YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2026; August Vol. 83 (No. 8): pp. 471–528.

Vojnosanitetski Pregled 2026 August Vol. 83 (No. 8): pp. 471–528.

Photo: Narodowe Archiwum Cyfrowe / Hirszfeld Institute (c. 1950)

Vol. 83, No. 8, 2026

VOJNOSANITETSKI PREGLED

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

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

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

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

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

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

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 83, br. 8, 2026

VOJNOSANITETSKI PREGLED

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

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

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

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

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

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

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

Photo: Narodowe Archiwum Cyfrowe / Hirszfeld Institute (c. 1950)

Ludwik Hirszfeld (1884–1954), born on August 5th 142 years ago, was a prominent Polish physician, immunologist, and microbiologist. In the world of medicine, he will be remembered for his fundamental contribution to discovering the principles of blood group inheritance (together with Emil von Dungern), introducing the ABO blood group nomenclature system, and establishing differences in the distribution of ABO blood group types among populations (together with his wife, Hanna Hirszfeld). In Serbia, he is remembered as a volunteer doctor who joined the Serbian Army in 1915 during World War I to help suppress the epidemic of typhus, which by some estimates claimed around 300,000 lives. Under those extremely difficult circumstances, Ludwik Hirszfeld discovered one of the causative agents of paratyphoid fever, the bacterium *Salmonella paratyphi C*, later named *Salmonella hirszfeldi*.

Ludvik Hiršfeld (1884–1954), rođen 5. avgusta pre 142 godine, bio je istaknuti poljski lekar, imunolog i mikrobiolog. U svetu medicine će ostati upamćen po fundamentalnom doprinosu otkrića principa nasleđivanja krvnih grupa (zajedno sa Emil von Dungern), uvođenju nomenklaturnog ABO sistema krvnih grupa i utvrđivanju razlika u zastupljenosti ABO tipova krvnih grupa među ljudima (zajedno sa suprugom Hanom Hiršfeld). U Srbiji ga pamte kao lekara dobrovoljca koji se srpskoj vojsci u Prvom svetskom ratu priključio 1915. godine kako bi pomogao u suzbijanju epidemije pegavog tifusa, u kojoj je po nekim procenama stradalo oko 300 000 ljudi. U tim izuzetno teškim okolnostima, Ludvik Hiršfeld je otkrio jedan od uzročnika paratifusa, bakteriju *Salmonella paratyphi* C kasnije nazvanu *Salmonella hirszfeldi*.

CURRENT TOPIC

UDC: 616.441-002:618.146-006.6:[616.98:578.82/.83
DOI: https://doi.org/10.2298/VSP250518046B

Endocrine-immune interactions in Hashimoto's thyroiditis and human papillomavirus–induced cervical cancer: the role of Epstein-Barr, hepatitis C, and hepatitis D viruses in shared pathogenesis

Endokrino-imunološke interakcije u Hašimotovom tireoiditisu i karcinomu grlića materice indukovanom humanim papiloma virusom: uloga virusa Epstein-Barr, hepatitis C i hepatitis D u zajedničkoj patogenezi

Danijela Bubanja-Petrović[*], Mirjana Tanić Slavić[†]

[*]Health Center "Dr. Nika Labović" Berane, Montenegro; [†]Clinical Hospital Center, Kosovska Mitrovica, Serbia

Abstract

Hashimoto's thyroiditis (HT) and human papillomavirus (HPV)-induced cervical cancer (CC) are distinct clinical entities; however, their etiologies share several pathogenetic mechanisms related to immune dysregulation, endocrine disturbances, and chronic inflammation. HT is an autoimmune disease characterized by lymphocytic infiltration of the thyroid gland, autoantibody production, and progressive tissue damage resulting from a persistent inflammatory response. In contrast, the development of HPV-induced CC depends on the virus's ability to evade host immune surveillance, thereby enabling long-term persistence of the infection. Increasing evidence suggests a potential role of other viral infections, particularly Epstein-Barr virus and hepatitis C virus, in the development of autoimmune thyroid diseases. The underlying mechanisms include chronic inflammation, molecular mimicry, and impaired immune tolerance. Furthermore, thyroid hormones and the sex hormones estrogen and progesterone play a significant role in modulating innate and adaptive immune responses and may influence the persistence of viral infections. Although there is an overlap in the immunological and endocrine mechanisms involved in these conditions, current evidence remains insufficient to confirm a direct association between HT and an increased risk of developing CC. Further experimental and epidemiological studies are required to elucidate the potential relationship between HT and HPV-induced CC.

Keywords:
autoimmune diseases; hashimoto disease; hepatitis viruses; human papillomavirus viruses; risk assessment; uterine cervical neoplasms.

Apstrakt

Hašimotov tireoiditis (HT) i humanim papiloma virusom (HPV)-indukovan karcinom grlića materice (*cervical cancer* – CC) različiti su klinički entiteti, ali u etiologiji obe bolesti je nekoliko zajedničkih patogenetskih mehanizama povezanih sa poremećajima imunskog i endokrinog sistema i hroničnom inflamacijom. HT je autoimunska bolest koju karakterišu limfocitna infiltracija štitaste žlezde, produkcija autoantitela i progresivno oštećenje tkiva žlezde usled perzistentnog inflamacijskog odgovora. Nasuprot tome, razvoj CC indukovanog HPV-om zavisi od sposobnosti virusa da izbegne imunološki nadzor domaćina, što omogućava dugoročnu perzistenciju infekcije. Sve više dokaza ukazuje na moguću ulogu drugih virusnih infekcija u nastanku autoimunskih bolesti štitaste žlezde, posebno Epstein-Barr-ovog virusa i virusa hepatitisa C. Osnovni mehanizmi uključuju hroničnu upalu, molekularnu mimikriju i poremećenu imunološku toleranciju. Osim toga, hormoni štitaste žlezde i polni hormoni estrogen i progesteron imaju značajnu ulogu u modulaciji urođenog i stečenog imunskog odgovora i mogu uticati na perzistenciju virusnih infekcija. Mada postoji preklapanje u imunološkim i endokrinim mehanizmima uključenim u ta stanja, trenutno nema dovoljno dokaza koji potvrđuju direktnu povezanost HT sa povišenim rizikom od nastanka CC. Potrebna su dodatna eksperimentalna i epidemiološka istraživanja, kojima bi se razjasnila potencijalna povezanost HT i CC indukovanog HPV-om.

Ključne reči:
autoimunske bolesti; tireoiditis, limfomatozni; hepatitis, virus; papillomavirus, humani; rizik, procena; grlić materice, neoplazme.

Correspondence to: Danijela Bubanja-Petrović, "Dr. Nika Labović" Health Center, Miljana Tomičića bb, Berane, Montenegro. E-mail: drdanijelabubanjapetrovic@gmail.com

Introduction

Hashimoto's thyroiditis (HT) and cervical cancer (CC) induced by human papillomavirus (HPV) are distinct diseases; however, both are influenced by immune dysregulation and chronic inflammatory processes. Evidence suggests that viral agents, including Epstein-Barr virus (EBV), may contribute to autoimmune thyroid disorders by modulating immune responses (IRs), while HPV-driven carcinogenesis is strongly dependent on host immune surveillance and evasion mechanisms [1, 2]. Understanding the endocrine and immune mechanisms of HT and HPV infections is crucial for clarifying potential common pathogenic pathways [2, 3]. This knowledge may improve diagnostic and therapeutic approaches and enhance our understanding of the pathogenesis of both diseases [4]. In addition to HPV, EBV and hepatitis C virus (HCV) may also contribute to the development of autoimmune thyroid diseases (AITDs), indicating a broader spectrum of viruses involved [1, 5].

Immunopathogenesis of Hashimoto's thyroiditis

HT is an autoimmune disorder in which the immune system erroneously targets thyroid tissue, leading to progressive glandular damage and dysfunction [4, 6, 7]. The main pathogenic mechanisms include infiltration of T lymphocytes, cytokine-mediated immune activation, and the development of chronic inflammatory processes within the thyroid gland [4, 7]. In HT, the immune system produces autoantibodies, most commonly anti-thyroid peroxidase – anti-TPO and anti-thyroglobulin – anti-TG, which contribute to thyroid tissue destruction by targeting key proteins involved in thyroid hormone synthesis and storage [4, 7]. This persistent immune-mediated inflammation results in progressive impairment of thyroid function and reflects a state of immune dysregulation that may influence broader immune homeostasis [4, 7]. In addition, AITDs have been associated with infectious and environmental triggers that may contribute to immune system activation and dysregulation [6], highlighting the complex interplay between genetic susceptibility, IRs, and external factors in disease pathogenesis. Some studies suggest an association between HT and an increased risk of thyroid malignancies, particularly papillary thyroid carcinoma; however, evidence regarding associations with malignancies in other organs remains limited and inconclusive [8].

Human papillomavirus, immune response, and hormonal modulation in cervical cancer

HPV is the leading cause of CC, responsible for over 99% of cases, with HPV types 16 and 18 being particularly oncogenic [9]. HPV induces malignant transformation primarily by evading host immune surveillance, thereby reducing IR against infected cells. This immune evasion occurs due to HPV's ability to inhibit T lymphocyte and natural killer cell activity [2].

Hormonal factors, particularly estrogen and progesterone, play key roles in the progression of HPV infection. Estrogen enhances the expression of cellular factors that facilitate HPV entry, promoting infection [2, 9]. On the other hand, progesterone reduces T lymphocyte activity, leads to immune dysregulation, and weakens the IR to HPV [2, 10]. These hormonal influences, combined with HPV's immune evasion mechanisms, create a favorable environment for the development of cancer [11]. Additionally, hormonal fluctuations during the menstrual cycle can affect HPV persistence and IR, emphasizing the importance of hormonal regulation in CC development [2, 9, 12, 13].

Immune evasion mechanisms by viruses: Epstein-Barr, hepatitis C, and hepatitis D

Viruses such as EBV, HCV, and hepatitis D virus (HDV) contribute to immune evasion, reducing the immune system's ability to identify and eliminate infected cells. Chronic infections with these viruses can increase the risk of developing malignancies. For instance, EBV can establish latent infections and induce T cell exhaustion, contributing to immune dysregulation, promoting autoimmune diseases (ADs), including HT. Similarly, HCV and HDV induce chronic inflammation, impairing immune tolerance and the function of regulatory T cells, which are essential for preventing ADs. By leading to immune dysregulation, these viruses foster environments that increase the risk of both ADs and malignancies, including those linked to HPV infection [14–16].

Viruses and the development of Hashimoto's thyroiditis

Chronic viral infections, particularly EBV, play a significant role in the development of HT. Limited evidence suggests a possible association between EBV infection and AITDs, including HT, primarily through mechanisms of immune dysregulation and molecular mimicry. Similarly, HCV induces chronic inflammation that triggers auto IR, increasing susceptibility to HT, while HDV may also contribute to immune dysfunction and thyroid diseases [14–18].

Endocrine regulation, immune dysfunction, and hormonal influence

Thyroid hormones and sex hormones, particularly estrogen and progesterone, play an important role in modulating IRs. Estrogen has been shown to influence both innate and adaptive immunity, potentially enhancing susceptibility to persistent viral infections such as HPV, while progesterone may exert immunosuppressive effects on cellular IRs [19, 20].

In the context of HT, altered thyroid hormone levels may contribute to immune imbalance rather than generalized immunodeficiency. This endocrine–immune interaction may influence cytokine production and immune cell activity [21].

However, the extent to which the interplay between thyroid and sex hormones contributes to viral persistence and

malignancy risk remains insufficiently clarified, and current evidence is still limited [20, 21].

Common pathogenetic mechanisms and potential interactions between human papillomavirus, Hashimoto's thyroiditis, and other viruses

Although HT and HPV infection are distinct clinical entities, several overlapping mechanisms, involving immune dysregulation and chronic inflammation, may be implicated. In HT, the primary feature is not generalized immune suppression but rather dysregulation of immune tolerance, including altered regulatory T-cell function and persistent inflammatory response [22].

HPV, on the other hand, employs well-established immune evasion mechanisms that allow viral persistence and progression toward malignancy [23]. The coexistence of immune dysregulation in HT and viral immune evasion in HPV infection may theoretically contribute to prolonged infection and increased oncogenic potential; however, direct causal relationships have not been clearly established.

The role of additional viral infections, such as EBV and HCV, has been more extensively studied in the context of immune modulation and chronic inflammation [24]. In contrast, evidence regarding HDV remains limited and should be interpreted with caution.

Currently, there is a lack of robust epidemiological data directly linking HT with increased risk of CC. Therefore, proposed interactions between these conditions should be considered hypothesis-generating and require further investigation.

Conclusion

The endocrine-immune interactions between Hashimoto's thyroiditis and human papillomavirus infections, along with the involvement of viruses such as Epstein-Barr, hepatitis C, and hepatitis D, suggest common pathogenetic mechanisms that may increase the risk of malignancies. Reduced immune efficiency, hormonal fluctuations, and immune evasion strategies contribute to the development of both conditions. This understanding provides opportunities for improving diagnostic approaches and therapeutic strategies.

REFERENCES

1. *Janegova A, Janega P, Rychly B, Kuracinova K, Babal P.* The role of Epstein-Barr virus infection in the development of autoimmune thyroid diseases. Endokrynol Pol 2015; 66(2): 132–6. DOI: 10.5603/EP.2015.0020.
2. *Stanley M.* Immune responses to human papillomavirus. Vaccine 2006; 24 Suppl 1: S16–22. DOI: 10.1016/j.vaccine.2005.09.002.
3. *Caturegli P, De Remigis A, Rose NR.* Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 2014; 13(4–5): 391–7. DOI: 10.1016/j.autrev.2014.01.007.
4. *Shukla SK, Singh G, Ahmad S, Pant P.* Infections, genetic and environmental factors in pathogenesis of autoimmune thyroid diseases. Microb Pathog 2018; 116: 279–88. DOI: 10.1016/j.micpath.2018.01.004.
5. *Tomer Y, Huber A.* The etiology of autoimmune thyroid disease: a story of genes and environment. J Autoimmun 2009; 32(3–4): 231–9. DOI: 10.1016/j.jaut.2009.02.007.
6. *Ajjan RA, Weetman AP.* The pathogenesis of Hashimoto's thyroiditis: further developments in our understanding. Horm Metab Res 2015; 47(10): 702–10. DOI: 10.1055/s-0035-1548832.
7. *Wang L, Zhu X, Xu S, Zhou B, Wu Y, Li Z,* et al. Hashimoto's thyroiditis: from pathogenesis to clinical management. Front Endocrinol (Lausanne) 2026; 17: 1729316. DOI: 10.3389/fendo.2026.1729316.
8. *Ralli M, Angeletti D, Fiore M, D'Aguanno V, Lambiase A, Artico M,* et al. Hashimoto's thyroiditis: an update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential malignant transformation. Autoimmun Rev 2020; 19(10): 102649. DOI: 10.1016/j.autrev.2020.102649.
9. *Burd EM.* Human papillomavirus and cervical cancer. Clin Microbiol Rev 2003; 16(1): 1–17. DOI: 10.1128/CMR.16.1.1-17.2003.
10. *Münz C.* Latency and lytic replication in Epstein–Barr virus-associated oncogenesis. Nat Rev Microbiol 2019; 17(11): 691–700. DOI: 10.1038/s41579-019-0249-7.
11. *Vargas-Uricoechea H.* Autoimmune thyroid disease and differentiated thyroid carcinoma: a review of the mechanisms that explain an intriguing and exciting relationship. World J Oncol 2024; 15(1): 14–27. DOI: 10.14740/wjon1728.
12. *Zignego AL, Ferri C, Pileri SA, Caini P, Bianchi FB.* Extrahepatic manifestations of hepatitis C virus infection: a general overview and guidelines for a clinical approach. Dig Liver Dis 2007; 39(1): 2–17. DOI: 10.1016/j.dld.2006.06.008.
13. *Rizzetto M.* Hepatitis D virus: introduction and epidemiology. Cold Spring Harb Perspect Med 2015; 5(7): a021576. DOI: 10.1101/cshperspect.a021576.
14. *Sevvana M, Keck Z, Foung SK, Kuhn RJ.* Structural perspectives on HCV humoral immune evasion mechanisms. Curr Opin Virol 2021; 49: 92–101. DOI: 10.1016/j.coviro.2021.05.002.
15. *Oberhardt V, Hofmann M, Thimme R, Neumann-Haefelin C.* Adaptive immune responses, immune escape and immune-mediated pathogenesis during HDV infection. Viruses 2022; 14(2): 198. DOI: 10.3390/v14020198.
16. *Duclos-Vallée JC, Johanet C, Trinchet JC, Deny P, Laurent MF, Duron F,* et al. High prevalence of serum antibodies to hepatitis C virus in patients with Hashimoto's thyroiditis. BMJ 1994; 309(6958): 846–7. DOI: 10.1136/bmj.309.6958.846.
17. *Thomas D, Karachaliou F, Kallergi K, Vlachopapadopoulou E, Antonaki G, Chatzimarkou F,* et al. Herpes virus antibodies seroprevalence in children with autoimmune thyroid disease. Endocrine 2008; 33(2): 171–5. DOI: 10.1007/s12020-008-9068-8.
18. *Weetman AP.* An update on the pathogenesis of Hashimoto's thyroiditis. J Endocrinol Invest 2021; 44(5): 883–90. DOI: 10.1007/s40618-020-01477-1.
19. *Collins MK, McCutcheon CR, Petroff MG.* Impact of estrogen and progesterone on immune cells and host–pathogen interactions in the lower female reproductive tract. J Immunol 2022; 209(8): 1437–49. DOI: 10.4049/jimmunol.2200454.
20. *Tan IJ, Peeva E, Zandman-Goddard G.* Hormonal modulation of the immune system – a spotlight on the role of progestogens. Autoimmun Rev 2015; 14(6): 536–42. DOI: 10.1016/j.autrev.2015.02.004.
21. *Rubingh J, van der Spek A, Fliers E, Boelen A.* The role of thyroid hormone in the innate and adaptive immune response during infection. Compr Physiol 2020; 10(4): 1277–87. DOI: 10.1002/cphy.c200003.

22. *Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR,* et al. The biology and life-cycle of human papillomaviruses. Vaccine 2012; 30 Suppl 5: F55–70. DOI: 10.1016/j.vaccine.2012.06.083.
23. *Hammerstad SS, Blackard JT, Lombardi A, Owen RP, Concepcion E, Yi Z,* et al. Hepatitis C Virus Infection of Human Thyrocytes: Metabolic, Hormonal, and Immunological Implications. J Clin Endocrinol Metab 2020; 105(4): 1157–68. DOI: 10.1210/clinem/dgz241.
24. *Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC.* Human papillomavirus and cervical cancer. Lancet 2013; 382(9895): 889–99. DOI: 10.1016/S0140-6736(13)60022-7.

Received on May 18, 2025
Revised on April 2, 2026
Revised on May 29, 2026
Accepted on June 10, 2026
Online First July 2026

ORIGINAL ARTICLES

UDC: 618.33-056.7-07:616.12-039
DOI: https://doi.org/10.2298/VSP260119044Z

Diagnostic value of prenatal ultrasound parameters combined with serum apolipoprotein C1 and lamin A levels in the detection of fetal cardiac structural abnormalities and chromosomal abnormalities

Dijagnostički značaj kombinacije parametara prenatalnog ultrazvučnog pregleda i nivoa apolipoproteina C1 i lamina A u serumu u otkrivanju anomalija strukture srca fetusa i hromozomskih abnormalnosti

[1]Lin Zheng*, [1]Juan Wu†, Jianshe Zhang‡

***Guiyang Maternal and Child Health Hospital, Department of Eugenics and Genetics, Guiyang, Guizhou, China; †Sihong First People's Hospital, Department of Clinical Laboratory, Suqian, Sihong, Jiangsu, China; ‡The Second People's Hospital of Tongxiang, Department of Ultrasound, Jiaxing, Zhejiang, China**

[1]the two authors contributed equally to this study

Abstract

Background/Aim. Early screening for fetal cardiac structural abnormality (CSA) is crucial for improving prenatal outcomes. The aim of the study was to evaluate the diagnostic value of prenatal ultrasound parameters, combined with serum apolipoprotein C1 (APOC1) and lamin A (LMNA) levels, for detecting fetal CSA and chromosomal abnormality (CA). **Methods.** The research included 146 pregnant women (PW) admitted from July 2022 to March 2024, who were divided into two groups. The study group comprised 86 PW with a fetal CSA diagnosis, while the control group consisted of 60 PW with normal fetal cardiac structures who underwent prenatal examination during the same period. **Results.** Patients in the study group had lower ventricular systolic velocity (S), ventricular diastolic velocity (D), atrial systolic velocity (A), maximum A-wave velocity (TA_{max}), and serum LMNA levels, alongside a higher pulsatility index (PI) and serum APOC1 levels compared to the control group ($p < 0.001$). Serum APOC1 levels were negatively correlated with S, D, A, and TA_{max} ($r < 0$, $p < 0.001$) but positively correlated with PI ($r > 0$, $p < 0.001$). Conversely, serum LMNA levels were positively correlated with S, D, A, and TA_{max} ($r > 0$, $p < 0.001$), and negatively correlated with PI ($r < 0$, $p < 0.001$). For the diagnosis of CA, the areas under the curve – AUCs for S, D, A, TA_{max}, PI, serum APOC1, and serum LMNA levels alone, as well as the combination of all indicators reached, were 0.750, 0.817, 0.823, 0.802, 0.835, 0.796, 0.831, and 0.911, respectively. **Conclusion.** In the serum of PW carrying a fetus with CSA and CA, APOC1 levels were elevated, whereas LMNA levels were reduced. Combining prenatal ultrasound parameters with serum APOC1 and LMNA levels yields a significantly higher diagnostic value, which could be used for the early screening of high-risk pregnancies.

Keywords:
apolipoproteins c; biomarkers; chromosome aberrations; diagnosis; heart defects, congenital; ultrasonography, prenatal.

Apstrakt

Uvod/Cilj. Rani skrining strukturnih anomalija srca (*cardiac structural abnormality* – CSA) fetusa od ključnog je značaja za poboljšanje prenatalnih ishoda. Cilj rada bio je da se proceni dijagnostički značaj prenatalnih ultrazvučnih parametara u kombinaciji sa serumskim nivoima apolipoproteina C1 (APOC1) i lamina A (LMNA) u detekciji CSA fetusa i hromozomskih abnormalnosti (*chromosomal abnormality* – CA). **Metode.** Istraživanjem je obuhvaćeno 146 trudnica, hospitalizovanih od jula 2022. do marta 2024. godine, podeljenih u dve grupe. Ispitivanu grupu činilo je 86 trudnica sa dijagnozom CSA fetusa, dok je kontrolnu grupu činilo 60 trudnica sa normalnom strukturom srca fetusa, kojima je urađen prenatalni pregled u istom periodu. **Rezultati.** Kod ispitanica u ispitivanoj grupi utvrđeni su niža sistolna brzina komora (S), dijastolna brzina komora (D), sistolna brzina pretkomora (A), maksimalna brzina A-talasa (TA_{max}) i nivo serumskog LMNA, kao i viši pulsatilni indeks (*pulsatility index* – PI) i nivo serumskog APOC1, u odnosu na ispitanice

Correspondence to: Jianshe Zhang, The Second People's Hospital of Tongxiang, Department of Ultrasound, Jiaxing 314500, Zhejiang Province, China. E-mail: zhangjstsph@sdsch.cn

kontrolne grupe ($p < 0,001$). Utvrđena je negativna korelacija serumskog APOC1 sa S, D, A i TA_{max} ($r < 0, p < 0,001$), ali pozitivna korelacija sa PI ($r > 0, p < 0,001$). Nasuprot tome, nivoi serumskog LMNA bili su u pozitivnoj korelaciji sa S, D, A i TA_{max} ($r > 0, p < 0,001$) i u negativnoj korelaciji sa PI ($r < 0, p < 0,001$). Za dijagnozu CA, *areas under the curve* – AUCs za S, D, A, TA_{max}, PI, nivoe serumskog APOC1 i LMNA, pojedinačno, kao i kombinaciju svih pokazatelja, iznosile su 0,750, 0,817, 0,823, 0,802, 0,835, 0,796, 0,831 i 0,911, redom. **Zaključak.** U serumu trudnica sa CSA i CA fetusa, nivoi APOC1 su bili povišeni, dok su nivoi LMNA bili sniženi. Kombinacija parametara prenatalnog ultrazvučnog pregleda i serumskih nivoa APOC1 i LMNA imala je veću dijagnostičku vrednost, što bi moglo biti od koristi za rani skrining trudnoća sa visokim rizikom.

Ključne reči:
apolipoproteini c; biomarkeri; hromosomi, aberacije; dijagnoza; srce, kongenitalne mane; ultrasonografija, prenatalna.

Introduction

Cardiac structural abnormality (CSA), a common type of fetal malformation, mainly refers to an abnormal cardiac structure during fetal development in the uterus, and its diagnosis in early pregnancy and pathogenesis have always been the focus of research in the field of obstetrics and gynecology [1]. Fetal CSA produces no obvious subjective symptoms in the mother initially, and it is mostly detected during prenatal examination, which has become an important factor influencing pregnancy outcomes and causing neonatal death, seriously reducing the quality of birth [2]. Therefore, early screening for CSA during fetal development is crucial, and identifying effective screening modalities is of primary importance. With the recent continuous development of clinical medical technology, prenatal ultrasound (PUS) has become a common method for diagnosing fetal CSA. It has been reported that cardiac function is closely related to bloodstream changes, and fetuses with CSA often develop hemodynamic abnormalities [3]. Nevertheless, blood flow spectral parameters obtained from PUS examination may serve as useful screening indicators for fetal cardiac structural abnormalities [4]. In addition, maternal serum biomarker testing, which is minimally invasive and timely, may complement ultrasound-based prenatal screening [5].

At present, no specific biological indicators are available for the clinical diagnosis of fetal cardiac abnormality. In recent years, protein molecules have been highly valued in the study of pregnancy markers, which can be used in the screening for fetal structural abnormalities [6]. Serum apolipoprotein C1 (APOC1) and lamin A (LMNA) are common protein molecules that have been confirmed to be associated with congenital cardiac malformations [7]. APOC1 is involved in lipid transport and lipid metabolism, and abnormal maternal lipid metabolism may influence placental-fetal metabolic homeostasis and embryonic cardiovascular development [5]. *LMNA* encodes lamin A/C, a nuclear lamina protein involved in nuclear architecture, chromatin organization, gene-expression regulation, and cardiomyocyte differentiation. Therefore, APOC1 and LMNA were selected as candidate maternal serum biomarkers because they may reflect biological changes related to abnormal fetal cardiac development [8]. Fetal growth and development is a dynamic process generally under the influence of a variety of factors, and chromosomal abnormality (CA) is recognized as one of the risk factors for fetal cardiac malformations [9]. Following the diagnosis of fetal CSA, whether to terminate the pregnancy should be determined according to the specific etiology. Surgery can be performed after birth if simple CSA occurs, while termination of pregnancy is usually recommended if it is caused by CA [10]. Therefore, prenatal diagnosis of CA is also important. Nevertheless, the diagnostic value of PUS parameters, combined with serum APOC1 and LMNA levels, for fetal CSA and CA has been rarely reported.

Therefore, the diagnostic value of PUS parameters combined with serum APOC1 and LMNA levels for fetal CSA and CA was investigated in this study, aiming to guide the early screening of high-risk pregnancies.

Methods

Subjects

The study was approved by the Ethics Committee of the Tongxiang Second People's Hospital, Tongxiang, Zhejiang, China (from July 12, 2022). Written informed consent was obtained from all participants.

The study included a total of 146 pregnant women (PW) admitted to our hospital from July 2022 to March 2024, who were divided into two groups. The first group, selected as a study group (SG), consisted of 86 PW carrying a fetus with CSA, while the control group (CG), selected as a healthy group, included 60 PW with normal fetal cardiac structure and received prenatal examination during the same period. The sample size was based on the consecutive enrollment of all eligible PW during the study period.

Inclusion and exclusion criteria

The inclusion criteria were as follows: PW who had abnormal results in PUS examination in SG; those who had normal results in PUS examination in CG; those with singleton pregnancy; those with natural pregnancy; and those who voluntarily signed the informed consent form.

The exclusion criteria involved the following: PW with severe liver or kidney dysfunction; those with concurrent gestational diabetes; those with hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, or pre-existing chronic hypertension; those with an abnormal mental state; those with a history of threatened abortion; those with coagulation dysfunction; or those who had a history of alcohol consumption or smoking.

Prenatal ultrasound examination

PUS examination was performed at 11–13 weeks of gestation, as described in the text that follows. PW were instructed to lie in a supine position, and routine examinations were performed to detect the fetal heart rate, fetal movement, and development status using an E8 color Doppler ultrasound diagnostic instrument (GE, USA) equipped with a convex array probe (applied with couplant, frequency: 2–5 MHz). It was determined whether the examination results were consistent with the conditions at the corresponding gestational age (GA). Then the section was adjusted during a fetal resting state: at the sagittal plane of the midline of the upper abdomen, the long axis of the umbilical vein was displayed and traced toward the fetal head, and the umbilical venous duct was identified and measured for its length. Then, color Doppler flow imaging was performed at the entrance of the catheter (volume: 1–2 mm, beam-flow angle $< 30^{\circ}$), and the blood flow spectrum of more than 5 consecutive fetal cardiac cycles (< 5 min) was obtained. Finally, the following parameters were measured: ventricular systolic velocity (S), ventricular diastolic velocity (D), atrial systolic velocity (A), maximum A-wave velocity (TA_{max}), and pulsatility index (PI). A-wave reversal or disappearance suggested CSA. Fetuses with suspected CSA at 11–13 weeks of gestation were referred for detailed fetal echocardiography (FECG) during the second trimester. The final diagnosis of fetal CSA was based on detailed FECG and follow-up assessment. The type of cardiac anomaly, GA at first suspicion, GA at confirmation, chromosomal results, and pregnancy outcomes were recorded.

Detection of serum levels of APOC1 and LMNA

Fasting venous blood (3 mL) was drawn and placed into a sterile anticoagulant tube, followed by centrifugation using a TDZ5-WS centrifuge (Xiangyi Centrifuge Instrument Co., Ltd., Changsha, Hunan, China) for 10 min (radius: 20 cm, 3,000 revolutions *per* minute – rpm). The supernatant was harvested to determine serum levels of APOC1 and LMNA by the enzyme-linked immunosorbent assay – ELISA using kits provided by MultiSciences (Lianke) Biotech Co., Ltd., Hangzhou, Zhejiang, China. The analytical ranges were 3.2–50,000 pg/mL for APOC1 and 15.6–1,000 pg/mL for LMNA. After conversion to the fmol/mL units used in the present study, these ranges were 0.48–7,561 fmol/mL and 0.22–14.3 fmol/mL, respectively. The healthy PW in this study were used as the internal reference population for APOC1 and LMNA.

Diagnosis and confirmation of fetal cardiac structural abnormality

Fetal CSA was initially suspected based on PUS findings at the end of the first trimester. All suspected women were referred for detailed FECG and were re-evaluated during the second trimester. The final diagnosis of fetal CSA was established only when the anomaly was confirmed by second-trimester FECG and/or postnatal echocardiography or pathological examination after termination of pregnancy. Cases without confirmatory examination or incomplete follow-up information were excluded from the final analysis.

Chromosomal abnormality detection

PW in SG underwent genetic counseling after fetal CSA was suspected by PUS. Amniocentesis was performed during the second trimester to obtain fetal cells for chromosomal analysis. Chromosomal karyotyping was performed to determine the presence or absence of CA. The results of chromosomal karyotyping were used as the reference standard, and SG was further divided into two subgroups: the CA group and the chromosomally normal group.

Collection of clinical data

Age, GA, primipara/multipara, body mass index (BMI), PUS parameters (S, D, A, TA_{max}, and PI), and serum levels of APOC1 and LMNA were recorded and compared between the CA and chromosomally normal groups.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 software. Measurement data were expressed as mean ± standard deviation (SD) and analyzed using the independent-samples *t*-test. Count data were represented as numbers (percentages) and compared using the χ^2 test. Bivariate Pearson correlation analysis was conducted to evaluate the correlations between serum APOC1 and LMNA levels and PUS parameters. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic value of PUS parameters combined with serum APOC1 and LMNA levels for fetal CSA and CA. A value of $p < 0.05$ was considered statistically significant.

Results

In SG, PW were aged 25–34 years, with an average of 28.56 ± 2.34 years. The GA ranged from 11 to 13 weeks, with an average of 12.02 ± 0.25 weeks. There were 56 primiparas and 30 multiparas. The BMI was 22–27 kg/m^2, with an average of 23.48 ± 1.14 kg/m^2. In CG, PW were aged 26–34 years, with an average of 28.74 ± 2.41 years. GA ranged from 11 to 13 weeks, with an average of 12.00 ± 0.24 weeks. There were 41 primiparas and 19 multiparas. The BMI was 22–27 kg/m^2, with an average of 23.53 ± 1.20 kg/m^2. The general data were well balanced between the two groups ($p > 0.05$).

Characteristics of diagnosed fetal cardiac structural abnormalities

Among the 86 fetuses with CSA, the initial suspicion was made based on PUS findings at a mean GA of 12.04 ± 0.43 weeks. All cases were subsequently re-evaluated and

confirmed by detailed FECG during the second trimester at a mean GA of 21.36 ± 2.18 weeks. The diagnosed fetal cardiac anomalies included ventricular septal defect in 22 cases, atrial septal defect in 8 cases, atrioventricular septal defect in 10, tetralogy of Fallot in 9, double outlet right ventricle in 7, transposition of the great arteries in 6, hypoplastic left heart syndrome in 5, pulmonary stenosis or pulmonary atresia in 6, aortic stenosis, coarctation of the aorta, or interrupted aortic arch in 5 cases, and single ventricle or complex congenital heart disease in 8 cases.

CA were detected in 26 fetuses, whereas 60 fetuses had normal chromosomal findings. CA included trisomy 21 in 10 cases, trisomy 18 in 6, trisomy 13 in 3, Turner syndrome in 2, and other CA in 5 cases. Regarding pregnancy outcomes, 49 pregnancies continued to live birth, 31 pregnancies were terminated after multidisciplinary counseling, 3 cases resulted in intrauterine fetal death, and 3 were lost to follow-up.

Prenatal ultrasound parameters and serum APOC1 and LMNA levels in study and control groups

PW in SG had lower S, D, A, TA_{max} values and serum LMNA levels, and higher PI values and serum APOC1 levels than patients in CG ($p < 0.001$) (Table 1).

Correlations of serum APOC1 and LMNA levels with prenatal ultrasound parameters

The results of Pearson correlation analysis showed that APOC1 levels were negatively correlated with S, D, A, and TA_{max} ($r < 0$, $p < 0.001$) but positively correlated with PI ($r > 0$, $p < 0.001$). Serum LMNA levels were positively correlated with S, D, A, and TA_{max} ($r > 0$, $p < 0.001$), and negatively correlated with PI ($r < 0$, $p < 0.001$) (Table 2).

Diagnostic value of prenatal ultrasound parameters combined with serum APOC1 and LMNA levels for fetal cardiac structural abnormality

ROC curves were plotted with the presence or absence of fetal CSA as a state variable (abnormal/normal = 1/0) and S, D, A, TA_{max}, PI, and serum APOC1 and LMNA levels as test variables.

ROC curve and its areas under the curve (AUCs) values for S, D, A, TA_{max}, PI, serum APOC1 levels, and serum LMNA levels alone and the combination of all indicators were 0.812, 0.798, 0.685, 0.846, 0.781, 0.784, 0.811, and 0.915, respectively, for the diagnosis of fetal CSA (Table 3 and Figure 1).

Table 1

Prenatal ultrasound parameters values and serum levels of APOC1 and LMNA in the study and control groups

Group	S	D	A	TA_{max}	PI	APOC1	LMNA
Study (n = 86)	30.25 ± 1.99	22.35 ± 4.05	7.01 ± 2.53	18.76 ± 3.61	1.73 ± 0.75	32.25 ± 10.36	0.16 ± 0.07
Control (n = 60)	34.25 ± 2.18	27.56 ± 4.72	8.63 ± 1.82	26.15 ± 4.88	1.05 ± 0.46	21.56 ± 9.68	0.29 ± 0.14
t-test	11.488	7.142	4.250	10.517	6.247	6.300	4.879
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

APOC1 – apolipoprotein C1; LMNA – lamin A; S – ventricular systolic velocity; D – ventricular diastolic velocity; A – atrial systolic velocity; TA_{max} – maximum A-wave velocity; PI – pulsatility index; n – number.
All values are given as mean ± standard deviation.
Values are expressed as cm/s for S, D, A, and TA_{max}, and as fmol/mL for APOC1 and LMNA.

Table 2

Correlations of serum APOC1 and LMNA levels with prenatal ultrasound parameters

Variable	S	D	A	TA_{max}	PI
APOC1					
r	-0.512	-0.502	-0.613	-0.571	0.555
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
LMNA					
r	0.519	0.610	0.612	0.615	-0.542
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

For abbreviations, see Table 1.

Table 3

Diagnostic value of prenatal ultrasound parameters combined with serum APOC1 and LMNA levels for fetal cardiac structural abnormality

Index	AUC	SE	Cut-off	95% CI	*p*-value
S	0.812	0.035	31.856	0.743–0.881	< 0.001
D	0.798	0.038	24.361	0.724–0.872	< 0.001
A	0.685	0.043	7.356	0.600–0.770	< 0.001
TA_{max}	0.846	0.032	20.458	0.783–0.909	< 0.001
PI	0.781	0.037	1.426	0.708–0.854	< 0.001
APOC1	0.784	0.039	28.561	0.706–0.861	< 0.001
LMNA	0.811	0.041	0.261	0.730–0.891	< 0.001
Combination	0.915	0.027	-	0.862–0.968	< 0.001

AUC – area under the curve; SE – standard error; CI – confidence interval.
For other abbreviations, see Table 1.

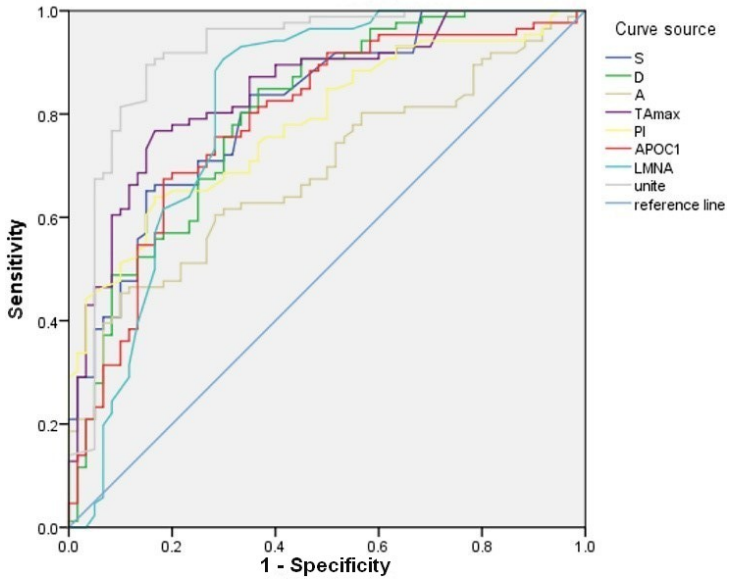


Fig. 1 – Receiver operating characteristic (ROC) curves of prenatal ultrasound parameters combined with serum APOC1 and LMNA levels for the diagnosis of fetal cardiac structural abnormality. For other abbreviations, see Table 1.

Table 4

Prenatal ultrasound parameters values and serum levels of APOC1 and LMNA among CG, chromosomally normal fetuses with CSA, and chromosomally abnormal fetuses with CSA

Variable	CG (n = 60)	Fetuses with CSA		*F*	Overall *p*-value	*Post-hoc* comparison
		chromosomally normal (n = 60)	chromosomally abnormal (n = 26)			
S	34.25 ± 2.18	33.26 ± 5.13	28.56 ± 3.85	19.667	< 0.001	abnormal < healthy and normal
D	27.56 ± 4.72	24.13 ± 3.02	20.42 ± 2.16	35.652	< 0.001	normal < healthy; abnormal < normal < healthy
A	8.63 ± 1.82	7.80 ± 1.20	6.21 ± 0.75	25.879	< 0.001	normal < healthy; abnormal < normal < healthy
TA_{max}	26.15 ± 4.88	21.26 ± 2.69	17.30 ± 2.51	57.288	< 0.001	normal < healthy; abnormal < normal < healthy
PI	1.05 ± 0.46	1.50 ± 0.27	1.85 ± 0.24	51.551	< 0.001	normal > healthy; abnormal > normal > healthy
APOC1	21.56 ± 9.68	28.26 ± 1.80	35.69 ± 2.74	46.358	< 0.001	normal > healthy; abnormal > normal > healthy
LMNA	0.29 ± 0.14	0.24 ± 0.06	0.14 ± 0.07	19.605	< 0.001	normal < healthy; abnormal < normal < healthy

CG – control group; CSA – cardiac structural abnormality; n – number. For other abbreviations, see Table 1.
All values are given as mean ± standard deviation.
Values are expressed as cm/s for S, D, A, and TA_{max}, and as fmol/mL for APOC1 and LMNA.

Prenatal ultrasound parameters and serum APOC1 and LMNA levels among the control group, chromosomally normal fetuses with cardiac abnormality, and chromosomally abnormal fetuses with cardiac abnormality

To further evaluate the diagnostic value of PUS parameters and serum APOC1 and LMNA levels in different fetal subgroups, SG was divided into chromosomally normal and CA fetuses with ultrasonographically detected CSA, and both subgroups were compared with CG. As shown in Table 4, there were significant overall differences among the three groups in S, D, A, TA_{max}, PI, serum APOC1 levels, and serum LMNA levels ($p < 0.001$). Compared with CG, chromosomally normal fetuses with CSA showed lower D, A, TA_{max}, and LMNA levels, and higher PI and APOC1 levels. Chromosomally abnormal fetuses with CSA showed more marked changes, including lower S, D, A, TA_{max}, and LMNA levels and higher PI and APOC1 levels than the other two groups. Overall, these findings suggest a stepwise alteration in fetal cardiac hemodynamic parameters and maternal se-rum biomarkers, progressing from healthy controls to chromosomally normal fetuses with CSA, and further to those with both CSA and CA (Table 4).

Diagnostic value of prenatal ultrasound parameters combined with serum APOC1 and LMNA levels for identifying chromosomal abnormality among fetuses with cardiac structural abnormality

ROC curves were further constructed to evaluate the ability of PUS parameters and serum APOC1 and LMNA levels to identify CA among fetuses with ultrasonographically detected CSA. As shown in Table 5 and Figure 2, the AUCs of S, D, A, TA_{max}, PI, APOC1, and LMNA were 0.750, 0.817, 0.823, 0.802, 0.835, 0.796, and 0.831, respectively. The combined model achieved the highest diagnostic performance, with an AUC of 0.911, indicating that the combined assessment may help further stratify the risk of CA in fetuses with CSA.

Discussion

With the continuous improvement of ultrasound technology, ultrasound, characterized by non-invasiveness, simple operation, and repeatability, has been widely employed in prenatal examination, which is safer than invasive examination and thus favored by PW. PUS examination can observe fetal development from multiple directions and sections, and take advantage of color Doppler blood flow imaging technology to visually display the distribution of blood flow in the heart and vessels [11, 12]. S, D, A, TA_{max}, and PI are common parameters of PUS, and cardiac condition can be reflected by measuring blood flow velocity of the umbilical venous duct [4]. Under normal physiological conditions, ductus venosus S-wave, D-wave,

Table 5

Diagnostic value of prenatal ultrasound parameters combined with serum APOC1 and LMNA levels for identifying chromosomal abnormalities among fetuses with CSA

Index	AUC	SE	Cut-off	95% CI	*p*-value
S	0.750	0.054	29.689	0.644–0.856	< 0.001
D	0.817	0.046	30.157	0.727–0.907	< 0.001
A	0.823	0.045	22.148	0.736–0.911	< 0.001
TA_{max}	0.802	0.048	19.057	0.707–0.896	< 0.001
PI	0.835	0.046	1.680	0.746–0.924	< 0.001
APOC1	0.796	0.062	31.172	0.675–0.917	< 0.001
LMNA	0.831	0.048	0.175	0.737–0.925	< 0.001
Combination	0.911	0.031	-	0.850–0.972	< 0.001

CSA – cardiac structural abnormality; AUC – area under the curve; SE – standard error; CI – confidence interval.
For other abbreviations, see Table 1.
Values are expressed as cm/s for S, D, A, and TA_{max}, and as fmol/mL for APOC1 and LMNA.

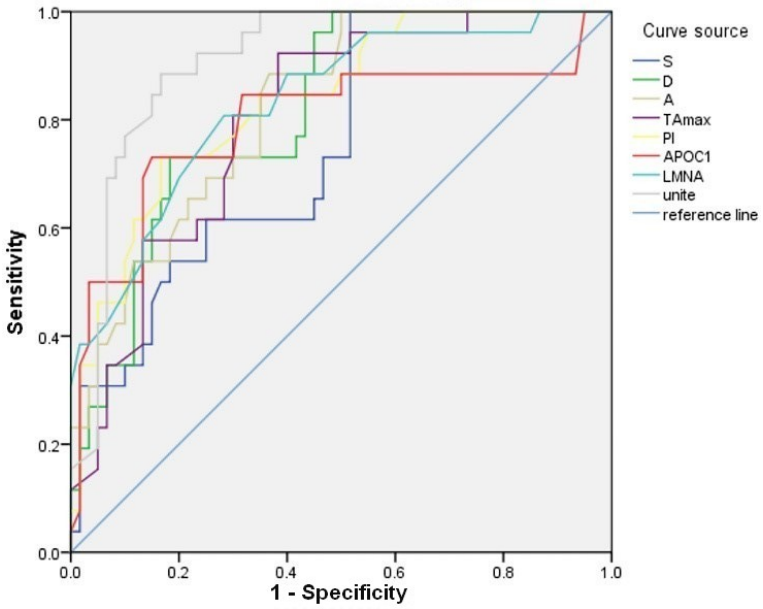


Fig. 2 – Receiver operating characteristic (ROC) curves of prenatal ultrasound parameters combined with serum APOC1 and LMNA levels for diagnosis of chromosomal abnormality.
For other abbreviations, see Table 1.

A-wave, and TA_{max} values show GA-related changes at 11–13+6 weeks of gestation. In contrast, PI for veins remains relatively stable during this gestational period [13]. In case of fetal CSA, the normal physiological needs cannot be met, resulting in hemodynamic abnormality and increased cardiac load [14]. Brickmann et al. [15] found that abnormal blood flow parameters of the umbilical venous duct were important indicators of fetal cardiovascular abnormalities, and that these parameters were most susceptible to right atrial structural abnormality. Chimoriya et al. [16] believe that fetal CSA would lead to changes in blood flow of the umbilical venous duct, mainly manifested as reduced blood flow velocity, and A-wave reversal or decline. In this study, S, D, A, and TA_{max} were lower, while PI was higher in SG than in CG, suggesting that PUS parameters are expected to be effective indicators for diagnosing fetal CSA. Because fetal cardiac anomalies are difficult to classify definitively by the end of the first trimester, the 11–13-week ultrasound findings in this study were considered screening results, and suspected cases were further confirmed by detailed FECG in the second trimester.

Peripheral blood screening for pregnancy-related markers is also an ideal method for prenatal examination. Proteomics can quantitatively analyze the expression of disease-related protein molecules, thereby guiding disease diagnosis and targeted therapy [17]. APOC1, an important apolipoprotein in plasma, can be implicated in plasma lipid transport. Increased APOC1 levels may reflect altered maternal-fetal lipid metabolism and inflammatory status, which may influence placental function and fetal cardiovascular development [5]. In the present study, APOC1 was negatively correlated with S, D, A, and TA_{max}, but positively correlated with PI, suggesting that increased APOC1 may be associated with impaired fetal hemodynamics. Research suggests that high serum APOC1 levels increase the risk of coronary heart disease and are closely associated with heart disease [18, 19]. It is inferred that serum APOC1 levels probably have a certain correlation with fetal CSA. In this study, serum APOC1 levels were higher in SG than in CG, indicating that serum APOC1 levels are elevated in PW carrying a fetus with CSA. However, its specific mechanism of action remains to be further verified. The results of Pearson correlation analysis showed that serum APOC1 levels were negatively correlated with S, D, A, and TA_{max}, but positively correlated with PI in PW carrying a fetus with CSA, demonstrating its association with fetal cardiac structure.

As a nucleoskeleton protein, LMNA plays an important role in the morphological development of the nuclear membrane. Its expression is induced during the differentiation of embryonic stem cells into neural cells and cardiomyocytes, thereby facilitating cardiomyocyte development and contributing to the pathogenesis of multiple cardiovascular diseases [20]. According to the results of this study, serum LMNA levels were lower in SG than in CG, suggesting that PW carrying a fetus with CSA exhibit decreased serum LMNA levels. LMNA is involved in nuclear lamina stability, chromatin organization, and cardiomyocyte differentiation. Decreased LMNA expression may disturb embryonic cardiovascular cell differentiation and normal cardiac morphogenesis [8, 21]. It is inferred that fetal CSA may be attributed to a structural disorder of the nuclear fiber layer induced by decreased LMNA expression, thereby inhibiting embryonic stem cell differentiation [8]. In addition, it was found that serum LMNA levels were positively correlated with S, D, A, and TA_{max}, and negatively correlated with PI, implying that serum LMNA levels are also closely linked to fetal CSA and involved with serum APOC1 in the process of cardiac structural changes. To further evaluate the diagnostic value of PUS parameters combined with serum APOC1 and LMNA levels for fetal CSA, ROC curve analysis was performed. For the diagnosis of fetal CSA, the results revealed that the AUCs for S, D, A, TA_{max}, PI, serum APOC1 levels, and serum LMNA levels, both alone and in combination, were 0.812, 0.798, 0.685, 0.846, 0.781, 0.784, 0.811, and 0.915, respectively. These findings further confirmed the high value of PUS parameters, combined with serum APOC1 and LMNA levels, in the diagnosis of fetal CSA.

In addition, chromosomes are an important player in fetal organ and functional development. CA can also lead to fetal cardiac developmental delay, also manifested as A-wave reversal or disappearance [22]. Following the diagnosis of fetal CSA, non-invasive deoxyribonucleic acid (DNA) or amniocentesis is often recommended to identify the etiology and the presence or absence of CA. However, non-invasive DNA testing is expensive, and amniocentesis is invasive and carries certain risks, so their widespread adoption and use as routine examinations are limited. In case of fetal CSA caused by CA, it is often necessary to terminate pregnancy by induction of labor to reduce the risk of neonatal malformations [23]. Therefore, it is particularly important to seek an effective screening method for CA. As revealed in this study, lowered S, D, A, TA_{max}, and serum LMNA levels, along with elevated PI and serum APOC1 levels, were detected in the abnormal group compared with those in the normal group, indicating that both PUS parameters and serum APOC1 and LMNA levels can serve as screening indicators for CA. Furthermore, AUCs of 0.750, 0.817, 0.823, 0.802, 0.835, 0.796, 0.831, and 0.911 corresponding to S, D, A, TA_{max}, PI, serum APOC1, and serum LMNA levels alone and the combination of all indicators were obtained for the diagnosis of CA. It can be seen that combining PUS parameters with serum APOC1 and LMNA levels yields a higher diagnostic value for CA.

Limitations of the study

This study has several limitations. First, this was a single-center observational study, and the sample size was based on consecutive enrollment rather than a prospective *a priori* sample-size calculation. Second, the diagnostic model requires further validation in larger multicenter cohorts.

Conclusion

Apolipoprotein C1 levels were high, and lamin A levels were low in pregnant women with fetal cardiac structural and chromosomal abnormalities, which were closely associated with prenatal ultrasound parameters. The combined assessment of these parameters exhibits a higher diagnostic value, making it a promising tool for the early screening of high-risk pregnancies.

Conflicts of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Tan CMJ, Lewandowski AJ.* The Transitional Heart: From Early Embryonic and Fetal Development to Neonatal Life. Fetal Diagn Ther 2020; 47(5): 373–86. DOI: 10.1159/000501906.
2. *Karim JN, Bradburn E, Roberts N, Papageorghiou AT; ACCEPTS study.* First-trimester ultrasound detection of fetal heart anomalies: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2022; 59(1): 11–25. DOI: 10.1002/uog.23740.
3. *Barris DM, Mikhno M, Kornblit M, Wang K, Duong S, Cohen J,* et al. Clinical utility of repeat fetal echocardiography in congenital heart disease. Ultrasound Obstet Gynecol 2023; 62(5): 695–700. DOI: 10.1002/uog.26240.
4. *Minnella GP, Crupano FM, Syngelaki A, Zidere V, Akolekar R, Nicolaides KH.* Diagnosis of major heart defects by routine first-trimester ultrasound examination: association with increased nuchal translucency, tricuspid regurgitation and abnormal flow in ductus venosus. Ultrasound Obstet Gynecol 2020; 55(5): 637–44. DOI: 10.1002/uog.21956.
5. *Rouland A, Masson D, Lagrost L, Vergès B, Gautier T, Bouillet B.* Role of apolipoprotein C1 in lipoprotein metabolism, atherosclerosis and diabetes: a systematic review. Cardiovasc Diabetol 2022; 21(1): 272. DOI: 10.1186/s12933-022-01703-5.
6. *Ling Y, Su J, Lin J, Wang S.* Screening of serum biomarkers of preeclampsia by proteomics combination with bioinformatics. Hypertens Pregnancy 2019; 38(3): 184–92. DOI: 10.1080/10641955.2019.1640246.
7. *Yin YN, Cao L, Wang J, Chen YL, Yang HO, Tan SB,* et al. Proteome profiling of early gestational plasma reveals novel biomarkers of congenital heart disease. EMBO Mol Med 2023; 15(12): e17745. DOI: 10.15252/emmm.202317745.
8. *Wang Y, Elsherbiny A, Kessler L, Cordero J, Shi H, Serke H,* et al. Lamin A/C-dependent chromatin architecture safeguards naïve pluripotency to prevent aberrant cardiovascular cell fate and function. Nat Commun 2022; 13(1): 6663. DOI: 10.1038/s41467-022-34366-7.
9. *Madazli R, Alıcı Davutoglu E, Alpay V, Kaymak D, Erenel H, Oztunc F.* Perinatal outcomes of pregnancies with prenatally diagnosed foetal congenital heart disease. J Obstet Gynaecol 2022; 42(5): 1079–84. DOI: 10.1080/01443615.2021.2000944.
10. *Zhao X, Shen Y, Kong D, Li W, Yao L, Li S,* et al. Correlation between types of ventricular septal defect and chromosomal abnormalities in low-risk non-invasive prenatal testing. Arch Gynecol Obstet 2024; 310(3): 1517–23. DOI: 10.1007/s00404-024-07566-3.
11. *Buijtendijk MF, Bet BB, Leeflang MM, Shah H, Reuvekamp T, Goring T,* et al. Diagnostic accuracy of ultrasound screening for fetal structural abnormalities during the first and second trimester of pregnancy in low-risk and unselected populations. Cochrane Database Syst Rev 2024; 5(5): CD014715. DOI: 10.1002/14651858.CD014715.pub2.
12. *Karim JN, Di Mascio D, Roberts N, Papageorghiou AT; ACCEPTS study.* Detection of non-cardiac fetal abnormalities on ultrasound at 11-14 weeks: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2024; 64(1): 15–27. DOI: 10.1002/uog.27649.
13. *Pruksanusak N, Kor-anantakul O, Suntharasaj T, Suwanrath C, Hanprasertpong T, Pranpanus S,* et al. A reference for ductus venosus blood flow at 11-13+6 weeks of gestation. Gynecol Obstet Invest 2014; 78(1): 22–5. DOI: 10.1159/000362273.
14. *Gregorovicova M, Lashkarinia SS, Yap CH, Tomek V, Sedmera D.* Hemodynamics During Development and Postnatal Life. Adv Exp Med Biol 2024; 1441: 201–26. DOI: 10.1007/978-3-031-44087-8_11. Erratum in: Adv Exp Med Biol 2024; 1441: C1–2. DOI: 10.1007/978-3-031-44087-8_68.
15. *Brickmann C, Zang FC, Klotz D, Kunze M, Lenz S, Hentschel R.* Emergency button cannula vs. umbilical catheter as neonatal emergency umbilical vein access - a randomized cross-over pilot study. J Perinat Med 2022; 51(1): 27–33. DOI: 10.1515/jpm-2022-0071.
16. *Chimoriya R, Awasthy N, Kumar G.* Temporary pacemaker implantation through umbilical vein in a low birth weight neonate with congenital complete heart block. Cardiol Young 2021; 31(10): 1687–9. DOI: 10.1017/S1047951121001220.
17. *Lim JH, Lim JM, Lee HM, Lee HJ, Kwak DW, Han YJ,* et al. Systematic Proteome Profiling of Maternal Plasma for Development of Preeclampsia Biomarkers. Mol Cell Proteomics 2024; 23(9): 100826. DOI: 10.1016/j.mcpro.2024.100826.
18. *Pinto-Souza CC, Kaihara JNS, Nunes PR, Mastella MH, Rossini BC, Cavecci-Mendonça B,* et al. Different Proteomic Profiles Regarding Antihypertensive Therapy in Preeclampsia Pregnant. Int J Mol Sci 2024; 25(16): 8738. DOI: 10.3390/ijms25168738.
19. *Lemesle G, Chouraki V, de Groote P, Turkieh A, Beseme O, Drobecq H,* et al. Apolipoprotein Proteomic Profiling for the Prediction of Cardiovascular Death in Patients with Heart Failure. Proteomics Clin Appl 2020; 14(6): e2000035. DOI: 10.1002/prca.202000035.
20. *Chen L, Xin Y, Wu Q, Wang Y, Zhang Y, Xue J,* et al. Maternal serum Lamin A is a potential biomarker that can predict adverse pregnancy outcomes. EBioMedicine 2022; 77: 103932. DOI: 10.1016/j.ebiom.2022.103932.
21. *Tiwari V, Alam MJ, Bhatia M, Navya M, Banerjee SK.* The structure and function of lamin A/C: Special focus on cardiomyopathy and therapeutic interventions. Life Sci 2024; 341: 122489. DOI: 10.1016/j.lfs.2024.122489.
22. *Ji X, Xia Y, Zhang H, Wang H, Shi R, Han Z.* Study on the application of ultrasonography in the diagnosis of fetal cardiac structural abnormalities and the relationship between fetal cardiac structural abnormalities with chromosome abnormalities in early pregnancy. Ann Transl Med 2021; 9(24): 1790. DOI: 10.21037/atm-21-6492.
23. *Benchikh S, Bousfiha A, Razoki L, Aboulfaraj J, Zarouf L, Elbakay C,* et al. Chromosome Abnormalities Related to Reproductive and Sexual Development Disorders: A 5-Year Retrospective Study. Biomed Res Int 2021; 2021: 8893467. DOI: 10.1155/2021/8893467.

Received on January 19, 2026
Revised on May 3, 2026
Revised on June 7, 2026
Accepted on June 24, 2026
Online First July 2026

ORIGINAL ARTICLE

UDC: 616.6-022.1-07-093
DOI: https://doi.org/10.2298/VSP260219042H

Analysis of the diagnostic value of fluorescence rapid on-site evaluation compared with bacterial culture in urinary tract infection: a retrospective study

Analiza dijagnostičke vrednosti brze procene na licu mesta fluorescentnom mikroskopijom u poređenju sa bakterijskom kulturom kod infekcije urinarnog trakta: retrospektivna studija

Feng Helian, Jianmei Song, Xu Wang, Jiaqi Li, Guoli Li, Xiaoyang Li

Capital Medical University, Beijing Chaoyang Hospital, Laboratory Department, Beijing, China

Abstract

Background/Aim. Prompt and precise detection of urinary tract infections (UTIs) is crucial for timely antibiotic administration and improved treatment outcomes. The aim of the study was to analyze the diagnostic efficacy of fluorescence rapid on-site evaluation (ROSE) compared to bacterial culture (BC) and to evaluate its clinical applicability for identifying UTIs. **Methods.** This retrospective study included 240 inpatients with clinically indicated UTIs evaluated between October 2024 and March 2025. All urine specimens were analyzed using both fluorescence ROSE testing and BC, with the latter serving as the reference standard. Diagnostic metrics–such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy–were calculated. Receiver operating characteristic (ROC) curve analysis was performed to determine the area under the curve (AUC). **Results.** BC revealed 179 (74.58%) positive cases. Fluorescence ROSE demonstrated a sensitivity of 95.53%, specificity of 85.25%, PPV of 95.00%, and NPV of 86.67%, resulting in an overall diagnostic accuracy of 92.92%. The ROC analysis produced an AUC of 0.90 (95% confidence interval: 0.86–0.94). The misclassification rate was minimal at 7.08%. *Escherichia coli* was the primary pathogen, constituting 54.19% of isolates. Furthermore, fluorescent ROSE significantly decreased diagnostic turnaround time compared to traditional culture. **Conclusion.** Fluorescence ROSE has improved diagnostic precision and fast detection capabilities for UTIs. As an adjunct to BC, it may improve early identification and facilitate rapid antibiotic therapy, especially in clinical settings requiring a swift response.

Keywords:
bacteriological techniques; diagnosis; microscopy, fluorescence; urinary tract infections.

Apstrakt

Uvod/Cilj. Brzo i precizno otkrivanje infekcija urinarnog trakta (*urinary tract infections* – UTI) ključno je za blagovremenu primenu antibiotika i poboljšanje ishoda lečenja. Cilj rada bio je da se analizira dijagnostička efikasnost brze procene na licu mesta (*rapid on-site evaluation* – ROSE) fluorescentnom mikroskopijom, u poređenju sa bakterijskom kulturom (BK) i da se proceni njena klinička primenljivost za identifikaciju UTI. **Metode.** Retrospektivnom studijom obuhvaćeno je 240 hospitalizovanih bolesnika, kojima je od oktobra 2024. do marta 2025. godine klinički utvrđena UTI. Svi uzorci urina analizirani su metodom ROSE fluorescentnom mikroskopijom i BK (koja je služila kao referentni standard). Izračunati su dijagnostički pokazatelji, kao što su osetljivost, specifičnost, pozitivna prediktivna vrednost (PPV), negativna prediktivna vrednost (NPV) i ukupna tačnost. Da bi se utvrdila *area under the curve* (AUC), sprovedena je analiza *receiver operating characteristic* – ROC krive. **Rezultati**. Korišćenjem BK, otkriveno je 179 (74,58%) pozitivnih slučajeva. Metoda ROSE fluorescentnom mikroskopijom pokazala je osetljivost 95,53%, specifičnost 85,25%, PPV 95,00% i NPV 86,67%, uz ukupnu dijagnostičku tačnost od 92,92%. Analizom ROC krive dobijena je AUC 0,90 (95% *confidence interval*: 0,86–0,94). Stopa pogrešne klasifikacije bila je minimalna i iznosila je 7,08%. *Escherichia coli* bila je primarni patogen, čineći 54,19% izolata. Pored toga, korišćenjem metode ROSE fluorescentnom mikroskopijom značajno je skraćeno vreme dijagnostičke obrade u odnosu na tradicionalnu kulturu. **Zaključak.** Korišćenjem metode ROSE fluorescentnom mikroskopijom, dijagnostička preciznost i mogućnosti brzog otkrivanja UTI bili su poboljšani. Kao dodatak BK, ova metoda može

Correspondence to: XiaoYang Li, Capital Medical University, Beijing Chaoyang Hospital, Laboratory Department, 100 041 Beijing, China. E-mail: xiaoyangli4321@gmail.com

poboljšati ranu identifikaciju i olakšati brzu antibiotsku terapiju, posebno u kliničkim uslovima, gde je potreban brz odgovor.

Ključne reči:
bakteriološke tehnike; dijagnoza; mikroskopja, fluorescencija; urinarni trakt, infekcije.

Introduction

Urinary tract infection (UTI) is a common, clinically encountered infectious disease of the urinary system. The timely and accurate diagnosis of UTI plays a crucial role in the rational selection of antibiotics, improvement of patient prognosis, reduction of complications, prevention of bacterial resistance, and other related aspects [1].

Rapid diagnosis enabled by accurate laboratory tests is critical for improving clinical outcomes in infectious diseases. The diagnostic workflow for infectious diseases integrates clinical history, physical examination, radiographic findings, routine laboratory data, and targeted microbiological/parasitological analyses [2].

Although traditional bacterial culture (BC) methods are recognized as the gold standard for diagnosing UTIs [3], they have multiple prominent limitations that restrict clinical application. First, the testing cycle is long, usually requiring 18–24 hrs of incubation and even longer for slow-growing pathogens, which delays the confirmation of diagnosis and the initiation of targeted treatment. Historically, the major drawback of conventional microbiological diagnostics has been the long turnaround time. Nevertheless, BC remains the undisputed gold standard against which all novel rapid assays are validated. While pathogen isolation *via* culture requires prolonged incubation, it remains the only method that enables complete serological, molecular, and biochemical identification of etiologic agents as well as antimicrobial susceptibility testing, which is irreplaceable for clinical treatment guidance. Second, the operation is relatively complex, involving inoculation, incubation, colony identification, and drug-sensitivity testing, which requires professional technical personnel and strict laboratory conditions [4, 5]. It cannot meet the needs of rapid diagnosis of acute infections: in cases of severe acute UTI (such as acute pyelonephritis with high fever), delays in culture results may lead to disease progression and severe complications [6]. Third, the method is susceptible to false-negative (FN) results. For instance, when the bacterial load in the urine sample (US) is low, and the pathogen is difficult to culture (such as some fastidious bacteria), this may lead to missed detection [7].

Microscopic examination—encompassing light, fluorescence, and electron microscopy—enables direct detection of microorganisms in clinical specimens and remains an irreplaceable rapid tool in microbiological diagnosis [8]. Common microscopic preparations include wet mounts, hanging-drop preparations, dark-field mounts, and stained smears. Wet mount techniques preserve microbial viability and allow real-time observation of leukocytes (indicating inflammation), erythrocytes, parasites, and motile bacteria. Non-specific stains, differential stains (e.g., Gram stain), and fluorescent dyes enhance visualization of pathogens. The Gram stain, despite moderate sensitivity, provides rapid morphological information about bacterial shape, arrangement, and cell wall properties. Fluorescent stains (non-antibody-based) have become increasingly valuable in rapid microbiological screening [9]. A major limitation of all manual microscopic methods is their high dependence on specialized expertise [10].

With the continuous advancement of medical technology, fluorescence rapid on-site evaluation (ROSE) has emerged as a new rapid detection technique, offering novel perspectives for the diagnosis of UTIs [11].

Compared with traditional chemical staining (e.g., Gram stain) and wet-mount microscopy, fluorescent ROSE provides higher signal intensity and contrast, reduces reliance on subjective microscopic judgment, shortens reading time, and lowers the requirement for extensive on-site expert experience. It enables more sensitive detection of low-burden pathogens and clearer distinction between microbial structures and cellular debris, thus improving differentiation between infection and contamination [12].

The aim of the study was to analyze diagnostic data of 240 clinically suspected UTI patients, comparing the diagnostic performance of fluorescence ROSE and BC in order to provide a basis for the rational application of fluorescence ROSE in clinical practice.

Methods

The study protocol was approved by the Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University, Beijing, China (No. 2452/UTI/08/2024, from October 12, 2024). This retrospective study collected and analyzed clinical data and inspection results from patients who were admitted to the hospital and underwent relevant inspections.

Research subjects

A total of 240 inpatients with clinically suspected UTI, admitted to our hospital from October 2, 2024, to March 31, 2025, were selected. USs were collected from these patients the morning after admission, and all samples were submitted for microbiological examination as specimens.Researchers could trace the entire case history of patients from the beginning of data collection onwards.

Inclusion and exclusion criteria

Inclusion criteria were as follows: patients with typical acute UTI symptoms, including frequent urination, urgency, dysuria, lower abdominal pain, and fever (body temperature ≥ 38.5 °C); routine urine examination demonstrating elevated white blood cells (WBC) count [≥ 10 cells *per* high-power

field (HPF)] or positive nitrite test; adult patients aged 18–80 years; patients with first-episode acute UTI or acute exacerbation of chronic UTI diagnosed with complete clinical data such as detailed medical history, physical examination results, laboratory test results, and treatment records; USs collected prior to the initiation of antibiotics (confirmed by clinical medication records); diagnostic basis consistent with the diagnostic criteria for UTI in the Guidelines for the Diagnosis and Treatment of Urinary Tract Infections (2023 Edition) [13].

Exclusion criteria were the following: patients with infections in other sites (such as respiratory tract infection, abdominal infection) or systemic infections (such as sepsis); patients who used antibiotics within 7 days before sampling; pregnant or lactating women; patients with severe heart, liver, kidney, or other organ dysfunction or immune system diseases (such as rheumatoid arthritis, acquired immune deficiency syndrome – AIDS); patients with urinary tract anatomical abnormalities (such as hydronephrosis, ureteral stenosis) or recent urinary tract surgery (within 1 month); patients with chronic asymptomatic bacteriuria without acute infection symptoms; USs with obvious contamination (such as visible turbidity, foreign bodies) or improper collection (such as non-midstream urine).

Research methods

All USs underwent fluorescence ROSE testing and microbial culture, with the latter serving as the gold standard for evaluating the diagnostic performance of fluorescence ROSE. Each US was divided into two parts: one part was used for fluorescence ROSE detection and the other for microbial culture, to compare the diagnostic indicators of the two methods.

Fluorescence rapid on-site evaluation technique

The techniques for collecting and analyzing USs were performed as follows. First, the collected midstream US was placed in a centrifuge and spun at 2,500 revolutions *per* minute (rpm) for 10 min. Next, a small amount of the precipitate was removed, spread evenly on a glass slide, and allowed to air-dry naturally. Finally, the sample was stained for 5 min using a commercial fluorescence staining kit.

Under a fluorescence microscope, an artificial intelligence (AI) algorithm automatically scans and identifies microorganisms by analyzing morphological features such as size, shape, and fluorescence intensity, enabling preliminary classification of common pathogens (e.g., bacilli vs. cocci, Gram-negative vs. Gram-positive-like patterns).

After AI identification, attending physicians with professional microbiology experience manually review all images. They confirm pathogen morphology and inflammatory cell infiltration, and distinguish true infection from sample contamination based on cell quantity, distribution, and background characteristics. The physicians also observe cell morphology, microbial morphology, and the presence of inflammatory cell infiltration to determine whether bacteria, fungi, or other pathogens are present. Fluorescence ROSE mainly provides preliminary morphological classification rather than definitive species-level identification. Final species confirmation and antimicrobial susceptibility testing still rely on conventional BC. The result is judged positive if specific fluorescent-labeled pathogens are detected and negative otherwise.

Bacterial culture

The USs were inoculated onto blood agar plates and China blue lactose agar media with an inoculation volume of 100 μL, followed by incubation at 35 °C, in a 5% CO_2 incubator for 18–24 hrs. Colony growth was then observed: if the colony count was $\geq 10^5$ colony-forming units (CFU)/mL, bacterial identification and antibiotic sensitivity testing were performed using an automatic microbial identification system (VITEK® 2 Compact system, bioMérieux, Marcy-l'Étoile, France). If the colony count was $< 10^5$ CFU/mL, the culture was considered negative (excluding contaminated colonies). BC results served as the gold standard for UTI diagnosis, which was defined as positive if pathogenic bacteria were identified and negative otherwise.

Observation indicators

Primary indicators were sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of fluorescence ROSE (with BC as the gold standard). Secondary indicators referred to the positive detection rates of the two methods and the detection times (from sample collection to result reporting) for fluorescence ROSE and BC.

Evaluation methods

Diagnostic performance metrics, including sensitivity, specificity, PPV, and NPV, were calculated using standard formulas: Sensitivity = true positive (TP) / [TP + FN] × 100%; specificity = true negative (TN) / [TN + false positive (FP)] × 100%; PPV = TP / (TP + FP) × 100%; NPV = TN / (TN + FN) × 100%; TP: fluorescence ROSE positive + BC positive; FP: fluorescence ROSE positive + BC negative; TN: fluorescence ROSE negative + BC negative; FN: fluorescence ROSE negative + BC positive.

Statistical analysis

Data were analyzed using SPSS Statistics version 27. Categorical variables were represented as frequencies and percentages and analyzed using the χ^2 test. Diagnostic metrics such as sensitivity, specificity, PPV, and NPV were derived from the contingency table, along with their respective 95% confidence intervals (CIs). The concordance among fluorescence ROSE and BC was measured using the Kappa statistic. A two-tailed p-value < 0.05 was deemed statistically noteworthy.

Results

Patient demographic and clinical characteristics

A total of 240 inpatients with clinically suspected UTI were included in this retrospective study (Table 1). The mean age was 56.8 ± 15.2 years (range: 18–80 years). Females accounted for 61.25% of the study population.

The most common presenting symptoms were urinary frequency/urgency (73.33%), dysuria (62.08%), and lower abdominal pain (42.50%). Fever (≥ 38 °C) was observed in 31.67% of patients. Regarding comorbidities, 32.50% had diabetes mellitus (DM) and 35.00% had hypertension. Indwelling urinary catheters were present in 23.75% of patients. Elevated urine WBCs (≥ 10 cells/HPF) were detected in 88.75% of cases, and nitrite positivity was observed in 55.83%.

Diagnostic performance and receiver operating characteristic curve analysis

Using BC as the reference standard, fluorescence ROSE correctly identified 171 TP and 52 TN cases, with 9 FPs and 8 FNs among the 240 patients (Table 2). Accordingly, fluorescence ROSE demonstrated a sensitivity of 95.53% (171/179) and a specificity of 85.25% (52/61). The PPV was 95.00%, while the NPV reached 86.67%. The overall diagnostic accuracy was 92.92% (223/240), indicating high reliability in detecting UTIs. Receiver operating characteristic (ROC) curve analysis produced an area under the curve (AUC) of 0.90 (95% CI: 0.86–0.94) (Figure 1). Furthermore, the Youden index was 0.81. Collectively, these results indicate that fluorescence ROSE exhibits high overall diagnostic performance according to established thresholds for clinical diagnostic tests.

Distribution of uropathogens identified by bacterial culture

Among the 179 (74.58%) culture-positive USs, *Escherichia coli* was the predominant pathogen, accounting for 54.19% of all isolates. This was followed by *Klebsiella* spp. (17.32%) and *Enterococcus* spp. (11.73%). Less frequently isolated organisms included *Proteus* spp. (6.70%) and *Pseudomonas aeruginosa* (5.03%), while other pathogens collectively represented 5.03% of cases (Table 3).

Overall, Gram-negative bacteria constituted the majority of isolates, highlighting their primary role in the etiology of UTI within the study population. The predominance of *Escherichia coli* aligns with well-established epidemiological patterns of UTIs and supports the external validity of the present findings.

Table 1

Baseline demographic and clinical characteristics of patients (n = 240)

Characteristic	Value
Age, years	56.8 ± 15.2
Age ≥ 65 years	81 (33.75)
Gender	
male	93 (38.75)
female	147 (61.25)
Body temperature ≥ 38 °C	76 (31.67)
Dysuria	149 (62.08)
Urinary frequency/urgency	176 (73.33)
Lower abdominal pain	102 (42.50)
Indwelling urinary catheter	57 (23.75)
Diabetes mellitus	78 (32.50)
Hypertension	84 (35.00)
Recurrent UTI history	67 (27.92)
Elevated urine WBC (≥ 10 cells/HPF)	213 (88.75)
Positive nitrite test	134 (55.83)

n – number of patients; UTI – urinary tract infection; WBC – white blood cells; HPF – high-power field.
Values are given as numbers (percentages) or mean ± standard deviation.

Table 2

Diagnostic performance of fluorescence ROSE compared with bacterial culture

Fluorescence ROSE	Bacterial culture		Total
	positive	negative	
Positive	171 (TP)	9 (FP)	180
Negative	8 (FN)	52 (TN)	60
Total	179	61	240

ROSE – rapid on-site evaluation; TP – true positive; FP – false positive; FN – false negative; TN – true negative. Values are given as numbers.

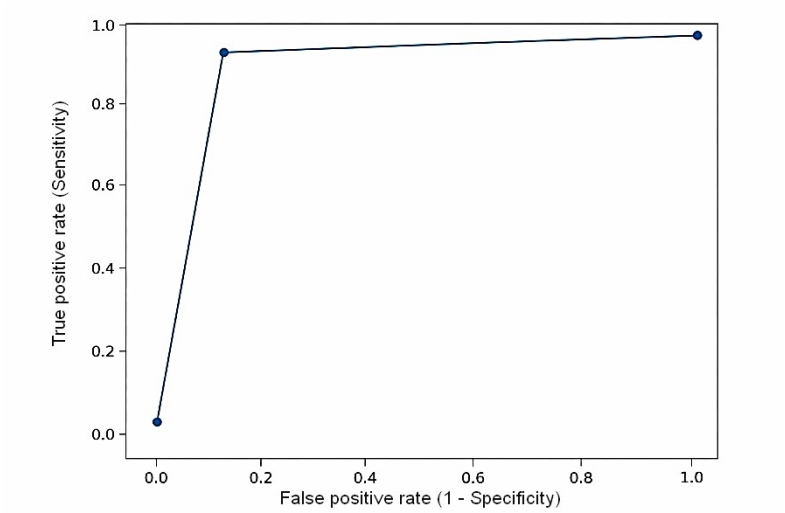


Fig. 1 – Receiver operating characteristic (ROC) curve for fluorescence rapid on-site evaluation (ROSE) in the diagnosis of urinary tract infection.

Table 3

Distribution of uropathogens identified by bacterial culture (n = 179)

Pathogens	Value
Escherichia coli	97 (54.19)
Klebsiella spp.	31 (17.32)
Enterococcus spp.	21 (11.73)
Proteus spp.	12 (6.70)
Pseudomonas aeruginosa	9 (5.03)
Other	9 (5.03)

n – number of patients.
All values are given as numbers (percentages).

False-positive and false-negative analysis

Among the 240 samples, 9 (5.00%) cases were FPs and 8 (3.33%) cases were FNs (Table 2), resulting in an overall misclassification rate of 7.08%.

Slide preparation lasted for 3.0 ± 0.3 min. The AI image reading requires approximately 4.0 ± 0.5 min.

Clinical utility

Fluorescence ROSE significantly reduced diagnostic turnaround time compared with conventional BC. The average processing time was approximately 7 min, whereas culture required approximately 24 hrs, representing a reduction of more than 95%.

The rapid availability of microbiological information may support earlier clinical decision-making and timely initiation of antimicrobial therapy. Fluorescence ROSE may therefore serve as a useful adjunct to conventional culture, particularly in clinical settings requiring prompt diagnosis.

Discussion

Fluorescence ROSE is a new type of rapid microbial detection technology that combines fluorescence staining, AI image recognition, and microscopic observation [14]. Its core principle is to centrifuge USs to enrich pathogens, perform specific fluorescence staining on the precipitates, and then use AI algorithms for automatic recognition and detection under a fluorescence microscope with physician review to confirm the presence of pathogens [15]. Fluorescence ROSE offers multiple technological advantageous features.

Although slide preparation and image analysis require only several minutes, the complete reporting workflow in clinical practice is generally completed within 2–4 hrs. This timeline is substantially shorter than the 18–24 hrs required for BC [16]. Furthermore, the high sensitivity of specific fluorescence labeling allows for the effective identification of pathogens even at low concentrations, thereby reducing the rate of missed detections [17]. Additionally, the operation is relatively simple and can be completed entirely in a laboratory [18]. It requires neither complex equipment nor long-term cultivation, allowing it to provide rapid, preliminary pathogen identification (such as distinguishing between bacteria and fungi) to help clinicians formulate initial, targeted treatment plans [19]. Consequently, in clinical practice, fluorescence ROSE is mainly used for rapid screening of acute infectious diseases, especially for scenarios requiring urgent diagnosis and treatment (such as emergency UTI patients and severe infection patients in intensive care units), to compensate for the limitations of BC in rapid diagnosis [20].

Comorbidities such as DM and indwelling urinary catheters are known to alter the urinary microenvironment and increase the risk of colonization, which may influence both microbiological culture yields and rapid test performance. Although these factors were recorded as baseline characteristics, the present study did not perform subgroup analyses due to limited sample size.

By analyzing USs from 240 clinically suspected acute UTI patients, this study found that fluorescence ROSE had high sensitivity (95.53%) and good specificity (85.25%) in detecting common UTI pathogens, which is consistent with the research results of Zhang et al. [20] and Trang et al. [21]. Wang et al. [22] applied fluorescence ROSE to the diagnosis of pulmonary infections and found that its sensitivity for pathogen detection was 93.1%, which is close to the sensitivity observed in this study, indicating stable performance across different infectious diseases. Mirham et al. [23] reported that slide preparation can be completed within 4 min, which is comparable to the 3.0 ± 0.3 min reported in this study. Chen et al. [24] noted that AI image reading requires approximately 4–5 min, consistent with the 4.0 ± 0.5 min observed in our experiment. These findings confirm the rapid detection capability of fluorescence ROSE. Fluorescence ROSE demonstrated excellent sensitivity and specificity, achieving an overall diagnostic accuracy of 92.92% and an AUC of 0.90, signifying exceptional discriminative capacity for distinguishing infected from non-infected patients. The Youden index of 0.81 indicates a robust equilibrium between sensitivity and specificity. The enhanced sensitivity suggests that fluorescence ROSE may be particularly effective as a rule-out test for patients with suspected UTIs, while its commendable specificity supports its use as a supplementary diagnostic tool alongside traditional BC.

The high sensitivity of fluorescence ROSE may be attributed to two main reasons. First, pathogens in urine samples are relatively homogeneous, facilitating morphological recognition under fluorescence microscopy [20]. Second, fluorescence staining enhances pathogen visualization, particularly when bacteria adhere to epithelial cells or are phagocytosed by leukocytes [25]. Regarding specificity, fluorescence ROSE uses direct immunofluorescence technology to bind specifically to microbial structures, generating characteristic fluorescence signals under specific wavelengths [26], which improves diagnostic precision. However, non-standardized sample collection or processing, such as contamination or suboptimal preparation, may lead to FP and FN results [27], which may partially explain the occurrence of FP cases observed in this study.

Urine BC remains the gold standard for UTI diagnosis, allowing pathogen identification and antimicrobial susceptibility testing [3]. In this study, BC identified 179 positive cases, of which 171 were consistent with fluorescence ROSE results, demonstrating good agreement ($\kappa = 0.803$). However, BC requires prolonged incubation, and empirical antibiotic therapy is often initiated before culture results are available, potentially contributing to inappropriate antibiotic use and antimicrobial resistance [28]. Fluorescence ROSE provides preliminary pathogen information within a short time frame, supporting earlier adjustment of empirical therapy [29].

A significant discovery of this investigation is the considerable decrease in diagnostic turnaround time. Fluorescence ROSE produced results in minutes compared to the roughly 24 hrs required for bacterial growth, indicating a reduction exceeding 95%. Timely pathogen identification may enable rapid initiation of antimicrobial therapy, enhance antibiotic stewardship, and reduce unnecessary exposure to broad-spectrum antibiotics. This swift diagnostic skill is especially crucial in emergency and inpatient settings where prompt treatment decisions are essential.

Compared with Wu et al. [30], fluorescence ROSE in this study demonstrated higher sensitivity but slightly lower specificity, possibly due to differences in staining protocols and AI recognition algorithms. Zhou et al. [31] emphasized that rapid diagnostic technologies must balance sensitivity and specificity, and further optimization of staining techniques and AI algorithms may improve specificity. The investigation has several strengths. Initially, it offers an extensive examination of fluorescence ROSE using various diagnostic metrics, including sensitivity, specificity, predictive values, ROC analysis, agreement testing, and misclassification evaluation [32]. Secondly, it mirrors actual clinical practice by examining hospitalized patients with probable UTIs [1]. These aspects augment the clinical relevance and translational significance of the results.

In this study, subgroup analysis by individual pathogens was not performed, so diagnostic differences among specific organisms remained unclear, in contrast to the differentiation observed in some other infections [33].

Limitation of the study

The study has several limitations. First, as a single-center retrospective study with a limited sample size (240 cases), it may introduce selection bias and limit generalizability. Second, subgroup analysis of individual pathogens was not performed. Third, special populations such as children, pregnant women, and patients with complicated infections were not included, and further validation is required [34]. Fourth, a detailed analysis of FP mechanisms was not conducted. FP results may be related to the detection of nonviable bacteria, cellular debris, and specimen contamination that does not yield growth in culture. Suppressed bacterial viability from prior antimicrobial exposure may also contribute to culture-negative yet fluorescence-positive findings. FN results were infrequent and may be associated with low bacterial load, uneven microbial distribution within the specimen, and technical factors during slide preparation and imaging. The low FN rate supports the utility of fluorescence ROSE as a rapid screening method, while positive findings should be interpreted alongside confirmatory culture testing. Fifth, the impacts of major comorbidities such as DM and indwelling urinary catheters on test performance were not quantitatively analyzed.

To address these limitations, our future research will focus on several key areas. First, we plan to expand the sam-

ple size and conduct multicenter prospective studies to evaluate pathogen-specific diagnostic performance, and include special patient populations. Second, we aim to evaluate pathogen-specific diagnostic performance and optimize AI recognition algorithms to reduce FP rates. Third, we will explore the integration of fluorescence ROSE with molecular diagnostic technologies, such as polymerase chain reaction (PCR), to further enhance diagnostic accuracy. Finally, future studies will assess diagnostic performance in clinical subgroups stratified by DM, urinary catheterization, and other key comorbidities.

Conclusion

Fluorescence ROSE has considerable potential as a rapid adjunctive diagnostic tool that might enhance the early detection and clinical management of UTIs. Fluorescence ROSE demonstrated superior effectiveness in diagnosing urinary tract infection, with elevated sensitivity, commendable specificity, and robust overall precision compared with bacterial culture. The area under the receiver operating characteristic curve validated the model's strong discriminative capacity. Fluorescence ROSE considerably decreased diagnostic turnaround time, enabling swift preliminary microbiological identification and aiding quicker clinical decision-making. While bacterial culture is crucial for definitive pathogen identification and antibiotic susceptibility testing, fluorescence ROSE serves as an effective and reliable screening technique. Integrating it into standard clinical processes may enhance diagnostic efficiency, facilitate the prompt initiation of antimicrobial treatment, and perhaps promote more judicious antibiotic use. Additional multicenter prospective trials are necessary to confirm its wider clinical usefulness.

Acknowledgement

We would like to express our gratitude to the higher officials for providing facilities.

Conflicts of interest

The authors declare no conflict of interest.

Funding

The project was supported by the Natural Science Foundation of Beijing, China (No. 7254352).

REFERENCES

1. *Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C.* Urinary Tract Infections: The Current Scenario and Future Prospects. Pathogens 2023; 12(4): 623. DOI: 10.3390/pathogens12040623.
2. *Mizusawa M.* Updates on Rapid Diagnostic Tests in Infectious Diseases. Mo Med 2020; 117(4): 328–37.
3. *Xu R, Deebel N, Casals R, Dutta R, Mirzazadeh M.* A New Gold Rush: A Review of Current and Developing Diagnostic Tools for Urinary Tract Infections. Diagnostics (Basel) 2021; 11(3): 479. DOI: 10.3390/diagnostics11030479.
4. *Yamin D, Uskoković V, Wakil AM, Goni MD, Shamsuddin SH, Mustafa FH,* et al. Current and Future Technologies for the Detection of Antibiotic-Resistant Bacteria. Diagnostics (Basel) 2023; 13(20): 3246. DOI: 10.3390/diagnostics13203246.
5. *Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D.* Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015; 28(1): 208–36. DOI: 10.1128/CMR.00110-14.
6. *Marantidis J, Sussman RD.* Unmet Needs in Complicated Urinary Tract Infections: Challenges, Recommendations and Emerging Treatment Pathways. Infect Drug Resist 2023; 16: 1391–405. DOI: 10.2147/IDR.S382617.
7. *Moreland RB, Brubaker L, Tinawi L, Wolfe AJ.* Rapid and accurate testing for urinary tract infection: new clothes for the emperor. Clin Microbiol Rev 2025; 38(1): e0012924. DOI: 10.1128/cmr.00129-24. Erratum in: Clin Microbiol Rev 2025; 38(2): e0010325. DOI: 10.1128/cmr.00103-25.
8. *Hazelton PR, Gelderblom HR.* Electron microscopy for rapid diagnosis of infectious agents in emergent situations. Emerg Infect Dis 2003; 9(3): 294–303. DOI: 10.3201/eid0903.020327.
9. *Demeke G, Fenta A, Dilnessa T.* Evaluation of Wet Mount and Concentration Techniques of Stool Examination for Intestinal Parasites Identification at Debre Markos Comprehensive Specialized Hospital, Ethiopia. Infect Drug Resist 2021; 14: 1357–62. DOI: 10.2147/IDR.S307683.
10. *Miswan N, Singham GV, Othman N.* Advantages and Limitations of Microscopy and Molecular Detections for Diagnosis of Soil-transmitted Helminths: An Overview. Helminthologia 2022; 59(4): 321–40. DOI: 10.2478/helm-2022-0034.
11. *Zhang L, Wang B, Yin G, Wang J, He M, Yang Y,* et al. Rapid Fluorescence Sensor Guided Detection of Urinary Tract Bacterial Infections. Int J Nanomedicine 2022; 17: 3723–33. DOI: 10.2147/IJN.S377575.
12. *Shang M, Guo J, Guo J.* Point-of-care testing of infectious diseases: Recent advances. Sens Diagn 2023; 2(5): 1123–44. DOI: 10.1039/d3sd00092c.
13. *Al Lawati H, Blair BM, Larnard J.* Urinary Tract Infections: Core Curriculum 2024. Am J Kidney Dis 2024; 83(1): 90–100. DOI: 10.1053/j.ajkd.2023.08.009.
14. *Tian Y, Zhang J, Rao X, Zhou B, Zhao X, He Y,* et al. AI-assisted rapid on-site evaluation ROSE of EUS-FNA cytopathology for pancreatic solid lesions: A two-stage deep learning approach. Endosc Ultrasound 2025; 14(6): 314–27. DOI: 10.1097/eus.0000000000000154.
15. *Kim HJ, Kim M, Zhang H, Kim HR, Jeon JW, Seo Y,* et al. Artificial Intelligence in Diagnostics: Enhancing Urine Test Accuracy Using a Mobile Phone-Based Reading System. Ann Lab Med 2025; 45(2): 178–84. DOI: 10.3343/alm.2024.0304.
16. *Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Mitic Culafic D,* et al. Antimicrobial Susceptibility Testing: A Comprehensive Review of Currently Used Methods. Antibiotics (Basel) 2022; 11(4): 427. DOI: 10.3390/antibiotics11040427.
17. *Yoon SA, Park SY, Cha Y, Gopala L, Lee MH.* Strategies of Detecting Bacteria Using Fluorescence-Based Dyes. Front Chem 2021; 9: 743923. DOI: 10.3389/fchem.2021.743923.
18. *Bourbeau PP, Ledeboer NA.* Automation in clinical microbiology. J Clin Microbiol 2013; 51(6): 1658–65. DOI: 10.1128/JCM.00301-13.
19. *Yang S, Rothman RE.* PCR-based diagnostics for infectious diseases: uses, limitations and future applications in acute-care

settings. Lancet Infect Dis 2004; 4(6): 337–48. DOI: 10.1016/S1473-3099(04)01044-8.
20. *Zhang L, Wang B, Yin G, Wang J, He M, Yang Y,* et al. Rapid Fluorescence Sensor Guided Detection of Urinary Tract Bacterial Infections. Int J Nanomedicine 2022; 17: 3723–33. DOI: 10.2147/IJN.S377575.
21. *Trang VAV, Truyen TTTT, Nguyen MT, Mai HP, Phan TC, Phan SH,* et al. Development of a novel risk score for diagnosing urinary tract infections: Integrating Sysmex UF-5000i urine fluorescence flow cytometry with urinalysis. PLoS One 2025; 20(5): e0323664. DOI: 10.1371/journal.pone.0323664.
22. *Wang X, Ding Q, Groleau RR, Wu L, Mao Y, Che F,* et al. Fluorescent Probes for Disease Diagnosis. Chem Rev 2024; 124(11): 7106–64. DOI: 10.1021/acs.chemrev.3c00776.
23. *Mirham L, Naugler C, Hayes M, Ismiil N, Belisle A, Sade S,* et al. Performance of residents using digital images versus glass slides on certification examination in anatomical pathology: a mixed methods pilot study. CMAJ Open 2016; 4(1): E88–94. DOI: 10.9778/cmajo.20140075.
24. *Chen Y, Wang Y, Wüstenberg T, Kizilcec RF, Fan Y, Li Y,* et al. Effects of generative artificial intelligence on cognitive effort and task performance: study protocol for a randomized controlled experiment among college students. Trials 2025; 26(1): 244. DOI: 10.1186/s13063-025-08950-3.
25. *Cseresnyes Z, Hassan MIA, Dahse HM, Voigt K, Figge MT.* Quantitative Impact of Cell Membrane Fluorescence Labeling on Phagocytosis Measurements in Confrontation Assays. Front Microbiol 2020; 11: 1193. DOI: 10.3389/fmicb.2020.01193.
26. *Gest AMM, Sahan AZ, Zhong Y, Lin W, Mehta S, Zhang J.* Molecular Spies in Action: Genetically Encoded Fluorescent Biosensors Light up Cellular Signals. Chem Rev 2024; 124(22): 12573–660. DOI: 10.1021/acs.chemrev.4c00293.
27. *Goumas G, Vlachothanasi EN, Fradelos EC, Mouliou DS.* Biosensors, Artificial Intelligence Biosensors, False Results and Novel Future Perspectives. Diagnostics (Basel) 2025; 15(8): 1037. DOI: 10.3390/diagnostics15081037.
28. *Allel K, Peters A, Furuya-Kanamori L, Spencer-Sandino M, Pitchforth E, Yakob L,* et al. Impact of inappropriate empirical antibiotic therapy on in-hospital mortality: a retrospective multicentre cohort study of patients with bloodstream infections in Chile, 2018-2022. BMJ Public Health 2024; 2(2): e001289. DOI: 10.1136/bmjph-2024-001289.
29. *Santonocito R, Avnet S, Tuccitto N, Musso N, Cavallaro A, Spinello M,* et al. Rapid detection of Staphylococcus aureus using a fluorescent PoC array for perioperative guidance for infection. Biosens Bioelectron 2026; 294: 118199. DOI: 10.1016/j.bios.2025.118199.
30. *Wu E, Bieniosek M, Wu Z, Thakkar N, Charville GW, Makky A,* et al. ROSIE: AI generation of multiplex immunofluorescence staining from histopathology images. Nat Commun 2025; 16(1): 7633. DOI: 10.1038/s41467-025-62346-0.
31. *Zhou J, Park S, Dong S, Tang X, Wei X.* Artificial intelligence-driven transformative applications in disease diagnosis technology. Med Rev (2021) 2025; 5(5): 353–77. DOI: 10.1515/mr-2024-0097.
32. *Umemneku Chikere CM, Wilson K, Graziadio S, Vale L, Allen AJ.* Diagnostic test evaluation methodology: A systematic review of methods employed to evaluate diagnostic tests in the absence of gold standard - An update. PLoS One 2019; 14(10): e0223832. DOI: 10.1371/journal.pone.0223832.
33. *Li Y, Li C, Fang Y, Zhang L, Ying X, Ren R,* et al. Comprehensive Analysis of Pathogen Diversity and Diagnostic Biomarkers in Patients with Suspected Pulmonary Tuberculosis Through Metagenomic Next-Generation Sequencing. Infect Drug Resist 2025; 18: 2215–27. DOI: 10.2147/IDR.S504587.
34. *Bridwell RE, Carius BM, Long B, Oliver JJ, Schmitz G.* Sepsis in Pregnancy: Recognition and Resuscitation. West J Emerg Med 2019; 20(5): 822–32. DOI: 10.5811/westjem.2019.6.43369.

Received on February 19, 2026
Revised on April 28, 2026
Revised on May 26, 2026
Accepted on June 10, 2026
Online First July 2026

ORIGINAL ARTICLE

UDC: 615.099:546.262.3-31]:616.152.11
DOI: https://doi.org/10.2298/VSP260608041J

Association between pure high anion gap metabolic acidosis and adverse outcomes in acute carbon monoxide poisoning: a preliminary retrospective cohort study

Povezanost čiste metaboličke acidoze sa visokim „anjonskim zjapom“ i nepovoljnih ishoda akutnog trovanja ugljen monoksidom: preliminarna retrospektivna kohortna studija

Ke Junjie*†, Wu Hao*, Yin Lin*, Zhu Decai*†

***Bengbu Medical University, Bengbu, Anhui, China; †Bozhou People's Hospital, Department of Emergency Medicine, Bozhou, Anhui, China**

Abstract

Background/Aim. Acute carbon monoxide poisoning (ACOP) is a medical emergency, which causes a lack of oxygen in the tissues. The aim of the study was to determine the clinical characteristics of different acid-base disorder phenotypes in ACOP patients, focusing on the association between pure high anion gap (AG) metabolic acidosis and adverse outcomes (AOs). **Methods**. This retrospective study included 124 ACOP patients classified into three phenotypes according to the acid-base analysis: Type 1 (pure high AG metabolic acidosis), Type 2 (mixed acid-base disorders), and Type 3 (other conditions). Clinical indicators and outcomes were compared across groups. Multivariable logistic regression identified independent risk factors for AOs. **Results**. Type 1 phenotype patients [22 (17.74%)] exhibited significantly lower acid-base balance parameters (pH, HCO_3^-, standard base excess) and higher metabolic stress markers (AG, lactate; all $p < 0.05$). They had higher rates of systemic inflammatory response syndrome (SIRS) (72.73% vs. 46.67% and 38.60%) and intensive care unit admission (45.45% vs. 17.78% and 19.30%) than the Type 2 and 3 groups (all $p < 0.05$). Multivariable analysis confirmed the Type 1 phenotype as an independent risk factor for both SIRS [odds ratio (OR): 6.36, 95% confidence interval (CI): 1.486–27.213] and intensive care unit admission (OR: 4.71, 95% CI: 1.102–20.151). **Conclusion**. This preliminary study identifies pure high AG metabolic acidosis as a strong predictor of AOs in ACOP. This readily available arterial blood gas parameter provides a novel perspective for early risk stratification in emergency settings, though its clinical utility requires validation in prospective studies.

Keywords:
acidosis; blood gas analysis; carbon monoxide poisoning; intensive care units; risk assessment; systemic inflammatory response syndrome.

Apstrakt

Uvod/Cilj. Akutno trovanje uglen-monoksidom (*acute carbon monoxide poisoning* – ACOP) je urgentno medicinsko stanje, koje izaziva nedostatak kiseonika u tkivima. Cilj rada bio je da se utvrde kliničke karakteristke različitih fenotipova acido-baznih poremećaja kod pacijenata koji su pretrpeli ACOP, sa fokusom na povezanost između čiste metaboličke acidoze sa visokim „anjonskim zjapom“ (*anion gap* – AG) i nepovoljnih ishoda. **Metode.** Ova retrospektivna studija obuhvatila je 124 pacijenta koja su pretrpela ACOP, svrstana prema rezultatima analiza acido-baznog statusa u tri tipa fenotipa: tip 1 (čista metabolička acidoza sa visokim AG), tip 2 (mešoviti acido-bazni poremećaji) i tip 3 (ostala stanja). Upoređivani su klinički pokazatelji i ishodi između grupa. Za identifikaciju nezavisnih faktora rizika od nastanka nepovoljnih ishoda korišćena je multivarijabilna logistička regresija. **Rezultati.** Pacijenti fenotipa tip 1 [22, (17,74%)] imali su značajno niže vrednosti parametara acido-baznog statusa (pH, HCO_3^-, standardni bazni eksces) i više vrednosti markera metaboličkog stresa (AG, laktat; svi $p < 0,05$). Imali su veću učestalost sindroma sistemskog inflamacijskog odgovora (*systemic inflammatory response syndrome* – SIRS) (72,73% vs. 46,67% i 38,60%, redom) i prijema u jedinicu intenzivne nege (45,45% vs. 17,78% i 19,30%, redom) u odnosu na grupe tipa 2 i tipa 3 (svi $p < 0,05$). Multivarijabilnom analizom potvrđen je fenotip tipa 1 kao nezavisan faktor rizika kako za nastanak SIRS [*odds ratio* (OR): 6,36, 95% *confidence interval* (CI): 1,486–27,213], tako i za prijem u jedinicu intenzivne nege (OR: 4,71, 95% CI: 1,102–20,151). **Zaključak.** Ovom

Correspondence to: Zhu Decai, Bozhou People's Hospital, 616 Duzhong Road, Bozhou 236 800, Anhui, China. E-mail: zhudcgn@163.com

preliminarnom studijom identifikovana je čista metabolička acidoza sa visokim AG kao snažan prediktor nepovoljnih ishoda kod ACOP. Kao lako dostupan parametar iz analize gasova arterijske krvi, on u hitnim situacijama pruža novu mogućnost za ranu stratifikaciju rizika u urgentnim stanjima, mada njegova klinička primena zahteva validaciju u prospektivnim studijama.

Ključne reči:
acidoza; krv, analiza gasova; trovanje ugljenmonoksidom; intenzivna nega, odeljenja; rizik, procena; sindrom sistemske zapaljenske reakcije.

Introduction

Acute carbon monoxide (CO) poisoning (ACOP) represents a significant global public health challenge, characterized by persistently high morbidity and mortality rates. ACOP typically results from exposure to CO generated by the incomplete combustion of carbon-containing fuels in enclosed or poorly ventilated spaces. Common sources include malfunctioning domestic gas heaters, coal stoves, charcoal grills, portable generators, vehicle exhaust in garages, and occupational exposure in industrial settings [1–2]. The 2021 Global Burden of Disease Study reported approximately 28,900 deaths globally attributable to unintentional ACOP that year, with significant regional variations in mortality highlighting the ongoing severity of the situation and the urgent need for effective control strategies [3]. The pathophysiological core of ACOP is tissue hypoxia. CO not only reduces the blood's oxygen-carrying capacity by forming carboxyhemoglobin (COHb) but also directly inhibits the mitochondrial respiratory chain, triggering oxidative stress (OS) and energy metabolism dysfunction, ultimately leading to internal environment disturbances [4–6]. In this context, arterial blood gas (ABG) analysis is a key tool for assessing acid-base balance, with parameters such as pH, bicarbonate, and the anion gap (AG) becoming crucial for determining the prognosis of critically ill patients [7–8].

High AG metabolic acidosis (MA) is a common metabolic disturbance in ACOP, primarily due to lactate accumulation from tissue hypoxia [9]. However, current research has predominantly focused on evaluating the overall acid-base status or the prognostic value of individual biochemical markers [10, 11], lacking a refined differentiation of acid-base disorder phenotypes. A key knowledge gap exists regarding whether there are essential differences in clinical characteristics and prognostic significance between pure high AG MA and mixed acid-base disorders [12, 13]. Although the Glasgow Coma Scale (GCS) score and COHb level are classic indicators for assessing severity, it remains to be elucidated whether the specific phenotype of pure high AG MA remains an independent predictor of adverse outcomes (AOs) after adjusting for these known risk factors [14, 15]. This limitation results in a lack of precise evidence in current guidelines for individualized risk stratification and intervention based on different metabolic phenotypes [4].

To address this gap, this study aims to employ the clinically commonly used three-step method for analyzing acid-base disorders to finely classify the acid-base status of ACOP patients at admission. It will focus specifically on pure high AG MA to clarify its clinical characteristics and its association with AOs. The study hypothesizes that in ACOP patients, pure high AG MA, compared to mixed acid-base disorders, can more independently and strongly predict the development of systemic inflammatory response syndrome (SIRS) and the need for intensive care unit (ICU) admission, thereby providing a novel perspective for early risk stratification that goes beyond traditional indicators.

Therefore, the aim of this study was to generate evidence to improve the identification of critically ill ACOP patients and guide precise management.

Methods

Study design and participants

This single-center retrospective cohort study was approved by the Ethics Committee of Bozhou People's Hospital, Bozhou, China (No. BoYiLunShen 263, from November 03, 2025) and conducted in accordance with the Declaration of Helsinki and relevant ethical guidelines. Consecutive adult patients with ACOP admitted to the Emergency Department of our Hospital between December 2022 and December 2024 were enrolled.

Inclusion criteria were as follows: age ≥ 18 years; confirmed ACOP diagnosis based on a clear history of CO exposure, clinical symptoms, and laboratory tests; COHb level > 5% at admission; and availability of complete ABG analysis, electrolyte panel, lactate measurement, clinical data, laboratory results, and follow-up outcomes obtained immediately upon admission.

Exclusion criteria were: pre-existing chronic organ dysfunction (e.g., chronic heart failure, chronic renal failure, cirrhosis), chronic metabolic diseases (e.g., diabetic ketoacidosis, renal tubular acidosis), or autoimmune diseases; prehospital treatments that could significantly alter acid-base balance or laboratory parameters (e.g., high-flow oxygen therapy, mechanical ventilation, fluid resuscitation for acid-base correction); concomitant conditions such as other toxin poisonings, major trauma, acute infections, or recent surgery, and incomplete clinical, laboratory, or outcome data precluding acid-base phenotyping or statistical analysis.

A final cohort of 124 patients was included.

Acid-base disorder phenotyping

Acid-base disorder phenotyping was performed *via* a standardized three-step physiological algorithm using admission pre-oxygenation ABG analysis and electrolyte panel data to characterize acid-base patterns that support early, rapid emergency decision-making [16, 17]. First, the primary acid-base disorder has been determined based on pH, partial pres-

sure of arterial carbon dioxide ($PaCO_2$), and hydrogen carbonate (HCO_3^-). Subsequently, AG is calculated as AG = $Na^+ + K^+ - Cl^- - HCO_3^-$, with AG > 16 mmol/L defining high-AG MA. Finally, the adequacy of respiratory compensation is assessed using Winter's formula. If the measured $PaCO_2$ fell within the range of the predicted $PaCO_2 \pm 2$ mmHg, respiratory compensation was deemed appropriate, and the case was classified as Type 1 (pure high AG MA). If the measured value fell outside this range, it indicated the presence of a mixed respiratory component, and the case was classified as Type 2 (mixed disorder). Accordingly, the final classification was: Type 1 (pure high AG MA) – elevated AG with appropriate respiratory compensation; Type 2 (mixed disorder) – presence of any two or more primary acid-base disorders; Type 3 (other) – all other simple disorders and cases without significant acid-base derangement, excluding Type 1. To ensure objectivity and reliability, all phenotyping results were independently and blindly assessed by two clinicians. Any discrepancies were resolved by a third senior physician for final adjudication. This classification method adheres to established physiological principles [18, 19].

Data collection and outcome definition

Data were extracted from the hospital electronic medical records and were classified into several categories: baseline characteristics, clinical and laboratory parameters, and clinical outcomes. Baseline characteristics included age, sex, height, weight, smoking history, alcohol use, and comorbidities (e.g., hypertension, coronary artery disease, and diabetes mellitus). Clinical and laboratory parameters on admission consisted of the following: vital signs (temperature, heart rate, respiratory rate, blood pressure); GCS score (calculated as the average of independent assessments by two emergency physicians); ABG parameters (pH, $PaCO_2$, HCO_3^-); electrolytes (Na^+, Cl^-, K^+); AG, lactate, COHb, complete blood count, liver and kidney function tests; and myocardial injury, which was defined as elevated cardiac troponin I (cTnI ≥ 0.05 ng/mL). The final category included clinical outcomes. Primary outcomes were the occurrence of SIRS and the need for ICU admission. SIRS was diagnosed when at least two of the following criteria were met [20]: temperature > 38 °C or < 36 °C; heart rate > 90 beats/min; respiratory rate > 20 breaths/min or $PaCO_2$ < 32 mmHg; white blood cell count > 12×10^9/L or < 4×10^9/L, or > 10% immature bands. SIRS was monitored continuously during the first 6 hrs of hospitalization, and its occurrence was recorded as a binary outcome. ICU admission was defined as transfer to the ICU for intensive monitoring and treatment due to critical illness within 6 hrs of emergency department presentation.

Treatment protocol

All patients received uniform institutional care: high-flow oxygen (10–15 L/min) immediately upon arrival, followed by hyperbaric oxygen therapy as routine standard care for all confirmed ACOP cases. No patient received bicarbonate or alkalinizing agents to correct acidosis. Mechanical ventilation and vasopressors were used only for standard indications. Treatment modality was identical across all phenotype groups and was not analyzed as a covariate.

Statistical analysis

As an exploratory retrospective cohort study analyzing associations between acid-base phenotypes and outcomes in ACOP, the sample size was determined by the consecutive eligible cases available during the study period. We acknowledged that the sample size, particularly for the Type 1 subgroup, might limit statistical power. A *post-hoc* power analysis using Demidenko's formula [21] was performed to contextualize the findings. For the primary outcome (ICU admission), based on an odds ratio (OR) of 3.60 from univariate analysis, an alpha of 0.05, and 80% power, the calculated required sample size was approximately 180. With 124 included patients, the achieved power was approximately 64%. For the secondary outcome (SIRS), the required sample size was approximately 128, corresponding to an achieved power of 79%. Analyses were performed using SPSS 26.0. Normally distributed continuous data are presented as mean ± standard deviation and compared using one-way analysis of variance – ANOVA (with *post-hoc* testing if variances were equal); non-normally distributed data are presented as median (first quartile – Q1, third quartile – Q3) and compared using the Kruskal-Wallis *H* test. Categorical data are presented as numbers (percentages) and compared using the chi-square (χ^2) or Fisher's exact test. Correlations were assessed using Spearman's rank correlation. Variables with $p < 0.1$ in univariate logistic regression were entered into multivariable logistic regression models (using the stepwise method; entry $\alpha = 0.05$; removal $\alpha = 0.10$) to identify independent risk factors. A two-sided $p < 0.05$ was considered statistically significant.

Results

Comparison of baseline characteristics and clinical indicators across the three groups

The 124 ACOP patients were classified into three acid-base phenotype groups: Type 1 – pure high AG MA [22 (17.74%)], Type 2 – mixed acid-base disorders [45 (36.29%)], and Type 3 – other conditions [57 (45.97%)]. No statistically significant differences were observed among the three groups regarding age, sex, BMI, vital signs, or baseline laboratory indicators (all $p > 0.05$), indicating comparability at baseline.

Significant differences were found among the groups in acid-base balance, metabolic, and inflammatory markers (all $p < 0.05$). Specifically, patients with the Type 1 phenotype exhibited characteristic features of severe MA with a high AG: they had the lowest pH and HCO_3^- levels, and the highest AG values (e.g., pH: Type 1: 7.33 vs. Type 2: 7.39 vs. Type 3: 7.41; AG: Type 1: 25.20 mmol/L vs. Type 2: 20.99 mmol/L vs. Type 3: 16.78 mmol/L). Concurrently, lactate levels were significantly higher in the Type 1 group com-

pared to the other two groups (median: 5.20 mmol/L vs. 3.80 mmol/L vs. 2.20 mmol/L, respectively) (Table 1).

Analysis of clinical outcomes revealed that the Type 1 phenotype group had the highest incidence of adverse events. Both the incidence of SIRS (72.73%) and the rate of ICU admission (45.45%) were significantly higher in the Type 1 group compared to the Type 2 (SIRS: 46.67%; ICU admission: 17.78%) and Type 3 groups (SIRS: 38.60%; ICU admission: 19.30%) (intergroup comparisons, all $p < 0.05$) (Table 1).

Correlation analysis of major clinical indicators

Spearman correlation analysis revealed significant associations among key clinical indicators (Table 2). The Type 1

Table 1

Comparison of baseline characteristics and clinical indicators among ACOP patients with different acid-base phenotypes

Variable	Reference ranges	Type 1 (n = 22)	Type 2 (n = 45)	Type 3 (n = 57)	*p*-value
Age, years	–	55.0 (44.5, 71.8)	58.0 (50.0, 70.0)	55.0 (46.0, 68.0)	0.741
Gender					
male	–	7 (31.80)	22 (48.90)	30 (52.60)	0.246
BMI, kg/m²	–	23.95 (22.98, 26.53)	23.53 (21.48, 26.84)	24.22 (22.04, 27.04)	0.646
Temperature, °C	36.0–37.4	36.50 (36.50, 36.60)	36.60 (36.40, 36.80)	36.5 (36.50, 36.60)	0.585
Heart rate, bpm	60.0–100.0	95.00 (86.00, 104.50)	82.00 (76.00, 108.00)	85.00 (78.00, 98.00)	0.284
Respiratory rate, breaths/min	12.0–20.0	19.00 (16.30, 20.00)	18.00 (16.00, 21.00)	18.00 (15.00, 20.00)	0.622
Systolic BP, mmHg	90.0–139.0	118.00 (104.50, 129.50)	129.00 (118.00, 144.00)	127.00 (116.00, 139.00)	0.081
Hypertension	–	6 (27.30)	14 (31.10)	15 (26.30)	0.862
Diabetes mellitus	–	3 (13.60)	3 (6.70)	6 (10.50)	0.635
GCS score	–	11.00 (8.00, 13.00)	12.00 (8.00, 14.00)	13.00 (9.00, 15.00)	0.158
COHb, %	–	28.40 (20.20, 40.70)	21.20 (13.20, 34.80)	27.50 (14.30, 33.40)	0.352
pH	7.35–7.45	7.33 (7.31, 7.36)	7.39 (7.34, 7.43)	7.41 (7.38, 7.44)	< 0.001
HCO_3^-, mmol/L	22.00–27.00	15.45 (14.10, 17.20)	19.50 (16.40, 23.50)	21.10 (20.00, 23.00)	< 0.001
AG, mmol/L	8.00–16.00	25.20 (23.08, 26.46)	20.99 (18.67, 25.18)	16.78 (15.44, 18.37)	< 0.001
Lactate, mmol/L	0.50–2.20	5.20 (2.92, 8.38)	3.80 (2.00, 5.50)	2.20 (1.70, 3.30)	< 0.001
WBC count, $\times 10^9$/L	4.00–10.00	14.12 (10.94, 21.23)	11.74 (8.97, 15.81)	10.43 (8.21, 13.55)	0.030
Hemoglobin, g/L	130.0–175.0	143.50 (124.00, 154.00)	136.00 (120.00, 155.00)	133.00 (125.00, 144.00)	0.563
Creatinine, μmol/L	57.0–97.0	56.60 (47.70, 79.50)	65.30 (48.90, 94.10)	59.40 (48.30, 69.30)	0.391
ALT, U/L	10.0–40.0	25.00 (20.00, 40.50)	30.00 (20.00, 47.00)	21.00 (17.00, 33.00)	0.050
Albumin, g/L	40.0–55.0	45.90 (40.80, 48.00)	43.90 (39.90, 45.90)	41.50 (39.30, 45.30)	0.048
Myocardial injury	–	14 (63.60)	20 (44.40)	22 (38.60)	0.133
SIRS	–	16 (72.73)	21 (46.67)	22 (38.60)	0.024
ICU admission	–	10 (45.45)	8 (17.78)	11 (19.30)	0.026

ACOP – acute carbon monoxide poisoning; n – number; BMI – body mass index; bpm – beats *per* minute; BP – blood pressure; GCS – Glasgow Coma Scale; COHb – carboxyhemoglobin; AG – anion gap; WBC – white blood cell; ALT – alanine aminotransferase; SIRS – systemic inflammatory response syndrome; ICU – intensive care unit.

***Note*: Data are presented as median (first quartile, third quartile) or as numbers (percentages). Comparisons among groups were performed using the Kruskal-Wallis *H* test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. For detailed definitions of Types 1–3, see the Methods section.**

Table 2

Spearman correlation coefficient matrix of major clinical indicators and outcomes in patients with ACOP

Variable	SIRS	Type 1	ICU admission	GCS Score	COHb	Lactate
SIRS	–					
Type 1	0.231*	–				
ICU admission	0.237**	0.241**	–			
GCS score	-0.416**	-0.127	-0.506**	–		
COHb	0.082	0.117	0.653**	-0.428**	–	
Lactate	0.484**	0.280**	0.423**	-0.467**	0.478**	–

ACOP – acute carbon monoxide poisoning; SIRS – systemic inflammatory response syndrome; ICU – intensive care unit; GCS – Glasgow Coma Scale; COHb – carboxyhemoglobin; n – number.

***Note*: The data in the table represent Spearman correlation coefficients (ρ). *$p < 0.05$, ** $p < 0.01$ (two-tailed test). For brevity, sample size (n = 124) and non-significant *p*-values are not shown in the table. "Type 1" refers to the pure high anion gap metabolic acidosis phenotype.**

acid-base phenotype (pure high AG MA) showed significant positive correlations with the occurrence of SIRS (ρ = 0.231, $p < 0.05$), ICU admission (ρ = 0.241, $p < 0.01$), and lactate levels (ρ = 0.280, $p < 0.01$, more precise: $p = 0.002$). This indicates a significant association between this phenotype and systemic inflammatory response, critical outcomes, and the degree of tissue hypoxia. However, no significant correlation was found between the Type 1 phenotype and the GCS score (ρ = -0.127, $p = 0.162$) or COHb levels (ρ = 0.117, $p = 0.199$), suggesting that the critical nature of the Type 1 phenotype appears to be independent of traditional indicators of consciousness impairment and poisoning severity. In contrast, traditional critical illness indicators such as lactate, GCS score, and COHb showed stronger correlations with each other and with both SIRS and ICU admission (all $p < 0.001$).

Analysis of risk factors for intensive care unit admission

Univariate logistic regression identified multiple variables associated with ICU admission ($p < 0.1$) (Table 3). Multivariable analysis further identified three independent predictors: Type 1 phenotype [OR: 4.71, 95% confidence interval (CI): 1.102–20.151, $p = 0.037$], GCS score (OR: 0.76, 95% CI: 0.618–0.940, $p = 0.011$), and COHb (OR: 1.12, 95% CI: 1.054–1.200, $p < 0.001$). Hemoglobin showed a trend (OR: 1.03, 95% CI: 0.997–1.067, $p = 0.077$). Heart rate and white blood cell count did not demonstrate independent predictive value.

Analysis of risk factors for systemic inflammatory response syndrome occurrence

Univariate logistic regression analysis demonstrated that the Type 1 phenotype (pure high AG MA) was significantly associated with the development of SIRS in patients with ACOP ($p < 0.012$). Variables with $p < 0.1$ in the univariate analysis were subsequently included in a multivariable logistic regression model. After multivariable adjustment, Type 1 phenotype remained the strongest independent predictor (OR: 6.36, 95% CI: 1.486–27.213, $p = 0.013$), alongside GCS score (OR: 0.79, $p = 0.008$), hypertension (OR: 0.14, $p = 0.006$, protective), hemoglobin (OR: 1.03, $p = 0.050$), creatinine (OR: 1.02, $p = 0.025$), ALT (OR: 1.04, $p = 0.033$) and myocardial injury (OR: 3.76, $p = 0.023$). The magnitude of this association is presented in Table 4, indicating that this specific acid-base disorder phenotype possesses independent predictive value for the onset of SIRS.

Table 3

Risk factors for intensive care unit admission

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Type 1 phenotype	3.64	1.372–9.662	0.009	4.71	1.102–20.151	0.037
GCS score	0.68	0.582–0.796	< 0.001	0.76	0.618–0.940	0.011
COHb, %	1.15	1.089–1.219	< 0.001	1.12	1.054–1.200	< 0.001
Gender						
male	2.16	0.918–5.057	0.078	–	–	–
Heart rate, bpm	1.03	1.006–1.054	0.012	–	–	–
WBC count, $\times 10^9$/L	1.10	1.029–1.178	0.006	–	–	–
Hemoglobin, g/L	1.05	1.022–1.076	< 0.001	1.03	0.997–1.067	0.077
Albumin, g/L	1.08	1.012–1.161	0.022	–	–	–
Age, years	1.00	0.973–1.020	0.748	–	–	–
BMI, kg/m²	0.98	0.883–1.091	0.725	–	–	–
Temperature, °C	1.00	0.282–3.562	0.997	–	–	–
Respiratory rate, breaths/min	1.02	0.936–1.101	0.708	–	–	–
Systolic BP, mmHg	0.99	0.976–1.013	0.552	–	–	–
Hypertension	0.45	0.157–1.298	0.140	–	–	–
Diabetes mellitus	1.10	0.278–4.375	0.890	–	–	–
Creatinine, μmol/L	1.00	0.992–1.015	0.578	–	–	–
ALT, U/L	1.00	0.984–1.021	0.809	–	–	–
Total bilirubin, μmol/L	1.06	0.985–1.132	0.128	–	–	–
Myocardial injury	1.69	0.732–3.910	0.218	–	–	–

OR – odds ratio; CI – confidence interval; GCS – Glasgow Coma Scale; COHb – carboxyhemoglobin; bpm – beats *per* min; WBC – white blood cell; BMI – body mass index; BP – blood pressure; ALT – alanine aminotransferase.

***Note*: Variables with $p < 0.1$ in univariate analysis were entered into multivariate analysis using the stepwise method (entry $\alpha = 0.05$, removal $\alpha = 0.10$). Blood gas parameters (pH, $PaCO_2$, HCO_3^-, AG, lactate) were excluded from regression analysis due to causal inclusion relationships with the metabolic acidosis phenotype. A dash (–) indicates the variable was not included in the final multivariate model.**

Table 4

Risk factors for systemic inflammatory response syndrome

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Type 1 phenotype	3.66	1.323–10.119	0.012	6.36	1.486–27.213	0.013
GCS score	0.76	0.676–0.864	< 0.001	0.79	0.662–0.941	0.008
Gender						
male	2.49	1.208–5.137	0.013	–	–	–
Hypertension	0.39	0.171–0.895	0.026	0.14	0.035–0.562	0.006
Hemoglobin, g/L	1.05	1.025–1.072	< 0.001	1.03	1.000–1.060	0.050
Creatinine, μmol/L	1.03	1.014–1.049	< 0.001	1.02	1.003–1.046	0.025
ALT, U/L	1.05	1.024–1.083	< 0.001	1.04	1.003–1.070	0.033
Albumin, g/L	1.10	1.024–1.188	0.010	–	–	–
Myocardial injury	6.45	2.940–14.140	< 0.001	3.76	1.201–11.747	0.023
Diabetes mellitus	0.33	0.086–1.296	0.113	–	–	–
COHb, %	1.02	0.987–1.046	0.278	–	–	–
Age, years	1.00	0.976–1.016	0.691	–	–	–
BMI, kg/m²	1.01	0.920–1.098	0.911	–	–	–
Systolic BP, mmHg	1.00	0.980–1.012	0.645	–	–	–
Total bilirubin, μmol/L	1.02	0.954–1.084	0.601	–	–	–

OR – odds ratio; CI – confidence interval; GCS – Glasgow Coma Scale; ALT – alanine aminotransferase; COHb – carboxyhemoglobin; BMI – body mass index.

***Note*: Variables with $p < 0.1$ in univariate analysis were entered into multivariate analysis using the stepwise method (entry $\alpha = 0.05$, removal $\alpha = 0.10$). Blood gas parameters (pH, $PaCO_2$, HCO_3^-, AG, lactate) were excluded from regression analysis due to causal inclusion relationships with the metabolic acidosis phenotype. Systemic inflammatory response syndrome (SIRS) component variables (temperature, heart rate, respiratory rate, white blood cell count) were excluded from SIRS-related regression analyses due to definitional overlap with the outcome. A dash (–) indicates the variable was not included in the final multivariate model.**

Discussion

This preliminary study indicates that ACOP patients presenting with pure high AG MA upon admission face a significantly elevated risk of developing SIRS and requiring ICU admission. Despite the limited sample size, the core value of this study lies in being the first to utilize standardized physiological phenotyping to reveal the independent prognostic role of compensatory failure in ACOP. We have clearly identified this phenotype as a critical clinical manifestation in ACOP. Multivariable analysis demonstrated that even after adjusting for factors like GCS score and COHb level, it remained a strong independent risk factor for both SIRS (OR: 6.36) and ICU admission (OR: 4.71). This directly links the core pathophysiology of ACOP–tissue hypoxia and metabolic failure–to clinical risk, providing a key metabolic indicator for identifying high-risk patients in the emergency setting [5, 9].

The Type 1 phenotype signifies that the patient has entered a vicious cycle of tissue hypoxia and compensatory failure. CO not only reduces oxygen-carrying capacity by forming COHb but also directly inhibits mitochondrial cytochrome c oxidase, leading to lactate accumulation, the primary cause of elevated AG [4–6]. The weak positive correlation observed between the Type 1 phenotype and lactate levels ($\rho = 0.280$, $p = 0.002$) in univariate analysis supports this pathological mechanism. Its "pure" nature–indicating appropriate respiratory compensation–suggests that despite maximal compensatory effort, the acidosis remains uncorrected, pointing to the extreme severity of the hypoxia and metabolic disturbance [16, 17]. Multivariable analysis further confirmed this phenotype as an independent predictor of AOs, consistent with findings from Wang et al. [9] and Kimmel and Alscher [13].

A key aspect of this study is its distinction between the prognostic implications of pure vs. mixed high-AG MA. The "pure" nature of the Type 1 phenotype implies that respiratory compensation has reached its maximum capacity yet fails to correct the acidosis, indicating that the MA has exceeded physiological compensatory limits, leading to a decompensated vicious cycle of hypoxia-lactate accumulation-worsening acidosis. In contrast, the Type 2 (mixed) phenotype encompasses various scenarios such as over-compensation, under-compensation, or combination with other acid-base disorders, resulting in complex pathological significance that may dilute the critical signal of a high AG. Consequently, its prognostic value is considerably lower than that of the pure phenotype.

This study addresses a gap in previous research, which often focused on overall acid-base status or single parameters [10, 11], by confirming the independent prognostic value of pure high AG MA. This aligns with findings in critical care: Mochizuki et al. [22] reported higher mortality with metabolic acidemia than with respiratory acidemia, and Canova et al. [23] confirmed MA as an independent risk factor for in-hospital mortality. Our study focuses this understanding specifically on ACOP, deepening the knowledge of acid-base disorders in this condition. Furthermore, the high incidence of SIRS in Type 1 patients is consistent with the systemic inflammatory state seen in septic patients in the emergency department [24] and

supports the mechanism proposed by Simonsen et al. [5] wherein CO poisoning triggers OS and inflammatory responses, thereby completing the pathophysiological chain of ACOP.

In clinical practice, rapid acid-base phenotyping using admission blood gas analysis is simple, immediate, and incurs no additional cost, making it well-suited for emergency department use. Identifying patients with the Type 1 phenotype should prompt initiation of more intensive monitoring and treatment, including early hemodynamic monitoring, assessment of eligibility for advanced respiratory support, optimization of hyperbaric oxygen therapy schedules, and lactate clearance therapy, thereby enabling precise intervention. Future research should involve large-scale, multicenter prospective studies to validate the predictive power of this phenotype, explore targeted personalized treatment strategies, and clarify its impact on improving patient outcomes, thereby providing an evidence base for updating clinical guidelines.

Limitation of the study

This study has several limitations. First, its single-center retrospective design and relatively small sample size limit statistical power, resulting in wide CIs for the ORs in the multivariable analysis. The conclusions should therefore be considered hypothesis-generating and require validation in larger prospective multicenter studies. Second, the retrospective design is susceptible to unmeasured confounding factors. Specifically, data on CO exposure duration, transport time to hospital, and prehospital treatments (e.g., oxygen administration by emergency medical services, fluid resuscitation) were not systematically documented in electronic medical records and thus could not be included in the analysis. These factors may significantly influence the severity of poisoning and subsequent outcomes. Their absence reflects an inherent limitation of retrospective emergency department studies, and future prospective studies should incorporate standardized prehospital data-collection protocols. Third, the study focused only on short-term outcomes (SIRS, ICU admission) and lacked follow-up on long-term neurological sequelae and mortality [12], preventing a comprehensive assessment of the phenotype's long-term value. Finally, constrained by data availability in the emergency setting, SIRS rather than the sequential organ failure assessment – SOFA score was used to define the inflammatory response; biomarkers of inflammation and OS were not measured [5, 9]; the efficacy of different treatments across phenotypes was not analyzed [15]; and the generalizability of the single-center data requires verification [14].

Conclusion

Ultimately, pure high anion gap metabolic acidosis represents a critical phenotype in acute carbon monoxide poisoning and is a strong independent predictor for the development of systemic inflammatory response syndrome and the need for intensive care unit admission. This readily available parameter from routine blood gas analysis provides a crucial metabolic perspective for identifying high-risk patients, stratifying risk, and guiding intensive treatment in the emergency department, holding significant clinical value. This study enriches the evidence base concerning acid-base disorders in acute carbon monoxide poisoning, provides a reference for refining clinical guidelines, and lays the groundwork for subsequent prospective research.

REFERENCES

1. *Bleecker ML.* Carbon monoxide intoxication. Handb Clin Neurol 2015; 131: 191–203. DOI: 10.1016/B978-0-444-62627-1.00024-X.
2. *Weaver LK.* Carbon monoxide poisoning. Undersea Hyperb Med 2020; 47(1): 151–69. DOI: 10.22462/01.03.2020.17.
3. *GBD 2021 Carbon Monoxide Poisoning Collaborators.* Global, regional, and national mortality due to unintentional carbon monoxide poisoning, 2000-2021: results from the Global Burden of Disease Study 2021. Lancet Public Health 2023; 8(11): e839–49. DOI: 10.1016/S2468-2667(23)00185-8.
4. *Hampson NB, Piantadosi CA, Thom SR, Weaver LK.* Practice recommendations in the diagnosis, management, and prevention of carbon monoxide poisoning. Am J Respir Crit Care Med 2012; 186(11): 1095–101. DOI: 10.1164/rccm.201207-1284CI.
5. *Simonsen C, Magnusdottir SO, Andreasen JJ, Wimmer R, Rasmussen BS, Kjaergaard B,* et al. Metabolic changes during carbon monoxide poisoning: An experimental study. J Cell Mol Med 2021; 25(11): 5191–201. DOI: 10.1111/jcmm.16522.
6. *Dent MR, Rose JJ, Tejero J, Gladwin MT.* Carbon Monoxide Poisoning: From Microbes to Therapeutics. Annu Rev Med 2024; 75: 337–51. DOI: 10.1146/annurev-med-052422-020045.
7. *Sanagustín MN, Osredkar J.* Blood gas analysis: Clinical applications, interpretation and future directions (Review). Med Int (Lond) 2025; 6(1): 7. DOI: 10.3892/mi.2025.291.
8. *Castro D, Patil SM, Zubair M, Keenaghan M.* Arterial Blood Gas. In StatPearl [Internet]. Treasure Island (FL): StatPearls Publishing; 2026.
9. *Wang W, Huang H, Xiong X, Ye C, Huang J, Ling YA.* Multidimensional metabolic profiling in carbon monoxide poisoning: acid-base disturbances correlate with neurological severity. Biomarkers 2025; 30(5): 363–70. DOI: 10.1080/1354750X.2025.2551303.
10. *Aykut A, Günsoy E, Karabulut BÖ, Aktaş RS, Öncül MV, Karabulut AE.* Serum lactate and carboxyhemoglobin as predictors of hyperbaric oxygen therapy in carbon monoxide poisoning: a retrospective study. BMC Emerg Med 2025; 25(1): 252. DOI: 10.1186/s12873-025-01410-w.
11. *Doğruyol S, Akbaş I, Tekin E, Doğruyol M.* Carbon monoxide intoxication in geriatric patients: How important are lactate values at admission? Hum Exp Toxicol 2020; 39(6): 848–54. DOI: 10.1177/0960327120903484.
12. *Rahimi M, Aghabiklooei A, Nasouhi S, Mashayekhian M, Ghoochani A, Yousefi Y,* et al. A 5-year Assessment on Carbon Monoxide Poisoning in a Referral Center in Tehran-Iran. Int J Prev Med 2019; 10: 116. DOI: 10.4103/ijpvm.IJPVM_338_18.
13. *Kimmel M, Alscher MD.* Disorders of the acid-base balance and the anion gap. Dtsch Med Wochenschr 2016; 141(21): 1549–54. (German) DOI: 10.1055/s-0042-109042.
14. *Mattiuzzi C, Lippi G.* Worldwide epidemiology of carbon monoxide poisoning. Hum Exp Toxicol 2020; 39(4): 387–92. DOI: 10.1177/0960327119891214.

15. *Liao WC, Cheng WC, Wu BR, Chen WC, Chen CY, Chen CH,* et al. Outcome and prognostic factors of patients treated in the intensive care unit for carbon monoxide poisoning. J Formos Med Assoc 2019; 118(4): 821–7. DOI: 10.1016/j.jfma.2018.09.005.
16. *Berend K, de Vries AP, Gans RO.* Physiological approach to assessment of acid-base disturbances. N Engl J Med 2015; 372(2): 193–5. DOI: 10.1056/NEJMc1413880.
17. *Morikawa MJ, Ganesh PR.* Acid-Base Interpretation: A Practical Approach. Am Fam Physician 2025; 111(2): 148–55.
18. *Hopkins E, Sanvictores T, Sharma S.* Physiology, Acid Base Balance. In StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
19. *Cao Y, Wang M, Yuan Y, Li C, Bai Q, Li M.* Arterial blood gas and acid-base balance in patients with pregnancy-induced hypertension syndrome. Exp Ther Med 2019; 17(1): 349–53. DOI: 10.3892/etm.2018.6893.
20. *Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M,* et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315(8): 801–10. DOI: 10.1001/jama.2016.0287.
21. *Demidenko E.* Sample size determination for logistic regression revisited. Stat Med 2007; 26(18): 3385–97. DOI: 10.1002/sim.2771.
22. *Mochizuki K, Fujii T, Paul E, Anstey M, Uchino S, Pilcher DV,* et al. Acidemia subtypes in critically ill patients: An international cohort study. J Crit Care 2021; 64: 10–7. DOI: 10.1016/j.jcrc.2021.02.006.
23. *Canova TJ, Lipps K, Dahiya G, Hillerson DB, Kashani KB, Jentzer JC.* Admission Acid-Base Status and Mortality in Cardiac Intensive Care Unit Patients. J Intensive Care Med 2026; 41(7): 670–82. DOI: 10.1177/08850666251399182.
24. *Henning DJ, Puskarich MA, Self WH, Howell MD, Donnino MW, Yealy DM,* et al. An Emergency Department Validation of the SEP-3 Sepsis and Septic Shock Definitions and Comparison With 1992 Consensus Definitions. Ann Emerg Med 2017; 70(4): 544–52.e5. DOI: 10.1016/j.annemergmed.2017.01.008.

Received on February 23, 2026
Revised on June 8, 2026
Revised on June 21, 2026
Accepted on July 8, 2026
Online First July 2026

ORIGINAL ARTICLE

UDC: 616.32-008.1-053.9
DOI: https://doi.org/10.2298/VSP251019047Y

Head flexion, trunk sway, and self-reported strategies in older adults screening positive for oropharyngeal dysphagia: an exploratory mixed-methods study

Fleksija glave, njihanje trupa i samoprijavljene strategije kod starijih osoba sa pozitivnim skriningom na orofaringealnu disfagiju: istraživačka studija mešovitih metoda

Ziya Yildiz*, Ahmet Ali Süzen†

Isparta University of Applied Sciences, *Uluborlu Vocational School, †Department of Computer Engineering, Isparta, Türkiye

Abstract

Background/Aim. Older adults with oropharyngeal dysphagia (OD) residing in nursing homes without clinical guidance may spontaneously adopt compensatory strategies through adjustments in eating and swallowing. However, evidence regarding the spontaneous emergence of head flexion (HF) and postural adaptations remains limited. The aim of the study was to investigate adaptive head posture and trunk sway (TS) during swallowing in older adults screening positive for OD (spOD). **Methods**. Based on the presence of a swallowing disorder determined by the 100 mL water swallow test, a total of 45 older adults were classified into two groups: a group with spOD (spOD group) and a group without spOD (non-spOD group). Head posture was assessed using a smartphone-based accelerometer, and TS was evaluated with a Swaymeter. Data were analyzed using image processing techniques. Group comparisons were performed using multivariate analysis of covariance – MANCOVA with Bonferroni correction, followed by a confirmatory multivariable linear regression. Semi-structured interviews were conducted with the spOD group to explore their recognition of spontaneous strategies. **Results**. The spOD group (n = 21) showed significantly greater maximum HF during swallowing compared to the non-spOD group (n = 24) (p = 0.0002). No significant group differences were observed in TS parameters (p > 0.00625). Linear regression indicated a positive association between spOD and maximum HF: B = 13.46, 95% confidence interval: 6.50–20.41, p < 0.001. In interviews, the participants reported various conscious strategies (e.g., small bites, water use, breath-holding), but none mentioned HF or trunk sway. **Conclusion**. Despite the HF and TS results obtained in older individuals, this cross-sectional study cannot determine whether HF serves a compensatory function or is merely associated with OD. Therefore, no clinical recommendations can be drawn from these findings.

Keywords:
aged; deglutition disorders; head movements; nursing home residents; oropharynx.

Apstrakt

Uvod/Cilj. Starije osobe sa orofaringealnom disfagijom (OD), koje borave u domovima za stare bez kliničkog vođstva, mogu spontano, kroz prilagođavanje ishrani i gutanju, usvojiti kompenzacione strategije. Međutim, ograničeni su dokazi o spontanoj pojavi fleksije glave (*head flexion* – HF) i posturalnih adaptacija. Cilj rada bio je da se kod starijih osoba sa pozitivnim skriningom na OD (*screening positive for OD* – spOD) istraži adaptivni položaj glave i njihanje trupa (*trunk sway* – TS) tokom gutanja. **Metode.** Na osnovu prisustva poremećaja gutanja, utvrđenog testom gutanja 100 mL vode, ukupno 45 starijih osoba klasifikovano je u dve grupe: grupa spOD i skrining negativnu grupu (*non*-spOD grupa). Položaj glave procenjivan je pomoću akcelerometra na „pametnom“ telefonu, dok je TS evaluiran primenom Swaymeter-a. Podaci su analizirani korišćenjem tehnika obrade slika. Upoređivanje rezultata grupa izvršeno je korišćenjem multivarijantne analize kovarijanse (*multivariate analysis of covariance* – MANCOVA), sa Bonferonijevom korekcijom, nakon čega je sprovedena potvrdna multivarijabilna linearna regresija. Da bi se istražilo prepoznavanje spontanih strategija, sprovedeni su polustrukturalni intervjui sa ispitanicima spOD grupe. **Rezultati.** Grupa spOD (n = 21) pokazala je značajno veću maksimalnu HF tokom gutanja u poređenju sa *non*-spOD grupom (n = 24) (p = 0,0002). Nisu primećene značajne razlike između grupa u parametrima TS

Correspondence to: Ahmet Ali Süzen, Isparta University of Applied Sciences, Department of Computer Engineering, 32 050, Isparta, Türkiye. E-mail: ahmetsuzen@isparta.edu.tr

($p > 0,00625$). Linearnom regresijom pokazana je pozitivna veza između spOD i maksimalne HF: B = 13,46, 95% *confidence interval*: 6,50–20,41, $p < 0,001$. Učesnici su u intervjuima prijavili različite svesne strategije (npr. mali zalogaji, upotreba vode, zadržavanje daha), ali niko nije pomenuo HF ili TS. **Zaključak**. Uprkos rezultatima za HF i TS kod starijih osoba, ovom studijom preseka ne može se utvrditi da li je HF kompenzatorna funkcija ili je samo povezana sa OD. Shodno tome, na osnovu ovih nalaza se ne mogu doneti kliničke preporuke.

Ključne reči:
stare osobe; gutanje, poremećaji; glava, pokreti; staračke domovi, stanari; orofarinks.

Introduction

Oropharyngeal dysphagia (OD), which impairs the safety and efficiency of swallowing from the oral cavity to the oesophagus, is common among older adults in nursing homes and is associated with a poor prognosis [1]. OD is common in older adults, particularly after stroke and in neurological diseases such as Parkinson's and Alzheimer's disease [2]. However, in healthy older adults, age-related physiological changes in swallowing can occur and may contribute to OD [3]. In older adults, OD can lead to serious complications, including dehydration, malnutrition, aspiration pneumonia, hospitalization, and even death [4]. It is also strongly associated with poor quality of life, social isolation, anxiety, and depression [5]. Despite these severe consequences, OD often goes unrecognized in older adults living in nursing homes. This is largely because individuals tend to perceive swallowing difficulties as a natural part of ageing and therefore do not report them to healthcare professionals. Moreover, clinicians frequently fail to detect OD, which leads to an underestimation of its true prevalence [6]. Although prevalence rates vary, a recent review reported that OD affects approximately 52.2% of nursing home residents [95% confidence interval (CI): 33.3–67.2] [7].

Clinicians often recommend standard compensatory strategies such as dietary adjustments, oral hygiene, postural modification and swallowing manoeuvres to manage OD [8, 9]. Because follow-up care for dysphagia is often inadequate, and rehabilitation programs are not fully tailored to individual needs, many people with OD attempt to manage the condition independently [10]. Qualitative studies report some techniques that elderly residents of nursing homes develop spontaneously before seeking clinical support when they have difficulty swallowing. These methods include slowing down eating, taking small bites, tapping the chest or back during choking, drinking water after each bite, and maintaining oral hygiene [4]. Similarly, community-dwelling older people often use diet-based strategies to cope with OD, such as avoiding hard-to-swallow foods, rinsing the mouth with water, or softening hard foods with warm liquids [11]. Some of these self-reported strategies–such as maintaining oral hygiene, taking small bites, and swallowing with water–partially align with the compensatory methods recommended by clinicians. Although head flexion (HF) [12] and other postural compensation (e.g., upright sitting, head rotation) [8] are frequently recommended in clinical settings and have been reported as experiential practices among individuals with paralysis [13], there is no clear evidence that older adults with OD in nursing homes adopt these strategies on their own.

This study aimed to evaluate spontaneous head and trunk movement patterns during water swallowing in older adults screening positive for OD (spOD) compared to those without spOD.

Methods

Study design and ethical approval

This study employed a cross-sectional and mixed-method explanatory sequential design. Qualitative data were used to enrich the interpretation of quantitative findings and to explore participants' lived experiences [14].

In the first stage of the study, demographic information, dependence levels, and cognitive status of the participants were recorded. Participants were then classified into two groups based on the presence or absence of spOD, as determined by the 100 mL water swallow test. Each participant was instructed to swallow 10 mL of thickened water, during which head posture (HP) and trunk sway (TS) were assessed. TS was measured using a Swaymeter, and HP was evaluated using an accelerometer. Subsequently, qualitative data were gathered through semi-structured interviews.

Ethical approval for the study was obtained from the Ethics Committee of Isparta University of Applied Sciences, Isparta, Türkiye (No. 02, from April 05, 2024). The study was conducted in accordance with the principles of the Declaration of Helsinki, and written informed consent was obtained from all participants.

Participants

A power analysis was conducted to determine the required sample size for the study. Based on the study by Nagura et al. [12], the sample size was calculated using GPower 3.1 software. Assuming $\alpha = 0.05$, power $(1-\beta)$ of 0.95, and an effect size of 1.15, the minimum sample size was estimated to be n = 18 participants *per* group. The inclusion criteria for the study were as follows: patients aged 65–80 years; those residing in a nursing home for at least 1 year; those receiving full oral nutrition; those with no cognitive impairment; patients with no prior clinical support for a swallowing disorder; and those volunteering to participate in the study. The study's exclusion criteria included the following: a diagnosed neurological disorder; any orthopedic limitation affecting head/neck mobility or sitting posture; a history of oncological disease or treatment; a history of head and neck surgery; and dependence in activities of daily living.

Participants were recruited from three nursing homes located in Isparta Province, southern Türkiye. Based on the inclusion and exclusion criteria, 45 out of 171 older adult residents were eligible for the study. Reasons for their exclusion were age over 80 years (n = 38), residence less than 1 year (n = 7), inability to meet nutritional needs through oral intake (n = 19), cognitive impairment (n = 24), history of neurological disease (n = 16), prior neck surgery (n = 2), dependence in daily living activities (n = 5), and current or past oncological treatment (n = 15).

Evaluation of cognitive, functional status, and swallowing function

Demographic information, existing chronic conditions, surgical history, medication use, smoking and alcohol habits, dietary habits, and pulmonary infections in the past year were evaluated. Participants scoring below the cut-off on the Mini-Mental State Examination and the Barthel index [15], which assess cognitive and functional status, were excluded.

The 100 mL water swallow test is a practical and widely used screening method to assess swallowing function in older adults with spOD, with a reported sensitivity of 85.5% and specificity of 50% [16]. However, the test is a screening instrument, not a diagnostic gold standard [17]. Water was chosen because it is a standardized liquid bolus and, compared to thicker consistencies, it places greater demands on the swallowing mechanism, making it a more sensitive test for revealing OD risk in older adults [18]. No instrumental confirmation (Videofluoroscopic Swallow Study/Flexible Endoscopic Evaluation of Swallowing – VFSS/FEES) was performed. This test provides an objective measure of an individual's liquid-swallowing capacity and is clinically relevant for identifying spOD risk. Participants were asked to drink 100 mL of water as quickly as possible while seated upright. The test was discontinued if any signs of choking, coughing, suspected aspiration, or change in voice quality were observed. Swallowing speed was calculated by dividing the volume of water consumed (mL) by the elapsed time (sec), expressed as mL/sec. Participants exhibiting a swallowing speed below 10 mL/sec were classified as spOD [17].

Swaymeter-based sitting balance assessment and image processing

The Swaymeter test is a validated and reliable method for measuring postural sway in the horizontal plane, particularly in older adults [19]. One end of the Swaymeter rod was secured with a belt at the participants' axillary level while they were seated on a backless, armless chair. The other end, fitted with a ballpoint pen, was placed in contact with a sheet of paper marked with guidelines spaced 0.5 cm apart. Participants were instructed to hold 10 mL of water thickened to 1% consistency (Nestle® ThickenUp® Clear, Vevey, Switzerland) in their mouths and then swallow it as they normally would. At the onset of swallowing, the pen was brought into contact with the paper and lifted immediately after the swallow was completed. TS was recorded in four directions: right-anterior, right-posterior, left-anterior, and left-posterior (Figure 1). The number of swallows was also recorded by observing laryngeal elevation.

The obtained data were analyzed using a visual processing model in four regions (anterior-right, anterior-left, posterior-right, posterior-left) in terms of the number of pixels and the distance of the furthest pixel from the center point. In this study, Swaymeter analysis evaluated not only the maximum sway distances [19] but also pixel density, allowing assessment of the directional persistence and duration of TS. An image processing algorithm was developed using digital images captured with a camera (Figure 2a) to quantitatively evaluate traces recorded on paper with the Swaymeter. In this study, the Open Computer Vision Library – OpenCV and Numerical Python – NumPy were used for the automatic detection and quantitative analysis of objects in the red color spectrum in color images. In the first stage, the system automatically processed all images in the specified

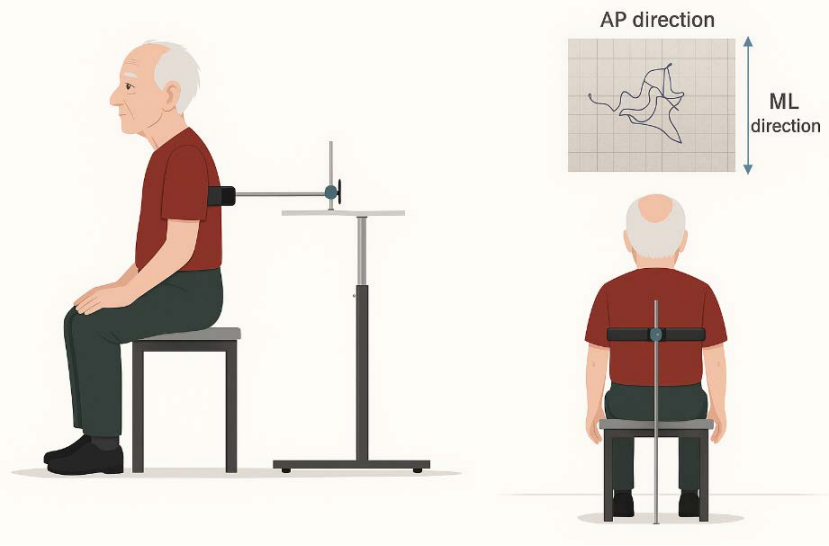
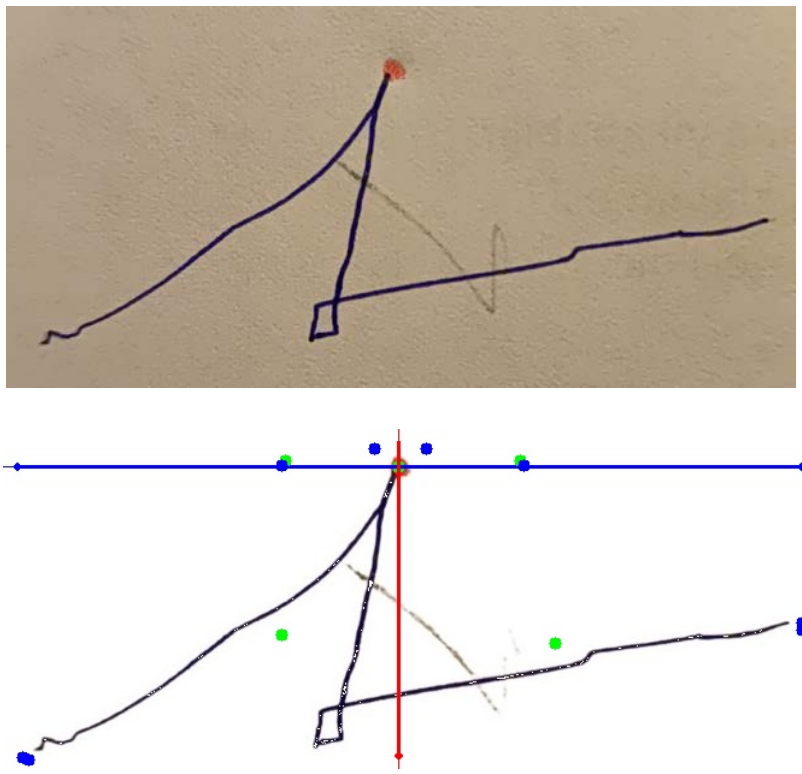


Fig. 1 – Illustration of the Swaymeter measurement procedure.
AP – anterior-posterior; ML – mediolateral.



a)

b)

Fig. 2 – a) Original digital image of data prepared by Swaymeter; b) Final image processed by the original image processing algorithm.

directory. Each image was first read and checked for potential reading errors. To facilitate artifact analysis in the image, all black pixels were first converted to white. Subsequently, red regions were detected by masking them with two separate thresholding ranges in the hue, saturation, and value (HSV) color space, and the largest red contour was selected. The geometric center of this red region was calculated using the method of moments, and reference axes, the center point, and arrowheads were drawn on the image for visual verification.

In the first stage, the segmentation performance was optimized by converting the black pixel clusters (red, green, and blue – RGB values ≤ 30) in the image to a white background. Then, the color space was converted from blue, green, red – BGR to HSV, and two adjacent ranges were defined for red tones: (H:0–10, S:100–255, V:100–255 and H:160–180, S:100–255, V:100–255). The pixel clusters in these intervals were merged, morphological operations (erode/dilate) were applied, and noise filtering was performed. When calculating the areas of red objects detected by contour analysis, their centers of gravity were determined using the method of moments (M[“m10”]/M[“m00”], M[“m01”]/M[“m00”]). In the subsequent analysis, the image was divided into four regions (upper right, lower right, upper left, lower left), and the coordinates of the colored pixels in each region were collected separately. For each region, we calculated center coordinates and the Euclidean distance from the center to the target red area (converted from pixels to centimeters). In addition, the coordinates and distance of the boundary point furthest from the center were recorded. These metrics were recorded for objective comparisons. The relevant analysis points and reference lines were visually added to each processed image for verification purposes (Figure 2b).

Assessment of head posture

During the 10 mL thickened water swallow test, participants’ head movements were recorded simultaneously with the Swaymeter measurement. HF-extension and lateral flexion were assessed using the Accell InstantView – AccelView application installed on an Android smartphone attached to the participant’s head. Android-based clinometer applications have demonstrated acceptable reproducibility and accuracy for evaluating head movements in the sagittal and frontal planes [20]. A systematic review and meta-analysis by Sedrez et al. [21] further confirmed that smartphones are valid and reliable tools for assessing cervical range of motion. Specifically, the meta-analysis showed very high correlation coefficients for the evaluation of cervical flexion, extension, and lateral flexion, as well as high correlation coefficients for intrarater and interrater reproducibility of all cervical movements. To avoid restricting spontaneous head movements, the head was not aligned to any fixed reference point. In the analysis, the moment when the participant began swallowing was defined as the zero point. This reference was set within the inclinometer application, and all measurements were evaluated relative to this point. Head movements were analyzed based on the maximum range of motion from the zero point to the final head position reached at the end of the swallowing task (Figure 3).

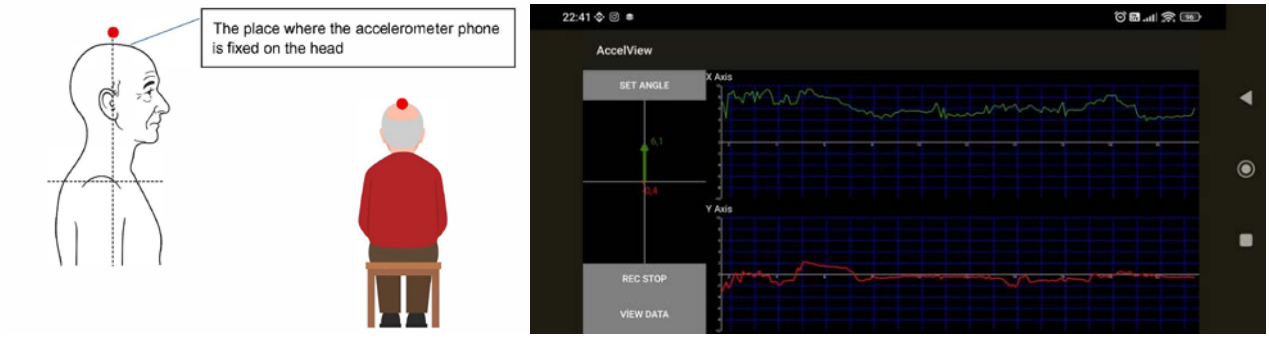


Fig. 3 – Placement of the phone above the head and screenshot of the Accell InstantView – AccelView application.

Collection of qualitative data

Face-to-face interviews were used as the qualitative data collection method in this study. The semi-structured interview guide was developed through a systematic five-phase process to ensure methodological rigor [22], which was described in the text that follows. The first phase involved identifying prerequisites. Semi-structured interviews were deemed appropriate as this method allows exploration of personally sensitive experiences and perceptions regarding swallowing difficulties while enabling participants to freely express their adaptive strategies. The second phase focused on retrieving and using previous knowledge. An initial draft pool of questions was directly informed by key themes identified in previous qualitative studies on the swallowing experiences of nursing home residents [4, 11], establishing a foundational theoretical framework for the interview guide. The third phase involved formulating the preliminary guide. Based on the literature review, a preliminary interview guide was developed with open-ended questions focusing on participants' swallowing experiences, self-developed strategies, and environmental adaptations. The guide included main themes with follow-up questions to encourage detailed responses. The fourth phase involved pilot testing. The preliminary interview guide underwent a rigorous, multi-stage validation process. Expert assessment was conducted by two academic physiotherapy specialists with over 10 years of geriatric rehabilitation experience. They evaluated each item on a 3-point Likert scale (1 = keep as is, 2 = needs revision, 3 = remove completely) while also providing open-ended suggestions for new questions. This expert feedback was then complemented by field-testing through cognitive interviews with eight community-dwelling older adults to assess question clarity, relevance, and feasibility, with revisions implemented based on this comprehensive evaluation. Finally, the fifth phase focused on finalizing the guide. Based on integrated feedback from both experts and participants, revisions were made to question wording and sequence. This iterative development process ensured that the final semi-structured interview form possessed robust content and face validity, making it a rigorous tool for capturing the lived experiences of older adults with swallowing difficulties.

Using this validated instrument, all interviews were audio-recorded. The resulting transcripts were analyzed using qualitative content analysis [23], with data saturation confirmed after conducting interviews with 12 participants identified with spOD.

Statistical analysis

The normality of continuous variables was assessed based on skewness and kurtosis values. Variables with skewness > |2| and kurtosis > |2| were considered non-normal. Normally distributed variables were reported as mean ± standard deviation and compared between groups (spOD vs. non-spOD) using the independent samples *t*-test. The Pearson chi-square test was used to compare categorical variables. These preliminary analyses were conducted to verify that the two groups were comparable with respect to demographic and clinical characteristics before testing the main hypotheses. Correlations between continuous variables were examined using Pearson correlation analysis.

To compare the head movement parameters and TS between groups while controlling for age, body mass index (BMI), and length of stay (LOS), a one-way multivariate analysis of covariance (MANCOVA) was performed. Prior to MANCOVA, the following assumptions were tested: homogeneity of covariance matrices (Box's M test, with $\alpha = 0.005$ due to small sample size), homogeneity of error variances (Levene's test), and homogeneity of regression slopes (group × covariate interactions). Box's M test was non-significant ($p > 0.005$), and the group × covariate interactions were all non-significant ($p > 0.05$), indicating that the assumptions were met. However, the Levene test was significant for some dependent variables ($p < 0.05$). Therefore, the robust Pillai's trace criterion was used for the multivariate test. The multivariate effect of group was considered significant at $p < 0.05$. Following a significant multivariate effect, univariate *F*-tests were performed for each dependent variable. To control for Type I error resulting from multiple comparisons, a Bonferroni correction was applied for the four head movement parameters, adjusting the univariate significance level to $\alpha = 0.0125$ (0.05/4). Partial eta squared (η^2) was reported as a measure of effect size. For TS parameters (eight dependent variables), a separate MANCOVA was conducted using the same

covariates. Because Box's M test was significant ($p < 0.001$), Pillai's trace was used, and Bonferroni correction was applied with $\alpha = 0.00625$ (0.05/8).

As a sensitivity analysis, a separate multivariable linear regression (enter method) was performed with maximum HF as the dependent variable and group, age, BMI, Barthel index, feeding time, and sex as independent variables to confirm the robustness of the MANCOVA finding for the primary outcome. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

The interview recordings were analyzed using qualitative content analysis [23]. Transcripts were first read holistically, then examined line by line to identify meaning units related to the eating experiences of individuals with spOD. No predefined coding scheme was used; instead, codes were developed inductively during the analysis process.

Results

Quantitative findings

A total of 45 older adults (28 men and 17 women) with a mean age of 73.71 ± 4.52 years participated in this study conducted in nursing homes. The average LOS in the nursing home was 40.44 ± 17.44 months (spOD: 40.19 ± 17.59; non-spOD: 40.66 ± 17.69). The mean Barthel index score was 96.33 ± 4.04 (spOD: 95.23 ± 4.02; non-spOD: 97.29 ± 3.89), and BMI was 27.57 ± 4.32 kg/m² (spOD: 26.59 ± 4.05; non-spOD: 28.43 ± 4.44). No significant differences were found between the groups in terms of these descriptive variables ($p > 0.05$). As expected, the mean swallowing speed was significantly higher in the non-spOD group (18.31 ± 2.73 mL/sec) compared to the spOD group (7.03 ± 1.09 mL/sec) ($p < 0.001$). However, the mean number of swallows was significantly greater in the spOD group (spOD: 5.28 ± 1.00; non-spOD: 3.12 ± 0.74; $p < 0.001$). A significant relationship was observed between feeding time and the presence of spOD ($\chi^2(1) = 7.87$; $p = 0.005$), whereas no significant association was found between sex and the presence of spOD ($\chi^2(1) = 0.002$; $p = 0.96$) (Table 1).

MANCOVA was conducted to determine whether the dependent variables (maximum HF/extansion, post-swallow HF/extansion, maximum lateral flexion, and post-swallow lateral flexion) differed between groups (spOD vs. non-spOD) after controlling for age, BMI, and LOS (Table 2). Preliminary assumption testing showed that the Box's M test for homogeneity of covariance matrices was non-significant, $F(10, 8464.30) = 1.63$, $p = 0.091$. Although Levene's test indicated violations of homogeneity of variance for some variables ($p < 0.05$), Pillai's trace and Wilks' lambda (Wilks' Λ) yielded parallel results; therefore, Wilks' Λ was reported. After controlling for covariates, the main effect of group was significant: Wilks' $\Lambda = 0.59$, $F(4.37) = 6.53$, $p < 0.001$, partial $\eta^2 = 0.41$. Among the covariates, BMI had a significant multivariate effect ($p = 0.023$), whereas age and LOS did not ($p > 0.05$). Following the significant multivariate result, univariate *F*-tests were performed to identify which dependent variables contributed to the difference. To control for Type I error due to multiple comparisons, a Bonferroni correction was applied, setting the significance level at $p < 0.0125$. Only maximum angle of HF/extension showed a statistically significant group difference, $F(1.40) = 15.69$, $p < 0.001$, partial $\eta^2 = 0.28$. Inspection of the adjusted means (M) revealed that the spOD group had significantly higher maximum angle of HF/extension scores [M = 15.87, standard error (SE) = 2.54] than the non-spOD group (M = 2.25, SE = 2.36). None of the other dependent variables reached statistical significance at the Bonferroni-corrected threshold (all $p > 0.0125$).

MANCOVA was conducted on eight TS parameters (pixel counts and maximum distances in four directions) with age, BMI, and LOS as covariates (Table 3). Preliminary assumption testing using Box's M test indicated a violation of the homogeneity of covariance matrices ($p < 0.001$); therefore, the more robust Pillai's trace criterion was used for the multivariate test. After controlling for age, BMI, and LOS,

Table 1

Descriptive statistics results between groups (spOD vs. non-spOD)

Variable	spOD (n = 21)		non-spOD (n = 24)		*t*-test	*p*-value†
	min–max	mean ± SD	min–max	mean ± SD		
Age, years	65–80	75.09 ± 4.43	65–80	72.5 ± 4.32	-1.98	0.054
BMI, kg/m²	19.9–31.7	26.59 ± 4.05	18.23–34	28.43 ± 4.44	1.45	0.15
LOS, month	13–70	40.19 ± 17.59	14–69	40.66 ± 17.69	0.09	0.92
Barthel index score	90–100	95.23 ± 4.02	90–100	97.29 ± 3.89	1.73	0.09
SwalSpe, mL/sec	5.4–9.52	7.03 ± 1.09	14.64–24.3	18.31 ± 2.73	17.69	< 0.001
nSwal	4–7	5.28 ± 1.00	2–4	3.12 ± 0.74	-8.26	< 0.001
	n (%)		n (%)		$\chi^2(df)$	*p*-value$^{\alpha}$
FeTi, min						
< 20	8 (38.1)		19 (79.2)		7.87 (1)	0.005
> 20	13 (61.9)		5 (20.8)			
Sex						
male	13 (61.9)		15 (62.5)		0.002 (1)	0.96
female	8 (38.1)		9 (37.5)			

spOD – screening positive for oropharyngeal dysphagia; non-spOD – screening negative for oropharyngeal dysphagia; n – number; min – minimum; max – maximum; SD – standard deviation; BMI – body mass index; LOS – length of stay; SwalSpe – swallowing speed; nSwal – number of swallows; *df* – degrees of freedom; FeTi – feeding time.

***Note*: † independent samples *t*-test; α Pearson chi-square test.**

Table 2

Univariate group comparisons for head movement parameters

Dependent variable/Group	Adjusted M (SE)	95% CI	*F*(1.40)	*p*-value	Partial η²
HeadAPM					
spOD	15.87 (2.54)	10.73 – 21.01	15.69	0.0002	0.28
non-spOD	2.25 (2.36)	-2.53 – 7.03			
HeadAPE					
spOD	3.52 (1.28)	0.93 – 6.11	1.83	0.184	0.04
non-spOD	-0.38 (1.19)	-2.79 – 2.03			
HeadLFM					
spOD	0.91 (1.75)	-2.63 – 4.45	1.37	0.249	0.03
non-spOD	-1.67 (1.63)	-4.97 – 1.63			
HeadLFE					
spOD	-0.48 (0.99)	-2.48 – 1.52	0.04	0.835	0.00
non-spOD	-0.91 (0.92)	-2.77 – 0.95			

M – mean; SE – standard error; CI – confidence interval; HeadAPM – head anterior/posterior maximum angle from the start; spOD – screening positive for oropharyngeal dysphagia; non-spOD –screening negative for oropharyngeal dysphagia; HeadAPE – head anterior/posterior end angle from the start; HeadLFM – head lateral flexion maximum angle from the start; HeadLFE – head lateral flexion end angle from the beginning.

***Note*: Controlling for covariates included age, body mass index, and length of stay [adjusted M (SE), 95% CI]. Bonferroni correction was applied ($\alpha = 0.0125$).**

Table 3

Univariate group comparisons for trunk sway parameters

Dependent variable/Group	Adjusted M (SE)	95% CI	*F*(1.40)	*p*-value	Partial η²
AntRP					
spOD	2,768.0 (1,641.0)	-544.2 – 6,080.2	0.45	0.505	0.011
non-spOD	3,831.4 (1,530.1)	737.5 – 6,925.3			
AntRM, cm					
spOD	3.25 (0.83)	1.57 – 4.93	1.55	0.22	0.037
non-spOD	4.68 (0.78)	3.10 – 6.26			
PostRP					
spOD	4,962.4 (817.1)	3,310.9 – 6,613.9	3.07	0.087	0.071
non-spOD	3,370.8 (762.1)	1,830.3 – 4,911.3			
PostRM, cm					
spOD	5.66 (1.07)	3.49 – 7.83	4.82	0.034	0.107
non-spOD	2.99 (1.00)	0.97 – 5.01			
AntLP					
spOD	2,781.2 (890.2)	981.7 – 4,580.7	2.02	0.163	0.048
non-spOD	4,424.8 (830.0)	2,746.9 – 6,102.7			
AntLM, cm					
spOD	2.89 (0.77)	1.33 – 4.45	1.27	0.267	0.031
non-spOD	4.08 (0.72)	2.62 – 5.54			
PostLP					
spOD	4,140.4 (1,259.5)	1,593.8 – 6,687.0	0.21	0.65	0.005
non-spOD	3,586.8 (1,174.2)	1,212.5 – 5,961.1			
PostLM, cm					
spOD	5.18 (0.99)	3.18 – 7.18	0.07	0.789	0.002
non-spOD	4.82 (0.92)	2.96 – 6.68			

M – mean; SE – standard error; CI – confidence interval; AntRP – anterior right pixel count; spOD – screening positive for oropharyngeal dysphagia; non-spOD – screening negative for oropharyngeal dysphagia; AntRM – anterior right maximum distance; PostRP – posterior right pixel count; PostRM – posterior right maximum distance; AntLP – anterior left pixel count; AntLM – anterior left maximum distance; PostLP – posterior left pixel count; PostLM – posterior left maximum distance.

***Note*: Controlling for covariates included age, body mass index, and length of stay [adjusted M (SE), 95% CI]. Bonferroni correction applied, significance threshold $p < 0.00625$.**

the multivariate main effect of group was not statistically significant: Pillai's trace = 0.15, $F(8.33) = 0.72$, $p = 0.67$, partial $\eta^2 = 0.15$. No significant difference was observed in the overall TS pattern between the spOD and non-spOD groups. Although the multivariate test was non-significant, univariate *F*-tests for each dependent variable were examined to provide descriptive information. To control for Type I error due to multiple comparisons, a Bonferroni correction was applied ($\alpha = 0.05/8 = 0.00625$). After correction, none of the eight variables showed a statistically significant group

difference (all $p > 0.006$). The smallest p-value was 0.034 (for posterior right max distance), which remained well above the adjusted threshold. Inspection of the adjusted means revealed similar TS values across groups. Taken together, these findings suggest that TS parameters were comparable between groups, and no evidence was found for an association between spOD and TS during swallowing.

To examine the robustness of the MANCOVA findings, a multivariable linear regression (enter method) was performed with maximum HF as the dependent variable and group, age, BMI, LOS, and sex as independent variables. The regression model was significant and explained 45% of the variance in maximum HF ($R^2 = 0.45$, adjusted $R^2 = 0.38$). As shown in Table 4, after controlling for age, BMI, LOS, and sex, spOD group status (spOD vs. non-spOD) remained significantly and positively associated with maximum HF (B = 13.46, SE = 3.44, 95% CI: 6.50–20.41, β = 0.505, $p < 0.001$). This confirmatory analysis is consistent with the primary MANCOVA finding and suggests that the association between spOD and greater HF during swallowing is independent of potential confounders.

According to the Pearson correlation analysis, age was significantly negatively correlated with swallowing speed ($r = -0.32$, $p = 0.03$) and positively correlated with the number of swallows ($r = 0.39$, $p = 0.007$) (Table 5). A significant negative correlation was also found between swallowing speed and both the number of swallows ($r = -0.81$, $p < 0.001$) and maximum HF in the sagittal plane ($r = -0.55$, $p < 0.001$). Additionally, a significant positive correlation was observed between the number of swallows and maximum HF ($r = 0.43$, $p = 0.003$). No other significant correlations were observed between the remaining variables ($p > 0.05$) (Table 5).

Qualitative findings

Analysis of the semi-structured interviews revealed that participants with spOD had independently developed a wide range of strategies, which were grouped into two main themes (Figure 4). The first main theme was pre-swallow preparation and food adaptation. This theme encompassed strategies employed before the swallowing act itself. Participants consistently reported nutrient bite control ("I always cut my food into very small pieces, like baby food."), mental and physical preparation ("I need to be in a quiet place and focus only on eating."), fluid support ("I take a sip of water after every bite to help it go down."), and nutrient diversion ("I avoid bread because it always gets stuck."). The second theme addressed spontaneous swallowing manoeuvres during swallowing. This theme included behavioral compensations used during the swallow. Participants described reflexive responses such as breath-holding and intentional coughing ("I have to hold my breath and then cough lightly to clear my throat."). Physical support strategies were commonly used, including stabilizing the body by holding onto the chair ("I grip the armrests tightly to keep my body steady.") and keeping shoulders fixed. Additionally, swallowing control manoeuvres such as multiple swallows *per* bite were frequently mentioned.

Table 4

Multivariable linear regression analysis for maximum head flexion

Independent variable	B (95% CI)	SE	β	p-value	Adj R^2	$F(df)$
Constant	-62.63 (-121.2 – -4.05)	28.96	–	0.037	0.38	6.41 (5.39)
Group (spOD vs. non-spOD)	13.46 (6.50 – 20.41)	3.44	0.505	< 0.001		
Age	0.75 (-0.02 – 1.53)	0.38	0.253	0.057		
BMI	-0.32 (-1.1 – 0.47)	0.39	-0.102	0.418		
Length of stay	0.13 (-0.06 – 0.32)	0.09	0.166	0.177		
Sex	-0.53 (-7.34 – 6.28)	3.37	-0.019	0.876		

CI – confidence interval; SE – standard error; Adj – adjusted; ; *df* – degrees of freedom; spOD – screening positive for oropharyngeal dysphagia; non-spOD – screening negative for oropharyngeal dysphagia; BMI – body mass index. *Note*: Dependent variable – maximum head flexion. Predictors were entered simultaneously (enter method). Adjusted R^2 and F value are for the full model.

Table 5

Correlation coefficients among study variables (n = 45)

Variable	Age	SwalSpe	nSwal	HeadAPM	Mean ± SD
Age					
p-value	–	0.03	0.17	0.007	73.71 ± 4.52
r	1	-0.32	0.2	0.39	
SwalSpe					
p-value		–	0.000†	0.000	13.05 ± 6.06
r		1	-0.81	-0.55	
nSwal					
p-value			–	0.003	4.13 ± 1.39
r			1	0.43	

n – number; SwalSpe – swallowing speed; nSwal – number of swallows; HeadAPM – head anterior/posterior maximum angle from the start; SD – standard deviation.

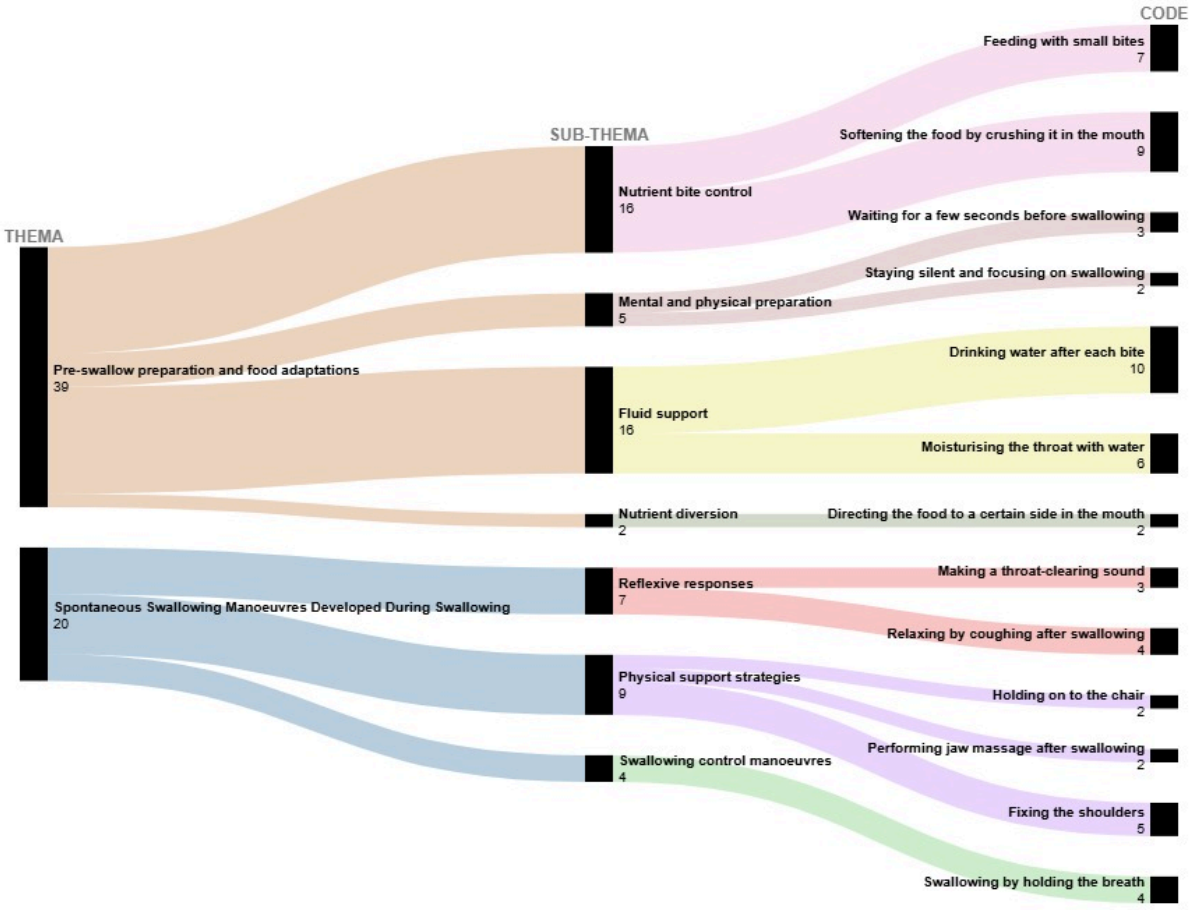


Fig. 4 – Theme-sub-theme-code-frequency distribution (numbers under codes indicate frequency).

Discussion

According to the Pearson correlation analysis, it was noted in the presented study that as age increases, swallowing speed tends to decrease, while the number of swallows increases. Based on the study results, it could also be suggested that lower swallowing speed is associated with a greater number of swallows and increased anterior HF.

This study used a mixed-methods approach to describe spontaneous HPs and TS as observed in older adults in nursing homes with spOD. During swallowing, a significant increase in HF was observed in the spOD group compared to the non-spOD group. After Bonferroni correction, no significant difference was found between the two groups in TS parameters and the angles of other head movements. A positive correlation was found between the maximum HF angle, age, and number of swallows. Additionally, the spOD group showed higher swallowing frequency and longer feeding time. Participants reported various strategies they used to manage swallowing difficulties, including reducing food intake, receiving fluid support, choosing a quiet environment, holding onto a fixed surface, keeping the shoulders level, breath-holding, and reflexive coughing.

Head and neck posture is known to affect swallowing function. Debucean et al. [24] reported that head hyperextension and forward HP are associated with swallowing disorders due to decreased oesophageal patency and changes in the position of the hyoid-related muscles. In contrast, HF and chin tuck (ChT) positions have been reported to enhance swallowing function by accelerating laryngeal closure, reducing pharyngeal residue, and increasing muscle activation [12, 25]. Based on these findings, clinical guidelines recommend that individuals with spOD be trained in HF and ChT positions under the supervision of a healthcare professional [8]. In our study, we observed that individuals with spOD exhibited greater HF angle during swallowing without clinical guidance. One possible interpretation is that this posture may have been acquired through experience; however, our data cannot determine whether it was learned consciously or whether it actually facilitates swallowing. Furthermore, the positive correlation between HF and the number of swallows suggests a relationship, but whether HF directly reduces swallowing effort or improves comfort remains unknown based on these findings.

Qualitative data showed that participants consciously used several physical strategies during swallowing. For instance, some participants fixed their shoulders (“I keep my body still while swallowing”; “I do not move my shoulders.”), held onto the chair (“I squeeze my hands on the armrests”; “I feel safer.”), or performed jaw massage (“I lightly

massage my jaw after swallowing"; "It feels like the food goes down more easily."). These reports reflect explicit, awareness-based coping behaviors. Interestingly, none of the participants mentioned HF during swallowing in the interviews. This is noteworthy because the quantitative part of the study found that the spOD group exhibited significantly greater HF compared to the non-spOD group. One possible reason for this discrepancy is that HF may occur at a less conscious level of motor control, whereas the strategies reported in the interviews are deliberate and easily verbalized. Another possibility is that the semi-structured interview did not include specific questions about HP, which could have led to underreporting of this particular movement. The present study cannot determine whether HF serves a compensatory function or whether it occurs without conscious awareness. However, the fact that HF was observed only in the spOD group and was not verbally reported by participants suggests that this movement may operate outside of deliberate awareness.

Ko et al. [26] reported that performing the ChT manoeuvre with each bite during swallowing may facilitate the swallowing process. The ChT position is considered either a combination of head and neck flexion or a form of isolated HF. In clinical practice, ChT is consciously taught to individuals with spOD [12]. In our study, however, participants with spOD exhibited greater HF during swallowing without any prior training or instruction. This spontaneously observed HF resembles the ChT posture in direction, although it may be a simpler, less refined movement. Our findings cannot determine whether this HF provides the same physiological benefits as the ChT manoeuvre; however, they may generate the hypothesis that spontaneous HF could serve a similar purpose, which should be tested in future studies using direct physiological measures. Although studies examining TS during swallowing are limited, it is well established that temporomandibular joint disorders, occlusal imbalances, tooth loss, and changes in tongue position–as well as simple oral activities such as breathing or chewing gum–can influence postural control and TS in individuals [27–31]. In our study, no significant difference was found in TS parameters of individuals with spOD during swallowing performed in the relaxed sitting position. One possibility is that participants limited their trunk movements, whether consciously or automatically. Qualitative data show that participants reported using physical strategies such as keeping their shoulders still and using the chair for support. These observations are consistent with the recommendations of Gampp et.al. [32], who propose holding onto the chair arms and leaning back to stabilize the trunk during swallowing. Although our data cannot determine whether this is conscious or automatic, qualitative reports of deliberate actions (e.g., holding the chair, fixing the shoulders) suggest that at least some stability is consciously achieved.

A review of the qualitative literature reveals that the spontaneous strategies developed by older adults are largely grounded in individual experiences and, in some cases, align with clinical recommendations. Previous studies have reported that personality and cognition-based approaches–such as imagining a favorite food during swallowing [13], intentionally slowing the swallowing pace [4], or traits like stubbornness [10]–can influence swallowing performance. Participants in our study implemented cognition preparation strategies by choosing a quiet environment and incorporating a brief pause before swallowing. Based on these findings, it can be inferred that cognitive approaches vary among individuals, and that clinicians should consider such experiences in the context of rehabilitation.

While previous qualitative studies have reported fluid-assisted approaches such as softening food with hot water [11] and swallowing with water [4], our study revealed different water-based strategies, including drinking water to moisten the throat and taking fluids regularly after each bite. In addition to reflexive responses reported in previous studies, such as breath-holding during swallowing, participants [4] in our study reported engaging in behaviors such as coughing and throat clearing after each bite. Our study's qualitative findings suggest that spontaneously used swallowing strategies are influenced by personal, cognitive, and environmental factors, and that some elderly people may develop such strategies without having received prior clinical instruction. However, it is considered necessary to support the impact of these participant-reported strategies on swallowing performance through quantitative research.

Limitations of the study

This study has several limitations. First of all, it included only older adults residing in a nursing home; therefore, the findings may not be generalizable to the elderly population living in the community. Since the qualitative data collection process relied on participants' ability to articulate their experiences, some strategies may have been unintentionally underreported. Since the study was designed as cross-sectional, it was not possible to observe changes over time. Frailty, medication burden, and detailed postural assessments were not evaluated; residual confounding cannot be completely ruled out. Classification of participants into spOD and non-spOD groups was based solely on the 100 mL water swallow test, which has a reported specificity of 50%. Consequently, some degree of participant misclassification cannot be excluded. Only a liquid bolus (water) was used. Water swallow tests do not assess the oral preparatory and oral phases, and findings may not generalize to other consistencies (e.g., puree, solid food). Despite these limitations, the study offers valuable insights into the spontaneously developed compensatory strategies of older adults. It quantitatively reveals how such strategies–particularly those related to HP and TS–are employed during swallowing. Findings should be interpreted as exploratory and hypothesis-generating.

Conclusion

This mixed-methods study examined head and trunk movements during swallowing in older adults living in a nursing home who screened positive for oropharyngeal dys-

phagia. Quantitative findings revealed an association between screening-positive oropharyngeal dysphagia and greater head flexion during swallowing. No significant group differences were observed for trunk sway parameters or other head movements.

In qualitative interviews, participants described various strategies aligned with clinical recommendations (e.g., small bites, drinking water with swallows, choosing a quiet environment). However, none of the participants mentioned head flexion. This discrepancy between the qualitative and quantitative findings raises the possibility that head flexion may occur without explicit awareness. Nevertheless, our study cannot determine whether this movement is conscious or contributes to swallowing safety.

These findings are hypothesis-generating regarding the association between screening-positive oropharyngeal dysphagia and head flexion during swallowing. Clinicians may find it useful to be aware of spontaneously exhibited head postures and self-reported strategies, but further research is needed to determine their potential relevance for rehabilitation. However, findings apply to individuals screening positive for oropharyngeal dysphagia and await confirmation in samples with instrumentally confirmed dysphagia.

REFERENCES

1. *Ribeiro M, Miquilussi PA, Gonçalves FM, Taveira KVM, Stechman-Neto J, Nascimento WV,* et al. The Prevalence of Oropharyngeal Dysphagia in Adults: A Systematic Review and Meta-analysis. Dysphagia 2024; 39(2): 163–76. DOI: 10.1007/s00455-023-10608-8.
2. *Takizawa C, Gemmell E, Kenworthy J, Speyer R.* A Systematic Review of the Prevalence of Oropharyngeal Dysphagia in Stroke, Parkinson's Disease, Alzheimer's Disease, Head Injury, and Pneumonia. Dysphagia 2016; 31(3): 434–41. DOI: 10.1007/s00455-016-9695-9.
3. *Ortega O, Martín A, Clavé P.* Diagnosis and Management of Oropharyngeal Dysphagia Among Older Persons, State of the Art. J Am Med Dir Assoc 2017; 18(7): 576–82. DOI: 10.1016/j.jamda.2017.02.015.
4. *Hsu WC, Tsai HH, Weng LC, Wang YW.* The experience of eating for older nursing home residents with dysphagia: A qualitative descriptive study. Int J Older People Nurs 2023; 18(6): e12566. DOI: 10.1111/opn.12566.
5. *Engh MCN, Speyer R.* Management of Dysphagia in Nursing Homes: A National Survey. Dysphagia 2022; 37(2): 266–76. DOI: 10.1007/s00455-021-10275-7.
6. *Forster A, Samaras N, Gold G, Samaras D.* Oropharyngeal dysphagia in older adults: A review. Eur Geriatr Med 2011; 2(6): 356–62. DOI: 10.1016/j.eurger.2011.08.007.
7. *Rivelsrud MC, Hartelius L, Bergström L, Løvstad M, Speyer R.* Prevalence of oropharyngeal dysphagia in adults in different healthcare settings: a systematic review and meta-analyses. Dysphagia 2023; 38(1): 76–121. DOI: 10.1007/s00455-022-10465-x.
8. *Chen S, Kent B, Cui Y.* Interventions to prevent aspiration in older adults with dysphagia living in nursing homes: a scoping review. BMC Geriatr 2021; 21(1): 429. DOI: 10.1186/s12877-021-02366-9.
9. *Melgaard D, Westergren A, Skrubbeltrang C, Smithard D.* Interventions for Nursing Home Residents with Dysphagia—A Scoping Review. Geriatrics (Basel) 2021; 6(2): 55. DOI: 10.3390/geriatrics6020055.
10. *Helldén J, Bergström L, Karlsson S.* Experiences of living with persisting post-stroke dysphagia and of dysphagia management – a qualitative study. Int J Qual Stud Health Well-being 2018; 13(sup 1): 1522194. DOI: 10.1080/17482631.2018.1522194.
11. *Howells SR, Cornwell PL, Ward EC, Kuipers P.* Client perspectives on living with dysphagia in the community. Int J Speech Lang Pathol 2021; 23(2): 201–12. DOI: 10.1080/17549507.2020.1765020.
12. *Nagura H, Kagaya H, Inamoto Y, Shibata S, Ozeki M, Otaka Y.* Effects of head flexion posture in patients with dysphagia. J Oral Rehabil 2022; 49(6): 627–32. DOI: 10.1111/joor.13322.
13. *Moloney J, Walshe M.* "I had no idea what a complicated business eating is…": a qualitative study of the impact of dysphagia during stroke recovery. Disabil Rehabil 2018; 40(13): 1524–31. DOI: 10.1080/09638288.2017.1300948.
14. *Sandelowski M, Voils CI, Barroso J.* Defining and designing mixed research synthesis studies. Res Sch 2006; 13(1): 29.
15. *Küçükdeveci AA, Yavuzer G, Tennant A, Süldür N, Sonel B, Arasil T.* Adaptation of the modified Barthel Index for use in physical medicine and rehabilitation in Turkey. Scand J Rehabil Med 2000; 32(2): 87–92.
16. *Doan TN, Ho WC, Wang LH, Chang FC, Nhu NT, Chou LW.* Prevalence and Methods for Assessment of Oropharyngeal Dysphagia in Older Adults: A Systematic Review and Meta-Analysis. J Clin Med 2022; 11(9): 2605. DOI: 10.3390/jcm11092605.
17. *Wu MC, Chang YC, Wang TG, Lin LC.* Evaluating swallowing dysfunction using a 100-ml water swallowing test. Dysphagia 2004; 19(1): 43–7.
18. *Brodsky MB, Suiter DM, González-Fernández M, Michtalik HJ, Frymark TB, Venediktov R,* et al. Screening accuracy for aspiration using bedside water swallow tests: a systematic review and meta-analysis. Chest 2016; 150(1): 148–63. DOI: 10.1016/j.chest.2016.03.059.
19. *Sturnieks DL, Arnold R, Lord SR.* Validity and reliability of the Swaymeter device for measuring postural sway. BMC Geriatr 2011; 11: 63. DOI: 10.1186/1471-2318-11-63.
20. *Ghorbani F, Kamyab M, Azadinia F.* Smartphone Applications as a Suitable Alternative to CROM Device and Inclinometers in Assessing the Cervical Range of Motion in Patients With Non-specific Neck Pain. J Chiropr Med 2020; 19(1): 38–48. DOI: 10.1016/j.jcm.2019.10.004.
21. *Sedrez JA, Furlanetto TS, Gelain GM, Candotti CT.* Validity and reliability of smartphones in assessing spinal kinematics: a systematic review and meta-analysis. J Manipulative Physiol Ther 2020; 43(6): 635–45. DOI: 10.1016/j.jmpt.2019.10.012.
22. *Kallio H, Pietilä AM, Johnson M, Kangasniemi M.* Systematic methodological review: developing a framework for a qualitative semi-structured interview guide. J Adv Nurs 2016; 72(12): 2954–65. DOI: 10.1111/jan.13031.
23. *Pope C, Mays N.* Qualitative research in health care. 3rd ed. USA: John Wiley & Sons; 2013. p. 168.
24. *Debucean D, Mihaiu J, Maghiar AM, Marcu F, Marcu OA.* A Multidisciplinary Approach to Swallowing Rehabilitation in Patients with Forward Head Posture. Medicina (Kaunas) 59(9): 1580. DOI: 10.3390/medicina59091580.
25. *Matías Ambiado-Lillo M.* Impact of Head and Neck Posture on Swallowing Kinematics and Muscle Activation: A Systematic Review. Dysphagia 2025; 40(5): 1049–54. DOI: 10.1007/s00455-025-10821-7.
26. *Ko JY, Shin DY, Kim TU, Kim SY, Hyun JK, Lee SJ.* Effectiveness of Chin Tuck on Laryngeal Penetration: Quantitative As-

sessment. Dysphagia 2021; 36(6): 1054–62. DOI: 10.1007/s00455-020-10238-4.

27. *Peng CH, Pauloski BR*. Ultrasonography as Biofeedback to Increase Muscle Activation During the Mendelsohn Maneuver in Healthy Adults. Dysphagia 2023; 38(4): 1156–68. DOI: 10.1007/s00455-022-10542-1.
28. *Alghadir AH, Zafar H, Iqbal ZA*. Effect of tongue position on postural stability during quiet standing in healthy young males. Somatosens Mot Res 2015; 32(3): 183–6. DOI: 10.3109/08990220.2015.1043120.
29. *Fadilliogˇlu C, Kanus L, Möhler F, Ringhof S, Hellmann D, Stein T*. Influence of controlled stomatognathic motor activity on sway, control and stability of the center of mass during dynamic steady-state balance - an uncontrolled manifold analysis. Front Hum Neurosci 2022; 16: 868828. DOI: 10.3389/fnhum.2022.868828.
30. *Goto F, Kushiro K, Tsutsumi T*. Effect of chewing gum on static posturography in patients with balance disorders. Acta Otolaryngol 2011; 131(11): 1187–92. DOI: 10.3109/00016489.2011.607846.
31. *Schmid M, Conforto S, Bibbo D, D'Alessio T*. Respiration and postural sway: detection of phase synchronizations and interactions. Hum Mov Sci 2004; 23(2): 105–19. DOI: 10.1016/j.humov.2004.06.001.
32. *Gampp Lehmann K, Sticher H*. Posture and Function: What Helps Us Swallow. In: *Nusser-Müller-Busch R, Gampp Lehmann K*, editors. Facial-Oral Tract Therapy (FOTT): For Eating, Swallowing, Nonverbal Communication and Speech. Cham: Springer; 2021. pp 45–72. DOI: 10.1007/978-3-030-51637-6_4.

Received on October 19, 2025
Revised on May 10, 2026
Revised on June 28, 2026
Accepted on July 8, 2026
Online First August, 2026

CASE REPORT

UDC: 616.24-006.6-07/-08::618.2
DOI: https://doi.org/10.2298/VSP260316043D

Maternal and fetal outcomes in pregnant women with lung cancer: a case report

Maternalni i fetalni ishodi kod trudnica obolelih od karcinoma pluća

Suzana Drobnjak*, Nataša Karadžov Orlić*†, Aleksandra Vasić*, Jelica Uljarević*, Svetlana Janković*†

***Clinic for Gynecology and Obstetrics "Narodni Front", Department of High-Risk Pregnancy, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia**

Abstract

Introduction. Lung cancer (LC) during pregnancy is rare, rarer than other types of malignancy. We present the case of a pregnant patient with advanced-stage LC, highlighting the diagnostic approach, pregnancy management, delivery, and postpartum course. **Case report.** A 46-year-old primiparous patient, 30 weeks pregnant with *in vitro* fertilization, was admitted to the hospital with right-sided chest pain and cough. The patient was a former smoker with a 15-year history of smoking. Due to the patient's general condition and reported symptoms, an X-ray and computed tomography of the chest were performed. A massive necrotic lobulated tumor was revealed in the right lobe of the lung, involving the pleura and almost the entire lung parenchyma. A tru-cut biopsy of the lesion in the right lung was immediately performed. Histopathological examination of the biopsy specimen revealed a poorly differentiated neuroendocrine tumor of the small cell LC. At 32 weeks of gestation, signs of fetal hypoxemia were diagnosed, and the pregnancy was terminated by elective cesarean section. A male fetus weighing 1,550 g was born, with an Apgar score of 7/8, and after stabilization was referred to the Institute for Neonatology, Belgrade, Serbia. The placenta was sent for histopathological examination, and metastatic poorly differentiated neuroendocrine carcinoma was confirmed. The patient was presented to the Gynecological, Pulmonology, and Oncologic Councils, and radiotherapy was initiated immediately after delivery, but unfortunately, she died 7 days after delivery. **Conclusion.** The diagnosis and treatment of LC in pregnancy is challenging. That includes timely detection, treatment decisions and efficacy, as well as the potential risks for the fetus.

Keywords:
biopsy, needle; diagnosis; lung neoplasms; pregnancy; radiotherapy; treatment outcome.

Apstrakt

Uvod. Karcinom pluća (*lung cancer* – LC) tokom trudnoće je redak, ređi od drugih vrsta maligniteta. Prikazujemo trudnicu sa uznapredovalim LC, sa posebnim osvrtom na dijagnostički pristup, vođenje trudnoće, porođaj i postporođajni tok. **Prikaz bolesnika.** Prvorotkinja, starosti 46 godina, u 30. nedelji trudnoće nakon vantelesne oplodnje, primljena je u bolnicu zbog bola u grudnom košu sa desne strane i kašlja. Bolesnica je bila bivši pušač, sa 15-godišnjom istorijom pušenja. Zbog opšteg stanja i prijavljenih simptoma, urađeni su rendgenski snimak i kompjuterizovana tomografija grudnog koša. U desnom režnju pluća otkriven je masivni nekrotični lobulirani tumor, koji je zahvatao pleuru i skoro ceo plućni parenhim. Odmah je izvršena *tru-cut* biopsija lezije u desnom plućnom krilu. Histopatološkim pregledom biopsije uzorka utvrđen je slabo diferencirani neuroendokrini sitnoćelijski LC. U 32. nedelji gestacije dijagnostikovani su znaci fetalne hipoksemije i trudnoća je prekinuta elektivnim carskim rezom. Rođen je muški fetus, težine 1 550 g, Apgar skora 7/8 i posle stabilizacije upućen je na Institut za neonatologiju, Beograd, Srbija. Placenta je poslata na histopatološki pregled, i potvrđen je metastatski slabo diferenciran neuroendokrini karcinom. Bolesnica je prikazana na ginekološkom, pulmološkom i onkološkom konzilijumu i radioterapija je započeta odmah posle porođaja, ali je, nažalost, preminula 7 dana posle porođaja. **Zaključak.** Dijagnoza i lečenje LC u trudnoći predstavljaju poseban izazov. To podrazumeva rano otkrivanje, odluke o lečenju i njegovu efikasnost, kao i rano otkrivanje potencijalnih rizika za fetus.

Ključne reči:
biopsija iglom; dijagnoza; pluća, neoplazme; trudnoća; radioterapija; lečenje, ishod.

Correspondence to: Svetlana Janković, Clinic for Gynecology and Obstetrics "Narodni Front", Department of High-Risk Pregnancy, Kraljice Natalije 62, 11 070 Belgrade, Serbia. E-mail: svetlanajankovic.r@gmail.com

Introduction

The incidence of malignant diseases during pregnancy is around 0.1% [1]. Lung cancer (LC) during pregnancy is rare, with only 133 cases reported in the literature from 1953 to 2024 [2]. The incidence of LC is on the rise due to the rising number of smokers and the trend of delayed childbearing. Among all cancers in pregnancy, it carries the highest mortality rate (64.3%) and increases the risk of placental abnormalities, preterm birth, and low birth weight [2, 3]. Diagnosis is usually made at an advanced stage of the disease [1] due to nonspecific symptoms that are often overlooked by both pregnant patients and their physicians, leading to delayed diagnosis and a poor prognosis [3, 4]. Distant metastases are often present, and in rare cases, metastases may also be found in placental tissue, which can negatively affect fetal outcomes [1].

We present the case of a pregnant patient with advanced-stage LC (stage IV), highlighting the diagnostic approach, pregnancy management, delivery, and postpartum course.

Case report

A primigravida, 46-year-old medical worker and radiologist, in the 30th week of pregnancy, achieved through the fourth attempt at *in vitro* fertilization (donor egg), presented to the Clinic for Gynecology and Obstetrics "Narodni Front" Belgrade, Serbia, due to right-sided chest pain radiating to the spine, tachycardia, and shortness of breath. The pain and shortness of breath had been present for the past 2 weeks, while tachycardia had been ongoing for several months (treated with Presolol® 10 mg prescribed by a primary care internist). The patient also reported an occasional dry cough but denied other respiratory symptoms. She denied weight loss (with a total weight gain of 3 kg during pregnancy). She was a former smoker with a history of smoking for 15 years (20 cigarettes *per* day), but had quit a year prior.

Upon hospital admission, clinical, ultrasound, and laboratory evaluations were performed.

Laboratory results included: leukocytes 9.8 × 10^9/L [reference range (RR): 3.4–9.7 × 10^9/L]; hemoglobin 98 g/L (RR: 119–157 g/L); platelets 593 × 10^9/L (RR: 158–424 × 10^9/L); C-reactive protein 147.9 mg/L (RR: less than 5 mg/L); potassium 5.0 mmol/L (RR: 3.5–5.1 mmol/L); lactate dehydrogenase 789 U/L (RR: 0–241 U/L); D-dimer 0.5 mg/L (III trimester cut-off 2.12 mg/L); erythrocyte sedimentation rate 70 mm/h (RR: less than 50 mm/h). After consultation with an internist, due to the patient's general condition and reported symptoms, a chest and heart X-ray was indicated.

The chest X-ray showed a massive right-sided effusion up to the anterior margin of the second rib, with the effusion shadow superimposed on a clearly contoured oval-shaped tumor shadow in the right hemithorax (Figure 1).

Due to these X-ray findings and the patient's symptoms, urgent further diagnostics were performed, including a computed tomography (CT) scan of the chest.

CT imaging showed a very large lobulated necrotic tumor in the right lung in continuity with the rib, extending into the pleura, and involved almost the entire right lung parenchyma. Only the apical segment of the upper lobe (segment I) and a small part of the posterior segments of the middle and lower lobes remained unaffected. After contrast

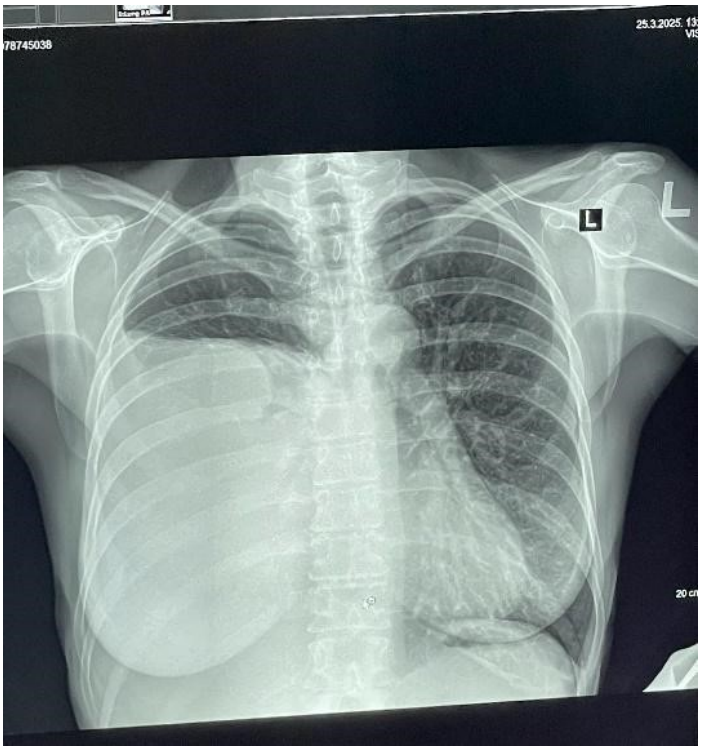


Fig. 1 – X-ray image of the heart and lungs of a woman at 30 weeks of gestation showing massive right pleural effusion extending to the anterior margin of the second rib, with a well-defined oval tumor shadow in the right hemithorax and involving almost the entire right lung parenchyma.

administration, the lesion demonstrated heterogeneous opacification and contained several central microcalcifications, measuring approximately 15 × 14 × 17 cm [anteroposterior × transverse × craniocaudal (AP × LL × CC)]. The tumor caused a marked shift of the mediastinal structures toward the left. It demonstrated compression and infiltration of the right pulmonary artery, the right main bronchus and its branches, with suspected involvement of the superior *vena cava*. A loculated pleural effusion containing clear fluid, measuring up to 1.5 cm in thickness, was present. In addition, a small thrombus was identified within the right pulmonary veins, resulting from compression by the tumor. Enlarged mediastinal lymph nodes were noted: 2R – 15 × 11 mm, 4R – 12 × 10 mm. Bilateral reactive axillary lymph nodes were present, measuring up to 13 × 10 mm. No evidence of tumor infiltration was identified in the remaining lung parenchyma. Cross-sections through the upper abdomen revealed multiple hypodense hepatic lesions, the largest located subcapsularly in segments II/III, measuring 22 × 17 mm, with central contrast enhancement. Additionally, a 3 × 2 cm osteolytic soft-tissue lesion was identified in the L1 vertebral body, consistent with a probable metastatic deposit.

A tru-cut biopsy of the lesion in the right lung was indicated and subsequently performed. Histopathological (HP) examination of the biopsy revealed: Histology and immunohistochemistry (IHC): tumor cells are CK AE1/AE3+ cytoplasmic dot-like, CD56 diffuse ++, synaptophysin diffuse+++, Pax7+, TTF-1-, vimentin -, CD99-, Nkx 2.2-, ERG1 -, Fli1-, pRb IHC nuclear expression observed in about 50% of tumor cells, Ki-67 shows nuclear immunopositivity in about 40% of tumor cells. The described morphological changes and IHC profile of the tumor cells are consistent with small cell LC (Figure 2).

With the HP findings, the patient was presented to the Gynecological Council at Obstetric and Gynaecology Clinic "Narodni Front", the Pulmonology Council of the Clinic for Pulmonology, University Clinical Center of Serbia, Belgrade, Serbia, and the Lung Council at the Institute for Oncology and Radiology of Serbia, Belgrade.

A discussion was held with the patient regarding the possibility of preterm termination of the pregnancy. Corticosteroid therapy was prescribed to promote artificial maturation of the fetal lungs, aiming to allow the earliest possible start of treatment for the underlying disease [systemic chemotherapy according to the EP protocol (includes etoposide and cisplatin), which the above-mentioned councils were initially reluctant to apply to a pregnant patient].

Additional diagnostics were performed to prepare for the treatment of the underlying disease: magnetic resonance imaging (MRI) of the brain showed an expansive lesion in the left cerebellum, mostly cystic with a small solid component, surrounded by a wide zone of perilesional edema, most likely secondary deposit and less likely of other etiology, along with signal alteration in the pons of unclear etiology. MRI of the cervical and thoracic spine indicated polydiscopathy with protrusions of the disc-osteophyte complex at the C4–5 intervertebral space dorsolaterally on the right, with mild irritation of the right C5 nerve root, and at C5–6 dorsally circumferentially with minor caudal migration of disc material medially and paramedially bilaterally, compromising the C7 nerve roots bilaterally, in close relation to the ventral aspect of the spinal cord without intramedullary myelopathy and with relative spinal canal stenosis (AP diameter ~9 mm), along with spondylosis and early spondyloarthrosis. MRI of the abdomen showed multiple focal liver lesions highly suspicious for secondary deposits.

The patient was closely monitored at the Department of High-Risk Pregnancy, Clinic for Gynecology and Obstetrics "Narodni Front" from March 25, 2025, to April 10, 2025. Ultrasound showed a mildly growth-restricted fetus (small for gestational age) without visible anomalies, in breech presentation, amniotic fluid index (AFI) 100 mm, placenta located on the posterior wall, 27 mm thick, grade II maturity by Grannum with central cord insertion. Doppler indices of uteroplacental and fetoplacental circulation were normal. The biophysical profile was normal (8/8). Daily ultrasound monitoring and non-stress cardiotocography were performed.

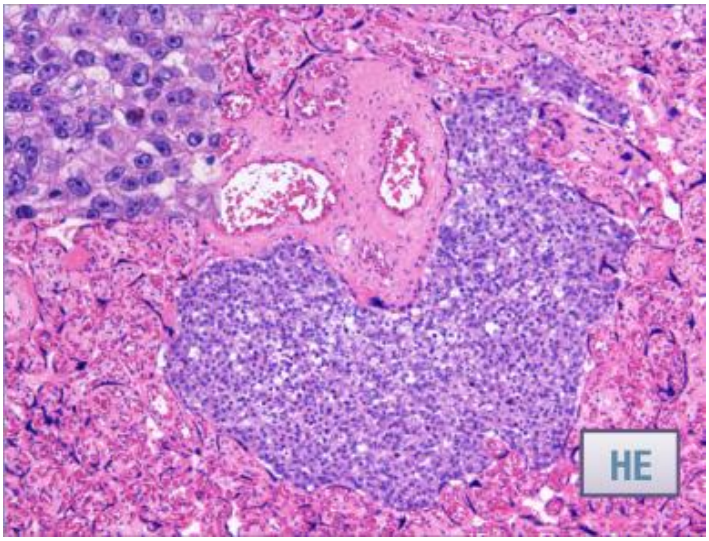


Fig. 2 – The immunohistochemical profile of the patient is consistent with small cell lung cancer (HE staining, magnification ×60). HE – hematoxylin-eosin.

The patient received antibiotic, symptomatic, supportive, and corticosteroid therapy and was evaluated by an internist, hematologist, and anesthesiologist.

At 32 weeks of gestation, a sudden decrease in AFI to 50 mm was diagnosed, with changes in fetoplacental and fetal circulation (cerebro/umbilical ratio < 1), indicating fetal hypoxemia. Given the conception *via* assisted reproductive technology, the diagnosis of LC, and the breech presentation of the fetus, it was decided to terminate the pregnancy by elective cesarean section.

The patient was medically, hematologically, and anesthesiologically prepared, and on April 10, 2025, an elective cesarean section was performed at 31+6 weeks of gestation. A viable male infant was delivered, weighing 1,550 g, with Apgar scores of 7/8. After stabilization in the neonatal Intensive Care Unit, the infant was transferred to the Institute for Neonatology, Belgrade, Serbia.

In the postoperative period, while the patient was in the Intensive Care Unit, she received one unit of blood of the appropriate blood type due to severe anemia, which she tolerated well. Prophylaxis for deep vein thrombosis and thromboembolic complications was administered, along with antibiotic and antiulcer therapy. The placenta was sent for HP examination.

Placental HP examination revealed the following macroscopic findings: the placenta measured 200 × 180 × 30 mm, with preserved fetal surface, a paramarginal cord insertion (150 mm), and a three-vessel cord. More than 70% of the placental tissue showed grayish-yellow areas. Histology and IHC showed synaptophysin diffusely positive, chromogranin A diffusely positive, CD56 positive, TTF-1 positive, Ki-67 ~80%, and CK7 focally positive. The conclusion stated that the findings were metastatic in nature and consistent with small cell LC.

On April 14, 2025, the patient was discharged from the Clinic in stable general condition with normal gynecological and ultrasound findings. In coordination with the team of physicians from the Institute for Oncology and Radiology of Serbia, the patient was presented to the Council the following day to plan the initiation of therapy for the underlying disease.

The patient passed away on April 17, 2025, after one round of radiation therapy to the affected part of the spinal column.

Discussion

Epidemiological data on cancer mortality show a decreasing trend for most malignant diseases across Europe, owing to the progress in prevention, screening, and treatment. However, mortality from LC is increasing, particularly among women [5]. LC is the second most common cancer in the world and has the highest mortality rate [6]. It is the second most common cancer in men after prostate cancer, and the fourth most common cancer in women after breast cancer, colorectal cancer, and thyroid cancer [2]. Malignancy is the second leading cause of death among women aged 25 to 39 years [6].

Cancer during pregnancy is rare, with an incidence of approximately 1 in 1,000 pregnancies. These numbers are expected to increase in the coming decades due to delayed childbearing [6, 7]. The most common cancers diagnosed during pregnancy are breast cancer, cervical cancer, hematological malignancies (such as lymphomas and acute leukemias), and melanoma [7].

Early diagnosis is extremely important. The 5-year survival rate varies greatly depending on the stage of diagnosis, ranging from 57% for localized disease to only 5% for patients with metastatic disease [8]. The two most effective strategies for reducing LC mortality are reducing tobacco use and exposure to tobacco smoke [9] and early diagnosis through LC screening programs [10].

Smoking remains the leading risk factor for LC, considering that 18.4% of the population in the European Union still smokes. Smoking cessation is therefore a key strategy for the prevention and management of LC. Tobacco control policies such as increasing tobacco taxes, creating smoke-free environments, educating the population, especially women and pregnant women, organizing health campaigns, and providing accessible smoking cessation counseling are expected to have a significant impact on public health [11].

Clinical screening studies, including the 2020 study by De Koning et al. [9] published in the New England Journal of Medicine, have shown that low-dose CT effectively reduces mortality, shifts LC diagnosis toward earlier stages of the disease, and improves survival rates and quality of life [9, 12–14].

The diagnosis of malignant tumors during pregnancy has attracted increasing attention due to its relatively higher prevalence and its negative impact on outcomes for both the mother and the newborn.

The incidence of LC during pregnancy is lower compared to breast cancer, cervical cancer, and Hodgkin lymphoma, but it has been increasingly documented over the last two decades [2]. The first case of LC during pregnancy was described in 1953 by Barr [15], and more than 90 additional cases have been reported since then [2–4].

LC during pregnancy has been rarely reported, but an increase in incidence over the past 20 years is expected due to a higher number of young female smokers [1, 4]. In pregnant women, LC is usually diagnosed at an advanced stage, most commonly stage III or IV, due to nonspecific or absent symptoms [2–4]. The most commonly reported nonspecific symptoms include chest pain, cough, sputum production, and shortness of breath. Diagnosis during pregnancy is often limited due to potential risks to the fetus or embryo. Because of this, these symptoms are often attributed to pneumonia or asthma, which delays accurate diagnosis and treatment [1, 4].

A study by Zhou et al. [4] from 2023 analyzed 93 cases of LC during pregnancy between 1953 and 2022. The average age of the pregnant women was 34 years, and the average gestational age at diagnosis was 26 weeks. Unfortunately, nonspecific symptoms such as cough, shortness of breath, and chest pain, as seen in our patient as well, often lead to

delayed diagnosis and disease progression to advanced stages. Metastatic disease was identified in 35.5% of all cases. All reported cases had a long history of nicotine exposure. HP analysis showed that 81 cases were non-small cell LC, with placental metastases detected in 35.6% of cases. Metastases were also diagnosed in two newborns [4].

Non-small cell LC accounts for approximately 85% of LCs and is more commonly associated with older populations, with smoking as the primary risk factor [16].

Metastatic transmission of LC to the fetus and placenta is rare, with 11 reported cases of placental metastases and 3 cases of fetal metastases described in the literature [1–4]. In 27% of cases, mothers died within the first 24 hrs after delivery, while in 33.3% of cases, survival was approximately 12 months [4]. Our patient died 7 days after delivery. Similar results were reported in a study by Jacobson et al. [2].

There is currently no established protocol for the diagnosis and treatment of LC during pregnancy. According to available guidelines, oncological therapy during pregnancy is generally avoided. Chemotherapy is often avoided because pregnancy can change drug metabolism and because of potential toxicity to the fetus. The general risks associated with chemotherapy include premature birth, low birth weight, transient neonatal tachypnea, and temporary neonatal leukopenia [17, 18]. Chemotherapy and radiotherapy are typically initiated after delivery [1, 18–20].

In general, the golden rule in treating pregnant women with cancer is that treatment should not differ from treatment given to non-pregnant patients [21]. The main goal of treatment during pregnancy is to improve maternal survival by treating the underlying disease while continuously protecting the fetus from the harmful effects of therapy.

Conclusion

Delayed childbearing and long-term smoking habits have contributed to an increase in lung cancer incidence over the past 10 years. The diagnosis and treatment of lung cancer during pregnancy are challenging. It involves early detection, making treatment decisions, and balancing the health of the pregnant woman, effective tumor treatment, and the potential risks to the fetus. A multidisciplinary approach is essential to achieve the best possible outcome for both the patient and her pregnancy.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Bade BC, Dela Cruz CS*. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med 2020; 41(1): 1–24. DOI: 10.1016/j.ccm.2019.10.001.
2. *Jacobson S, Badeghiesh A, Baghlaf H, Margolese N, Dahan MH*. Maternal and Fetal Outcomes in Pregnant Women With Lung Cancer: A Population-Based Study on 9 Million Pregnancies and 40 Cases of Lung Cancer. BJOG 2025; 132(6): 834–7. DOI: 10.1111/1471-0528.18090.
3. *Heimovaara JH, Huis In 't Veld EA, Lok CAR, Garcia AC, Halaska MJ, Boere I; International Network on Cancer Infertility and Pregnancy*. Maternal death by cancer in pregnancy: A descriptive study of the International Network on Cancer, Infertility and Pregnancy. BJOG 2024; 131(12): 1694–704. DOI: 10.1111/1471-0528.17894.
4. *Zhou JP, Wang Y, Lin YN, Sun XW, Ding YJ, Yan YR,* et al. Clinical Features and Management of Lung Cancer During Pregnancy: A Narrative Review Based on Reported Cases. Women's Health Rep (New Rochelle) 2023; 4(1): 544–50. DOI: 10.1089/whr.2023.0085.
5. *Malvezzi M, Santucci C, Boffetta P, Collatuzzo G, Levi F, La Vecchia C,* et al. European cancer mortality predictions for the year 2023 with a focus on lung cancer. Ann Oncol 2023; 34(4): 410–9. DOI: 10.1016/j.annonc.2023.01.010.
6. *World Health Organization*. Global cancer observatory: cancer today [Internet]. France: WHO; 2020 [cited 2023 Apr 11; accessed 2026 May 15]. Available from: https://gco.iarc.fr/today
7. *Rothschild SI*. Lung Cancer in Pregnancy—A Forgotten Disease Entity. J Thorac Oncol 2016; 11(9): 1376–8. DOI: 10.1016/j.jtho.2016.07.009.
8. *Siegel RL, Miller KD, Jemal A*. Cancer statistics, 2020. CA Cancer J Clin 2020; 70(1): 7–30. DOI: 10.3322/caac.21590.
9. *De Koning HJ, van der Aalst CM, de Jong PA, Scholten ET, Nackaerts K, Heuvelmans MA,* et al. Reduced lung-cancer mortality with volume CT screening in a randomized trial. N Engl J Med 2020; 382(6): 503–13. DOI: 10.1056/NEJMoa1911793.
10. *National Lung Screening Trial Research Team*. Lung cancer incidence and mortality with extended follow-up in the national lung screening trial. J Thorac Oncol 2019; 14(10): 1732–42. DOI: 10.1016/j.jtho.2019.05.044.
11. *Eurostat*. Daily smokers of cigarettes by sex, age, and educational attainment level [Internet]. Luxembourg: Eurostat; 2022 [cited 2026 May 15]. DOI: 10.2908/hlth_ehis_sk3e. Available from: https://ec.europa.eu/eurostat/databrowser/view/hlth_ehis_sk3e/default/table?lang=en
12. *The National Lung Screening Trial Research Team*. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011; 365(5): 395–409. DOI: 10.1056/NEJMoa1102873.
13. *EUR-Lex*. Council Recommendation of 9 December 2022 on strengthening prevention through early detection: a new EU approach on cancer screening replacing Council Recommendation 2003/878/EC2022 [Internet]. Luxembourg: EUR-Lex; 2022 [cited 2026 May 15]. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=oj:JOC_2022_473_R_0001
14. *Fernandes MGO, Dias M, Santos R, Ravara S, Fernandes P, Firmino-Machado J,* et al. Recommendations for the implementation of a national lung cancer screening program in Portugal—A consensus statement. Pulmonology 2024; 30(6): 625–35. DOI: 10.1016/j.pulmoe.2024.04.003.
15. *Barr JS*. Placental metastases from a bronchial carcinoma. J Obstet Gynaecol Br Emp 1953; 60(6): 895–7. DOI: 10.1111/j.1471-0528.1953.tb07292.x.
16. *Simons EA, Patil T, Camidge DR*. Pregnancy and pathways to motherhood in oncogene-driven lung cancer: a single institution experience. Clin Lung Cancer 2023; 24(2): e55–9. DOI: 10.1016/j.cllc.2022.11.003.
17. *Cardonick E, Iacobucci A*. Use of chemotherapy during human pregnancy. Lancet Oncol 2004; 5(5): 283–91. DOI: 10.1016/S1470-2045(04)01466-4.
18. *Silverstein J, Post AL, Chien AJ, Olin R, Tsai KK, Ngo Z,* et al. Multidisciplinary management of cancer during pregnancy.

JCO Oncol Pract 2020; 16(9): 545–57. DOI: 10.1200/OP.20.00077.

19. *Ji Y, Schwartz J, Hartford A, Ramsey J, Phillips J, Verschraegen C.* Successful treatment of non-small cell lung cancer with erlotinib throughout pregnancy. JAMA Oncol 2015; 1(6): 838–40. DOI: 10.1001/jamaoncol.2015.1300.
20. *Azim HA Jr, Scarfone G, Peccatori FA.* Carboplatin and weekly paclitaxel for the treatment of advanced non-small cell lung cancer (NSCLC) during pregnancy. J Thorac Oncol 2009; 4(4): 559–60. DOI: 10.1097/JTO.0b013e31819c8674.
21. *Soares A, Dos Santos J, Silva A, Magalhães H, Estevinho F, Sottomayor C.* Treatment of lung cancer during pregnancy. Pulmonology 2020; 26(5): 314–7. DOI: 10.1016/j.pulmoe.2020.05.004.

Received on March 16, 2026
Revised on April 21, 2026
Revised on May 13, 2026
Accepted on May 27, 2026
Online First July 2026

INSTRUCTIONS FOR AUTHORS

Before submitting a manuscript for consideration for publication in the journal *Vojnosanitetski pregled* (VSP), authors are required to carefully read the Instructions for Authors in order to prepare the manuscript in accordance with the journal's guidelines.

A manuscript that does not meet the requirements of these instructions cannot be considered and will be returned to the authors for completion and correction.

Upon acceptance of the manuscript for publication in VSP, the authors transfer their copyright to the journal's publisher, the Ministry of Defense of the Republic of Serbia, University of Defense.

VSP adheres to the recommendations of the International Committee of Medical Journal Editors (ICMJE), *Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals* (available at https://www.icmje.org/recommendations/).

VSP is available in open access mode. All articles may be downloaded free of charge from the journal's website and used in accordance with the Creative Commons Attribution–ShareAlike (CC BY-SA) license (https://creativecommons.org/licenses/by-sa/4.0/deed.en).

SENDING MANUSCRIPTS

The manuscript and all accompanying materials should be submitted **as a single document** (with all appendices incorporated into the text and placed at the end of the manuscript after the References section), exclusively in electronic form *via* the Aseestant submission system. In order to preserve quality, submitting the images as separate files is also recommended to avoid image compression and potential loss of quality, since Word may reduce their resolution. All authors and reviewers must be registered system users with a unique email address. Registration is possible at: http://aseestant.ceon.rs/index.php/vsp/user. The technical guide for using the electronic submission system is available at: https://aseestant.ceon.rs/index.php/vsp/about/submissions.

If you encounter any problems submitting your manuscript *via* the Aseestant platform, you can contact the journal's Editorial Office for assistance by sending an email to: vsp@vma.mod.gov.rs.

GENERAL INSTRUCTIONS

VSP publishes manuscripts that have not been previously published, in whole or in part, and that are not under consideration for publication or have not been accepted for publication in any other journal.

VSP does not consider manuscripts that have been previously published as preprints.

The journal accepts manuscripts whose results have been previously presented at scientific or professional meetings and published as abstracts, provided that these results have not been published with a DOI (e.g., an extended abstract in a journal supplement).

If part of the results presented in the submitted manuscript has previously been reported at a scientific/professional meeting or is part of a doctoral dissertation, the Submission Letter to the Editorial Board must specify the official name of the meeting, its location and date, and indicate whether the presented results have been published and in what form (e.g., same or different title or abstract). This information should also be clearly stated in a Note at the end of the manuscript.

Manuscripts are published in English. Certain categories of articles (e.g., history of medicine/dentistry/pharmacy) may, at the discretion of the VSP Editorial Board, be published in Serbian. All manuscript categories, except for Editorials, Letters to the Editor, Research Letters, Book Reviews, and Reports from Scientific or Professional Meetings, must include abstracts in both Serbian and English (as part of the manuscript). For details on the structure and length of the abstract, see the Abstract section of these Instructions.

Manuscripts should be prepared using Microsoft Word, with Times New Roman font, size 12 pt, and line spacing 1.5. Set the page size to A4, with a left margin of 4 cm and the remaining three margins 2 cm each. Text should be typed without hyphenation, and only one space should follow each punctuation mark. If special characters (symbols) are used in the text, the Symbol font should be applied.

References cited in the text should be indicated with Arabic numerals in superscript, in the order in which they appear.

Pages should be numbered consecutively in the bottom right corner, starting from the first page (excluding the title page).

When writing in English, follow the American English language standard. The International System of Units (SI) must be used, except for blood pressure (mm Hg) and temperature (°C).

Use standard abbreviations throughout the text. Avoid abbreviations in the title and abstract unless necessary. At their first mention, provide the full term followed by the abbreviation in parentheses; thereafter, use only the abbreviation in both the abstract and the main text. Do not use abbreviations in the Conclusion section (excluding the abstract).

Do not use commercial names of drugs or other products; if necessary, the generic name must also be provided. Devices and equipment should be referred to by their trade names, with manufacturer details (name and location) provided in round brackets. If labels combining letters and numbers are used in the text, ensure that superscript or subscript numbers are written precisely.

Avoid using bold or italic fonts, as they are reserved for subheadings. Exceptions include terms that must be italicized, such as gene names or foreign words in Latin.

Study groups must be clearly defined and consistently named throughout the manuscript. Use a single, consistent term for the same concept. In the Results section, avoid sentences beginning with phrases like "Table X shows" or "Figure X illustrates". Instead, the sentence should describe the result, with the table or figure reference placed in parentheses at the end of the sentence. Sentences should not begin with abbreviations, numbers, or dates. Avoid overly long sentences that reduce clarity; favor short, clear sentences. The Conclusion should be written with new sentences, without repeating previously stated ones. Translation of manuscripts into English using Google Translate may lead to misunderstandings and is therefore not recommended.

When selecting keywords, use Medical Subject Headings – MeSH (https://www.nlm.nih.gov/mesh/meshhome.html). Keywords in the accepted manuscript cannot be altered by the authors, as they are descriptors from the Thesaurus used by professional indexers.

REQUIRED ACCOMPANYING DOCUMENTS

AUTHOR STATEMENT AND AUTHORSHIP

For every manuscript submitted for consideration for publication in VSP, the author(s) must provide an Authorship Statement Form (ASF) confirming that the work has not been previously published and is not simultaneously under consideration for publication in another journal. The ASF must also confirm that all authors meeting the authorship criteria have read and approved the manuscript, and provide contact information for all authors (email address and mobile phone number). In this form, authors must declare any potential conflicts of interest or the absence thereof. All authors must sign the ASF by hand.

For additional information on different types of conflicts of interest, see the recommendations of the World Association of Medical Editors – WAME: http://www.wame.org.

VSP follows the authorship criteria recommended by ICMJE (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). Authorship is based on fulfilling all four given criteria: substantial contributions to the conception of the work, acquisition of data or analysis/interpretation of data; critical revision of the manuscript for important intellectual content; approval of the final version of the manuscript to be published; accountability for all aspects of the published work. All other contributors who participated in the work but do not meet the authorship criteria should be listed in the Acknowledgements, specifying their contribution. Individuals mentioned in the Acknowledgements must provide written consent.

ETHICAL APPROVAL

All research involving humans and/or human material must be conducted in accordance with the ICMJE recommendations (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/protection-of-research-participants.html) and the Declaration of Helsinki, 2024 revision (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Authors are required to submit a scanned copy of the Ethics Committee (EC) approval from the competent institution that approved the study, showing the date of issuance and the subject of the research, along with the manuscript. The EC approval should be submitted in the original language and in English (a certified copy is acceptable).

The Methods section must indicate that the study was approved by the relevant EC, including the name of the institution and the approval number, and that it was conducted in accordance with ethical principles for research involving humans and/or human material.

Patient anonymity must be protected following ICMJE recommendations. For research involving patient data that could allow direct or indirect identification, authors must obtain written informed consent from the patient, state in the manuscript that consent was obtained, and provide it to the Editorial Board if requested.

For research involving animals, authors must provide approval from the relevant EC ensuring compliance with international standards for the use of laboratory animals in research.

The Editorial Board reserves the right to reject manuscripts deemed not conducted in accordance with international ethical standards.

REPRODUCTION OF PREVIOUSLY PUBLISHED OR UNPUBLISHED THIRD-PARTY COPYRIGHTED MATERIAL

If previously published illustrations (photographs, diagrams, etc.) are used, the source must be cited, and permission must be obtained from the journal in which they were originally published, granting approval for their use in VSP. If unpublished third-party illustrations (photographs, diagrams, etc.) are used, permission must be obtained from the original author(s) for publication in VSP.

PLAGIARISM

Since 2012, all manuscripts submitted to VSP are checked for potential (self-)plagiarism using SCIndeks Assistant – Cross Check (iThenticate). Manuscripts found to contain (self-)plagiarism will be rejected. Depending on the extent and type of detected (self-)plagiarism, authors may be banned from publishing in VSP for varying durations. Relevant authorities at the authors' institutions and appropriate professional associations will also be notified.

USE OF AI

Generative artificial intelligence (AI) or AI-assisted technologies may be used only in compliance with the principles of transparency (the use of AI must be clearly stated in the manuscript), accountability (authors remain fully responsible for the accuracy and originality of the content), verifiability (all participants in the publishing process must verify that AI has not introduced fabricated data, citations, or claims), and confidentiality (authors and reviewers are prohibited from uploading manuscripts submitted to VSP to public AI services).

The use of AI tools is permitted only for limited linguistic and technical interventions in the manuscript text: grammar and spelling correction, stylistic refinement of the authors' text, assistance with formatting, and technical assistance (such as code correction). Authors may use AI tools exclusively to create AI-assisted, but not AI-generated content.

Authors who have used AI-assisted content are required to fully and accurately disclose the use of AI tools (the exact name of the AI tool, date of access, prompts used, and purpose of use), guarantee the originality of the

scientific contribution, avoid any fabrication or manipulation, and comply with the rules of scientific ethics. Information on AI use should be stated in the Methods or Acknowledgements section.

Using AI tools is prohibited for the following: generating substantial portions of the manuscript content; creating scientific ideas, data, or results; analyzing or interpreting results; formulating conclusions; modifying images, tables, or graphs (including graphical abstracts); altering data or references.

Any unequivocally established improper use of AI will result in rejection of the manuscript.

AI may not under any circumstances be an author or co-author, nor may it be cited as an author in the References section.

To protect confidentiality, no part of unpublished research submitted to VSP may be entered into a large language model by authors or reviewers.

Authors who have used any AI tools are required to submit an AI Use Statement when submitting the manuscript.

TYPES OF MANUSCRIPTS

VSP publishes the following categories and types of manuscripts and communications: Editorial, Original Article, Preliminary Report, Short Report, Case Report and Case Series, General (Narrative) Literature Review, Mini-Review, Systematic Literature Review, Meta-Analysis, Systematic Literature Review with Meta-Analysis, Current Topic, In Focus, Article on the History of Medicine/Dentistry/Pharmacy, Letter to the Editor, Research Letter, Clinical Research, Congress and Scientific Meeting Report, Book Review, In Memoriam, and other contributions.

ORIGINAL ARTICLE

An Original Article presents new and significant findings in a specific field, with a detailed description of the research methods used, the results obtained, and the conclusions drawn. The reference list should include the most recent and most relevant references in the field.

PRELIMINARY REPORT

A Preliminary Report presents research that has not yet been completed, with findings that require further investigation and validation before final conclusions can be drawn, but where the obtained information is of interest to the scientific and professional community. It contains all sections of an Original Article, but in a substantially abbreviated form. Authors are encouraged to subsequently publish a full Original Article with complete, validated data and a comprehensive analysis.

SHORT REPORT

A Short Report presents a completed research study that is small in scope, narrowly focused, and has clear conclusions based on the presented results. It includes all sections of an Original Article, but in a substantially abbreviated form. It is considered the final publication of that specific, limited study and cannot be republished as a full-length article (although follow-up research building on it is encouraged).

REVIEW ARTICLES

GENERAL (NARRATIVE) LITERATURE REVIEW

A General (Narrative) Literature Review provides a review, critical analysis, and synthesis of existing scientific knowledge on a selected topic. Authors cover all available relevant literature over a defined time period, present the results of relevant studies, identify gaps, limitations, or controversies, and indicate directions for future research, offering their own perspective on the issue in the form of concluding remarks.

Authors of this category of article should have published at least five papers in peer-reviewed journals (M20) in the field of the review topic.

MINI-REVIEW ARTICLE

A Mini-Review provides a concise overview of the existing literature and the most recent advances within defined aspects of a particular research field, as well as its new and/or current directions of development.

SYSTEMATIC LITERATURE REVIEW

A Systematic Literature Review synthesizes previously published studies on a specific topic using clearly defined and pre-established methodological procedures for study selection and evaluation. The author must use relevant databases, define inclusion and exclusion criteria, and apply a transparent methodology.

META-ANALYSIS

A Meta-Analysis uses statistical methods to combine quantitative data from multiple primary studies in order to identify overall trends and assess the strength of evidence on a specific topic. Authors must use relevant databases, define inclusion and exclusion criteria, and apply a transparent and reproducible methodology. The research question must be clearly defined using the PICOS framework, and selection guidelines and a study flow diagram (PRISMA) must be provided.

SYSTEMATIC LITERATURE REVIEW WITH META-ANALYSIS

A Systematic Literature Review with Meta-Analysis combines qualitative and quantitative synthesis, using statistical techniques to summarize quantitative results and qualitative synthesis for descriptive/narrative findings. Authors must use relevant databases, clearly define inclusion and exclusion criteria, and apply a transparent and reproducible methodology. The research question must be clearly defined according to the PICOS framework, with specification of the reporting guidelines used (e.g., PRISMA) and inclusion of a PRISMA flow diagram showing study selection.

CURRENT TOPIC

A Current Topic addresses a contemporary, unresolved, or controversial issue of theoretical and practical importance, presenting the authors' own research results or the most recent important data from the literature. The structure of the article is flexible, and brief concluding remarks with a clear message are encouraged.

IN FOCUS

An In Focus article provides a thematic, focused analysis or a brief overview of a scientific issue within the journal's scope, addressing a topic of significance for the scientific community and broader professional audience.

CASE REPORTS

CASE REPORT and CASE SERIES (≥4, ≤9)

Case reports or case series present cases with rare or unusual diagnoses, diagnostic processes, treatment strategies, clinical courses, or treatment outcomes that may be useful for clinical practice and medical education. The CARE guidelines should be followed when preparing the manuscript (https://www.care-statement.org/writing-a-case-report). Written informed consent from the patient is mandatory.

EDITORIAL

Editorials are non–peer-reviewed texts written by the Editor-in-Chief and/or members of the Editorial Board, intended to announce a new volume, special issues, or content of significance for the profession and/or institutions served by the journal, as well as invited editorial texts. Editorials should not contain unpublished or original data, and must include a statement of conflict of interest.

LETTER TO THE EDITOR

A non–peer-reviewed comment or critique of a paper published in VSP. It is written in a free format, with optional citation of relevant literature, and must not contain unpublished results. It is published at the discretion of the Editor-in-Chief.

RESEARCH LETTER

A Research Letter is a short report of original research, containing Introduction, Methods, Results, and Discussion in a condensed form (without separate sections or subheadings) and up to 2 supplementary items (tables/figures). It does not include an abstract or keywords, but must meet all general manuscript requirements for consideration, including the peer-review process.

HISTORY OF MEDICINE/STOMATOLOGY/PHARMACY

Manuscripts presenting material relevant to elucidating specific events and/or portraying notable figures in the history of medicine/stomatology/pharmacy, with particular emphasis on military medicine/stomatology/pharmacy.

CLINICAL RESEARCH

Clinical Research includes original randomized controlled trials and observational studies assessing the impact of one or more interventions or measures on human health outcomes, clinical practice, or health policy.

Manuscripts must be prepared in accordance with international guidelines (e.g., CONSORT – https://www.consort-statement.org/ or STROBE – https://www.strobe-statement.org/) and be registered in a recognized public registry (e.g., ClinicalTrials.gov).

BOOK REVIEW

A Book Review includes bibliographic details of the publication (authors, original title, publisher, place, and year of publication), a brief summary, and critical comments on the content, style, and significance of the book in the relevant field. The manuscript must not exceed 2 pages.

SCIENTIFIC MEETING REPORT

A Scientific Meeting Report presents the activities of a scientific or professional meeting, highlighting the most important presentations, conclusions, or recommendations relevant to the wider readership of VSP.

MANUSCRIPT LENGTH

A complete manuscript consists of: title page, abstracts in Serbian and English with keywords, main text, acknowledgements (if applicable), reference list, and supplementary material (tables, figures, charts, diagrams, drawings).

For Original Article, General (Narrative) Literature Review, Systematic Literature Review, Meta-Analysis, and Systematic Literature Review with Meta-Analysis, the manuscript length may not exceed 5,000 words.

For Mini-Review, Preliminary Report, Short Report, Case Report, Case Series, Current Topic, Clinical Research, and History of Medicine/Stomatology/Pharmacy, the manuscript length may not exceed 3,000 words.

Manuscripts in other categories/sections may have a maximum of 1,500 words.

MANUSCRIPT PREPARATION

TITLE PAGE

The first page of the manuscript should include the following:

1. Title of the manuscript without abbreviations;
2. Full names of all authors (without academic titles, but with ORCID numbers included for those who have them) with symbols assigned in the following order: ***, †, ‡, §, ‖, ¶, **, ††**… etc.;
3. Full official names of the institutions where the authors work, including city and country of the institution (the symbols *, †, ‡, §, ‖, ¶, **, †† … etc. correspond to the institutions of each author);
4. At the bottom of the page, provide the name and surname, postal address, email address, and phone number (mobile/Viber or WhatsApp) of the author responsible for correspondence.

ABSTRACT

The abstract and keywords should be provided on the second page of the manuscript. The abstract should be written in short and clear sentences. For the

categories Original Article, Preliminary Report, Short Report, Systematic Literature Review, Systematic Literature Review with Meta-Analysis, Meta-Analysis, and Clinical Research, the abstract must be structured and include the following sections: Introduction/Aim, Methods, Results, Conclusion. Each section should be written as a separate paragraph beginning with a bolded heading. The most important results should be presented, including numerical values and the level of statistical significance. The conclusion must be directly related to the study results. The abstract must not exceed 300 words.

For the categories Case Report and Case Series, the abstract should have the following structure: Introduction (with the aim stated in the last sentence), Case Report, Conclusion. Each section should be written as a separate paragraph beginning with a bolded heading. The abstract must not exceed 250 words.

For all other manuscript categories: General (Narrative) Literature Review, Mini Review, Current Topic, In Focus, and History of Medicine/Stomatology/Pharmacy, the abstract is unstructured and must not exceed 200 words.

Care should be taken in ensuring that the Serbian and English versions of the abstract are accurate and precise translations of each other. No sentence may appear in one version without being translated into the other.

KEYWORDS

Below the abstract, list five to seven relevant keywords or phrases that indicate the content of the manuscript. It is recommended to avoid repeating words from the title of the paper. When selecting keywords, use Medical Subject Headings (MeSH) (https://www.nlm.nih.gov/mesh/meshhome.html).

STRUCTURE OF THE MAIN TEXT

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

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

Manuscripts in the categories Case Report and Case Series should include the following sections: Introduction (the aim of the paper should be stated in the final paragraph of the Introduction), Case Report (the patient's identity must remain anonymous), Discussion, and References.

A Case Report must not have more than five authors.

QUESTIONNAIRES

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

TABLES AND FIGURES

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

Tables

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

In the legend below the table, all abbreviations used in the table and all symbols (e.g., superscript letters or bolded values) must be explained. In addition, the applied statistical methods must be clearly specified.

Figures (Illustrations)

Figures include all forms of graphical material (photographs, drawings, diagrams, and graphs). Figures should be embedded in the manuscript at the end of the text, after the References and after the Tables (if any). Figures should be numbered with Arabic numerals in the order in which they appear in the text. Capital letters A, B, C, etc., should be used to designate parts of multipart figures. Letters, numbers, and symbols must be clear, consistent, and of sufficient size to remain legible after reduction. All elements shown in figures must be saved as images (not as editable graphic objects) so that their position cannot be altered, ensuring the accuracy of the data presented. Only digital images with a minimum resolution of 300 dpi and in JPEG, PNG, or PDF format are accepted. Figures that do not meet these requirements will not be accepted for publication. The dimensions of submitted figures should be approximately the same as the dimensions at which they will be published. If authors are unable to provide digital photographs, original images should be scanned at a resolution of 300 dpi and at their original size and submitted in that form. All text in diagrams and graphs should be written in a sans-serif font for better readability (e.g., Arial, Helvetica), with a font size of no less than 10 pt. Measurement units and scales must be clearly indicated. Decimal numbers in graphs must be presented with a decimal point, and thousands should be separated by a comma (e.g., 1,234.56).

Video supplements (illustrations of the manuscript) may last 1–3 minutes and should be submitted in AVI or MP4 (FLV) format. A separate still image representing the video (video thumbnail) must also be provided for use in the electronic edition and publication in the printed edition, along with a link to the platform where the video is already hosted.

In the legend below each illustration, all abbreviations, symbols, numbers, or letters used to explain individual parts of the figure must be defined. For graphs, the applied statistical methods should be specified where appropriate; for photomicrographs, details of the staining method and magnification must be provided.

If photographs of persons (patients) are presented, the face must be blurred or written consent must be obtained from the person depicted. In imaging materials (X-rays, CT scans, ultrasound images, etc.), all information that could identify the patient must be removed. If a figure has been previously published, the source must be cited, and written permission must be obtained if the material is protected by copyright.

ABBREVIATIONS

Abbreviations should be used only when necessary, primarily for very long names of chemical compounds or for terms that are already widely recognized in abbreviated form (e.g., DNA). For each abbreviation—except standard units of measurement—the full term must be given at its first occurrence in the text (including the abstract). The use of abbreviations should be avoided in the title and abstract; in the title, abbreviations should be used only if absolutely necessary. For terms mentioned more than 3 times in the text, introducing appropriate abbreviations is recommended.

DECIMAL NUMBERS

In manuscripts written in English, decimal numbers should be written with a decimal point (e.g., 22.7), whereas in manuscripts written in Serbian, a comma should be used (e.g., 22,7). Whenever possible, numbers should be rounded to one decimal place and reported consistently throughout the manuscript (e.g., if one value is 32.2, all others should also be rounded to one decimal place, e.g., 32.0).

UNITS OF MEASUREMENT

Length, height, weight, and volume should be expressed in metric units (meter – m, kilogram (gram) – kg (g), liter – L) or their subunits. Temperature should be expressed in degrees Celsius (°C), and blood pressure in millimeters of mercury (mm Hg). Results of clinical and biochemical measurements should be reported in metric units according to the International System of Units (SI).

ACKNOWLEDGEMENTS

The contributions of individuals who should be acknowledged but do not meet the criteria for authorship should be stated. Financial support (sponsorships, grants, equipment, etc.) should be disclosed, as well as the name of the project within which the research was conducted.

STATISTICAL ANALYSIS

In the Methods section, the applied statistical methods should be described in sufficient detail to allow verification of their correct use and reproduction of the analysis. Results must be presented numerically and clearly, with appropriate measures of variability and reliability (e.g., standard deviation, standard error, confidence interval). The type of study should be specified, and the manner in which it was conducted should be described. Inclusion and exclusion criteria must be stated. The software and the version of the computer program used for statistical data analysis should be reported. In the Results section, as well as in the legends of tables and/or figures, the statistical method used to analyze the presented results must be indicated. The p values should always be written with a leading zero (e.g., $p > 0.05$, not $p > .05$).

REFERENCES

References should be numbered with Arabic numerals according to the order of their first appearance in the text (including tables and figure legends). It is recommended that the majority of cited references be published within the last ten years. At least 80% of the cited references should be original research articles, while books, book chapters, and review articles should account for no more than 20% of the total number of references. All references, regardless of the language of the original source, must be cited in English, with the original language indicated in parentheses after the reference.

All data on the references must be accurate, and the cited works should be easily accessible to readers. A DOI number must be provided for each reference. Citation of articles published in journals indexed in Current Contents, Index Medicus (MEDLINE), Excerpta Medica, Scopus, and Web of Science is recommended.

Citation of abstracts, secondary publications, oral communications, unpublished works, official or confidential documents, Wikipedia, preprints and in press articles, retracted articles, and articles published in predatory journals is not permitted.

When citing websites, the homepage must not be cited; instead, the specific webpage from which the information was obtained must be referenced. Each cited reference must be available for online verification. If a reference is not available online (e.g., archival material), the author must provide the source from which the cited material was obtained, or submit a photographed or scanned copy of the document by emailing it to: strliteratura@gmail.com.

References should be formatted according to the Vancouver style established by the ICMJE (https://connect.ebsco.com/s/article/Citing-Articles-in-Vancouver-ICMJE-Style?language=en_US).

Citation examples:

Article with 1 to 6 authors

*Nikolić A, Bioč*anin *V, Rančić N, Dušpara M, Đurić D.* Serbian translation and validation of the SF-36 for the assessment of quality of life in patients with diagnosed arterial hypertension. EABR Exp Appl Biomed Res 2023; 24(3): 227–34. DOI: 10.2478/sjecr-2020-0073

Article with more than 6 authors

Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med 2017; 13(3): 479–504. DOI: 10.5664/jcsm.6506

Volume with a Supplement

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

Issue with a Supplement

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

Volume with Part (Pt)

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

Issue with Part (Pt)

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

Issue with no Volume

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

No Volume or Issue

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

Pagination with Roman numerals

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

Book

Printed Book

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

Book in electronic format

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

Chapter

In an edited book

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

In an edited electronic (online) book

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

Website

Homepage

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

Part of a website

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

Conference Proceedings

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

Article from Conference Proceedings

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

Dissertation

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

Other published articles

News article

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

Holy Bible

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

Dictionaries and similar references

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

Other examples of citing publications can be seen at https://www.nlm.nih.gov/bsd/uniform_requirements.html

ARTICLE PUBLICATION FEES

The publication fee is to be paid after the manuscript has been accepted for publication. Decision on accepting the manuscript must be confirmed at a VSP Editorial Board meeting. Manuscripts for which the fee has not been paid will not be published.

Payment of the fee does not guarantee that the manuscript will be published in VSP.

All authors are required to pay the Article Processing Charge to cover the costs of language editing, as well as professional and technical processing of the manuscript, and its publication. Domestic authors pay 5,000 RSD, and international authors pay 150 EUR.

Institutional subscription (10,000 RSD) applies only to institutions that wish to receive physical copies of the journal's monthly issues. In the year the subscription is paid, authors can submit an unlimited number of manuscripts to VSP.

Copies of the payment receipt must be sent to strliteratura@gmail.com for record-keeping.

The following are exempt from the subscription fee: VSP reviewers (a manuscript ID or title of the reviewed paper must be provided), doctoral students (with proof of enrollment in the current academic year), and editors. The Editorial Board reserves the right to reduce or waive the subscription fee upon a justified request from the author.

For any additional information, you can contact the addresses and phone numbers listed below.

***Vojnosanitetski pregled* Editorial Office**
17 Crnotravska St
11 000 Belgrade
Serbia
Phone number: (+381 11) 3608-997
E-mail: vsp@vma.mod.gov.rs
E-mail for subscription information: strliteratura@gmail.com
Website: www.vsp.mod.gov.rs
ISSN 0042-8450
ISSN Online 2406-0720 OPEN ACCESS

УПУТСТВО ЗА АУТОРЕ

Пре подношења рукописа за разматрање за објављивање у часопису „Војносанитетски преглед“ (ВСП) неопходно је да аутори пажљиво прочитају Упутство за ауторе, како би рукопис припремили у складу са пропозицијама часописа.

Рад који не испуњава услове овог упутства не може бити разматран и биће враћен ауторима да га допуне и исправе**.**

Аутори рада преносе своја ауторска права на издавача часописа Министарство одбране Републике Србије, Универзитет одбране **након прихватања рада за објављивање у ВСП**.

ВСП се придржава препорука Међународног комитета уредника медицинских часописа (*International Committee of Medical Journal Editors* – ICMJE), *Препоруке за спровођење, извештавање, уређивање и публиковање научних радова у медицинским часописима* (доступно на https://www.icmje.org/recommendations/).

ВСП је доступан у режиму отвореног приступа. Сви чланци могу се бесплатно преузети са сајта часописа и користити у складу са лиценцом **Creative Commons Autorstvo-Deliti pod istim uslovima (CC BY-SA) (**https://creativecommons.org/licenses/by-sa/4.0/deed.en**).**

СЛАЊЕ РУКОПИСА

Рукопис рада и сви прилози уз рад достављају се **као један документ** (прилози су инкорпорирани у текст и позиционирани на крају рукописа иза одељка Литература) искључиво електронски преко система за пријављивање *Aseestant*. Ради очувања квалитета фотографија, препоручује се достављање слика и као посебних фајлова, јер Word може смањити њихову резолуцију, како би се избегла компресија слика и евентуални губитак квалитета.

Сви аутори и рецензенти морају бити регистровани корисници система са јединственом е-маил адресом. Регистрацију је могуће извршити на: http://aseestant.ceon.rs/index.php/vsp/user. Техничко упутство за коришћење система електронске пријаве доступно је на: https://aseestant.ceon.rs/index.php/vsp/about/submissions.

Уколико имате проблем са подношењем рукописа путем платформе *Aseestant* можете се обратити за помоћ Редакцији часописа слањем е-мејла на адресу: vsp@vma.mod.gov.rs.

ОПШТА УПУТСТВА

ВСП објављује радове који до сада нису претходно објављени (у целини или делом), који се не разматрају за објављивање нити су прихваћени за објављивање у неком другом часопису.

ВСП не разматра радове који су претходно објављени као препринт верзије.

Часопис прихвата и радове чији су резултати претходно приказани на научним или стручним скуповима и објављени у виду апстракта, под условом да ти резултати нису објављени са DOI бројем (нпр. проширени апстракт у додатку неког часописа).

Уколико је део резултата поднетог рукописа претходно саопштен на научном/стручном скупу или је део докторске дисертације, у Пропратном писму Уредништву потребно је навести званичан назив скупа, место и време одржавања, и да ли су саопштени резултати публиковани и у којој форми (нпр. исти или другачији наслов или сажетак), а у Напомени на крају рукописа то треба посебно назначити.

Радови се објављују на енглеском језику. Поједине категорије радова (нпр. историја медицине/стоматологије/фармације) се по одлуци Уредништва ВСП могу објавити и на српском језику. Све категорије рукописа осим категорија уводник, писмо уреднику, истраживачко писмо, приказ књиге, извештај са научног или стручног скупа се објављују са апстрактима на српском и енглеском језику (у склопу рукописа). О структури и обиму апстракта видети детаљније у одељку Апстракт овог Упутства.

За писање рукописа користити програм *Word*, фонт *Times New Roman*, величину слова 12, проред 1,5. Величину странице подесити на формат А4, са левом маргином од 4 цм а преостале три 2 цм. Текст куцати без дељења речи (хифенације), а после сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*.

Подаци о коришћеној литератури у тексту означавају се арапским бројевима у суперскрипту, редоследом којим се појављују у тексту.

Странице нумерисати редом у доњем десном углу, почев од прве стране (изузимајући насловну страну).

При писању текста на енглеском језику придржавати се језичког стандарда *American English*. Обавезно је коришћење међународног система мера (SI). Изузетак чине крвни притисак (mm Hg) i temperatura (^{o}C).

Приликом писања користе се стандардне скраћенице. Избегавати скраћенице у наслову и апстракту осим уколико је неопходно. Пун назив са скраћеницом у загради наводи се у њеном првом помињању, а даље у тексту само скраћенице, како у апстракту тако и у главном тексту. У закључку рада (не апстракта) нема скраћеница.

Не користити комерцијална имена лекова и других препарата, а уколико је то неопходно уз њихове називе обавезно навести и генеричка имена. Уређаји (апарати) се означавају фабричким називима, а податке о произвођачу (назив и место) навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или субскрипту.

Избегавати фонтове *bold* и курзив (*italic*) јер су резервисани за поднаслове. Изузеци су обавезно писање курзивом оних назива који се тако морају писати (нпр. гени или стране речи - латински).

Групе испитаника морају бити јасно дефинисане и доследно именоване кроз цео рад. За исти појам користити један, јединствен термин кроз цео рад. У одељку Резултати избегавати реченице које почињу са: „Табела X показује “ или „Слика X приказује“. Реченица треба да опише резултат, а ознака табеле или слике да стоји у загради на крају описа. Реченице не би требало почињати скраћеницом, бројем или датумом. Избегавати предугачке реченице које умањују јасноћу текста и дати предност краћим јасним реченицама. Закључак формулисати новим реченицама, без преписивања већ изречених. Превод радова на енглески језик посредством *Google Translate* може изазвати неразумевање текста и стога се не препоручује.

У избору кључних речи користити *Medical Subject Headings – MeSH* (https://www.nlm.nih.gov/mesh/meshhome.html). Кључне речи у прихваћеном рукопису не подлежу ауторској коректури, пошто су оне дескриптори из Тезауруса које одређују стручни индексери.

ОБАВЕЗНА ПРАТЕЋА ДОКУМЕНТА

ИЗЈАВА АУТОРА И АУТОРСТВО

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

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

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

ЕТИЧКА САГЛАСНОСТ

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

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

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

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

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

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

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

ПЛАГИЈАРИЗАМ

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

КОРИШЋЕЊЕ AI

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

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

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

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

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

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

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

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

ТИПОВИ РУКОПИСА

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

ОРИГИНАЛНИ ЧЛАНАК

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

ПРЕТХОДНО САОПШТЕЊЕ

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

КРАТКО САОПШТЕЊЕ

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

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

Преглед, критичка анализа и синтеза постојећих научних сазнања о изабраној теми. Аутори обухватају сву доступну припадајућу литературу за одређени временски период, приказују резултате релевантних истраживања, идентификују недостатке, ограничења или контроверзе и указују на правце будућих истраживања, дајући своје виђење проблема у виду закључног става. Аутори чланка ове категорије могу бити они који су објавили минимално пет радова публикованих у часописима с рецензијом (M20) из области прегледног рада.

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МЕТА-АНАЛИЗА

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

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

АКТУЕЛНА ТЕМА

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

У ФОКУСУ

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

КАЗУИСТИКА

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

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

УВОДНИК

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

ПИСМО УРЕДНИКУ

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

ИСТРАЖИВАЧКО ПИСМО

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

ИСТОРИЈА МЕДИЦИНЕ/СТОМАТОЛОГИЈЕ/ФАРМАЦИЈЕ

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

КЛИНИЧКО ИСТРАЖИВАЊЕ

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

ПРИКАЗ КЊИГЕ

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

ИЗВЕШТАЈ СА НАУЧНОГ ИЛИ СТРУЧНОГ СКУПА

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

ОБИМ РУКОПИСА

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

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

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

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

ПРИПРЕМА РАДА

НАСЛОВНА СТРАНА

На првој страници рукописа треба навести следеће:

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

АПСТРАКТ

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

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

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

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

КЉУЧНЕ РЕЧИ

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

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

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

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

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

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

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

ПРИЛОЗИ

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

Табеле

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

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

Слике (илустрације)

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

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

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

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

СКРАЋЕНИЦЕ

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

ДЕЦИМАЛНИ БРОЈЕВИ

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

ЈЕДИНИЦЕ МЕРА

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

ЗАХВАЛНИЦА

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

СТАТИСТИЧКА АНАЛИЗА

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

ЛИТЕРАТУРА

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

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

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

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

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

Примери цитирања:

Чланак са 1 до 6 аутора

Nikolić A, Biočanin V, Rančić N, Dušpara M, Đurić D. Serbian translation and validation of the SF-36 for the assessment of quality of life in patients with diagnosed arterial hypertension. EABR Exp Appl Biomed Res 2023; 24(3): 227–34. DOI: 10.2478/sjecr-2020-0073

Чланак са више од 6 аутора

Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med 2017; 13(3): 479–504. DOI: 10.5664/jcsm.6506

Волумен са суплементом

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

Свеска са суплементом

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

Волумен са делом (Pt)

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

Свеска са делом

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

Свеска без волумена

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

Без волумена и свеске

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

Пагинација римским бројевима

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

Књига

Штампана књига

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

Књига у електронском формату

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

Поглавље

У едитованој књизи

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

У едитованој електронској (online) књизи

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

Веб страница

Интернет страница

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

Део интернет странице

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

Зборник радова са конгреса

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

Рад из зборника

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

Дисертација

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

Остали објављени чланци

Новински чланак

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

Свето писмо

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

Речници и сличне референце

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

Остале примере навођења публикација можете видети на https://www.nlm.nih.gov/bsd/uniform_requirements.html

НАКНАДЕ ЗА ОБЈАВЉИВАЊЕ ЧЛАНКА

Накнада за објављивање рада се плаћа након прихватања рукописа за објављивање. Одлука о прихватању мора бити потврђена на седници Уредништва ВСП. Радови за које нису плаћене накнаде неће бити објављени.

Уплата није гаранција да ће рад бити објављен у ВСП.

Сви аутори дужни су да плате *Article Processing Charge* за покриће трошкова језичке, стручне и техничке обраде рукописа, као и његовог објављивања. Домаћи аутори плаћају износ од 5 000 динара, а инострани 150 еура.

Институционална претплата (10 000 динара) важи искључиво за институције (установе) које желе да добијају физичке копије месечних бројева часописа.

У години у којој су уплатили претплату аутори могу поднети неограничен број радова у ВСП.

Копије уплатнице неопходно је доставити на е-мејл адресу: strliteratura@gmail.com ради евиденције уплате.

Од претплате су ослобођени рецензенти ВСП (потребно је доставити ID број рада или наслов рада који су рецензирали), студенти на докторским студијама (уз потврду о уписаној текућој години), уредници. Уредништво задржава право да може умањити износ претплате или ослободити ауторе плаћања претплате на образложену молбу аутора.

За све додатне информације, можете се обратити на доле наведене адресе и бројеве телефона.

Редакција часописа „Војносанитетски преглед“
Ул. Црнотравска 17
11 000 Београд
Србија
Телефон: (+381 11) 3608-997
Е-пошта: vsp@vma.mod.gov.rs
Е-пошта за информације у вези са претплатом: strliteratura@gmail.com
Интернет страница: www.vsp.mod.gov.rs
ISSN 0042-8450
ISSN Online 2406-0720 OPEN ACCESS