such as daratumumab and isatuximab, can bind to CD38 antigens that are expressed at low levels on red blood cells (RBCs) [4]. This binding interferes with antibody screening and crossmatching tests by causing panagglutination in the Indirect Antiglobulin Test (IAT) [4, 5]. Such interference complicates the detection and identification of alloantibodies, as well as the provision of antigen-negative blood, which is vital for maintaining transfusion safety. This issue is particularly significant for MM patients who often require multiple transfusions throughout their treatment, thereby increasing the risk of alloimmunization and subsequent hemolytic transfusion reactions. In this report, we describe two cases involving complex compatibility testing in patients with multiple myeloma undergoing mAbs therapy, and we outline the strategies employed to address these challenges and ensure the safe administration of transfusions.

Case Report

The first case concerns a 49-year-old woman admitted to the Clinic of Hematology at the University Clinical Center of Vojvodina with clinical manifestations of anemia, a complication of her underlying multiple myeloma. The patient had no prior history of blood transfusions. Pretransfusion testing, conducted by the Blood Transfusion Institute of Vojvodina (BTIV), included the following steps to ensure the selection, matching, and reservation of appropriate red cell components for the transfusion recipient: 1) Blood Typing: The patient's blood group was identified as type A, RhD-positive, with Rh phenotype CcDee and negative for the Kell antigen; 2) Crossmatch Testing: A crossmatch was performed using the patient's serum against a sample of RBCs from the selected transfusion unit. The crossmatch involved incubation at 37°C and an antiglobulin phase, and was conducted on commercial Liss/Coombs card with a gel matrix containing anti-IgG and anti-C3d (Diamed GmbH, Switzerland). The result was positive. Further testing included antibody screening by IAT using commercial reagent RBCs (ID-DiaCell I+II, Bio-Rad, DiaMed GmbH, Switzerland) with Liss/Coombs cards (DiaMed GmbH, Switzerland), which returned a positive result with 1+ agglutination (grading 1-4). However, when the same reagent RBCs were enzyme-treated, the antibody screening was negative. The direct antiglobulin test (DAT) and auto control were also negative. Antibody identification using an 11-cell panel (Bio-Rad Diamed GmBH, Switzerland) revealed panreaction with 1+ agglutination. Upon consultation with the hematology team, it was confirmed that the patient had received Daratumumab (Darzalex) three months earlier as part of the DRd protocol (darzalex, lenalidomide, dexamethasone). After treating the RBCs from the transfusion unit with dithiothreitol (DTT), the crossmatch test turned negative. Consequently, the patient was transfused with Rh- and Kell-matched RBC units.

The second case involves a 66-year-old woman who was admitted to the Bone Marrow Transplantation Department of the Clinic of Hematology at the University Clinical Center of Serbia (UCCS) for ongoing treatment of multiple myeloma following autologous hematopoietic stem cell transplantation. The patient reported fatigue and bone pain localized in the cervical and lumbosacral spine, shoulders, and hips. A complete blood count indicated the need for a blood transfusion. The patient was receiving Darzalex as part of her therapeutic regimen. Pretransfusion testing was carried out at Hospital Blood Bank, Department of Pretransfusion Testing in the Emergency Center of UCCS. Blood typing identified the patient as type A, RhD-positive, using DiaMed ID-Gel Card (Diaclon ABO/D + reverse grouping, BioRad, Switzerland) on an automated immunohematology analyzer (IH-1000, BioRad, Switzerland). Crossmatches performed using commercial Liss/Coombs cards (Diamed GmbH, Switzerland) were all positive. Antibody screening by the IAT method, using commercial reagent RBCs (ID-DiaCell I+II, Bio-Rad, DiaMed GmbH, Switzerland), showed a 2+ reaction, while the DAT and auto control were negative. Antibody identification with an 11-cell panel (Bio-Rad Diamed GmBH, Switzerland) displayed panreaction with 2+ agglutination. Given that the UCCS information system confirmed the patient's ongoing

mAbs therapy, the panagglutination was attributed to the effect of the therapy. Subsequently, the patient's Rh and Kell phenotypes were determined, and a compatible RBCs unit was successfully provided.

Discussion

Panagglutination occurs when a patient's serum reacts with all RBCs used in both antibody screening and identification panel cells. Recently, this phenomenon has become increasingly prevalent in transfusion services, particularly among patients with multiple myeloma undergoing mAbs therapy [6]. Anti-CD38 mAbs bind to CD38 antigens present on the surface of RBCs, resulting in interference with routine pretransfusion testing procedures [7, 8]. The CD38 protein is also expressed on various lymphoid and myeloid cells, as well as certain non-hematopoietic cells [9]. Western blot analyses of CD38 on human RBCs have demonstrated an increased expression of CD38 on the membranes of RBCs in patients with tumors, compared to its lower expression in healthy individuals [10]. It is suggested that cytokines secreted by cancer cells might induce this heightened CD38 expression [10, 11]. The presence of these antibodies can lead to false-positive results in crossmatching and antibody screening, complicating the identification of clinically significant alloantibodies. Anti-CD38 mAbs cause panreactivity with commercial reagent RBCs, showing agglutination strengths ranging from 1+ to 3+ in screening or antibody identification tests using the IAT. The intensity of this reaction depends on the time elapsed since the last dose of Darzalex, with interference potentially lasting up to six months after the last administration [12]. In our cases, pretransfusion testing conducted within six months of the last dose revealed agglutination strengths of 1+ and 2+ using Liss/Coombs cards.

While the reactions caused by anti-CD38 mAbs can resemble those of autoantibodies, other diagnostic tests such as autocontrol, DAT, and eluate, are often negative, which may lead to the mistaken assumption that it is an alloantibody against a high-frequency antigen [9]. To prevent adverse transfusion events, several strategies should be considered. Ideally, before initiating mAb therapy, patient samples should be sent to the transfusion service for comprehensive testing, including ABO/RhD group typing, antibody screening, DAT, and phenotyping or genotyping (if patient received transfusion up to three months before) for Rh (CcEe), Kell, Kidd, Duffy, and Ss antigens [9, 12]. Unfortunately, blood samples are often only collected after the administration of mAb therapy, leading to interference with pretransfusion testing. When pretransfusion testing is affected by ongoing or recent mAb therapy, DTT treatment of RBCs is the most commonly used method to reduce interference in IAT [13]. DTT denatures the CD38 antigen on RBCs, preventing anti-CD38 antibodies from binding and causing agglutination [14]. However, this approach also destroys Kell antigens, which potentially complicates the detection of anti-K alloantibodies, necessitating the consideration of K-negative blood for transfusion [13, 14]. Moreover, DTT treatment is a time-intensive process, typically requiring 2-4 hours to complete. Hosokawa et al. have made a notable contribution by reducing the time required for antibody screening and cross-match. They achieved this by lowering the DTT concentration from 0.2 mol/L to 0.01 mol/L and employing an IAT tube technique with an automated cell washing centrifuge [15]. This modification also reduces the extent of K antigen denaturation.

An alternative strategy involves masking CD38 on the RBC surface using the DaraEx reagent, which contains anti-CD38 antibodies without the human Fc region [12]. When RBCs are treated with the DaraEx, the reagent binds to CD38 and blocks the epitopes to which Darzalex would typically attach. Tenorio et al. demonstrated that its effectiveness of DarEx is compatible to that of DTT treatment, with the added advantage of being simpler and faster [12]. However, this reagent is not yet available in Serbia. In cases where panagglutination persists despite treatment or reagent modification, RBC phenotyping or molecular genotyping can be utilized to assess the risk of alloimmunization [16]. This approach is particularly valuable for patients who may have developed alloantibodies before the initiation of mAb therapy.

Conclusion

Panagglutination in multiple myeloma patients undergoing monoclonal antibody therapy, particularly with agents like Darzalex, poses a significant challenge in pretransfusion testing. The non-specific agglutination triggered by these therapies can lead to delays in transfusion, obscure the detection of clinically significant alloantibodies, and increase the risk of transfusion-related complications. Although current strategies – such as dithiothreitol treatment, the use of DaraEx reagent, and genotyping – provide some solutions, further research is crucial to enhance patient safety. Close collaboration between clinics and blood transfusion laboratories, the establishment of standardized guidelines, and investment in innovative testing methods will be the key to overcoming these challenges in the future.

References

1. Rodriguez-Otero P, Paiva B, San-Miguel JF. Roadmap to cure multiple myeloma. Cancer Treat Rev. 2021;100:102284.

2. Bila J, Sretenović A, Marković O, Stanisavljević N, Vlaisavljević N, Savić I. Real-world evidence in diagnostics and treatment of patients with multiple myeloma. Med Pregl. 2022; 75(Suppl 1):115-20.

3. Moreau P, San Miguel J, Sonneveld P, Mateos MV, Zamagni E, Avet-Loiseau H, et al. Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(Suppl 4):iv52-61.

4. Gozzetti A, Ciofini S, Simoncelli M, Santoni A, Pacelli P, Raspadori D, et al. Anti CD38 monoclonal antibodies for multiple myeloma treatment. Hum Vaccin Immunother. 2022;18 (5):2052658.

5. Song J, Fu R. Review: effects of anti-CD38 monoclonal antibodies on red blood cell transfusion and interventions. J Clin Lab Anal. 2021;35(12):e23832.

6. Kokoris SI, Kalantzis D, Moschandreou D, Papaioannou K, Grouzi E. Panagglutination on the indirect antiglobulin test... this is the challenge! Asian J Transfus Sci. 2022;16(2):257-62.

7. Du C, Sui W, Huang H, Zhang Y, Ding X, Gao C, et al. Effect of clinical application of anti-CD38 and anti-CD47 monoclonal antibodies on blood group detection and transfusion therapy and treatment. Leuk Res. 2022;122:106953.

8. Grimaldi V, Pagano M, Moccia G, Maiello C, De Rosa P, Napoli C. Novel insights in the clinical management of hyperimmune patients before and after transplantation. Curr Res Immunol. 2023;4:100056.

9. Lee HC. Structure and enzymatic functions of human CD38. Mol Med. 2006;12(11-12):317-23.

10. Albeniz I, Demir-Coşkun O, Türker-Şener L, Baş A, Asoğlu O, Nurten R. CD38 expression as response of hematopoietic system to cancer. Oncol Lett. 2011;2(4):659-64.

11. Hartman WR, Pelleymounter LL, Moon I, Kalari K, Liu M, Wu TY, et al. CD38 expression, function, and gene resequencing in a human lymphoblastoid cell line-based model system. Leuk Lymphoma. 2010;51(7):1315-25.

12. Tenorio M, Jimenez GM, Gutierrez VG, Jimenez A, Blanchard MJ, Valles A, et al. Validation od Daraex to resolve daratumumab-induced interferences in pre-transfusion screen tests. Blood. 2019;134(Suppl 1):4983.

13. Wagner FF. Antibody testing in patients treated with anti-CD38: there is still room for improvement. Blood Transfus. 2020;18(4):244-6.

14. Li Y, Li C, Zhang L, Li J, Li Q, Ouyang H, et al. Long-term storage protocol of reagent red blood cells treated with 0.01M dithiothreitol (DTT) for pre-transfusion testing of patients receiving anti-CD38 therapy, daratumumab. Hematology. 2023; 28(1):2186037.

15. Hosokawa M, Kashiwagi H, Nakayama K, Sakuragi M, Nakao M, Morikawa T, et al. Distinct effects of daratumumab on indirect and direct antiglobulin tests: a new method employing 0.01 mol/L dithiothreitol for negating the daratumumab interference with preserving K antigenicity (Osaka method). Transfusion. 2018;58(12):3003-13.

16. Shih AW, Yan MTS, Elahie AL, Barty RL, Liu Y, Berardi P, et al. Utilising red cell antigen genotyping and serological phenotyping in sickle cell disease patients to risk-stratify patients for alloimmunisation risk. Transfus Med. 2020;30(4):263-74.

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Report on Delirium Awareness Day, March 15, 2024

The World Delirium Day event took place on March 15, 2024 at the Museum of the Yugoslav Film Archive. The gathering featured a range of experts, including anesthesiologists and neuropsychiatrists, who explored various psychological manifestations of delirium, with a particular focus on sleep disturbance as a significant predictive factor for its onset.

The event consisted of sixteen segments, each presented as a distinct lecture. Delirium is recognized as one of the most common postoperative complications in intensive care units, often leading to a prolonged recovery process. If not promptly identified and adequately treated, including fatal outcomes. The symposium addressed the different forms of delirium: hyperactive, hypoactive, and mixed. Lectures analyzed predisposing factors and assessed degrees severity using various rating scales. They emphasized the importance of early rehabilitation in preventing delirium, especially in relation to different types of surgeries.

Intraoperative pain management was highlighted as a crucial aspect of delirium prevention, with recommendations to use the lowest effective doses of opioid analgesics. For major surgical procedures, a combination of general and regional anesthesia was suggested for optimal postoperative pain control. Dexmedetomidine was identified as the sedative of choice for patients requiring both invasive and non-invasive ventilation in intensive care units. The speakers advised minimizing the use of benzodiazepines in premedication to reduce the risk of delirium.

Specific discussions were also dedicated to particular forms of delirium observed in pediatric patients and cases of postpartum psychosis in mothers. In children, delirium often presents challenges in establishing verbal communication and cooperation due to age-related factors. Additionally, folate deficiency and homocysteine accumulation were noted as contributing factors to delirium in pregnant women.

Creating patient-friendly conditions in intensive care units was underscored as essential for preventing delirium. Recommendations included dimming lights at night, providing glasses and hearing aids, encouraging patient mobility and engagement, and allowing family visits. These adaptable conditions were deemed critical in supporting patient well-being and reducing the risk of delirium.

Overall, delirium remains a significant medical and socio-economic issue in all intensive care unit settings. Key preventive strategies involve tailoring the hospital environment to meet the needs of the patient, considering their overall health status and the nature of their surgical procedure, as along with initiating early rehabilitation and ensuring effective pain management.

Dr Milica Gojković

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IN MEMORIAM
IN MEMORIAM

Prof. dr
TEODOR KOVAČ
(1923–2024)

Prof. dr Teodor Kovač je rođen 1923. godine u Novom Kneževcu od oca Arpada i majke Olge. Osnovnu školu završio je u rodnom mestu, a potom nastavio školovanje u Novom Sadu gde je i maturirao. Zbog svoje nacionalne pripadnosti tokom okupacije je bio proganjan i rat je proveo po logorima, zatvorima i u ilegali. Oslobođenje je dočekao zahvaljujući Crvenoj armiji, nedaleko od Budima.

Posle rata nastavio je školovanje u Beogradu. Medicinski fakultet je završio u Beogradu 1951. godine, a potom specijalizirao internu medicinu 1963. godine. Na Medicinskom fakultetu u Novom Sadu habilitovao je 1968. godine, a na istom fakultetu doktorirao 1972. godine. Uža specijalnost mu je bila endokrinologija, a posebno dijabetologija. Usavršavao se u Hamburgu i Cirihu. Obavljao je funkciju rukovodioca Endokrinološke službe Interne klinike u Novom Sadu, a kasnije je postao i upravnik Odeljenja za endokrinologiju. U zvanju redovnog profesora na Medicinskom fakultetu u Novom Sadu bio je od 1983. godine. Autor je preko 300 naučnih radova, a sa prof. dr Lazarom Lepšanovićem napisao je tri knjige, a koautor je još u devet monografija i udžbenika. U domaćoj i stranoj literaturi citiran je više desetina puta.

Bio je član je SLD i redovni član Akademije medicinskih nauka SLD i Endokrinološke sekcije SLD. Osnovao je Endokrinološku sekciju DLV i bio njen prvi predsednik. Obavljao je funkciju predsednika podružnice DLV. Od stranih asocijacija treba istaći da je bio član nemačkog, švajcarskog i austrijskog dijabetološkog društva, kao i počasni član Mađarskog društva za dijabetes. Do odlaska u penziju bio je član Izvršnog odbora Udruženja dijabetologa podunavskih zemalja sa sedištem u Beču.

Angažovao se i u radu brojnih stručnih časopisa, kao što su Srpski arhiv i Medicinski pregled, gde je bio je predsednik i urednik Redakcijskog odbora.

Dobitnik je brojnih priznanja strukovnih udruženja, kao i ordena Zasluga za narod sa srebrnim vencem. Srpsko lekarsko društvo mu je dodelio Nagradu na naučnoistraživački rad i Nagradu za životno delo.

Boraveći u inostranstvu na usavršavanju upoznao se sa, za to vreme novim, organizacionim modelima za zbrinjavanje bolesnika sa dijabetesom. Po povratku osnovao je Antidijabetesnu službu pri Klinici za endokrinologiju koja je pružala kompletnu zdravstvenu zaštitu obolelima od šećerne bolesti. Aktivno je učestvovao u popularisanju medicine kroz brojna predavanja i članke sa temom o šećernoj bolesti. Napisao je knjigu *Vodič kroz šećernu bolest* koja je namenjena obolelima od ove bolesti, ali i široj populaciji.

Pored angažovanja na unapređenju rada Endokrinološkog odeljenja aktivno je učestvovao u osnivanju Nuklearne medicinske laboratorije. Na taj način se stvorila mogućnost da se endokrinološka oboljenja dijagnostikuju na savremen i egzaktan način. Zajedno sa prof. dr Lazarom Lepšanovićem prepoznao je potrebu da se angažuje i na području drugih metaboličkih poremećaja, kao što su poremećaji metabolizma lipida i gojaznost. Ovome je doprinelo i osnivanje laboratorije za poremećaje lipida pri Zavodu za patološku fiziologiju na Medicinskom fakultetu u Novom Sadu. Kao kruna ovih aktivnosti je osnivanje Simpozijuma o hiperlipoproteinemijama pre više decenija koji se održavao svake četvrte godine.

Kao lekar bio je savestan, odgovoran, a to je zahtevao i od svojih saradnika. Uvek je težio boljem stručnom radu, posebno je insistirao na učešću svojih saradnika na sastancima Sekcije za endokrinologiju, dijabetes i bolesti metabolizma, na domaćim i stranim simpozijumima i kongresima. Škrt na pohvalama saradnicima, obično bi na povratku sa takvih skupova prokomentarisao na svoj način rečima: „Nismo se obrukali“. Kasnije smo shvatili i naučili da nas je u stvari pohvalio. Bio je pun ideja za daljni stručni i naučni rad svoje ekipe. Podržavao je svakog svog saradnika da se usavršava, vodio je brojne magistarske i doktorske disertacije. Zahvaljujući njegovim poznanstvima u inostranstvu omogućeno je nekolicini lekara Klinike za endokrinologiju usavršavanje na evropskim klinikama, a jednom neurohirurgu obuku za nove hirurške metode u lečenju tumora hipofize.

Podržavao je rad i stručno usavršavanje medicinskih sestara smatrajući ih važnim i nezamenljivim članovima radnog kolektiva.

I u dubokoj starosti, oslabljenog vida i sluha, ali mentalno svež, uvek je bio zanimljiv sagovornik, kako iz oblasti medicine, posebno istorije medicine u našim krajevima, tako i iz sfere svakidašnjih društvenih događanja. Sa odlaskom u penziju i dalje je pratio razvoj svoje matične klinike i voleo da čuje kako su i šta rade njegovi saradnici, pogotovo oni stariji koji su otišli u penziju posle njega.

Sećanje na njega čuvaće bolesnici koje je lečio, studenti i lekari koje je učio i medicinske sestre s kojima je radio.

Počivajte u miru profesore!

Prof. dr Tatjana Ivković Lazar

Prof. dr
VALERIJA
SEDLAK VADOC
(1946–2024)

Tiho, kako je i živela, 30. marta 2024. godine, napustila nas je prof. dr Valerija Sedlak Vadoc, redovni profesor Medicinskog fakulteta u Novom Sadu, u penziji.

Valerija Sedlak je rođena 18. decembra 1946. u Bačkoj Topoli. Roditelji su joj bili Petar i Marija Vadoc. U Bačkoj Topoli je završila osnovnu školu a gimnaziju u Subotici. Medicinski fakultet u Novom Sadu upisala je oktobra 1965. a diplomirala aprila 1971. godine. Zaposlila se na Institutu zajedničkih medicinskih službi, Zavodu za patološku fiziologiju. Na Medicinskom fakultetu u Novom Sadu počela je svoju veoma bogatu i uspešnu akademsku karijeru u zvanju asistenta predavača. Osvajala je pedagoško i naučno polje delovanja sticanjem akademskih titula, sve dok na kraju nije izabrana za punopravnog profesora (što je bila najviša akademska titula u tadašnjoj Jugoslaviji). Svoje stručno usavršavanje krunisala je trima završenim specijalizacijama.

Svoje bogato znanje iz oblasti biohemizma u organizmu i 1978. godine krunisala je specijalizacijom iz medicinske biohemije. U toku te prve specijalizacije, dr Sedlak je pokazala veliko interesovanje i za nuklearnu medicinu, koja je bila u povoju u tadašnjoj Jugoslaviji. Pokrajnska bolnica je tih godina nabavila gama kameru, koja je bila retkost u Jugoslaviji. Dr Sedlak je dala ogroman doprinos u razvoju nuklearne medicine, a posebno u oblasti nuklearne nefrourologije. Posebno se ističe njeno životno delo u scintigrafiji dinamike protoka radiofarmaka kroz bubreg kod bolesnika posle transplantacije bubrega i procene njegove funkcije. Kao već dokazan poznavalac nuklearne medicine, specijalizaciju iz ove oblasti završila je 1980. godine.

Nisu joj bile dovoljne dve specijalizacije, već je zbog potreba Zavoda za nastavnim kadrom iz patološke fiziologije, odabrala i treću specijalizaciju iz istoimenog predmeta, koju je završila 1986. godine. Samo godinu dana posle, odbranila je doktorsku disertaciju iz oblasti nuklearne medicine "Dijagnostika patofizioloških poremećaja opstruktivne uronefropatije". Rad na ovoj problematici je obeležio ceo njen radni vek čime je dala ogroman doprinos u oblasti neklearne nefrourologije, dece i odraslih.

Objavila je više od 160 stručnih i naučnih radova u zemlji i inostranstvu. Koautor je dve monografije, autor jednog udžbenika i tri izdanja priručnika praktičnih seminarskih vežbi.

Prof. Sedlak je 1998. godine uključena u organizaciju Mađarske akademije nauka – Mađarski istraživači u inostranstvu.

Uvek je bila slobodna i spremna za razgovor sa svojim studentima i saradnicima. Nesebično se davala i pružala svaku vrstu pomoći kome god je ona bila potrebna. Više puta je proglašavana omiljenim profesorom na Medicinskom fakultetu u Novom Sadu. Iz ličnih principa, odbila je rukovođenje Katedrom.

Vrednost profesionalnog doprinosa prof. dr Valerije Sedlak Vadoc zabeležena je i u izdanju Who Is Who? – Izvanredna dostignuća poslovnih i profesionalnih žena, Outstanding achievements of business and professional women (SAD) za 1999. i 2000. godinu. Bila je član raznih stručnih udruženja kao i brojnih specijalističkih sekcija SLD-DLV. U svom plodonosnom radu, bila je i predsednik stručne lekarske komisije Fitnes lige JSCG, Udruženja za zdrav način života. Glavni urednik Naučnog glasnika SCG Udruženja za zdrav način života – Nutrition & Health.

Koristivši svoje ogromno znanje iz tri oblasti medicine, u slobodno vreme, prof. dr Valerija Sedlak se bavila i oblastima primene farmakognozije, ortomolekularne medicine, optimalnom ishranom i suplementacijom i tradicionalnom kineskom medicinom, više od dvadeset godina. Bila je saradnik CaliVita International, skoro petnaest godina, kao predsednik stručne lekarske komisije. Prof. dr Sedlak Vadoc je takođe dobro poznata i po svojoj ekspertizi i predavanjima o prednostima primene integrativne medicine.

Bila je veoma obrazovana, širokih interesovanja, poliglota, govorila je pet jezika: maternji mađarski (otac joj je bio Mađar), češki (majka bila Čehinja) engleski, nemački i srpski.

Bila je udata i imala je dva fakultetski obrazovana sina: Roberta, diplomirang veterinara i Viktora, diplomiranog ekonomistu.

Prof. dr Valerija Sedlak je preminula u Budimpešti, a sahranjena je u rodnoj Bačkoj Topoli.

Sa izuzetnim poštovanjem čuvamo sećanje na uvaženu profesorku. Počivajte u miru!

Kedves Tanárnő, emleket tisztelettel őrizzűk. Nyugodjon békében.

Prof. dr Jasna Mihailović
Prof. dr Milan Ubavić

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