YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2025; August Vol. 82 (No. 8): pp. 465–546.

Vojnosanitetski Pregled 2025 August Vol. 82 (No. 8): pp. 465–546.

Vol. 82, No. 8, 2025

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
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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. 82, br. 8, 2025

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
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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

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World Humanitarian Day (WHD) has been observed since 2009, following a decision of the United Nations General Assembly on August 19 each year, to honor humanitarian workers. In response to global challenges, this day is an opportunity to affirm core humanitarian values, such as compassion, respect for human dignity, and interpersonal cooperation. This day should remind us of the necessity of solidarity and support for those in greatest need, and inspire us to work together to build a more just and humane society. The theme of this year's WHD, *Strengthening Global Solidarity and Empowering Local Communities*, highlights that humanitarian assistance has the most lasting impact when local communities are active drivers of change.

Svetski dan humanitarnog delovanja (SDHD) se obeležava od 2009. godine po odluci Generalne skupštine Ujedinjenih nacija svakog 19. avgusta, u cilju odavanja priznanja humanitarnim radnicima. U odgovoru na globalne izazove, ovaj dan je prilika da afirmišemo osnovne humanitarne vrednosti, kao što su saosećanje, poštovanje ljudskog dostojanstva i međuljudska saradnja. Ovaj dan bi trebalo da nas podseti na neophodnost solidarnosti i podrške onima kojima je pomoć najpotrebnija i motiviše da zajedničkim trudom izgradimo pravednije i čovekoljubivije društvo. Tema ovogodišnjeg SDHD, *Jačanje globalne solidarnosti i osnaživanje lokalnih zajednica*, naglašava da humanitarna pomoć ima najtrajniji učinak kada su lokalna društva aktivni nosioci promena.

EDITORIAL

UDC: 655.413:61]:[050:001.87(045)
DOI: https://doi.org/10.2298/VSP250415045R

When and how to write a "Case report" for a journal

Kada i kako napisati „Prikaz slučaja" za časopis

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At a time when an enormous number of papers are being written, accepted, and published daily around the world, the scientific community is faced with the task of accepting and publishing papers under the "Case reports" or "Case series reports" categories. These types of manuscripts should describe, in a concise and focused style, important, previously undetected or underreported clinical observations and their integration into a scientific context. They may include one or two cases, although some journals allow case reports to present up to three patients; likewise, case series reports may consist of three to four patient cases or more [1–5]. The essential conditions for this type of work to reach publication in this category are, as Sun [6] puts it, that it shows "a rare or unusual clinical condition, a previously unreported or unrecognized disease, unusual side effects to therapy or response to treatment, and unique use of imaging modalities or diagnostic tests to assist diagnosis of a disease".

Case reports may also become part of the literature when they bring to attention particularly bizarre cases. One such report is from 1991 [7], where *The New England Journal of Medicine* published the case of an 88-year-old man with Alzheimer's disease who had frequent cholesterol measurements ranging from 3.88 to 5.18 mmoL *per* liter despite consuming 20 to 30 eggs a day. His metabolism was studied after at least 15 years of the above daily egg intake. The purpose of publishing this case report is clear, as it is unlikely that there will be many patients like this one in the world with similar feeding habits; such an extreme diet could shed further light on cholesterol metabolism. In addition to being new or unusual, the report must, therefore, have a meaningful purpose.

The main components of this manuscript type are: introduction, case description, discussion, and conclusion [8]. Although preparing a case report is usually considered far easier than conducting any other elaborative research with a more complex design [9], writing a good case report is harder than it seems; this is because of the stringent need to present a case which, albeit isolated or in very limited numbers, must appear so relevant to the scientific community that it deserves publication. To that effect, expert-devised, now widely used guidelines, such as CARE [10, 11], have been developed to help authors write high-quality case reports that may be worthy of publication.

Case reports also contain parts of patients' medical records, such as images from radiological diagnostics, pathohistological findings, images of patient body parts, and other clinical findings. It is, therefore, paramount to obtain the patient's, parent's, or guardian's written consent and approval for publication of the case, pictures, or other visual forms in an anonymized version; this is increasingly important when images can be easily uploaded onto websites and quickly reach a vast audience. Approval should also be sought from the hospital's Institutional Review Board/Ethics Committee [12], particularly when slightly larger case series are presented (more than four cases).

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Case reports aim to advance knowledge by highlighting significant and unusual variations in the presentation of a clinical condition and/or in its diagnostic and therapeutic strategies [13]. It follows that, for case reports to be sufficiently interesting to a journal's readership, they should possess both clinical and educational value [14]. With this in mind, a thorough literature search is essential to prevent unnecessary writing efforts; what seems unique to the authors may not be as unique to the rest of the medical community. Today, with frequent advancements in medical science and practice, it has become increasingly difficult to find novel enough cases that can make a meaningful impact on clinicians' knowledge and daily practice. The key question that authors, as well as the reviewers of case reports or case series, should ask themselves is whether, by reading the manuscript, clinicians could learn something completely new that could help them in their practice.

Case reports are often considered a weak level of evidence [15, 16]. Additionally, their often low citation rate puts any journal at risk of seeing its impact factor decrease [17], which has led many editors to remove the "Case report" section from their journals and be more likely to accept this type of paper as "Letters to the editor" or in the "Correspondence" section. On the other hand, some journals are publishing only case reports, such as *Anesthesia and Analgesia Practice*, *Pain Medicine Case Reports*, *British Medical Journal Case Reports*, etc. This fact highlights the role that high-quality case reports can play in expanding current knowledge and enhancing learning [18]. It is also crucial to remember that case reports are often the first step for junior healthcare professionals on the publication ladder; therefore, they have an added educational value.

Undoubtedly, a case report rarely has the impact on clinical practice that double-blind randomized controlled trials, systematic literature reviews with meta-analysis, or clinical guidelines can have [6]. However, it can still be a valuable source of a different kind of meaningful knowledge for clinicians. This is because case reports provide a means for developing and discussing new hypotheses, such as those related to mechanisms of drug action, therapeutic avenues, or diagnostic tools, offering a springboard for the design of later studies and clinical trials [19]. However, the authors should avoid using case reports to prove that a certain therapeutic approach works.

While case reports may be considered second- or even third-tier publications, the fact that they may bring to the attention of the clinical community unusual and rare conditions or exceptional clinical events, sometimes repeating under certain circumstances, constitutes an opportunity for the scientific community to ponder these matters and refine existing knowledge. Whatever their 'ranking', any prospective publication should, of course, still satisfy certain requirements around value and quality, which would need to be reviewed, validated, and proofed with similar standards to other types of scientific publication.

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Received on April 15, 2025
Accepted on May 28, 2025
Online First June 2025

CURRENT TOPIC

UDC: 616.341-007-008-07-08
DOI: https://doi.org/10.2298/VSP250307055B

Diagnostic criteria and treatment of patients with short bowel syndrome – consensus statements of the National Society for Clinical Nutrition of Serbia (NUPENS)

Dijagnostički kriterijumi i lečenje bolesnika obolelih od sindroma kratkog creva – konsenzus izjave Nacionalnog Udruženja za kliničku ishranu Srbije (NUPENS)

Mihailo Bezmarević*[†], Ivan Palibrk[‡§], Mirjana Stojšić[‖¶], Natalija Vuković, Marija Djukanović[‡§], Danijela Jovanović[††‡‡], Igor Krdžić[§§], Dušica Simić[‡‖‖], Ivan Pantić*, Marina Panišić Šekeljić*, [¶¶]NUPENS Short Bowel Syndrome Multidisciplinary Expert Group**

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[¶¶]A list of the NUPENS Short Bowel Syndrome Multidisciplinary Expert Group and their institutional affiliations is provided in the Appendix

Key words:
diagnosis; enteral nutrition; intestinal failure; parenteral nutrition; parenteral nutrition, home; quality of life; short bowel syndrome.

Ključne reči:
dijagnoza; ishrana, enteralna; insuficijencija, intestinalna; ishrana, parenteralna; ishrana, parenteralna, kućna; kvalitet života; crevo, kratko, sindrom.

Introduction

Intestinal insufficiency is defined as a decrease in the function of the gastrointestinal tract below the minimum necessary for the absorption of macronutrients and/or water and electrolytes, to such an extent that intravenous (i.v.) supplementation (fluids and electrolites and nutrition therapy which includes macro- and micronutrients) is necessary to preserve life, health, and/or growth [1]. Chronic intestinal insufficiency or failure (CIF) occurs when intestinal insufficiency persists for months or years in metabolically stable patients who do not require hospital treatment. CIF is a consequence of severe gastrointestinal, benign systemic diseases, but it can also occur as a consequence of intra-abdominal malignant diseases [1, 2]. The basis of CIF therapy, which aims to preserve the health and life of patients, is home parenteral nutrition (HPN) [1, 3].

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CIF, as an organ dysfunction/failure, is the rarest organ failure. The prevalence of HPN due to CIF and resulting from benign diseases in Europe is observed in 5–80 patients/million inhabitants [4, 5]. CIF resulting from benign diseases is included in the European list of rare (orphan) diseases [6] and is registered under ORPHA 294422. In the International Classification of Diseases (ICD) – 10th revision (ICD-10), CIF is listed under the diagnosis code K63.8. In 2021, the definition of CIF given by the European Society for Clinical Nutrition and Metabolism (ESPEN) was accepted by the World Health Organization (WHO) and included in the 11th revision of the ICD (ICD-11) [7]. Based on all this, it can be concluded that CIF is a clearly defined disease that requires timely and proper diagnosis, appropriate monitoring, and therapy. The WHO and ESPEN have determined and defined official recommendations and guidelines for the diagnosis and treatment of CIF [2–4, 7].

According to the functional classification of intestinal insufficiency, CIF represents the third type. CIF is a chronic condition that requires i.v. supplementation for months and years, and the course of the disease can be reversible or irreversible. The most common cause of CIF is short bowel syndrome (SBS) [2]. SBS occurs as a result of congenital diseases of the small intestine and/or extensive surgical resections of the intestine. It denotes a clinical condition of patients where the residual length of the small intestine is less than 200 cm in continuity, with or without the colon [8, 9]. The code for this diagnosis is also included in the ICD-11 as DA96.04 - SBS [10]. If CIF or SBS are timely diagnosed or even suspected, with proper therapy, i.v. supplementation, parenteral nutrition (PN), and appropriate monitoring, long-term survival of these patients cannot be questioned. Nonetheless, in order for the patients to survive, long-term use of PN, either in hospital or at home, is necessary. In addition, it should be emphasized that the progress of the pharmaceutical industry in the 21st century has provided biological therapy for SBS patients. This biological therapy has made it possible to restore enteral autonomy and improve intestinal function, even many years after the loss of colon function. In this way, with biological therapy, most SBS patients become independent of i.v. supplementation for both water and electrolytes, as well as macronutrients, within a period of 6 to 8 months from the first administration [2, 11].

The number of CIF and SBS patients in the Republic of Serbia (RS) is unknown. Recognizing the need to identify and treat patients with CIF and SBS, the National Society for Clinical Nutrition of Serbia (NUPENS) formed two multidisciplinary expert teams for the implementation and realization of the HPN project at the ordinary meeting of the Board of Directors and the Supervisory Board on June 9, 2021, in Belgrade, Serbia. The expert teams cover both the pediatric and adult populations. After this initial step, NUPENS, in collaboration with the teams, conducted a cross-sectional study of patients with an indication for long-term PN. This study included seven healthcare institutions in the RS (six university centers and one general hospital). Data analysis led to the conclusion that in the RS, approximately 150 patients *per* year (pediatric and adult) need long-term PN. Half of these patients include those with SBS [12]. Based on unofficial data from team members employed in the above-mentioned health institutions, it was concluded that over 90% of these patients do not survive even one year after being diagnosed. Lethal outcomes in these patients occur due to malnutrition and dehydration caused by the inability to administer HPN. By the year 2024, there was a need to increase the number of physicians in both pediatric and adult expert teams. At the beginning of 2024, a group of clinicians and clinical scientists was appointed to perform a modified Delphi process, encompassing face-to-face meetings, e-mail communications, in-group discussions, literature reviews, and providing expert opinions on the treatment of patients with SBS in accordance with the capabilities of the health system in the RS. Overall, there were six major sections defined for the development of statements which were included: definitions and criteria for diagnosis and classification of SBS; the role of PN overall and HPN in patients with SBS; types and ways of oral/enteral nutrition in three types of SBS; conservative and drug therapy in SBS and therapy for improving and accelerating intestinal adaptation; criteria and indications for intestinal growth factor therapy in SBS patients and non-transplantation surgical procedures in the treatment of SBS. These six sections were chosen unanimously by all team members because they form the basis for understanding, diagnosing, and treating patients with SBS. After literature review, e-mail communications, and in-group discussions, the most important statements have been highlighted from the official guidelines and recommendations of ESPEN [2]. Also, some of these statements have been modified and adapted in a way that makes them easier to implement at the national level. A total of 32 statements were created, which were voted on for consensus at the joint conference of the Association of Patients with SBS and NUPENS on June 7, 2024, in Belgrade, Serbia. All statements received a consensus agreement of 100%. Thanks to the Ministry of Health of the RS, as well as the Republic Fund of Health Insurance of the RS, an initiative was launched to enable HPN during 2025.

The goal of NUPENS is to establish a registry of patients with CIF and SBS in our country, classify this entity as a rare disease, and educate healthcare professionals about SBS diagnostic procedures and recognition of this entity, its monitoring, and treatment. One of the results of the work of NUPENS, but also a step towards improving the treatment of patients with SBS, is the development of these consensus statements.

Definition, criteria for diagnosis, and classification of SBS

a) SBS is defined as a clinical condition associated with residual small bowel continuity of less than 200 cm.
b) The presence of clinical symptoms and signs of SBS despite residual small bowel length of more than 200 cm is defined as “functional SBS”.
c) Based on the anatomy of the residual intestinal continuity, SBS is classified as SBS with a terminal

small bowel ostomy (SBS type 1), SBS with jejuno-colic anastomosis (SBS type 2), and SBS with jejuno-ileal anastomosis with intact colon and the presence of ileocecal valve (SBS type 3).

Comment. In the adult population, the normal length of the small intestine measured from the duodenojejunal flexure (ligament of Treitz) is 275 to 850 cm. There is no precise analysis of the length of the small intestine in children, but it is known that a residual length of less than 25% of the anatomical length of the small intestine leads to SBS, in contrast to the adult population where a residual length of less than 67% of the anatomical length leads to the occurrence of this syndrome [8, 9, 11, 13–16]. A short intestine can be a consequence of extensive surgical resections or congenital anomalies of the small intestine. In adults, the most common causes of SBS are extensive resections related with Crohn's disease (about 20–40%), mesenteric ischemia (about 30%), complications related with surgical interventions (about 20%), radiation enteritis (about 7%), volvulus (about 4%), intestinal and perivisceral adhesions (about 3%), and other causes (about 10%). In the pediatric population, the causes of SBS are mesenteric ischemia (about 6%), volvulus (about 24%), intestinal malformations (about 28%), necrotizing enterocolitis (about 17%), and other causative factors (about 26%) [17–19]. Clinical characteristics of SBS include malabsorption, diarrhoea, fatty stools, malnutrition, and dehydration [2, 8, 9]. In some patients, SBS may be present even though the post-resection length of the small bowel exceeds 200 cm. This occurs due to inadequate function of the remaining bowel, such as accelerated motility or any form of mucositis/mucosal disease of the small bowel, resulting in a reduced absorptive capacity of the bowel below that expected for its remaining length [20]. This condition is described as "functional SBS" [8, 9, 19, 20]. The anatomical classification of SBS includes three types based on the anatomy of the remaining continuity of the gastrointestinal tract, as follows: SBS type 1 – terminal jejunostomy/ileostomy; SBS type 2 – jejuno-colic anastomosis where the remaining jejunum is continuous with part of the large bowel (most often with the transverse or left colon); SBS type 3 – jejuno-ileocolic anastomosis, where the ileocecal valve is preserved with an intact colon (Figure 1) [8, 9, 21]. The incidence of SBS types is approximately 60% for SBS type 1, 30.9% for type 2, and 9.1% for SBS type 3 [19]. The probability that with the applied therapy (special dietary regimen, conventional drug therapy, and non-transplant surgery) it will be possible to reduce or even completely wean off PN and develop full enteral autonomy is different in all three SBS types. In patients with SBS type 1, the rate of PN weaning is 20%, in SBS type 2, 40%, and in SBS type 3, 80% [22]. The likelihood of reversibility of bowel function in SBS is higher if there is a greater length of remaining bowel continuity. Thus, the reversibility of CIF in SBS is higher if the bowel length is greater than 100 cm in type 1, greater than 65 cm in type 2 (only if it is more than 50% of the colon length), and greater than 30 cm of small bowel in SBS type 3 (Figure 1) [13, 21, 22].

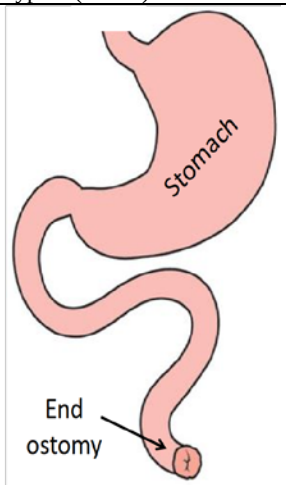
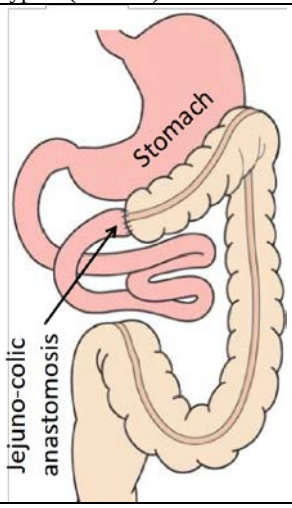
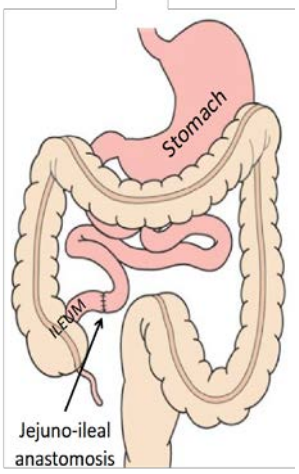
Classification	Type 1 (SBS-J)	Type 2 (SBS-JC)	Type 3 (SBS-JIC)
			
Description	Ileum, colon, and part of jejunum are resected; jejunostomy is created	Ileum, part of jejunum, and portion of colon (mainly right colon) are resected; remaining sections are joined (mainly jejunum and transversal or left colon)	Part of jejunum and ileum are resected, terminal ileum (at least 10 cm of terminal ileum) with ileocecal valve are present; remnant part of jejunum and terminal ileum are anastomosed
Probability of PN and intravenous supplementation dependence	Higher in patients with < 110–150 cm of jejunum remaining	Higher in patients with < 60–65 cm of jejunum remaining	Higher in patients with < 30–40 cm of small bowel remaining

Fig. 1 – Anatomical classification of SBS.
SBS – short bowel syndrome; SBS-J – SBS terminal jejunostomy/ileostomy; SBS-JC – SBS jejuno-colic anastomosis; SBS-JIC – SBS jejuno-ileocolic anastomosis; PN – parenteral nutrition.

The importance of PN and HPN in patients with SBS

a) PN is the first-line therapy that directly saves the life of a patient with SBS.
b) PN is the main therapy for patients with transient-reversible CIF, as well as for irreversible CIF, such as SBS.
c) In metabolically stable patients with SBS, PN can be administered at home because it improves the patient's quality of life (QoL), it is associated with a lower incidence of morbidity and mortality compared to PN in hospital settings, and it is more economical than PN in hospitals.
d) It is preferable to implement an HPN program by a trained and qualified team, as this would ensure the highest level of safety and effectiveness.

Comment. The primary outcome in patients with SBS depends exclusively on PN. Patients with SBS have a high probability of long-term survival if the primary therapy is PN, as it is the only way to provide appropriate amounts of macro- and micronutrients necessary for maintaining the functions of organs and organ systems, and for the growth and development of pediatric patients [3, 4, 18, 19]. In hospital settings and after diagnosing SBS (after surgical resection), the objective of the therapy is to repair electrolyte imbalance and metabolic state. Once the patients are metabolically stable without electrolyte imbalance and proper oral intake has been initiated (but still insufficient), they can be discharged for further home treatment with the use of PN at home. With proper training of patients and close relatives conducted by an expert team, the use of HPN ensures five-year survival in more than 80% of adult and more than 90% of pediatric SBS patients [23]. Properly implemented PN at home increases the QoL of SBS patients, as over 70% of patients achieve complete or almost complete social and occupational rehabilitation, as well as good quality family life [24]. On the other hand, even though the use of PN is also associated with complications, the most common of which is central venous access infection, patients within the HPN program have a significantly lower level of infectious complications compared to those with long-term use of PN in hospital settings [1, 3, 4, 24]. This primarily includes multidrug-resistant nosocomial infections with various bacterial strains in hospitalized patients with SBS. As the mortality rate in patients with long-term use of PN is mainly influenced by infectious complications [25], it may be said that HPN patients might have lower mortality and morbidity rates compared to those receiving PN in hospital settings. The occurrence of multidrug-resistant nosocomial infections and the costs of treating such complications, not counting the costs of hospital days, overall make HPN more economical compared to PN in hospital settings [1, 3, 24]. In order to maximally reduce possible complications, the engagement of a multidisciplinary or trained and qualified expert/expert team for the HPN program is of the utmost importance [2]. The total costs of the HPN program, when compared to the administration of PN in hospital settings, are significantly lower [2, 3]. In the RS, the HPN program still does not exist, but it would be substantially more economical compared to the administration of PN in hospital settings. Namely, if a patient receives PN three times a week, the costs of administering PN in hospital settings amount to approximately 51,120.00 RSD (440.00 EUR), in the case of commercial PN preparations. In the case of PN preparations made by the hospital itself (tailor-made), the cost of administration on a weekly basis, if the patient receives it three times a week, amounts to as much as 87,480.00 RSD (750.00 EUR). These amounts include: one day spent at the hospital ward (not semi-intensive and intensive care), working hours of a specialist, working hours of a medical technician, the costs of laboratory analyses, consumables and services for blood collection, consumables for additional therapy and the additional therapy itself, therapy and PN ordering services, hospital food and the PN preparations themselves. If, on the other hand, a patient receives PN three times a week at home, the cost of this therapy regimen *per* week is approximately 28,560.00 RSD (244.00 EUR), which is about 40% less than the cost of administering the therapy in a hospital setting. These calculations were made in Belgrade, Serbia, during the spring of 2022. Based on data on the safety and efficacy of HPN, it represents the primary form of therapy for CIF and SBS patients. In contrast, intestinal transplantation is a therapeutic approach for patients who are at risk of developing severe, fatal complications associated with PN in general or due to underlying gastrointestinal disease [23, 26].

Types of appropriate oral/enteral nutrition and criteria for oral intake in patients with SBS

a) Patients with SBS require dietary counseling by an experienced dietitian/nutritionist who is trained by or belongs to an expert team.
b) The dietary regimen should be individually tailored to each patient with SBS.
c) All patients with SBS should have their oral fluid intake monitored.
d) Oral fluid intake should be separated from meals in all patients with SBS.
e) The diet of all patients with SBS is based on the intake of solid foods, because liquid and mushy foods accelerate transit through the remaining part of the small intestine.
f) The recommendation for the intake of different macronutrients and their ratio in the meal (carbohydrates, fats, and proteins) differs in some types of SBS.
g) Soluble fiber supplementation (e.g., pectin) is not recommended in patients with SBS, as it does not improve intestinal absorption.
h) The use of enteral nutrition preparations in the form of oral nutritional supplements is indicated in patients with an expected increase in body weight, in order to reduce the volume and number of PN preparations, and should be prescribed and determined by an expert team.

i) In patients with mild dehydration with sodium loss, oral replacement with isotonic rehydration solutions rich in sodium is indicated.
j) In patients with a highly productive terminal jejunostomy, it is necessary to limit the oral intake of hypotonic fluids (water, tea, coffee, or alcohol), as well as hypertonic (fruit juices, carbonated drinks), in order to reduce the "output" at the stoma.
k) Patients who are severely dehydrated or sodium-depleted may be treated with isotonic oral rehydration solutions that are rich in sodium; however, i.v. fluids are the primary form of replacement.

Comment. Patients with SBS must compensate for malabsorption with hyperphagia, as this is the only way, besides PN, that can help with intestinal adaptation and preservation of the metabolic state [27]. The use of oral nutritional supplements, primarily isotonic, can help increase overall energy intake during the day. All patients with SBS should be encouraged to take a larger number of meals with the addition of certain oral nutritional supplements, which are preferably administered between meals. Understanding the physiology and pathophysiology of SBS is an indispensable factor in recommendations regarding the dietary regimen. In this regard, there are different types of counseling and diets, depending on the patient and the type of SBS. In those with a preserved colon, unabsorbed long-chain fatty acids accelerate intestinal transit and reduce water and electrolyte absorption. Fatty acids bind to calcium and magnesium and increase oxalate absorption with the consequent development of nephrolithiasis [2]. On the other hand, mono- and disaccharides draw fluid from the interstitium into the intestinal lumen by an osmotic gradient, which leads not only to accelerated transit but also to greater fluid loss, which is the case especially in SBS type 1. Due to the multifactorial effect of certain macronutrients and the pathophysiology of SBS itself, the optimal dietary regimen differs significantly in all three types of SBS [28]. In general, *per*-oral fluid intake should be limited, especially during the phases of intestinal adaptation. This primarily applies to SBS type 1 with high losses on the stoma. It is recommended that fluids be taken at least 60 min after the last meal [2, 29]. In SBS patients who are considered to benefit from enteral nutrition, either *via* tube feeding or oral nutritional supplements, standard formulas have a similar effect as polymeric formulas on nutrient absorption and water and electrolyte losses [30, 31]. Even though it has been proven that elemental (peptide) formula has better protein absorption in patients with SBS type 1 (90–150 cm of jejunal residuum), overall energy absorption cannot be significantly increased when compared to other types of formulas [32]. In all types of SBS, complex carbohydrate intake is recommended, as simple sugars increase intraluminal osmotic pressure, increase secretion, and accelerate transit, leading to greater losses [2]. It has been shown that maximal sodium absorption in humans occurs when an oral solution containing 120 mmol/L (2,160 mg) sodium chloride and 30 mmol/L (540 mg) glucose is administered [33]. Given that the large intestine has a significant capacity for water and electrolyte reabsorption, this type of oral rehydration is not particularly significant as it is indicated in patients with SBS type 1, and less commonly in those with type 2 [2]. Protein and energy requirements for CIF patients should be based on individual patient characteristics, specific needs, and the adequacy of the regimen should be regularly evaluated through clinical, anthropometric, and biochemical parameters. This refers not only to parenteral supplementation but also to oral/enteral intake. Generally, in stable SBS patients, the provision of 0.8–1.4 g of protein/kg/day is enough to meet daily requirements [1, 2], but this is mostly accomplished by PN. The diet of SBS patients with a preserved colon in continuity (types 2 and 3) can be high in complex carbohydrates, low in mono- and disaccharides, and low in fat [2]. Patients with a preserved colon, who are on the borderline of needing i.v. supplementation (PN and/or i.v. fluid replacement therapy), benefit from medium-chain triglycerides since they are easily hydrolyzed, do not require bile salts, and are easily absorbed across the intestinal mucosa and transported *via* portal vein to the liver [2, 34]. All SBS patients following a low-fat diet, or those in whom long-chain triglycerides have been replaced by medium-chain triglycerides, should be monitored for potential deficiencies in essential fatty acids and fat-soluble vitamins [1, 2]. In all three types of SBS, but especially in type 1, complex carbohydrates are the most important dietary carbohydrates. In SBS type 1, studies have shown that oral food can consist of any fat/carbohydrate ratio, provided that it has a low mono- and disaccharide content [1, 2, 34]. Enteral nutrition in combination with oral feeding can be prescribed in patients with CIF in whom the expected gain with enteral nutrition could allow weaning from HPN. Polymeric isotonic enteral diets may be the first choice [2]. The aim of continuous enteral nutrition is to provide better distribution and maximum exposure of the available intestinal surface area to nutrients while stimulating gastrointestinal secretions and endogenous hormonal secretions that are important for advancing intestinal adaptation [31, 32]. Compared to voluntary oral intake, it is more likely that enteral nutrition will increase intestinal absorption and accelerate adaptation in the immediate post-operative settings [2, 35]. On the other hand, an aggressive approach to enteral nutrition and enteral stimulation in type 1 SBS patients may aggravate gastric hypersecretion and intestinal fluid and electrolyte losses [36]. Regarding administration of oral fluids, as mentioned above, patients with type 1 SBS can use salt liberally and restrict the administration of oral fluids in relation to meals [2]. Those with borderline dehydration or sodium depletion can use an isotonic high-sodium oral rehydration solution to replace stoma sodium losses [1, 2]. In patients with high-output jejunostomy (SBS type 1), oral intake of low-sodium solutions, both hypotonic (e.g., water, tea, coffee, or alcohol) and hypertonic (e.g., fruit juices, carbonated beverages), should be limited in order to reduce the stoma output [2]. All SBS patients are at some risk of dehydration and electrolyte disturbances, especially those with reduced length of jejunum and jejunostomy. Many of these patients tend to secrete more sodium and fluid than they consume orally [29, 37]. Some of these patients even experience losses of water and

sodium when they take nothing by mouth (secretors) [37]. In addition, in these patients, oral intake of food and beverages increases the stomal losses of fluid and sodium. Some of these patients are also subject to magnesium deficiency. In these situations, they often describe an "insatiable thirst", and they are often tempted to compensate by increasing their oral beverage intake. However, since an increase in both hypotonic and hypertonic fluids may stimulate fluid secretion or increase the fluid and sodium influx into the lumen of the jejunum due to the leakiness of the epithelium, this would further aggravate stomal losses. Thus, a vicious cycle of chronic dehydration and excessive beverage intake is believed to be generated [2, 29, 33, 38, 39]. In order to halt this, the general advice has been that the patients should restrict excessive habitual beverages and instead drink oral rehydration solutions [1, 2]. The intestinal fluid and sodium absorption may be evaluated with measurements of 24-hr urine volume and urine sodium excretion. In addition to clinical evaluation, body weight, and standard blood biochemistry, urine sodium excretion may help assess the fluid balance of individual SBS patients and adjust oral fluid intakes [2].

The use of drug therapy in the treatment of patients with SBS, and therapies for improving and accelerating intestinal adaptation

a) Proton pump inhibitors and histamine receptor 2 antagonists are recommended in SBS cases because they reduce sodium excretion and stool loss (contents at the stoma), especially in the first six months after surgical intervention. This is very important if fecal loss is greater than 2 L *per* day.
b) Short-term use of octreotide may be of importance in patients with high-output jejunostomies, for better control of electrolyte imbalance. When using octreotide, proper monitoring of patients is necessary due to their side effects (negative impact on intestinal adaptation).
c) The use of loperamide is recommended in all patients with SBS, because it slows down transit and reduces fecal excretion of water and sodium. When compared to opiates (codeine phosphate, opium, etc.), loperamide is preferred. In SBS type 1, the administration and dosing of loperamide is not difficult, given the easy objectification of its effects.
d) SBS patients who have motility disorders, including those with segmental dilatation of the residual small intestine (e.g., appendix), as well as those with suspected excessive bacterial growth, may benefit from occasional antibiotic therapy, primarily with metronidazole.

Comment. All conditions occurring after small bowel resection are associated with gastric hypergastrinemia and hypersecretion [38]. The etiology of gastric gastrin hypersecretion lies in the loss of hormonal inhibitors in the ileum and colon. Gastric hypersecretion "washes out" the proximal parts of the small bowel, increasing intraluminal volume, decreasing absorption time, and contributing to greater losses. In addition, the associated hyperacidity accompanied by gastric hypersecretion leads to denaturation of pancreatic enzymes and disruption of intraluminal bile salt metabolism, which further reduces absorption [39]. On the other hand, undigested or partially digested nutrients increase intraluminal osmotic pressure and increase intestinal mucosal secretion, which leads to even greater losses at the stoma. Therefore, the administration of histamine receptor 2 antagonists and proton pump inhibitors is an indispensable therapy in patients with SBS [2]. The effects of octreotide and its analogues are reflected in the reduction of gastric, biliary, and pancreatic secretion, as well as the secretion of water and electrolytes (primarily sodium) in the jejunum and colon [40]. Furthermore, octreotide leads to an increase in the absorption of sodium and chlorine in the ileum with a decrease in intestinal motility (by inhibiting the synthesis of secretagogues) [41]. Although octreotide and its analogues can inhibit glucose absorption and reduce the absorption of macronutrients due to the inhibition of pancreatic enzyme secretion, it has been shown that it can temporarily help in the treatment of large losses at the jejunostomy, providing time for the correction of electrolyte and metabolic imbalance [2, 42]. The use of antidiarrheal drugs in SBS is widespread, and it aims at reducing water and electrolyte losses and minimizing symptoms resulting from diarrhoea. The use of these drugs can reduce the need for i.v. fluid and electrolyte replacement. In addition, indirectly, the reduction of the "output" at the jejunostomy prevents damage to the skin around the stoma and facilitates its care. Due to the central effects of some antidiarrheal drugs (codeine, diphenoxylate, opium), loperamide has a significant advantage in SBS. Loperamide inhibits the peristaltic activity of the small intestine, which slows intestinal transit. The result of this is an increase in absorption time not only of water and electrolytes, but also of macronutrients due to more appropriate intraluminal digestion [43]. The optimal dose of loperamide should be individually prescribed and ranges from 3–24 mg *per* day [2]. There is not enough data about excessive bacterial growth in the small bowel of patients with SBS. Patients with post-surgical small bowel dilatation, abdominal pain, and large jejunostomy losses may benefit from short-term metronidazole therapy. However, this therapy is not recommended in patients with preserved colon, i.e., those with SBS types 2 and 3 [2, 44, 45].

Criteria and indications for selection of patients with SBS for intestinal growth factor therapy

a) SBS patients who are absolutely dependent on PN (whether once or several times a week) for a period longer than 12–24 months after surgery should consider the introduction of intestinal growth factor therapy.
b) If intestinal adaptation has been achieved and the patient with SBS is still dependent on PN, the introduction of intestinal growth factor therapy should be considered.
c) Before the use of intestinal growth factors, it is necessary to conduct a thorough diagnosis of the gastrointes-

tinal tract and exclude the presence of malignant or premalignant disease.

d) Recent presence of malignant disease or the presence of malignant disease in patients with SBS is a contraindication for the use of intestinal growth factor therapy.
e) The indication for the use of intestinal growth factors must be determined by an expert team and experts in the field of CIF and SBS.
f) The first choice of intestinal growth factor therapy in SBS is the glucagon-like peptide-2 (GLP-2) analogue, the drug teduglutide, because it is the only one approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA).
g) The use of teduglutide requires monitoring of the SBS patient by an expert and/or an expert team.
h) The effectiveness of teduglutide therapy is reflected in the reduction of the need for PN over a period of 3–6 and 6–12 months from the initiation of therapy.

Comment. When all therapeutic options (drug therapy, dietary counseling and specific dietary regimens, prescribing certain enteral nutrition preparations, surgical reconstructive procedures, surgical procedures to increase the length of the small intestine and/or reduce transit through the small intestine) have been exhausted, with maximum intestinal adaptation achieved, and the patient with SBS is still dependent on PN for any period of time, then prescribing PN is the only therapeutic measure to preserve the patient's health and life. The period of intestinal adaptation varies, but it directly depends on the remaining length of the small intestine and the type of SBS. This period can be 6 or 12 months, but never more than 2 years [1–4]. Intestinal adaptation essentially refers to structural and functional changes in the intestinal mucosa, but also to the slowing down of transit and changes in the gastrointestinal microbiome [2]. This process is minimal in SBS type 1, unlike SBS type 3. It has been proven that such intestinal adaptation, which can lead to the independence of patients from PN, is possible only in 20% of patients with SBS type 1, 40% in type 2, and 80% in SBS type 3 [21, 22]. After the initial results in the conducted experimental studies, the clinical studies have shown that intestinal growth factors, primarily GLP-2, lead to increased absorption and reduced need for PN in patients with all types of SBS [46–49]. In addition, it has been proven that ending the therapy with GLP-2 analogues does not lead to a severe deterioration in the condition of patients with SBS, but rather that the effect is long-lasting. Also, treated patients can actually be cured, that is, their health and life do not depend on the use of PN [50]. In an extensive analysis of treated SBS patients [48], the use of teduglutide has been proven safe and effective (in the case of proper indication), and that it does not lead to an increase in the incidence and number of malignancies in treated patients [51, 52]. SBS patients with suspected or active malignant diseases, as well as those with anamnestic data for previous malignant disease of the gastrointestinal tract, including the hepatobiliary tract and pancreas, are not candidates for therapy with intestinal growth factors [2]. The main criteria for determining patients who are candidates for GLP-2 analogues would be the time elapsed since the last intestinal resection, as well as the absence of contraindications for this type of therapy. The time period must be long enough so that the maximum possible intestinal adaptation is ensured. The most reliable way we can use to define the parameters of intestinal adaptation and monitor an SBS patient who is a potential candidate for therapy with GLP-2 analogues is through the work and activities of an expert team [2]. The effectiveness of GLP-2 analogues therapy should be ensured on the basis of standardized protocols that monitor the balance of water, electrolytes, and energy. Teduglutide is the only recombinant analogue of the physiological GLP-2 analogue that is approved in Europe and America for the treatment of SBS in adults and children over the age of 1. The expected effects after initiating the therapy can be seen after 3, 6, 8, or 12 months, and they have a long-lasting effect in terms of complete cessation of PN and in terms of completely curing the SBS [2, 3, 53].

Non-transplantation surgical procedures in the treatment of SBS

a) In patients with SBS, reconstruction of intestinal continuity is indicated whenever possible, with the aim of reducing dependence on PN.
b) Surgical methods of "lengthening" and "augmenting" the intestine and slowing down transit may be considered in certain cases.

Comment. In case of surgical intervention, when extensive resection of the small intestine is indicated, with or without colonic resection, preserving as much of the small and/or large intestine as possible is strongly recommended. One of the main criteria for early identification of patients with CIF is measuring the remaining length of the healthy intestine at the time of surgical resection. The surgeon must register the length of the remaining small and/or large intestine, as well as indicate the exact anatomy of the gastrointestinal tract after surgical intervention [2]. Once the SBS patient is metabolically stable and without electrolyte imbalance, the reconstruction of the gastrointestinal tract and the creation of intestinal continuity between the stoma and the unused distal intestine must be the top priority. Reconstruction can be performed in over 80% of patients while hospitalized, and in 50% of "reconstructed" patients, the need for long-term PN administration can be significantly reduced [26, 54]. There are numerous surgical procedures that can be used for the surgical treatment of SBS. These procedures aim to slow intestinal transit, increase the mucosal surface area, and "lengthen" the remaining part of the intestine. These surgical procedures depend solely on the local condition of the abdomen, underlying diseases (Crohn's disease, radiation enteritis, etc.), the clinical condition of the patient, but also on a proper assessment of potential morbidity and benefits [2].

Conclusion

Bearing in mind that SBS and CIF are chronic diseases, the treatment of which requires a great deal of human, financial, and time commitment, and also the fact

that these are life-threatening diseases, it is absolutely necessary to recognize them as rare diseases and treat them properly as such. Fortunately, there is not a large number of SBS and CIF patients in our country, and therefore it is possible to devise and organize their treatment, primarily through HPN. With such action, the QoL of these patients would be at the highest possible level (outside the hospital, they are at home, active and fully functional). Moreover, such a program would lead not only to an improvement in the quality of their lives, but also that of their family members (the family as a whole becomes functional), and hospital capacities would also be freed up. Therefore, it is necessary to recognize SBS and CIF as rare diseases, to enable the existence and use of home enteral nutrition and HPN, and to ensure the availability of parenteral and enteral nutrition preparations to patients. Enteral and PN preparations should be free of charge and easily obtained from pharmacies (as well as the material for their administration). It is necessary to recognize the HPN program and home enteral nutrition as official legal procedures by the Republic Fund of Health Insurance of the RS. In addition, it is necessary to enable and organize appropriate services to support primarily the patients within the HPN program, but also those dependent on home enteral nutrition. Treatment of SBS requires multidisciplinary and multiprofessional activity. Vigilant monitoring and adequate treatment enable significant life extension of these patients.

Funding

This paper received no external funding.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

NUPENS wishes to thank the Association of Patients with Short Bowel Syndrome (UPSKC) and Ms. Mirjana Urukalo, the chairwoman of UPSKC, for exceptional cooperation, enthusiasm, and selfless assistance in all joint activities. NUPENS wishes to thank the Ministry of Health of the Republic of Serbia and the Republic Fund of Health Insurance of the Republic of Serbia (RFZO) for their understanding and support, and for enabling the implementation of home parenteral nutrition and providing adequate therapy to SBS patients.

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Received on March 7, 2025
Revised on May 13, 2025
Accepted on May 28, 2025
Online First August 2025

Appendix

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ORIGINAL ARTICLES

UDC: 616.24-008.4::612.821.7]:616.1
DOI: https://doi.org/10.2298/VSP250422053S

Challenges in assessing cardiovascular risk in obstructive sleep apnea-hypopnea syndrome: applicability of existing tools

Izazovi u proceni kardiovaskularnog rizika kod sindroma opstruktivne apneje-hipopneje u snu: primenljivost postojećih skorova

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Abstract

Background/Aim. Obstructive sleep apnea-hypopnea syndrome (OSAHS) is associated with an increased cardiovascular risk (CVR). The aim of this study was to examine CVR in hypertensive patients with OSAHS using the Systematic Coronary Risk Evaluation 2 (SCORE2), SCORE2-Diabetes, and American College of Cardiology/American Heart Association for atherosclerotic cardiovascular disease (ACC/AHA ASCVD) risk scores. **Methods.** Due to strict exclusion criteria, out of 410 consecutive OSAHS patients, 92 hypertensive patients with moderate or severe OSAHS were included in the study. All patients underwent CVR assessment using SCORE2, SCORE2-Diabetes, and ACC/AHA ASCVD risk scores. Additionally, all patients, except for seven individuals with extreme obesity (weight over 130 kg) who were unable to perform the test, underwent an exercise stress test, and six of them required further diagnostic assessment using stress echocardiography (three), computed tomography coronary angiography (two), and/or invasive coronary angiography (three). **Results.** The results showed a substantial burden of moderate to high CVR across all scores. Severe OSAHS was associated with a higher percentage of moderate to high CVR, particularly with the ACC/AHA ASCVD calculator. However, no significant correlation was found between the apnea-hypopnea index and CVR. Furthermore, in three patients, invasive coronary angiography showed multivessel disease requiring myocardial revascularization. **Conclusion.** General CVR calculators may inadequately represent the specific CVR in OSAHS patients, highlighting the need for tailored risk assessment and increased screening for coronary artery disease in this population. Our results emphasize the clinical relevance of screening for coronary artery disease in individuals with OSAHS.

Key words:
sleep apnea, obstructive; sleep apnea syndromes; coronary artery disease; hypertension; risk factors.

Apstrakt

Uvod/Cilj. Sindrom opstruktivne apneje-hipopneje u snu (*obstructive sleep apnea-hypopnea syndrome* – OSAHS) povezan je sa povećanim kardiovaskularnim rizikom (KVR). Cilj rada bio je da se ispita KVR kod obolelih od hipertenzije i OSAHS korišćenjem skorova rizika *Systematic Coronary Risk Evaluation 2* (SCORE2), SCORE2-Dijabetes i *American College of Cardiology/American Heart Association for atherosclerotic cardiovascular disease* (ACC/AHA ASCVD). **Metode.** Zbog strogih krterijuma isključenja od 410 uzastopnih bolesnika obolelih od OSAHS, 92 hipertenzivna bolesnika sa umerenim ili teškim OSAHS uključena su u studiju. Svi ispitanici podvrgnuti su proceni KVR-a korišćenjem SCORE2, SCORE2-Dijabetes i ACC/AHA ASCVD skorova rizika. Takođe, svi bolesnici, osim sedmoro sa ekstremnom gojaznošću (težina preko 130 kg) koji nisu mogli da urade test, podvrgnuti su testu fizičkim opterećenjem, a šestoro njih zahtevalo je dalju dijagnostičku procenu korišćenjem stres ehokardiografije (troje), kompjuterizovane tomografije koronarnih arterija (dvoje) i/ili invazivne koronarne angiografije (troje). **Rezultati.** Rezultati su pokazali značajno opterećenje umerenim do visokim KVR-om prema svim skorovima. Teški oblik OSAHS bio je povezan sa višim procentom umerenog do visokog KVR-a, posebno prema ACC/AHA ASCVD kalkulatoru. Nije pokazana značajna korelacija između indeksa apneje-hipopneje i KVR-a. Takođe, kod troje bolesnika, invazivna koronarna angiografija pokazala je višesudovnu koronarnu bolest koja je zahtevala revaskularizaciju miokarda. **Zaključak.** Opšti sistemi bodovanja KVR-a mogu neadekvatno prikazati specifični KVR kod obolelih od OSAHS, što naglašava

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potrebu za prilagođenom procenom rizika i pojačanim ranim otkrivanjem koronarne arterijske bolesti u ovoj populaciji. Naši rezultati naglašavaju klinički značaj detekcije koronarne arterijske bolest kod osoba sa OSAHS.

Ključne reči:
apneja u snu, opstruktivna; apneja, spavanje poremećaji, sindromi; koronarna bolest; hipertenzija; faktori rizika.

Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAHS) is the most common sleep-breathing disorder, affecting 34% of males and 17% of females aged 30 to 70 years [1]. However, the true prevalence of OSAHS is very difficult to estimate because it often remains undiagnosed and, consequently, untreated. It is characterized by intermittent episodes of partial or total airway collapse, which leads to snoring, night choking, arousals, and sleep fragmentation. Consequently, patients with OSAHS often experience daytime fatigue, reduced work productivity, increased sleepiness during the day, and a higher risk of motor vehicle accidents [2]. The diagnosis of OSAHS is established through a sleep study, and the severity of the disorder is typically quantified using the apnea-hypopnea index (AHI). The AHI is defined as the number of apneic or hypopneic episodes occurring during each hour of sleep [3].

There is a bidirectional connection between OSAHS and cardiovascular diseases (CVD), as OSAHS represents an independent risk factor for CVD, and patients with CVD frequently suffer from OSAHS [4]. Intermittent hypoxemia, autonomic dysfunction, and changes in intrathoracic pressure are the main reasons why patients with OSAHS have an increased risk for arterial hypertension (HTA), atrial fibrillation, pulmonary hypertension, heart failure, coronary artery disease (CAD), and sudden cardiac death. Furthermore, OSAHS and CVDs share many common risk factors such as age, male gender, obesity, and smoking [5].

Given the increased cardiovascular risk (CVR) in patients with OSAHS, accurate risk assessment is crucial in the prevention of CVDs. Assessing CVR is usually performed by CVR scores. Developed by European Society of Cardiology (ESC), the Systematic Coronary Risk Evaluation 2 (SCORE2) is an improved version of SCORE that is usually used in assessing the 10-year risk of fatal and non-fatal cardiovascular events in people aged 40 to 69 years without diabetes mellitus (DM), chronic kidney disease, extremely high cholesterol level, or preexisting CVD. While SCORE2 is designed for this specific age group, SCORE2-Older Persons (SCORE2-OP) is used in people who are 70 years old or older [6]. Furthermore, for patients with DM, the ESC recommends using SCORE2-Diabetes in assessing CVR [7]. On the other hand, the American College of Cardiology (ACC) and the American Heart Association (AHA) suggest using ACC/AHA atherosclerotic CVD (ASCVD) risk score in assessing individual's 10-year risk of having a first major atherosclerotic cardiovascular event (fatal and non-fatal stroke, non-fatal myocardial infarction, and fatal CAD death) [8]. However, the applicability and accuracy of these general CVR scores in the specific population of patients with OSAHS remain uncertain.

The aim of the study was to examine the applicability of general CVR assessment scores, such as SCORE2, SCORE2-Diabetes, and ACC/AHA ASCVD, in the specific population of hypertensive patients with OSAHS, and to highlight the potential need for tailored risk stratification strategies for this (potentially) high-risk group.

Methods

Out of 410 consecutive patients with OSAHS, 92 hypertensive patients were included in this prospective study due to strict exclusion criteria. Among them, 30 patients (32.6%) had moderate OSAHS [AHI 15–29 episodes *per* hour (15–29/hr)], while 62 patients (67.4%) had severe OSAHS (AHI ≥ 30/hr). The diagnosis of HTA was based on the latest guideline for the management of HTA published by the European Society of Hypertension [9]. OSAHS diagnosis was determined through full-night respiratory polygraphy (RPG) using the Alice NightOne device from Philips Respironics (Eindhoven, Netherlands). This assessment was conducted at the sleep laboratory of the Clinic for Lung Diseases, University Clinical Center Niš, Niš, Serbia, during the patients' typical sleep periods. The diagnostic criteria were based on the American Academy of Sleep Medicine Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea [10]. The RPG parameters investigated during research were AHI, oxygen desaturation index, the time spent with oxygen saturation below 90%, and the minimum, average, and maximum oxygen saturation levels. Participants also completed the Epworth Sleepiness Scale.

Individuals not diagnosed with OSAHS (AHI < 5/hr) or those with mild OSAHS (AHI 5–14/hr) were not included in the study. Furthermore, exclusion criteria encompassed patients with a prior diagnosis of CAD, heart failure, severe valvular disease or artificial heart valve, chronic kidney disease (defined as an estimated glomerular filtration rate under 60 mL/min/1.73 m^2), individuals younger than 40 or older than 80 years, and those with considerable physical or mental health issues.

Following the completion of the sleep study, all participants were admitted to the Department of Cardiovascular Diseases at the Institute for Treatment and Rehabilitation "Niška Banja", Niš. During their hospitalization, anthropometric measurements were taken, and laboratory analyses were conducted. CVR was then evaluated using SCORE2 (for non-DM individuals), SCORE2-Diabetes (for those with DM), and the ACC/AHA ASCVD risk score. Subsequently, all patients, except for seven individuals with extreme obesity (weight over 130 kg), who were unable to perform the test, underwent an exercise stress test (EST). These ESTs were conducted on a treadmill (3017 Full Vision Drive, Newton, Kansas, USA) following

the Bruce protocol. The exercise tests were terminated due to reaching a submaximal heart rate, the onset of limiting symptoms such as dyspnea, fatigue, chest pain, or dizziness, the presence of ischaemic changes on the electrocardiogram (horizontal or downsloping ST segment depression of ≥ 1 mm), complex ventricular arrhythmias (couplets of premature ventricular contractions or ventricular tachycardia), a hypertensive response defined as a rapid increase in systolic blood pressure (BP) to ≥ 220 mmHg or a decrease in systolic BP of > 10 mmHg, or at the patient's request.

The subjects' written consent was obtained, according to the Declaration of Helsinki. The study was approved by the Ethics Committee of the Institute for Treatment and Rehabilitation "Niška Banja", Niš (No. 3560/1, from March 29, 2023).

Statistical analysis

Data were analyzed using SPSS software version 20. Categorical data were expressed as frequencies and percentages, while quantitative data were presented as mean ± standard deviations. Data distribution was tested using the Kolmogorov-Smirnov test. Means of normally distributed data were compared using Student's *t*-test, while the Mann-Whitney *U* test was used for data whose distribution deviates significantly from normal distribution. For the comparison of frequencies, the Chi-square test was used. In correlation analysis, Pearson's correlation was used for normally distributed variables, while Spearman's rank correlation was used for data whose distribution deviates significantly from normal distribution. Statistical significance was set at $p < 0.05$.

Results

This study involved 92 hypertensive patients (average age 54.45 ± 9.61 years) diagnosed with either moderate [30 (32.6%)] or severe [62 (67.4%)] OSAHS. Initial analysis of anthropometric data revealed no significant differences in age, weight, or height. Patients with severe OSAHS had higher values of waist circumference, neck circumference (NC), body mass index, and systolic and diastolic BP compared to patients with moderate OSAHS. However, only NC reached statistical significance ($p = 0.040$) (Table 1).

Subsequently, risk factors for CVD were assessed. There were no significant differences in smoking status, physical activity (defined as walking at least 3 km a day), heredity, stress, and dyslipidemia. In contrast, DM (40.32% vs. 31.52%, $p = 0.007$) and obesity (93.55% vs. 86.96%, $p = 0.011$) were more prevalent in the group of patients with severe OSAHS. In addition, the number of risk factors was significantly higher in patients with severe OSAHS compared to those with moderate OSAHS (4.84 ± 1.13 vs. 4.27 ± 1.46, $p = 0.049$) (Table 2).

Upon admission to the Institute for Treatment and Rehabilitation "Niška Banja", a laboratory assessment was performed. No significant differences were observed in the investigated parameters between the groups, except for high-density lipoprotein, which was significantly higher in patients with moderate OSAHS (1.21 ± 0.30 vs. 1.08 ± 0.32, $p = 0.034$) (Table 3).

We used SCORE2 to assess the CVR in hypertensive OSAHS patients aged 40 to 69 years and without CVD and DM (Figure 1). Among the 55 investigated patients, the majority had high [30 (54.55%)] or moderate [23 (41.82%)] CVR. There were no statistically significant differences between the groups. In patients with DM, SCORE2-Diabetes was used. Of the 27 patients, 6 (22.22%) had very high CV risk, 15 (55.56%) had high, and 6 (22.22%) had intermediate CVR (Figure 2). Although all six patients with very high CVR had severe OSAHS, the investigated groups did not differ significantly. In six patients who were 70 years or older and without DM, SCORE-OP was performed, and all of them had high CVR. In four patients, cholesterol levels were either excessively high (above 9 mmol/L) or unusually low (below 3 mmol/L), rendering risk calculation using standard tools unfeasible.

Table 1

Anthropometric parameters

Parameters	Total		AHI 15–29		AHI ≥ 30		*p*-value
	n	mean ± SD	n	mean ± SD	n	mean ± SD	
Gender							
female, n (%)		17 (18.48)		7 (23.33)		10 (16.13)	0.287†
Body weight, kg	90	111.41 ± 19.81	29	107.86 ± 24.61	61	113.1 ± 17.06	0.244
Body height, cm	90	176.12 ± 9.03	29	175.66 ± 11.44	61	176.34 ± 7.73	0.737
Age, years	92	54.45 ± 9.61	30	55.07 ± 9.4	62	54.15 ± 9.78	0.669
Waist circumference, cm	89	121.24 ± 14.36	29	117.55 ± 16.82	60	123.02 ± 12.79	0.093
Neck circumference, cm	88	45.53 ± 3.92	28	44.29 ± 4.28	60	46.12 ± 3.63	**0.040**
Body mass index, kg/m^2	91	35.92 ± 6.33	30	34.4 ± 6.5	61	36.67 ± 6.17	0.109
Epworth scale	86	12.16 ± 4.87	28	12.36 ± 4.92	58	12.07 ± 4.88	0.799
Systolic blood pressure, mmHg	85	123.12 ± 12.1	28	121.07 ± 12.2	57	124.12 ± 12.03	0.277
Diastolic blood pressure, mmHg	85	79.47 ± 11.02	28	77.86 ± 6.44	57	80.26 ± 12.66	0.347

AHI – apnea-hypopnea index; SD – standard deviation; n – number. The bold value indicates a significance level of $p < 0.05$. *Note*: †The significance was obtained using the Chi-square test. All other significances were obtained using Student's *t*-test for two independent samples.

Table 2

Risk factors for cardiovascular diseases

Parameters	Total	AHI 15–29	AHI ≥ 30	*p*
Smoking	48 (52.17)	14 (46.67)	34 (54.84)	0.304
Physical activity	37 (40.22)	9 (30.00)	28 (45.16)	0.122
Obesity	80 (86.96)	22 (73.33)	58 (93.55)	**0.011**
Stress	20 (21.74)	8 (26.67)	12 (19.35)	0.295
Diabetes mellitus	29 (31.52)	4 (13.33)	25 (40.32)	**0.007**
Hyperlipidemia	67 (72.83)	22 (73.33)	45 (72.58)	0.574
Heredity	53 (57.61)	19 (63.33)	34 (54.84)	0.293
Number of risk factors	4.65 ± 1.27	4.27 ± 1.46	4.84 ± 1.13	**0.049**†

AHI – apnea-hypopnea index.
All values are given as numbers (percentages) and mean ± standard deviation. Bold values indicate a significance level of $p < 0.05$.
***Note:* †The significance was obtained using the Mann-Whitney *U* test. All other significances were obtained using the Chi-square test.**

Table 3

Laboratory assessment

Parameters	Total		AHI 15–29		AHI ≥ 30		*p*-value
	n	mean ± SD	n	mean ± SD	n	mean ± SD	
Cholesterol, mmol/L	92	5.65 ± 3.59	30	5.42 ± 1.14	62	5.77 ± 4.31	0.68
LDL, mmol/L	91	3.26 ± 0.97	29	3.22 ± 0.93	62	3.28 ± 1.00	0.798
HDL, mmol/L	92	1.12 ± 0.32	30	1.21 ± 0.30	62	1.08 ± 0.32	**0.034**
Triglyceride, mmol/L	92	2.09 ± 1.03	30	2.22 ± 1.40	62	2.03 ± 0.79	0.665
Glucose, mmol/L	92	5.91 ± 0.99	30	5.87 ± 1.29	62	5.93 ± 0.81	0.364
AST, U/L	92	21.89 ± 8.21	30	21.57 ± 6.57	62	22.05 ± 8.95	0.874
ALT, U/L	92	30 ± 16.07	30	28.73 ± 15.62	62	30.61 ± 16.37	0.521
Creatinine, μmol/L	92	93.97 ± 16.22	30	91.44 ± 17.55	62	95.19 ± 15.54	0.546
Urea, mmol/L	92	5.31 ± 1.36	30	5.11 ± 1.33	62	5.41 ± 1.37	0.334
Uric acid	92	376.21 ± 85.31	30	355.93 ± 77.35	62	386.02 ± 87.83	0.112
Sedimentation	92	20.75 ± 17.02	30	22.27 ± 21.10	62	20.02 ± 14.78	0.686
Leukocyte	92	7.41 ± 1.67	30	7.22 ± 1.75	62	7.5 ± 1.64	0.543
Erythrocyte	92	4.88 ± 0.44	30	4.86 ± 0.48	62	4.89 ± 0.43	0.819
Hematocrit	92	0.43 ± 0.04	30	0.43 ± 0.04	62	0.44 ± 0.03	0.418
Hemoglobin	92	143.03 ± 18.84	30	137.7 ± 27.39	62	145.61 ± 12.35	0.301
Thrombocyte	92	257.14 ± 55.2	30	267.53 ± 54.21	62	252.11 ± 55.40	0.184
eGFR	92	122.41 ± 32.69	30	115.9 ± 36.57	62	125.56 ± 30.46	0.073
Modified eGFR*	90	96.33 ± 22.89	29	93.45 ± 25.49	61	97.7 ± 21.63	0.263

AHI – apnea-hypopnea index; SD – standard deviation; n – number; LDL – low-density lipoproteins; HDL – high-density lipoproteins; AST – aspartate aminotransaminase; ALT – alanine aminotransaminase; eGFR – estimated glomerular filtration rate.
The bold value indicates a significance level of $p < 0.05$. All significances were obtained using the Mann-Whitney *U* test.
***Note:* *used for an overweight patient.**

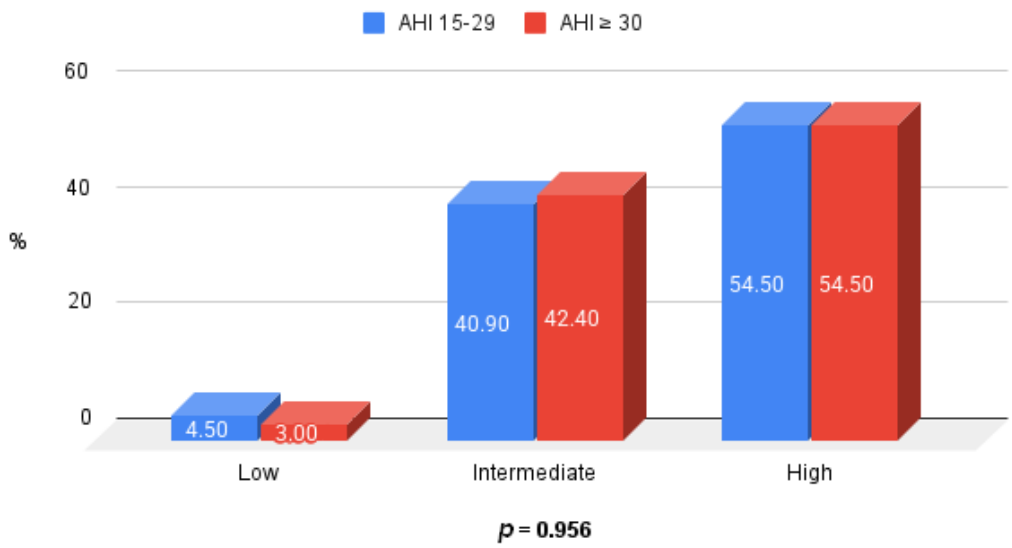

Fig. 1 – SCORE2 for assessing the CVR in hypertensive OSAHS patients.
CVR – cardiovascular risk; OSAHS – obstructive sleep apnea-hypopnea syndrome.
For other abbreviations, see Table 4.

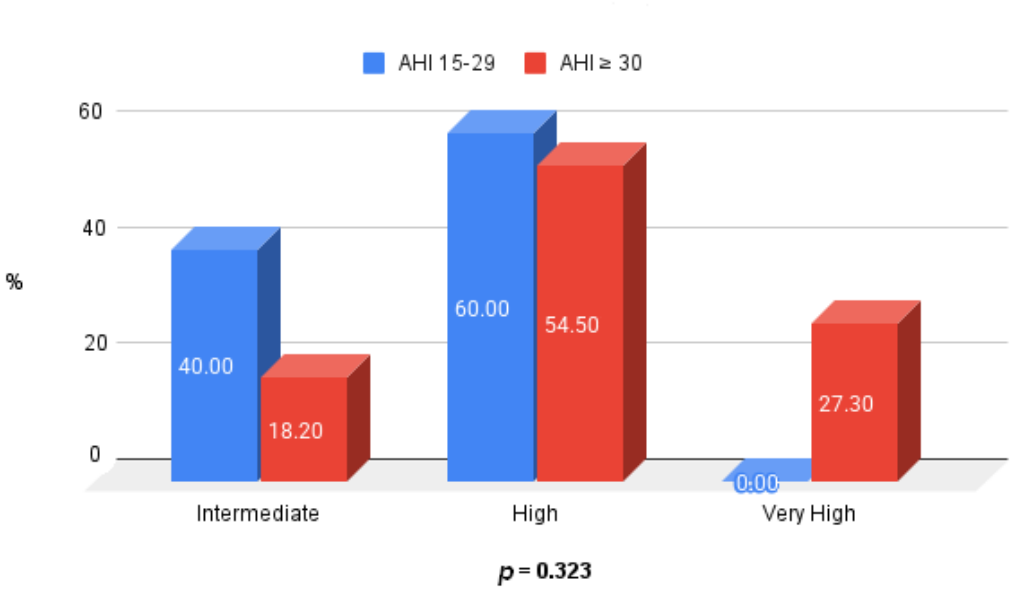

Fig. 2 – SCORE2-Diabetes in patients with diabetes mellitus for assessing the CVR in hypertensive OSAHS patients.
For abbreviations, see Table 4 and Figure 1.

The ACC/AHA ASCVD calculator was used in 91 patients. More than 70% of investigated patients had moderate or high CVR. This finding was even more pronounced in severe OSAHS, where 39.28% had moderate and 39.28% had high CVR. However, the investigated groups did not differ significantly (Figure 3). Finally, we performed a correlation between the AHI index and all three investigated scores and found no significant correlation (Table 4).

Patients with moderate OSAHS demonstrated better strain tolerance by achieving higher strain levels and longer duration of EST, but without statistical significance (Table 5). In contrast, patients with severe OSAHS reached submaximal heart rate more frequently than patients with moderate OSAHS (84.21% vs. 64.29%, $p = 0.038$).

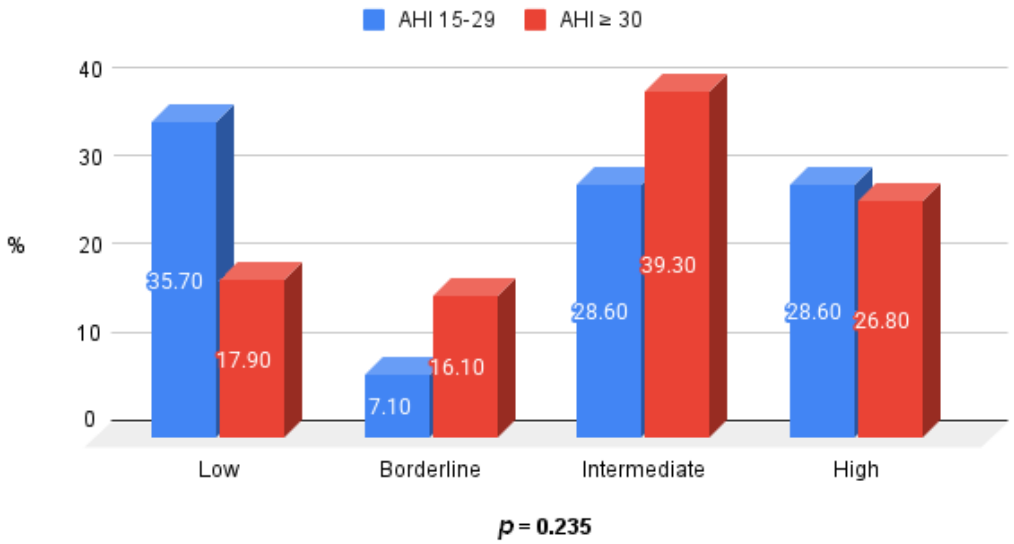

Fig. 3 – ACC/AHA ASCVD for assessing the CVR in hypertensive OSAHS patients. For abbreviations, see Table 4 and Figure 1.

Table 4

Correlation between AHI and risk SCORES

Risk scores	AHI		
	n	*r*	*p*
SCORE2	55	-0.013	0.926
SCORE2-Diabetes	27	0.062	0.760
ACC/AHA ASCVD	84	0.009	0.937

AHI – apnea-hypopnea index; SCORE2 – Systematic Coronary Risk Evaluation 2; ACC/AHA ASCVD – American College of Cardiology/American Heart Association for Atherosclerotic Cardiovascular Disease.
The Pearson correlation coefficient (*r*) was used.

Table 5

Exercise stress test

Parameters	Total	AHI 15–29	AHI ≥ 30	*p*-value
Level	2.68 ± 1.01	2.82 ± 1.22	2.61 ± 0.9	0.558
Duration	6.46 ± 2.99	6.9 ± 3.41	6.25 ± 2.77	0.403
Submaximal heart rate	66 (77.65)	18 (64.29)	48 (84.21)	**0.038**†
Ischemia	6 (7.06)	3 (10.71)	3 (5.26)	0.308†
Arrhythmias	8 (9.41)	1 (3.57)	7 (12.28)	0.188†
Double product before	9,556.82 ± 2,105.28	9,413.93 ± 1,695.78	9,627.02 ± 2,290.54	0.369
Double product after	23,293.41 ± 3,502.08	22,697.14 ± 3,269.47	23,586.32 ± 3,602.49	0.317

AHI – apnea-hypopnea index.
All values are given as numbers (percentages) or mean ± standard deviation.
The bold value indicates a significance level of $p < 0.05$.
***Note:* †Significance obtained using the Chi-square test. All other significances were obtained using the Mann-Whitney *U* test.**

Discussion

In the present study, OSAHS was more prevalent in males, which aligns with previous epidemiological studies [11]. Additionally, waist circumference and NC once again proved to be great indicators for diagnosing OSAHS [12]. Higher values of NC in severe compared to moderate OSAHS may indicate that NC positively correlates with the severity of OSAHS. Furthermore, this finding suggests that this simple anthropometric measurement may serve as a valuable clinical

tool for identifying patients at increased risk. In resource-limited settings like ours, where polysomnography may not be readily available, NC could serve as a cost-effective screening method. The study once again stressed the point that patients with OSAHS share many common risk factors with CVD [13]. Namely, the average number of risk factors was 4.65 ± 1.27, and patients with severe OSAHS had a higher number of risk factors compared to moderate OSAHS (4.27 ± 1.46 vs. 4.84 ± 1.13, $p = 0.049$). These findings are consistent with previous studies that have demonstrated a dose-dependent relationship between OSAHS severity and CVR [13]. Furthermore, obesity and DM were more prevalent in patients with severe compared to patients with moderate OSAHS, which also aligns with previous studies. Both obesity and DM contribute to increased CVR through mechanisms such as systemic inflammation, endothelial dysfunction, and autonomic dysregulation, which are further exacerbated by the intermittent hypoxemia and sleep fragmentation associated with OSAHS [14].

SCORE2, SCORE2-Diabetes, and ACC/AHA ASCVD are validated risk scores used for assessing the 10-year risk for CVD in individuals without established CVD [6–8]. SCORE2 is a relatively new score developed from ESC for assessing the 10-year risk for fatal and non-fatal CVD in individuals aged 40 to 69 years, without previous DM or CVD. It uses an algorithm that involves parameters such as gender, age, smoking status, total cholesterol, high-density lipoprotein cholesterol, and systolic BP. Importantly, the calibration of the risk calculator depends on the country. We used a calculator that was calibrated for Serbia and which uses the table for a very high overall risk for CVD. In the present study, a significant proportion of OSAHS patients aged 40–69 years, without CVD or DM (55 patients), exhibited high or intermediate CVR assessed by the SCORE2 calculator, which highlights the substantial cardiovascular burden in these patients.

For patients with DM and without CVD, we used SCORE2-Diabetes. Out of 27 patients, 6 (22.22%) had very high CV risk, 15 (55.56%) had high, and 6 (22.22%) had low CVR. All six patients with very high CVR were patients with severe OSAHS. In both groups, most patients had intermediate CVR. Notably, there was no single patient with low CVR, and the majority of patients with moderate or severe OSAHS had high or very high risk scores. However, in this group, as in the group of patients without DM, there was no significant correlation between CVR and OSAHS severity. Of note, SCORE2-OP was performed in six patients aged 70 years or older, and all six patients were found to have a high CVR. Three patients had cholesterol levels exceeding 9mmol/L, while one had a level below 3mmol/L. In these patients, the estimation of CVR could not be done.

The ACC/AHA ASCVD risk calculator uses parameters such as age, gender, race, systolic BP, total cholesterol, high-density lipoprotein cholesterol, smoking status, presence of DM, and treatment for HTA to estimate the 10-year risk of a first major atherosclerotic cardiovascular event (non-fatal myocardial infarction, coronary heart disease death, or stroke) in individuals aged 40–79 years without pre-existing ASCVD. More than 70% of investigated patients in our study had moderate or high CVR assessed by the ACC/AHA ASCVD calculator. This indicates that this score, as well as SCORE2 and SCORE2-Diabetes, highlights the high cardiovascular burden in hypertensive OSAHS patients. Once again, the group with severe OSAHS had a higher percentage of patients with moderate and high CVR compared to the group with moderate OSAHS. Nevertheless, this difference did not reach statistical significance ($p = 0.235$). Importantly, our investigation failed to show a significant correlation between the severity of OSAHS using AHI and all three scores: SCORE2, SCORE2-Diabetes, and ACC/AHA ASCVD. These findings are in contrast with previous studies employing other CVR scores. In these studies, CVR scores generally demonstrated a positive correlation with the severity of OSAHS. For instance, in the study by Archontogeorgis et al. [15], the severity of OSAHS was significantly associated with higher SCORE values ($p < 0.001$) and Framingham Risk Score values ($p < 0.001$). Furthermore, the authors reported significant positive correlations between the AHI and SCORE ($r = 0.251$, $p < 0.001$) and between AHI and Framingham Risk Score ($r = 0.291$, $p < 0.001$). The authors concluded that the 10-year risk of cardiovascular morbidity and mortality appeared to increase with OSAHS severity. Similarly, Borsini et al. [16] found a positive correlation between the ACC/AHA ASCVD risk score and OSAHS severity, as assessed by AHI.

Several factors may contribute to the aforementioned discrepancy. Firstly, these validated and generally accepted CVR scores do not consistently incorporate key CVR factors frequently present in patients with OSAHS, such as obesity [13, 17, 18] and metabolic syndrome [19]. Furthermore, important parameters derived from RPG, known to influence CVR, oxygen desaturation index [20], and hypoxic burden [21, 22], are not included in these scoring systems.

Patients with severe OSAHS showed better strain tolerance by achieving higher levels and longer duration of EST, which aligns with a previous study [23], but without statistical significance. On the other hand, a higher percentage of patients with severe OSAHS achieved submaximal heart rate compared to moderate OSAHS. This is partially due to higher values of heart rate and BP at the beginning of EST (the double product was higher in severe compared to moderate OSAHS). Notably, six patients had ischemia on EST. In three patients, a stress echocardiographic test was performed, two patients were sent for computer tomography coronary angiography, and one patient underwent invasive coronary angiography (ICA). The choice of diagnostic tool was made based on pre-test probability, risk factor-weighted clinical likelihood, and comorbidities. All three patients who were referred for a stress echocardiographic test showed no new wall-motion abnormalities. However, one of them survived a myocardial infarction during the trial and underwent ICA, which showed a significant multivessel disease. One of the two patients who underwent computed tomography coronary angiography was also found to have multivessel disease and, following ICA, was referred for cardiac surgery. Finally, the patient who was directly referred for ICA also had a significant multivessel disease that required percutaneous coronary intervention. Altogether, three (3.26%) patients had CAD, which required

revascularization. These results highlight the necessity of screening OSAHS patients for CAD [24, 25].

Study limitations

Several limitations should be considered when interpreting the present findings. First, the sample size was relatively small compared to some larger studies examining the assessment of CVR in patients with OSAHS [15, 16]. However, our study applied stricter inclusion and exclusion criteria, and to our knowledge, it is the first to investigate the usefulness of SCORE2, SCORE2-Diabetes, and ACC/AHA ASCVD risk scores in assessing CVR in patients with OSAHS. Second, RPG was utilized for OSAHS diagnosis instead of polysomnography, which is the established gold standard. This was necessitated by the limited availability of polysomnography facilities within our medical center. Despite its limitations, RPG remains a valuable and widely adopted tool for assessing respiratory events during sleep, particularly in settings with resource constraints.

Conclusion

Our study evaluated the cardiovascular risk profile of hypertensive patients with OSAHS using SCORE2, SCORE2-Diabetes, and ACC/AHA ASCVD risk scores. Our findings revealed a substantial burden of moderate to high cardiovascular risk across all three scores in our cohort. A higher percentage of patients with severe OSAHS exhibited moderate to high cardiovascular risk compared to those with moderate OSAHS across all risk scores, but without statistical significance. Importantly, our investigation failed to demonstrate a significant correlation between the apnea-hypopnea index and cardiovascular risk as assessed by any of the three scores. This finding suggests that these general risk calculators, primarily validated in the broader population, may not adequately capture the specific relationship between OSAHS severity and cardiovascular risk. Future research should investigate these findings in larger studies with diverse OSAHS populations and explore the potential for developing risk assessment tools that better account for the unique pathophysiological mechanisms linking OSAHS and cardiovascular diseases. Ultimately, these data emphasize the clinical relevance of screening for coronary artery disease in individuals with OSAHS.

Conflict of interest

The authors declare no conflict of interest.

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Received on April 22, 2025
Revised on May 23, 2025
Accepted on May 28, 2025
Online First July 2025

ORIGINAL ARTICLE

UDC: 616-036.22:[616.2-002.1-02:578.8
DOI: https://doi.org/10.2298/VSP250402052R

Sentinel surveillance of acute respiratory infections and distribution of SARS-CoV-2, influenza A and B, and respiratory syncytial virus in the post-COVID-19 period

Sentinelni nadzor nad akutnim respiratornim infekcijama i distribucija SARS-CoV-2, virusa gripa tipa A i B i respiratornog sincicijalnog virusa u post-COVID-19 periodu

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Abstract

Background/Aim. Acute respiratory infections (ARIs) remain a major global health concern. The aim of this study was to analyze the epidemiological and clinical characteristics of ARIs and detect the distribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, influenza B, and respiratory syncytial virus (RSV) among outpatients during the 2023/24 and 2024/25 seasons. **Methods.** A prospective study was conducted through sentinel surveillance, enrolling only patients with ARI symptoms/signs. Identification of one of the four viruses was performed using the CerTest SARS-CoV-2 + Flu A + Flu B + RSV rapid combined test. **Results.** In the 2023/24 season, 26.57% of samples tested positive for at least one virus, while in the 2024/25 season, a decrease in positivity for the tested viruses was observed, dropping to 14.24%. Influenza A dominated during the 2023/24 season, while the incidence of influenza B increased in early 2025, peaking in late February. SARS-CoV-2 showed moderate fluctuations throughout the study period, with a pronounced peak in September 2024, while RSV remained low during both seasons. The age distribution of patients varied: in the 2023/24 season, RSV and influenza B were most frequently recorded in young children aged 0–4 years, influenza A in children aged 0–4 and 5–14 years, while SARS-CoV-2 was most prevalent among adults aged 30–64 years. In the 2024/25 season, RSV and influenza A were most commonly diagnosed in children aged 0–4 years, influenza B in children aged 5–14 years, and SARS-CoV-2 in individuals over 30 years old. Elevated body temperature was the most common symptom regardless of the type of confirmed viral infection but was significantly less present in SARS-CoV-2 infections compared to other viruses. **Conclusion**. The results of this study indicate a shift in the dominance of viral causative agents between the observed seasons, with influenza A prevailing in 2023/24, while an increase in the incidence of influenza B virus was observed in 2024/25. SARS-CoV-2 circulated continuously but at a low level, while the impact of RSV was minimal in both seasons.

Key words:
epidemiology; influenza a virus; influenza b virus; respiratory syncytial viruses; respiratory tract infections; sars-cov-2.

Apstrakt

Uvod/Cilj. Akutne respiratorne infekcije (ARI) i dalje predstavljaju značajan globalni zdravstveni problem. Cilj rada bio je da se analiziraju epidemiološke i kliničke karakteristike ARI i detektuje distribucija koronavirusa 2 izazivača teškog akutnog respiratornog sindroma (*severe acute respiratory syndrome coronavirus 2* - SARS-CoV-2), virusa gripa tipa A, virusa gripa tipa B i respiratornog sincicijalnog virusa (RSV) među ambulantno lečenim bolesnicima tokom sezona 2023/24 i 2024/25. **Metode**. Sprovedena je prospektivna studija putem sentinelnog nadzora, a obuhvaćeni su samo bolesnici sa simptomima/znacima ARI. Identifikacija jednog od četiri virusa izvršena je korišćenjem brzog kombinovanog testa *CerTest SARS-CoV-2 + Flu A + Flu B + RSV*. **Rezultati**. U sezoni 2023/24, 26,57% uzoraka bilo je pozitivno na najmanje jedan virus, dok je u sezoni 2024/25 zabeležen pad pozitivnosti ispitivanih virusa na 14,24%. Infekcije virusom gripa A dominirale su tokom sezone 2023/24, dok je učestalost virusa gripa B porasla početkom 2025. godine, dostižući vrhunac krajem februara. SARS-CoV-2 pokazao je umerene fluktuacije tokom studijskog

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perioda, sa izraženim pikom u septembru 2024, dok je aktivnost RSV ostala niska tokom obe sezone. Uzrasna distribucija bolesnika je varirala: u sezoni 2023/24, RSV i virus gripa B najčešće su registrovani kod dece uzrasta 0–4 godine, virus gripa A kod dece uzrasta 0–4 i 5–14 godina, dok je SARS-CoV-2 bio najzastupljeniji među odraslima uzrasta 30–64 godine. U sezoni 2024/25, RSV i virus gripa A najčešće su dijagnostikovani kod dece uzrasta 0–4 godine, virus gripa B kod dece uzrasta 5–14 godina, a SARS-CoV-2 kod osoba starijih od 30 godina. Povišena telesna temperatura bila je najčešći simptom bez obzira na vrstu dokazane virusne infekcije, ali je bila značajno ređe prisutna kod infekcija SARS-CoV-2 u poređenju sa ostalim virusima. **Zaključak**. Rezultati ove studije ukazuju na promenu u dominaciji virusnih uzročnika između posmatranih sezona, pri čemu je virus gripa A bio dominantan u 2023/24, dok je u sezoni 2024/25 zabeležen porast učestalosti virusa gripa B. SARS-CoV-2 cirkulisao je kontinuirano, ali na niskom nivou, dok je uticaj RSV bio minimalan u obe sezone.

Ključne reči:
epidemiologija; grip a virus; grip b virus; respiratorni sincicijalni virusi; respiratorni trakt, infekcije; sars-cov-2.

Introduction

Acute respiratory infections (ARIs) are a major global health burden, contributing to medical costs, lost productivity, and antimicrobial resistance due to frequent antibiotic prescriptions [1–4]. Viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A and B, and respiratory syncytial virus (RSV) are key ARI pathogens, with overlapping clinical presentations that complicate diagnosis and highlight the need for timely testing [1, 5]. The coronavirus disease 2019 (COVID-19) pandemic accelerated molecular diagnostics, focusing on SARS-CoV-2 detection to curb transmission. Public health measures also reduced the circulation of other respiratory viruses, raising concerns about future outbreaks [5, 6]. SARS-CoV-2 has underscored the need to differentiate between viral pathogens due to its unique severity [7]. While fever and cough are common ARI symptoms, age-specific patterns vary: RSV predominantly affects young children, whereas influenza A is prevalent in school-aged children and young adults [1, 6, 7]. Coinfections, particularly with SARS-CoV-2 and influenza, may intensify disease burden, making rapid detection essential for effective management [8].

Seasonal variations shape the epidemiology of ARIs, with influenza and RSV peaking in winter [1, 9]. Understanding these patterns, alongside demographic and clinical characteristics, helps optimize preventive strategies, such as patient isolation and antiviral use [5–8]. Surveillance of respiratory infections is crucial for public health [9, 10].

The aim of this study was to analyze the epidemiology of ARI and the circulation patterns of four respiratory viruses in primary care across two consecutive seasons, to inform clinical practice and guide public health policies.

Methods

This study was designed as an observational, prospective clinical-epidemiological investigation analyzing the characteristics of patients presenting with one or more symptoms/signs of ARIs. A subset of patients treated by six physicians [three pediatricians and three general practitioners (GPs)] was included, comprising a total of 10,194 patients during the 2023/24 season and 8,726 patients during the 2024/25 season. In both seasons, patients were categorized into five age groups: 0–4 years, 5–14 years, 15–29 years, 30–64 years, and ≥ 65 years. The study was conducted at the Health Center Novi Sad, Novi Sad, Serbia. The 2023/24 season covered the period from December 21, 2023, to March 29, 2024 (15 consecutive weeks), while the 2024/25 season extended from September 18, 2024, to February 28, 2025 (24 consecutive weeks).

Inclusion/exclusion criteria

Patients of any age and gender presenting with one or more symptoms/signs of ARI – including fever (or subjective feverishness), cough, headache, malaise, myalgia, sore throat, or loss of appetite within the past seven days – during the two surveillance seasons and seeking medical care from paediatricians or GPs at their first visit, were included in the study. Given that six physicians in both seasons expressed willingness to participate, we included 2.8% and 2.4% of the total population under surveillance during the 2023/24 and 2024/25 seasons, respectively (Table 1) [11].

Patients were excluded if they did not present with at least one of the specified symptoms/signs during the

Table 1

Population under sentinel surveillance according to age groups

Season/Population	Age (years)					Total
	0–4	5–14	15–29	30–64	≥ 65	
Season 2023/24						
population of Novi Sad according to the 2023 estimated census	20,533	38,778	67,322	178,299	65,691	370,623
population under sentinel surveillance	1,184 (5.8)	2,165 (5.6)	1,958 (2.9)	3,086 (1.7)	1,801 (2.7)	10,194 (2.8)
Season 2024/25						
population of Novi Sad according to the 2024 estimated census	20,459	38,389	67,666	177,136	65,093	368,743
population under sentinel surveillance	1,503 (7.3)	2,606 (6.8)	1,436 (2.1)	1,839 (1.0)	1,342 (2.1)	8,726 (2.4)

All values are given as numbers (percentages).

surveillance periods, if their symptoms had persisted for more than seven days, if they sought medical care outside the designated study periods, or if they had consulted pediatricians or GPs more than once.

Patient survey

Patients were interviewed by a physician to complete the remaining sections of the questionnaire, which included information on age, gender, symptoms/signs associated with ARIs during the physician's visit, and the date of laboratory sampling.

Laboratory examination

Education on proper sampling techniques and sample handling procedures was provided to all participating physicians before the start of the study and lasted for five days. Nasopharyngeal swabs were collected for combined testing of SARS-CoV-2, Influenza A, Influenza B, and RSV to confirm or rule out infection with any of these four viruses. Since these were point-of-care tests, sample analysis was performed at the Health Center Novi Sad at the physicians' outpatient clinics.

CerTest SARS-CoV-2 + Flu A + Flu B + RSV (CerTest Rapid Test)

A total of 1,690 patients with one or more symptoms/signs of ARI were tested using the CerTest Rapid Test, with patient data collected prospectively. The CerTest Rapid Test one-step combo card is a chromatographic immunoassay designed for the simultaneous qualitative detection of SARS-CoV-2, influenza A, influenza B, and RSV antigens in nasopharyngeal or nasal samples from patients suspected of having these infections. The test contains four strips, each dedicated to detecting one of the target viruses: strip A – SARS-CoV-2, strip B – influenza A, strip C – influenza B, and strip D – RSV. Each strip contains monoclonal antibodies on the test (T) line to capture the virus-specific antigen and polyclonal antibodies on the control (C) line to verify test validity. If the sample contains viral antigens, they bind to a red-colored conjugate and form a visible red line at the T line, indicating a positive result. If no antigens are present or if levels are too low, only the C line appears. Testing is most effective within 5–7 days of symptom onset. A negative result does not rule out infection, and additional testing [e.g., polymerase chain reaction (PCR)] may be needed. The performance of the CerTest Rapid Test is shown in Table 2 [12].

Statistical analysis

The population under sentinel surveillance at the primary care level at the Health Center Novi Sad (Table 1) served as the denominator for calculating the weekly incidence of ARIs *per* 10,000 inhabitants, both in total and stratified by age group. The numerator represented the number of ARI cases recorded within this population during two distinct surveillance periods: from December 21, 2023, to March 29, 2024, and from September 18, 2024, to February 28, 2025.

Due to the small number of positive cases, the weekly incidence rates of SARS-CoV-2, influenza A, influenza B, and RSV were calculated *per* 100,000 inhabitants, based on the number of laboratory-confirmed cases of each virus, with adjustments made according to the denominator representing the population under sentinel surveillance.

Numerical data were expressed as measures of central tendency (arithmetic mean, median) and variability (interquartile range, mean ± standard deviation), while categorical data were presented as frequencies and percentages. The Chi-square test or Fisher's exact test was used to assess associations between categorical variables. Analysis of variance (ANOVA) was applied to compare mean values across multiple independent groups. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 21. Statistical significance was set at $p < 0.05$.

Ethical considerations

This investigation was classified as routine public health surveillance; therefore, approval from the Ethics Committee and written informed consent were not required in Serbia. However, before enrollment, oral informed consent was obtained from each participant or, in the case of participants under 15 years of age, from their parents or legal guardians. During the process of obtaining oral consent from participants or the parents/guardians of a child, a physician (either a GP or pediatrician) and a team nurse were present. Personal and confidential information was excluded, except for demographic data, including age, gender, symptoms/signs associated with ARI, and the date of sample collection. All data were anonymized before being accessed by the authors.

Results

The course of research and the laboratory testing results for four respiratory viruses over two consecutive seasons are presented in Figure 1. During the 2023/24 season, 186

Table 2

Evaluations for CerTest Rapid Test

Variable	SARS-CoV-2	Influenza type A	Influenza type B	RSV
Sensitivity	93.0 (86.1–97.1)	80.7 (73.8–86.5)	84.3 (75.0–91.1)	94.7 (74.0–99.9)
Specificity	99.8 (98.8–100.0)	99.2 (98.6–99.6)	99.5 (98.9–99.8)	100.0 (69.2–100.0)
PPV	98.9 (94.2–10.0)	92.2 (86.5–96.0)	92.6 (84.6–97.2)	100 (81.5–100.0)
NPV	98.5 (96.9–99.4)	97.8 (96.9–98.5)	98.8 (98.0–99.4)	90.9 (58.7–99.8)

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; RSV – respiratory syncytial virus; PPV – positive predictive value; NPV – negative predictive value.
All values are given as mean values (95% confidence interval).

(26.57%) out of 700 tested samples were positive for at least one of four viral pathogens. Influenza A was the most frequently detected virus, accounting for 113 (60.75%) cases. In the 2024/25 season, the proportion of positive samples decreased to 14.24% (141/990), with influenza B being the predominant virus [57 (40.43%)].

In the 2023/24 season, the incidence of ARI increased at the beginning of the year, peaking at 105 cases *per* 10,000 inhabitants from January 29 to February 2, 2024, followed by a subsequent decline. In the 2024/25 season, a gradual increase in incidence was observed starting in September, with peaks recorded between September 23 and 27, 2024 (75.6 cases *per* 10,000 inhabitants) and in the final weeks of January 2025 (76.8 cases *per* 10,000 inhabitants) (Figure 2).

Overall, across both seasons, the highest incidence rates of ARIs were observed in the youngest age group (0–4 years), while the lowest incidence rates were recorded in the oldest age group (≥ 65 years). Among children aged 0–4 years, a

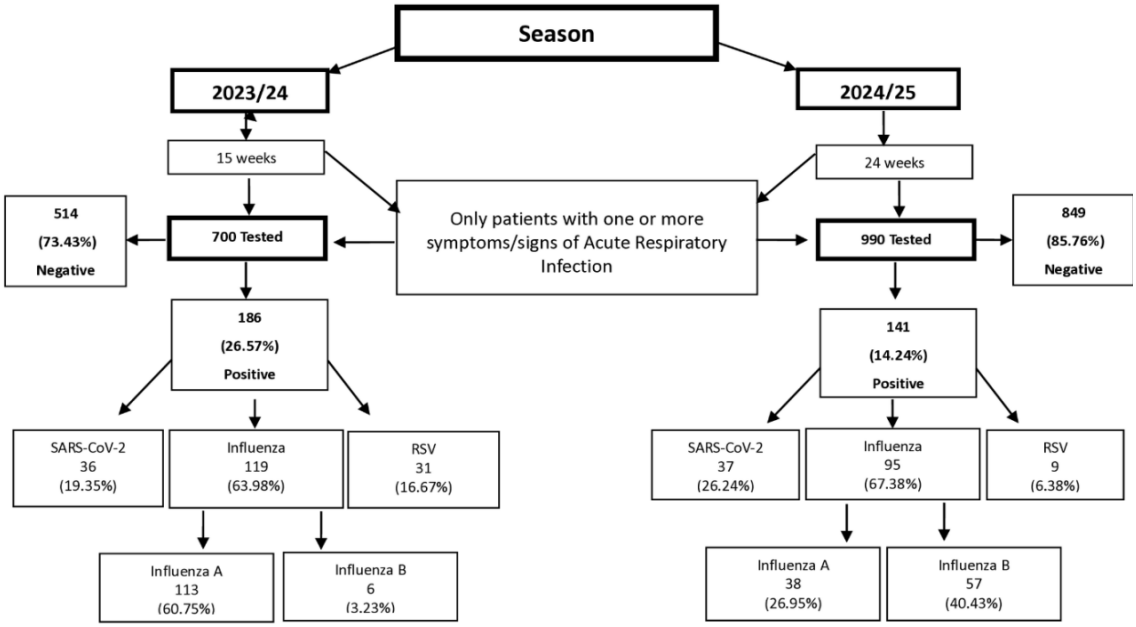


Fig. 1 – Algorithm for the sentinel surveillance and laboratory diagnosis of four different viruses using CerTest Rapid Test. For abbreviations, see Table 2.

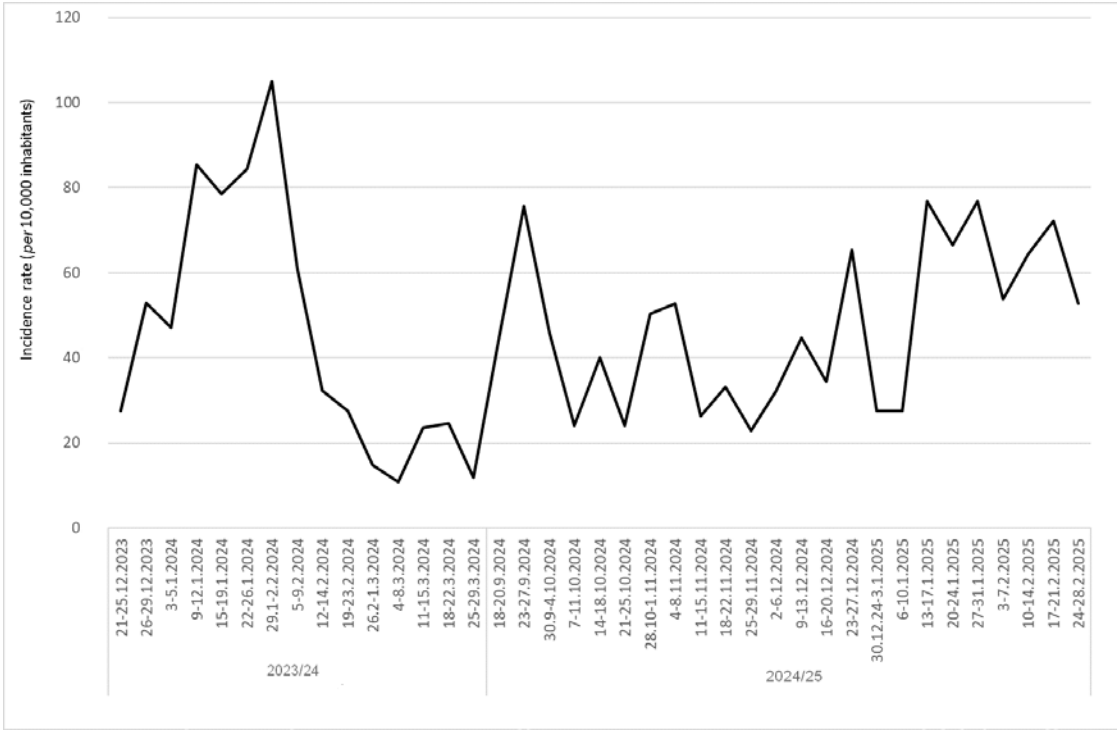


Fig. 2 – Weekly incidence rate of acute respiratory infection during the two seasons.

rapid increase in incidence was observed early in the 2023/24 season, peaking at over 200 cases *per* 10,000 inhabitants in early January 2024, followed by a sharp decline. In contrast, the 2024/25 season exhibited a different pattern, with a lower initial incidence but multiple peaks throughout the season, particularly in December 2024 and January 2025 (Figure 3a). Among school-aged children, incidence rates in the 2023/24 season peaked early, reaching approximately 140 cases *per* 10,000 inhabitants at the end of January 2024, followed by a marked decline. Conversely, the 2024/25 season exhibited a more gradual increase in incidence within this age group, characterized by multiple fluctuations and a peak in early 2025 (Figure 3b). In comparison, adults (15–29 years and 30–64 years) and the elderly population (≥ 65 years) consistently exhibited lower incidence rates across both seasons compared to younger age groups (Figures 3c–e).

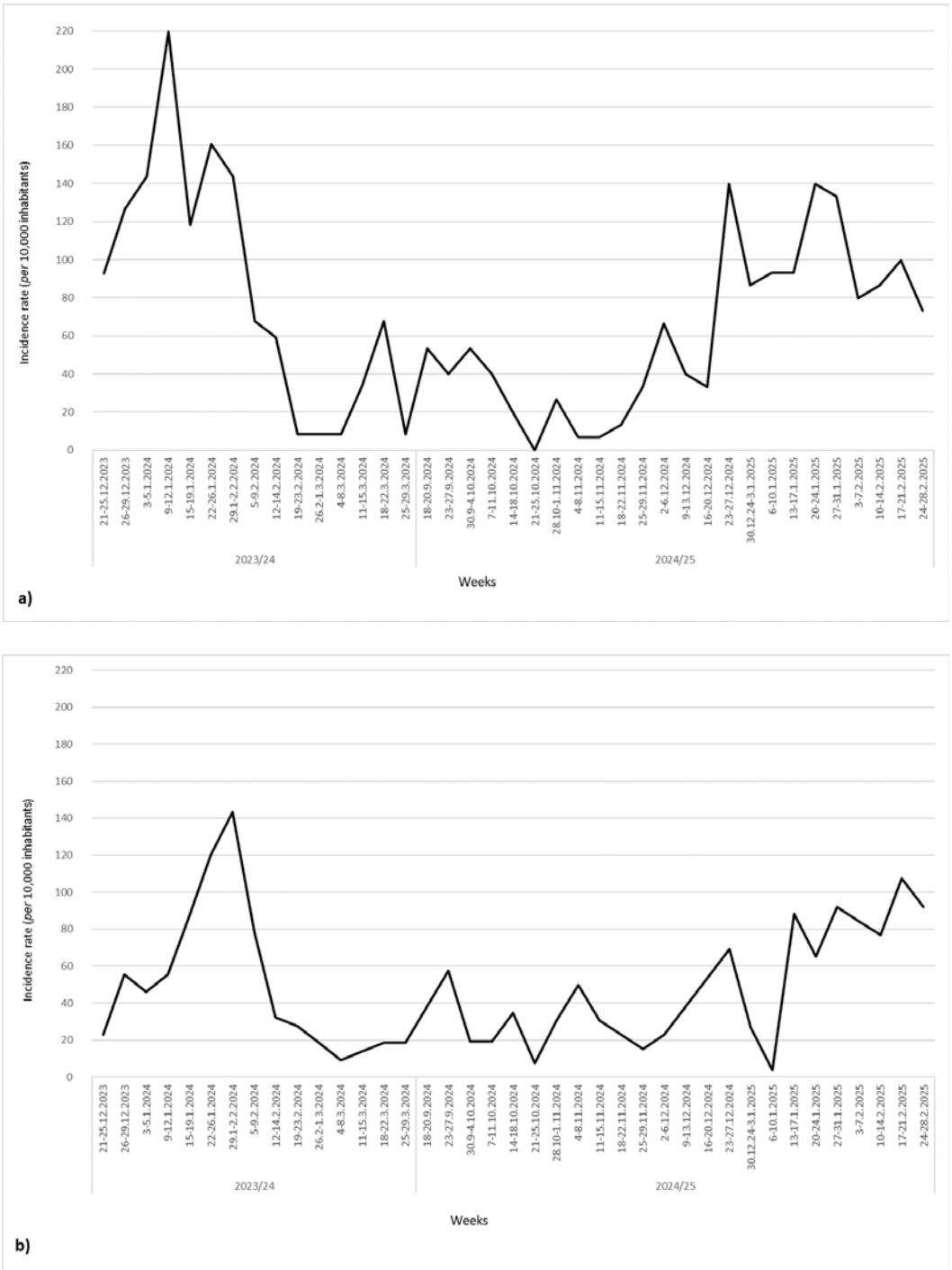
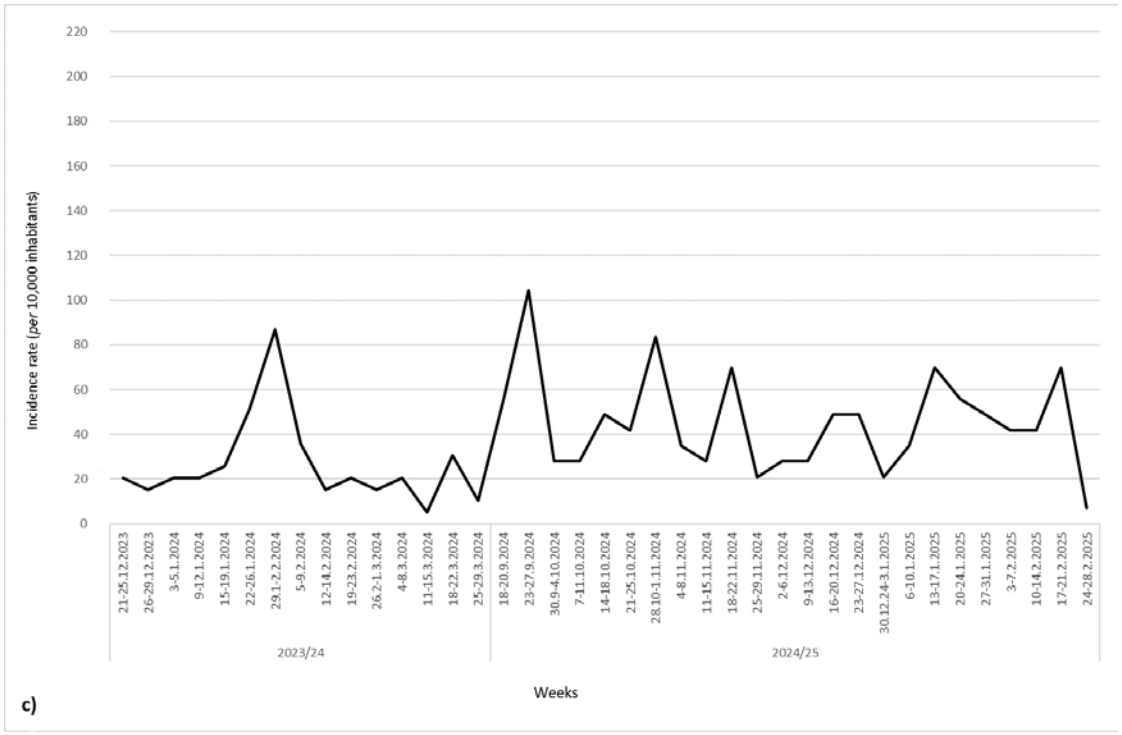


Fig. 3 – Incidence rates of acute respiratory infections *per* 10,000 inhabitants, stratified by surveillance week, for two consecutive seasons, and age: a) 0–4, b) 5–14, c) 15–29, d) 30–64, and e) ≥ 65 years.

***Note*: Fig. 3 continued on next page.**



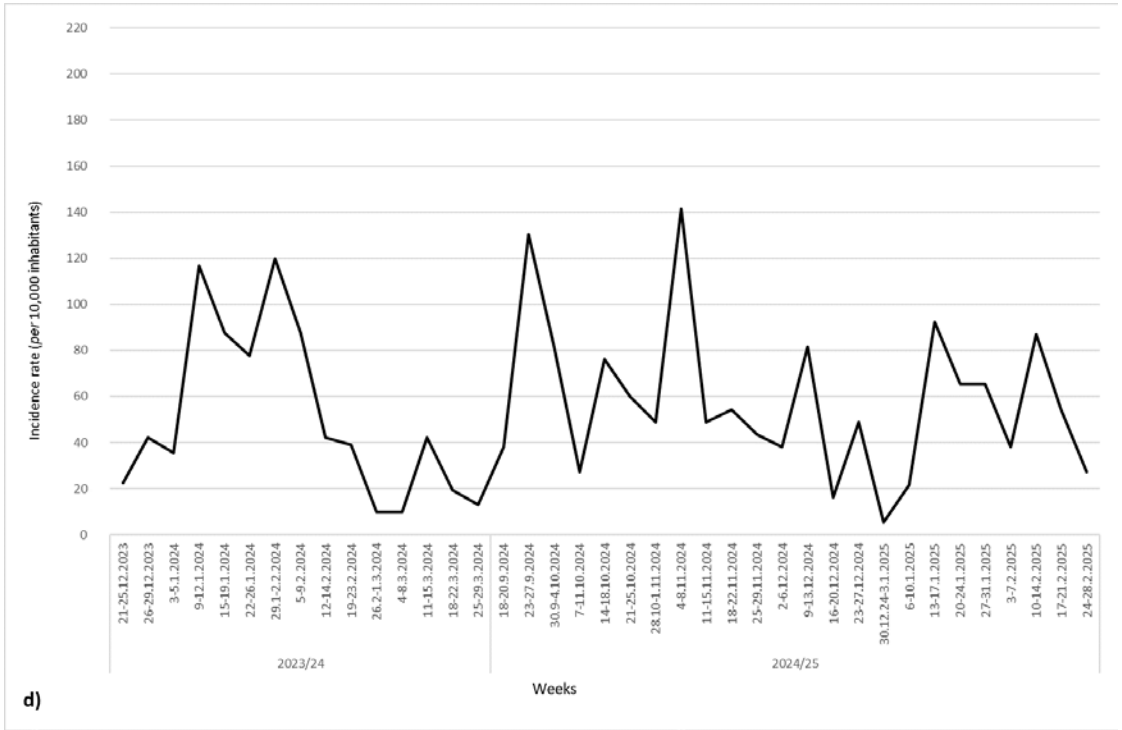


Fig. 3 (Continued) – Incidence rates of acute respiratory infections *per* 10,000 inhabitants, stratified by surveillance week, for two consecutive seasons, and age: a) 0–4, b) 5–14, c) 15–29, d) 30–64, and e) ≥ 65 years.

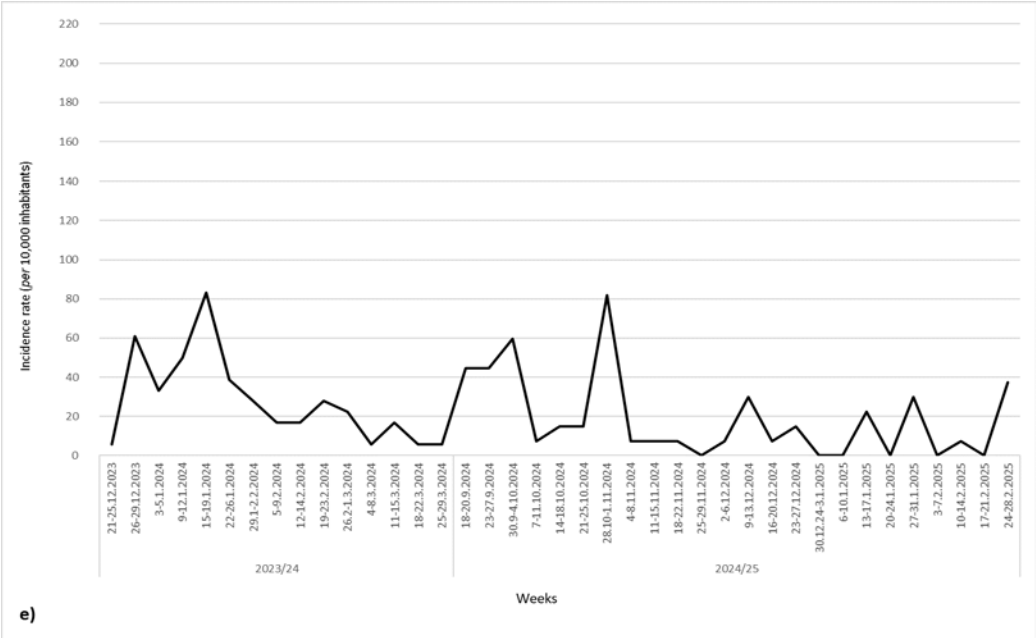


Fig. 3 (Continued) – Incidence rates of acute respiratory infections *per* 10,000 inhabitants, stratified by surveillance week, for two consecutive seasons, and age: a) 0–4, b) 5–14, c) 15–29, d) 30–64, and e) ≥ 65 years.

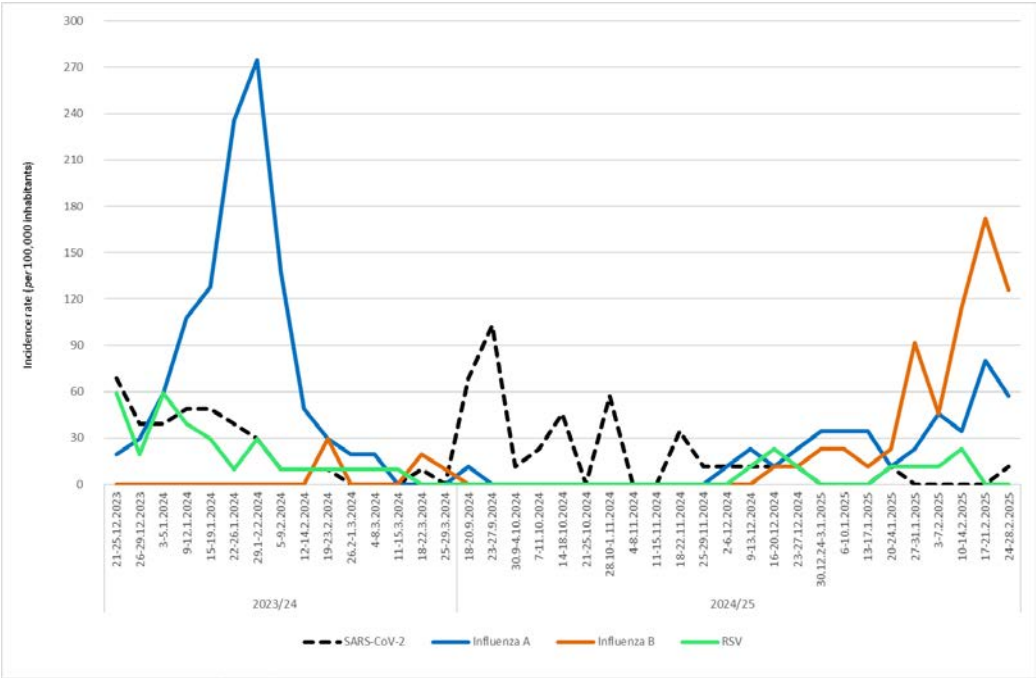


Fig. 4 – Weekly incidence rates of respiratory viruses (SARS-CoV-2, influenza A, influenza B, and RSV) during the two seasons. For abbreviations, see Table 2.

During the 2023/24 season, influenza A was the predominant virus, with a sharp increase in incidence observed from mid-January 2024, peaking in early February at approximately 270 cases *per* 100,000 inhabitants. Following this peak, the incidence of influenza A declined rapidly, reaching minimal levels by March 2024. Influenza B, SARS-CoV-2, and RSV exhibited relatively low activity during the same period, with sporadic detections and no significant peaks. In the 2024/25 season, influenza B showed a gradual increase in incidence starting from January 2025, peaking in late February with rates exceeding 170 cases *per* 100,000 inhabitants. Influenza A activity also rose during this period, though to a lesser extent compared to the previous season. SARS-CoV-2 exhibited moderate fluctuations throughout the season, with a notable peak (103.1 cases *per* 100,000 inhabitants) in late September 2024, while RSV activity remained consistently low, without any marked surges (Figure 4).

Figure 5a depicts the incidence rates of SARS-CoV-2 *per* 100,000 inhabitants during the 2023/24 and 2024/25 seasons. The data show fluctuations in incidence rates, with distinct peaks and variations across age groups. During the 2023/24 season, incidence rates were relatively low initially but increased in late January and early February 2024, particularly among young children (0–4 years) and the elderly (≥ 65 years). A sharp peak occurred in mid-February, predominantly among the elderly, followed by a decline. In the 2024/25 season, a resurgence occurred in late September, mostly affecting the youngest age group, followed by a peak in October 2024 among the 15–29 and ≥ 65 age groups. Sporadic increases were observed in the subsequent weeks, accompanied by minor fluctuations across age groups. A pronounced peak in influenza A incidence was recorded during the 2023/24 season, with the highest rates observed between late December 2023 and mid-February 2024. Children aged 0–4 and 5–14 years were the most affected groups, while incidence rates among individuals aged ≥ 65 years remained relatively low throughout this season. Following this peak, incidence rates declined sharply, with only sporadic detections in the later weeks of the season. During the 2024/25 season, in-

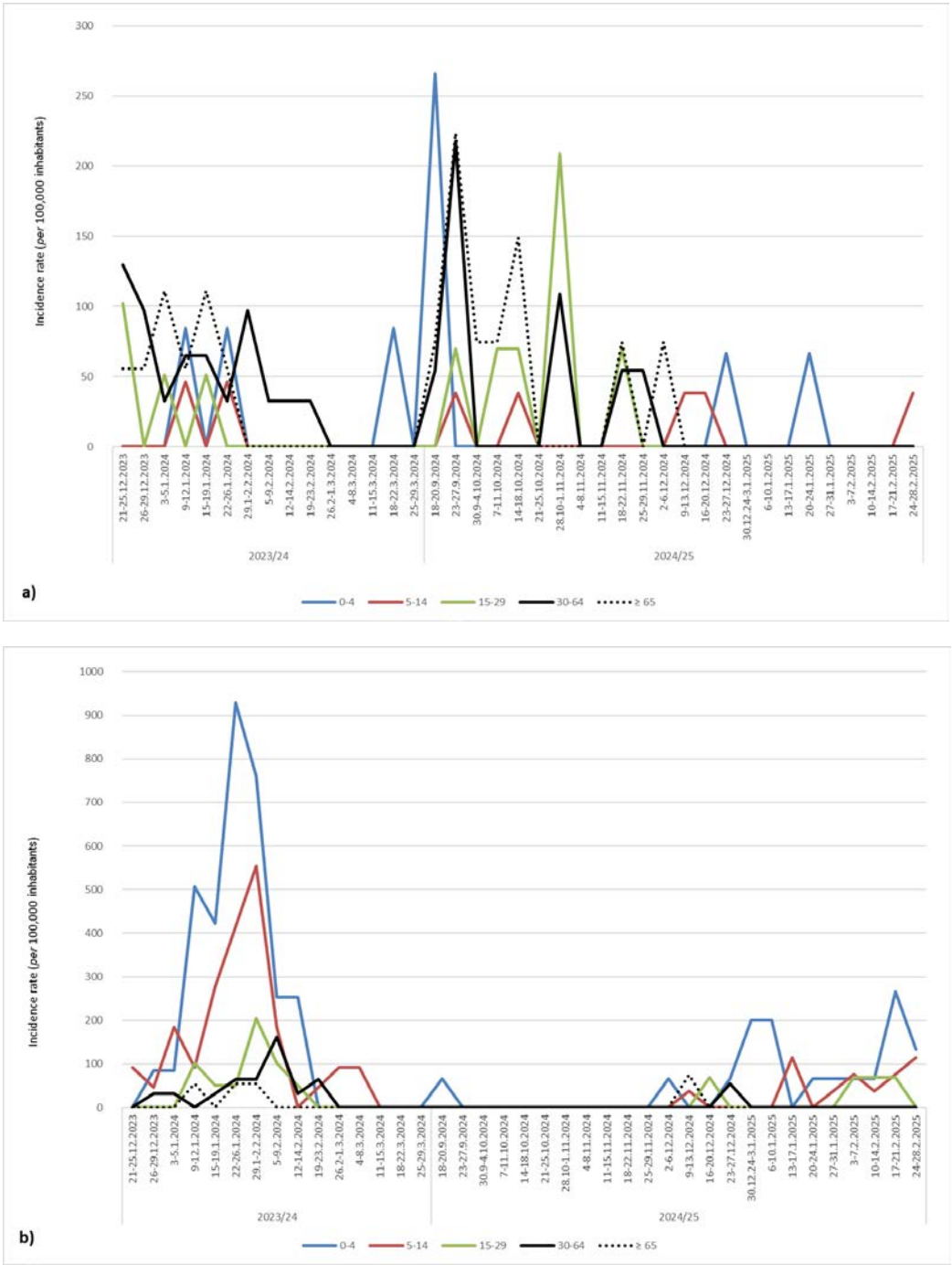


Fig. 5 – Incidence rates of: a) SARS-CoV-2, b) influenza A, c) influenza B, and d) RSV across age groups and surveillance week for two consecutive seasons. For abbreviations, see Table 2.

***Note*: Fig. 5 continued on next page.**

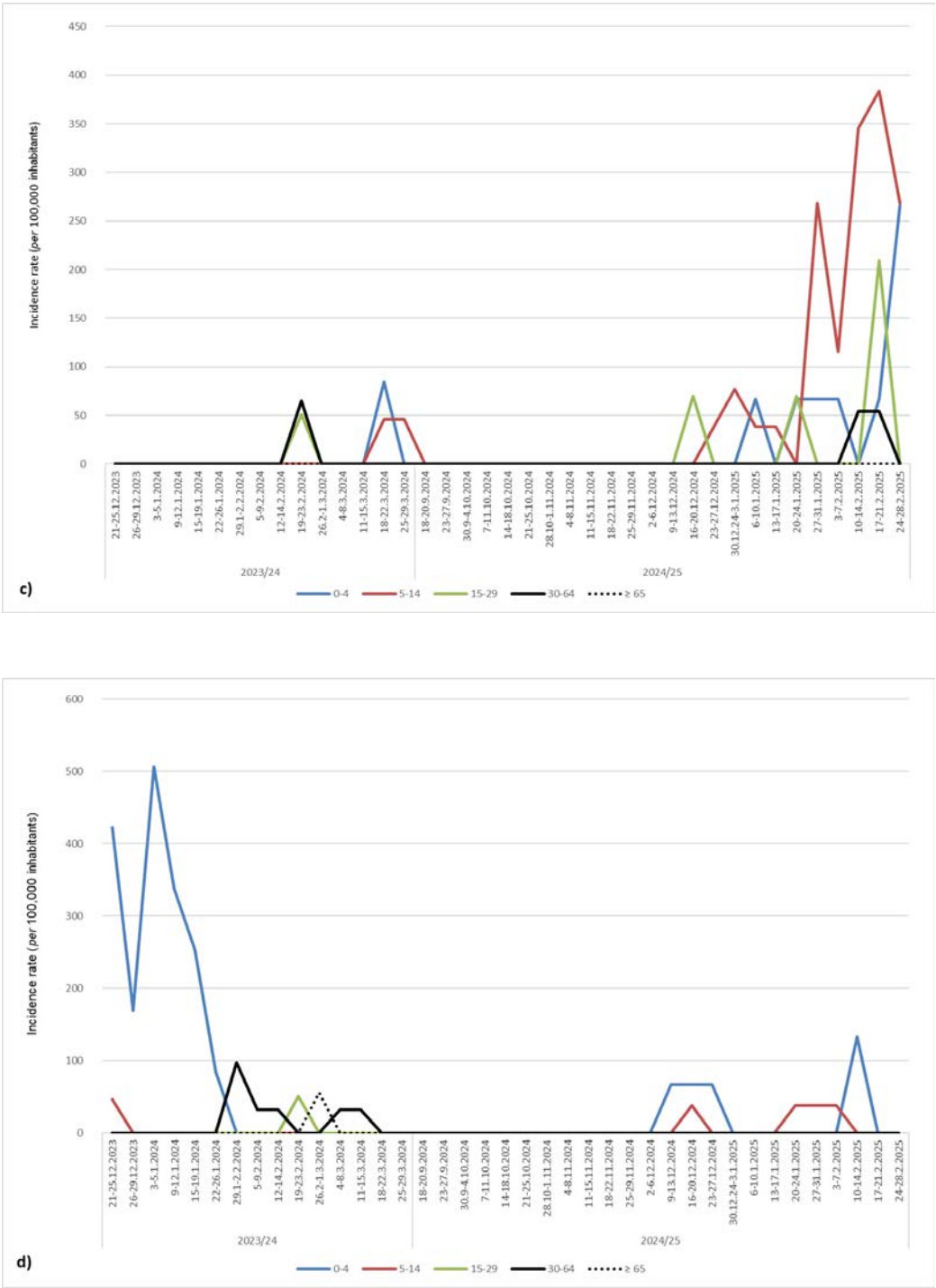


Fig. 5 (Continued) – Incidence rates of: a) SARS-CoV-2, b) influenza A, c) influenza B, and d) RSV across age groups and surveillance week for two consecutive seasons. For abbreviations, see Table 2.

fluenza A activity was markedly lower, with only minor increases observed in late December 2024 and early January 2025. Cases were detected across all age groups but with lower levels of incidence than in the previous season (Figure 5b). Figure 5c illustrates influenza B incidence rates across different age groups during the 2023/24 and 2024/25 seasons. Influenza B activity across age groups was minimal during the 2023/24 season, with only sporadic detections. In contrast, a significant increase in influenza B incidence was observed in the 2024/25 season, with a notable rise beginning in late December 2024, initially among individuals aged 15–29 years. The incidence peaked in February 2025, with the highest rates recorded among school-aged children (5–14 years), followed by young adults (15–29 years) and the youngest age group (0–4 years). Moderate incidence rates were reported among middle-aged adults (30–64 years), whereas the elderly population (≥ 65 years) exhibited the lowest incidence. During the 2023/24 season, the highest burden of RSV was observed in

the 0–4-year age group, with a peak in December 2023 and early January 2024, followed by a sharp decline. Incidence rates in other age groups were significantly lower, with only sporadic detections throughout the season. In the 2024/25 season, RSV activity remained low across all age groups, with low incidence rates primarily observed among children aged 0–4 and 5–14 years. No substantial RSV activity was detected in older age groups (≥ 15 years) (Figure 5d).

In the 2023/24 season, gender distribution did not differ significantly among the groups ($p = 0.4789$). However, a significant difference was observed in age distribution ($p < 0.0001$). RSV infections were predominantly observed in children aged 0–4 years (67.74%), whereas influenza A was most frequently detected in the 5–14-year age group (38.94%). SARS-CoV-2 infections were most prevalent among adults aged 30–64 years (52.78%) and those aged ≥ 65 years (25%). The highest mean age was recorded in SARS-CoV-2 patients (44.56 ± 23.14 years), while the lowest mean age was observed in patients with influenza B infection (9.17 ± 12.12 years) cases ($p < 0.0001$). The distribution of infections varied significantly by month of testing ($p < 0.0001$). SARS-CoV-2 cases peaked in January 2024 (50%), similar to influenza A (66.37%) and RSV (45.16%). Notably, 66.67% of influenza B cases were recorded in March 2024, whereas no SARS-CoV-2 cases were identified during that month. The median time from symptom onset to testing was comparable across the groups, ranging from 2 to 3 days, with no statistically significant difference ($p = 0.0810$). Fever (≥ 38 °C) was the most common symptom across all groups, with the highest prevalence in influenza A and influenza B cases (100.00% each). Fever was significantly less frequent in SARS-CoV-2 cases (80.56%) ($p = 0.0289$). Cough was prevalent across all infections, particularly in RSV patients (96.77%). Headache was most frequently reported in influenza B (83.33%) and SARS-CoV-2 (66.67%) cases, while it was rare in RSV infections (19.35%). Malaise and myalgia were most common in influenza cases, whereas sore throat was present in approximately half of the SARS-CoV-2 and influenza B cases. Loss of appetite was most frequently reported in RSV patients (58.06%) and was less common in other groups ($p < 0.05$) (Table 3).

Table 3

Demographic and clinical characteristics of patients with laboratory-confirmed SARS-CoV-2, influenza A, influenza B, and RSV infections during the 2023/24 season

Parameters	SARS-CoV-2 (n = 36)	Influenza A (n = 113)	Influenza B (n = 6)	RSV (n = 31)	*p*-value
	n (%)	n (%)	n (%)	n (%)	
Gender					
male	18 (50.00)	46 (40.71)	4 (66.67)	15 (48.39)	0.4789
female	18 (50.00)	67 (59.29)	2 (33.33)	16 (51.61)	
Age, years					
0–4	2 (5.56)	34 (30.09)	3 (50.00)	21 (67.74)	
5–14	2 (5.56)	44 (38.94)	2 (33.33)	1 (3.23)	
15–29	4 (11.11)	11 (9.73)	0 (0.00)	1 (3.23)	**< 0.0001**
30–64	19 (52.78)	15 (13.27)	1 (16.67)	7 (22.58)	
≥ 65	9 (25.00)	9 (7.96)	0 (0.00)	1 (3.23)	
mean ± standard deviation	44.56 ± 23.14	18.54 ± 23.68	9.17 ± 12.12	16.19 ± 23.85	**< 0.0001****
median (interquartile range)	43 (31–64)	9 (3–15)	5 (2–14)	2 (1–34)	
Month of testing					
December 2023	13 (36.11)	5 (4.42)	0 (0.00)	9 (29.03)	
January 2024	18 (50.00)	75 (66.37)	1 (16.67)	14 (45.16)	**< 0.0001**
February 2024	5 (13.89)	31 (27.43)	1 (16.67)	6 (19.35)	
March 2024	0 (0.00)	2 (1.77)	4 (66.67)	2 (6.45)	
Period of symptom onset to testing					
mean ± standard deviation	2.31 ± 1.05	2.61 ± 1.16	3.33 ± 1.70	2.90 ± 1.06	0.0810**
median time (interquartile range)	2 (2–3)	2 (2–4)	3 (2–5)	3 (2–4)	
Symptoms and signs*					
fever (≥ 38 °C)	29 (80.56)	113 (100.00)	6 (100.00)	27 (87.10)	
cough	27 (75.00)	99 (87.61)	4 (66.67)	30 (96.77)	
headache	24 (66.67)	64 (56.64)	5 (83.33)	6 (19.35)	
malaise	14 (38.89)	53 (46.90)	3 (50.00)	4 (12.90)	**0.0289**
myalgia	11 (30.56)	33 (29.20)	2 (33.33)	4 (12.90)	
sore throat	19 (52.78)	46 (40.71)	3 (50.00)	9 (29.03)	
loss of appetite	12 (33.33)	35 (30.97)	0 (0.00)	18 (58.06)	

n – number. For other abbreviations, see Table 2.

***Note*: *one patient could have one or more symptoms and signs; **ANOVA analysis of variance; values that differ significantly ($p < 0.05$) are marked in bold.**

In the 2024/25 season, gender distribution did not differ significantly among the groups ($p = 0.5098$). However, a statistically significant difference was observed in age distribution ($p < 0.0001$). RSV and influenza A infections were most prevalent among children aged 0–4 years (55.56% and 47.37%, respectively), whereas influenza B was predominantly detected in the 5–14-year age group (71.93%). SARS-CoV-2 infections primarily affected older individuals, with 27.03% of cases occurring in patients aged ≥ 65 years and 24.32% in those aged 30–64 years. The highest mean age was recorded among SARS-CoV-2 patients (37.97 ± 29.59 years), while the lowest was observed in RSV cases (4.11 ± 4.04 years) ($p < 0.0001$). The distribution of infections varied significantly by month of testing ($p < 0.0001$). SARS-CoV-2 cases were primarily detected in September (43.24%) and October 2024 (24.32%), whereas the majority of influenza A (50.00%) and influenza B (68.42%) cases were registered in February 2025. RSV cases were most frequently recorded in December 2024 (44.44%) and February 2025 (33.33%). The median time from symptom onset to testing was similar across all groups, ranging from 2 to 3 days, with no statistically significant difference ($p = 0.3650$). Analysis of clinical symptoms revealed that fever (≥ 38 °C) was highly prevalent in all groups, occurring in 100.00% of influenza A, influenza B, and RSV cases, and in 75.68% of SARS-CoV-2 cases, without statistically significant differences ($p = 0.2550$). Cough was most frequently reported in patients with influenza B (80.70%) and influenza A (78.95%). Headache was most common in SARS-CoV-2 (62.16%) and influenza B (59.65%) cases, but was absent in RSV infections. Malaise and myalgia were reported across all groups, with the highest frequency observed in SARS-CoV-2 and influenza B cases. Sore throat was present in approximately one-third of SARS-CoV-2 cases (37.84%) and 40.35% of influenza B cases, while it was rare in RSV infections (11.11%). Loss of appetite was most frequently reported in influenza A (55.26%) and influenza B (49.12%) cases, compared to 24.32% in SARS-CoV-2 and 33.33% in RSV cases (Table 4).

Discussion

To our knowledge, this is the first study using rapid combination tests for SARS-CoV-2, influenza A and B, and RSV in ARI sentinel surveillance in our country. The findings reveal seasonal patterns, demographic variations, and clinical characteristics over two consecutive seasons. Despite differences in surveillance duration (15 weeks in 2023/24 vs. 24 weeks in 2024/25), the latter showed a slower onset but a sharper rise later.

Table 4

Demographic and clinical characteristics of patients with laboratory-confirmed SARS-CoV-2, influenza A, influenza B, and RSV infections during the 2024/25 season

Parameters	SARS-CoV-2 (n = 37)	Influenza A (n = 38)	Influenza B (n = 57)	RSV (n = 9)	*p*-value
	n (%)	n (%)	n (%)	n (%)	
Gender					
male	14 (37.84)	17 (44.74)	30 (52.63)	5 (55.56)	0.5098
female	23 (62.16)	21 (55.26)	27 (47.37)	4 (44.44)	
Age, years					
0–4	6 (16.22)	18 (47.37)	9 (15.79)	5 (55.56)	
5–14	5 (13.51)	14 (36.84)	41 (71.93)	4 (44.44)	
15–29	7 (18.92)	3 (7.89)	5 (8.77)	0 (0.00)	**< 0.0001**
30–64	9 (24.32)	2 (5.26)	2 (3.51)	0 (0.00)	
≥ 65	10 (27.03)	1 (2.63)	0 (0.00)	0 (0.00)	
mean ± standard deviation	37.97 ± 29.59	10.79 ± 16.19	8.89 ± 6.54	4.11 ± 4.04	**< 0.0001****
median age (interquartile range)	30 (14–69)	5 (3–10)	7 (6–10)	2 (1–7)	
Month of testing					
September 2024	16 (43.24)	0 (0.00)	0 (0.00)	0 (0.00)	
October 2024	9 (24.32)	0 (0.00)	0 (0.00)	0 (0.00)	
November 2024	7 (18.92)	1 (2.63)	0 (0.00)	0 (0.00)	**< 0.0001**
December 2024	3 (8.11)	6 (15.79)	4 (7.02)	4 (44.44)	
January 2025	1 (2.70)	12 (31.58)	14 (24.56)	2 (22.22)	
February 2025	1 (2.70)	19 (50.00)	39 (68.42)	3 (33.33)	
Period of symptom onset to testing					
mean ± standard deviation	2.22 ± 0.93	2.16 ±1.31	2.53 (±1.11)	2.44 ± 0.68	0.3650**
median (interquartile range)	2 (2–3)	2 (1–3)	2 (2–3)	3 (2–3)	
Symptoms and signs*					
fever (≥ 38 °c)	28 (75.68)	38 (100.00)	57 (100.00)	9 (100.00)	
cough	20 (54.05)	30 (78.95)	46 (80.70)	7 (77.78)	
headache	23 (62.16)	16 (42.11)	34 (59.65)	0 (0.00)	
malaise	18 (48.65)	11 (28.95)	21 (36.84)	1 (11.11)	0.2550
myalgia	12 (32.43)	9 (23.68)	21 (36.84)	1 (11.11)	
sore throat	14 (37.84)	10 (26.32)	23 (40.35)	1 (11.11)	
loss of appetite	9 (24.32)	21 (55.26)	28 (49.12)	3 (33.33)	

n – number. For other abbreviations, see Table 2.

***Note:* *one patient could have one or more symptoms and signs; **ANOVA analysis of variance; values that differ significantly ($p < 0.05$) are marked in bold.**

The highest ARI incidence in the 2023/24 season (105 *per* 10,000 inhabitants) was recorded in late January, coinciding with school reopenings and cold weather. In contrast, the 2024/25 peak incidence (76.8 *per* 10,000 inhabitants) occurred in mid-to-late January [13], with a lower rate possibly influenced by school closures during public protests in Serbia. In the 2023/24 season, 73.43% of tested samples were negative, increasing to 85.76% in 2024/25. This suggests many ARI cases were unrelated to the tested viruses, possibly due to test limitations, other viral or bacterial pathogens, low viral loads, non-infectious conditions, or preexisting immunity [1, 2, 8, 10, 12]. The widespread circulation of SARS-CoV-2 significantly impacted the epidemiology of respiratory viruses [8–10, 14]. A study across 27 countries found SARS-CoV-2 in over 70% of hospitalized patients with viral infections between 2020 and 2022 [9]. Its dominance may be due to a higher basic reproduction number than influenza and RSV. While public health measures implemented during the COVID-19 pandemic reduced influenza and RSV transmission, they were less effective against the spread of SARS-CoV-2 [15, 16]. Rhinoviruses and enteroviruses persisted despite interventions like masking and school closures, likely due to their greater surface stability and different transmission routes [17, 18]. These viruses accounted for about 75% of viral detections in pediatric healthcare visits during the first year of the pandemic [17].

During the pandemic, SARS-CoV-2 dominated, with other respiratory viruses detected sporadically due to the implementation of widespread public health control measures [5, 14, 19]. Our findings indicate a shift in viral dominance during the 2023/24 and 2024/25 seasons. Influenza A, the most common virus in 2023/24 (60.75%), declined to 26.95% in 2024/25, while influenza B rose from 3.23% to 40.43%, suggesting a strain shift. SARS-CoV-2 remained stable, increasing slightly from 19.35% to 26.24%. RSV decreased from 16.67% to 6.38%, possibly due to seasonal dynamics or population immunity. These trends align with the resurgence of pre-pandemic influenza and RSV patterns in the European Union [20, 21]. Our findings emphasize seasonal variability in respiratory viruses and the need for ongoing surveillance. The earlier circulation of SARS-CoV-2 in 2024/25 and shifting influenza strains highlight the importance of timely public health interventions, including vaccination and preparedness measures [2, 4, 5, 7–10].

As the sentinel surveillance mainly covered preschool and school-aged children, ARIs disproportionately affected those aged 0–4 years, who had the highest incidence rates. RSV was most frequently detected in this group in both seasons. During the 2023/24 season, predominant influenza A suppressed other respiratory viruses. In 2024/25, SARS-CoV-2 initially dominated but was later overtaken by influenza. Throughout the study, influenza had a higher weekly incidence than SARS-CoV-2 and RSV, whose trends were similar. A study of 25 European countries found a similar pattern, with influenza peaking separately from SARS-CoV-2 and RSV [22].

In the 2023/24 season, influenza A was most common in preschool- and school-aged children (0–4 and 5–14 years), while influenza B was more frequent in children aged 0–4 years. The following season saw an inverse pattern. SARS-CoV-2 affected all age groups, especially adults (30–64 years) and the elderly, with a higher frequency in 2024/25. Similar age distribution trends have been observed in previous studies [5, 23, 24]. Once again, the distribution of respiratory viruses aligns with the restoration of pre-pandemic epidemiological patterns after the relaxation of COVID-19 preventive measures [2, 5, 7, 16, 18, 23].

The clinical presentations of ARIs showed significant overlap, with fever, cough, and headache as the most common symptoms. Fever was present in all cases of influenza A, influenza B, and RSV, but less so in SARS-CoV-2 infections. Similar patterns have been observed in other studies, where influenza and RSV were more strongly associated with fever than SARS-CoV-2 [1, 2, 8]. RSV infections also had lower frequencies of headache and malaise, but a higher prevalence of loss of appetite [17, 25], highlighting distinct clinical profiles.

The median time from symptom onset to testing was 2 to 3 days for all four viruses in both seasons, indicating consistent diagnostic practices. This highlights the importance of timely detection for effective clinical management and infection control. Patients with symptoms of any of the four viruses seek medical care at the primary healthcare level around the same time.

The shift in viral dominance and age-specific incidence trends highlights the need for targeted prevention, especially for young children. Enhanced RSV surveillance should be considered in both primary healthcare and hospital settings. Differentiating these viruses remains challenging due to overlapping symptoms, but multiplex molecular testing could aid early diagnosis, optimize antiviral use, and reduce unnecessary antibiotics. Seasonal variations emphasize the need for rapid, adaptive public health interventions, including timely vaccination and non-pharmaceutical measures to ease healthcare system burdens.

Our study had several limitations. First, it was conducted in a specific healthcare setting, limiting generalizability. ARI incidence rates were based on sentinel surveillance, which may miss mild or asymptomatic cases, leading to an underestimation of the true disease burden. The sample also had a lower participation rate among those aged 15 and above, especially in the 30–64 and ≥ 65 age groups. Future studies should include more sentinel physicians to better represent the age distribution. Second, we did not perform PCR testing to confirm rapid test results, and the sensitivity of the CerTest Rapid Test varied across pathogens, with lower sensitivity for influenza A and B, increasing the likelihood of false negatives. The test's performance for RSV also showed wide confidence intervals, suggesting variability in its accuracy. PCR remains essential for confirmation. Additionally, we lacked data on the test's limit of detection, which impacts its ability to detect low viral loads. Third, the study did not analyze viral co-detection, which may affect disease severity and transmission. Fourth, conducted over two seasons, the study offers short-term insights but limits conclusions on long-term trends. Finally, we did not assess vaccination status, which could influence infection risk and severity. Future

research should include vaccination data for a more comprehensive understanding. Despite the limitations, our study shows that ARI incidence of common respiratory viruses during the autumn-winter period can be effectively assessed with minimal burden on physicians and resources in the sentinel surveillance system.

Conclusion

Our data show a shift in viral dominance between the seasons, with influenza A prevailing in 2023/24 and influenza B becoming more prominent in 2024/25. SARS-CoV-2 circulated at low levels, while RSV had minimal impact. Despite using a limited set of indicators, our findings can be cautiously compared to broader research on viral pathogens. These results emphasize the need for ongoing surveillance, timely diagnostics, and targeted prevention strategies. Future research should explore the long-term effects of shifting viral patterns and the integration of rapid tests for multiple viruses in surveillance.

Acknowledgement

The authors would like to express their gratitude to all study participants for their involvement in this research, especially to Dušan Carević and Duško Pašić for their contributions.

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Received on April 2, 2025
Accepted on June 11, 2025
Online First July 2025

ORIGINAL ARTICLE

UDC: 355/359:61]:[355.23-057.875:159.947.5
DOI: https://doi.org/10.2298/VSP250402048P

What shapes cadets' decisions? Factors influencing the choice of the Faculty of Medicine of the Military Medical Academy

Šta oblikuje odluke kadeta? Faktori koji utiču na izbor Medicinskog fakulteta Vojnomedicinske akademije

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Abstract

Background/Aim. To create an effective promotional strategy for the Medical Faculty of the Military Medical Academy (MF MMA), it is essential to understand why young people decide to enroll in this military higher education institution (MHEI). The aim of this study was to identify the key factors and examine their influence on cadets' decisions to enroll in MF MMA. **Methods.** A cross-sectional study was conducted during the second semester of the 2023/2024 academic year with 121 participants (cadets and candidates for enrollment at the MF MMA). A qualitative phase, aimed at defining the research instrument, was implemented using the focus group method. Data collection was performed using an online questionnaire. Analysis was conducted using multiple linear regression. **Results.** The factors that have the greatest influence on cadets' decisions to enroll in the MF MMA are: "cultural capital" ($\beta = 0.260$; $p < 0.01$), "physical culture" ($\beta = 0.210$; $p < 0.05$), "quality of military medical education" ($\beta = 0.191$; $p < 0.05$), "career" ($\beta = 0.176$; $p < 0.05$), and "status" ($\beta = 0.171$; $p < 0.05$). **Conclusion.** The results indicate that cadets' decision to choose MF MMA is influenced by the combination of personal and institutional factors of a predominantly social nature. Candidates applying for enrollment at the MF MMA have strong personal affinities towards the profession of an army officer/military doctor, highly value the culture of the military organization, appreciate the educational offer of the MHEI, and recognize opportunities for professional and career development. These findings may contribute to defining the promotional strategy of MF MMA and ensure that the institution maintains a stable influx of high-quality and motivated candidates for education.

Key words:
education, medical; military medicine; serbia; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Da bi se kreirala efektivna promotivna strategija Medicinskog fakulteta Vojnomedicinske akademije (MF VMA), neophodno je razumeti razloge zbog kojih se mladi opredeljuju da upišu ovu vojnu visokoškolsku ustanovu (VVŠU). Cilj rada bio je da se utvrde ključni faktori i ispita njihov uticaj na odluku kadeta da upišu MF VMA. **Metode.** Sprovedena je studija preseka tokom drugog semestra školske 2023/2024. godine sa 121 ispitanikom (kadeti i kandidati za upis na MF VMA). Kvalitativna faza sa ciljem definisanja istraživačkog instrumenta realizovana je metodom fokus grupe. Prikupljanje podataka realizovano je putem *online* upitnika. Analiza je vršena primenom višestruke linearne regresije. **Rezultati.** Najveći uticaj na odluku kadeta da upišu MF VMA imaju faktori: „kulturni kapital" ($\beta = 0{,}260$; $p < 0{,}01$), „fizička kultura" ($\beta = 0{,}210$; $p < 0{,}05$), „kvalitet vojnog medicinskog obrazovanja" ($\beta = 0{,}191$; $p < 0{,}05$), „karijera" ($\beta = 0{,}176$; $p < 0{,}05$) i „status" ($\beta = 0{,}171$; $p < 0{,}05$). **Zaključak.** Rezultati ukazuju da na izbor MF VMA od strane kadeta utiče kombinacija ličnih i institucionalnih faktora pretežno socijalne prirode. Kandidati koji konkurišu za upis na MF VMA imaju naglašene lične afinitete prema profesiji oficira/vojnog lekara, visoko vrednuju kulturu vojne organizacije, cene obrazovnu ponudu VVŠU, i prepoznaju prilike za stručni i karijerni razvoj. Ova saznanja mogu doprineti definisanju promotivne strategije MF VMA i obezbediti da ta institucija ima stabilan priliv kvalitetnih i motivisanih kandidata za školovanje.

Ključne reči:
edukacija, medicinska; medicina, vojna; srbija; ankete i upitnici.

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Introduction

The marketing orientation of higher education institutions (HEIs) over the past few decades has become a key determinant of their institutional development in the context of increasing competition among universities and faculties [1, 2]. This trend has led to the fact that, despite their specific mission and role in the system of defence and the significant restrictions in the aspect of adopting marketing models of development, military HEIs (MHEIs) in the Republic of Serbia have to compete with their civilian counterparts for future students at the single market of higher education [3]. The Medical Faculty (MF) of the Military Medical Academy (MMA) – MF MMA educates future officers/military doctors, for the needs of the Ministry of Defence and the Serbian Armed Forces (SAF) through integrated academic medical studies and has an interest in ensuring a sufficient number of high-quality and motivated candidates for enrollment year by year.

Without understanding the factors and models that explain what influences potential candidates to enroll in a HEI, universities cannot predict the right manner to increase their efficiency in attracting new students, or they can overestimate the impact of what is done in that field [4]. Therefore, one of the key assumptions for creating an efficient promotional strategy for MHEIs in the Republic of Serbia – The University of Defense (UoD), the Military Academy (MA), and MF MMA – is to research the sets of influential factors which are crucial in cadets' decision to apply for them.

Although the influencing factors and models of HEI selection have been intensively studied since the mid-1970s, only one recent study can be found in major academic repositories that specifically addresses the factors influencing the selection of MHEIs, specifically the choice of MA in Turkey [5]. In contrast, research on the factors that influence the selection of medical faculty has not been published so far. The only study related to the selection factors of civilian HEIs in Serbia was published in 2020 [6]. This gap in the scientific literature indicates the need for a deeper understanding of the motivation and expectations of future students in the military educational context.

However, in most research, it is possible to establish certain regularities regarding the groups of key factors that determine the selection of a HEI. Firstly, researchers often suggest factors that directly relate to the student or candidate, as well as their immediate environment – the family. This group of influential factors can include: a) socioeconomic status, which includes income and ownership of movable and immovable property, parental professions and education, as well as educational aspirations and expectations that families and individuals have regarding education [7–9]; b) success in previous education [10]; c) career preferences [5, 11]; d) personal and family values [5, 12, 13]; e) the influence of close people – parents, relatives, and friends [5, 14, 15]; f) gender [16–18]. The second major group of influential factors, encompassing the institutional prerogatives of HEIs, is particularly significant from a marketing perspective, as these factors can be leveraged to influence the desired outcome of the selection process [19]. It often includes the following: a) institutional (academic) reputation [6, 20]; b) employment opportunities after the studies [6, 20]; c) career prospects [21]; d) expected salary (after graduation) [6, 22]; e) the quality of professors [11, 23]; f) infrastructure (facilities for accommodation, classes, sports, and extracurricular activities) [11, 20, 23]; g) complexity (difficulty) of enrollment [6]. The third group includes those factors that simultaneously relate to (future) students and the HEI and are referred to in the literature as interactional [7]: a) tuition costs [6, 24]; b) financial support (scholarships) [9]; c) location – geographical distance [6, 9, 25]; d) information and sources of information about the HEI [7, 21, 26].

Considerations and interpretations of the factors influencing higher education choices are primarily conceptualized within two model groups: economic models of human capital investment and economic models of status acquisition [27]. Economic or econometric models of HEI choice represent a theoretical framework that provides insight into how prospective students decide which HEI to attend based on economic parameters. Social models emphasize the importance of social connections, family environment, peer influence, and cultural norms in shaping educational aspirations and the choice of tertiary education institutions.

Hemsley-Brown and Oplatka [7] state that, despite their efforts, researchers have not yet succeeded in compiling a single list of factors that influence all potential students or in providing a definitive explanation for why students choose a particular university. Research findings in this field vary depending on the time period during which the study was conducted, the country or region, and the broader social, economic, political, and cultural context, as well as the characteristics of the population or sample and the applied methodology.

The aim of this study was to identify and analyze the key factors influencing the choice of MF MMA by relying on a review of existing literature and the results of research conducted with cadets of this MHEI.

Methods

A total of 121 respondents participated in the survey, out of which 112 were cadets from the first to the fourth year of study at MF MMA, Belgrade, Serbia (28 were in the first year, 24 in the second, 31 in the third, and 29 in the the fourth year of studies), which is more than two-thirds of the total number of cadets. There were also nine candidates for enrollment in MF MMA. Regarding gender, 31 respondents (25.6%) were male and 90 (74.4%) female. The gender structure of the sample corresponds to the gender structure of all cadets in the MF MMA. Considering the above facts, it may be concluded that the sample was adequate.

This research was conducted with the consent of the Rector of UoD and the Commander of MA during the second semester of the 2023/2024 school year.

The research was conducted in two phases. The first phase of qualitative research was conducted to develop a questionnaire for the subsequent quantitative empirical

research. To obtain a representative sample, 12 cadets from the first to the fourth year of studies at MF MMA (four males and eight females, equally distributed by year of study) participated in the qualitative research conducted using the focus group method [28]. The selection process considered gender parity and equal distribution across study years. During the session, cadets were encouraged to articulate their key reasons and motivations for enrolling in the MF MMA. In the first round, each participant shared one or two main reasons, followed by a moderated discussion to deepen insights and stimulate interaction. The discussion was guided using open-ended prompts focused on motivational and decision-making factors related to choosing a military medical education. The researcher diligently recorded the answers and attitudes expressed during the focus group. Content analysis was conducted by grouping statements based on frequency and similarity, which formed the basis for constructing the questionnaire items. This process ensured that the constructs reflected authentic cadet experiences and aligned with themes emerging from the qualitative data.

After processing and analyzing the data acquired using the focus group, based on the quality and frequency of given responses, a structured questionnaire was developed with a total of 39 questions (Appendix) – statements related to the reasons and motives for enrolling in MF MMA (34 statements) and the satisfaction with the decision to enroll (5 statements). The latter represents the outcome of the future model. All of the statements mentioned above were rated on a five-point Likert scale. The questionnaire also included questions regarding demographic data and information on socioeconomic status.

Based on a review of the scientific literature and the similarity of the responses given in the qualitative phase of the research, the constructs – factors concerning the decision to enroll in MF MMA were tested. The following constructs were formulated: "reputation", "benefits", "cultural capital", "career", "personal development", "quality of military medical education", "status", "physical culture", "influence of close people", and "self-perception". The "decision" scale, as the outcome in the future model, was formulated alongside these factors, measuring cadets' willingness and determination to enroll in MF MMA. The comprehensibility of the questionnaire was checked, and minor corrections to the wording of the statements were made with a group of cadets chosen by the commanders of the Cadet Brigade (the unit that includes all cadets of the MA and the MF MMA from the first to the fourth year of studies) using a random sample method. The number of subitems in each subconstruct is given in Table 1, while the details regarding the subitems are available upon request.

The second phase of the research was conducted using an online questionnaire (Google Forms). The survey was voluntary and completely anonymous. The questionnaire was distributed to all cadets from the first to the fourth year of studies by the management of the MF MMA. The respondents were informed that the purpose of the survey was scientific research and that the confidentiality of the data was guaranteed.

Statistical analysis

The statistical analysis in this study was conducted using IBM SPSS Statistics, version 26. Both descriptive and inferential statistical methods were applied to examine the factors influencing cadets' decisions to enroll in the MF MMA. Descriptive statistics included measures of central tendency and dispersion, with mean and standard deviation used to summarize continuous variables, while minimum and maximum values were reported to indicate the range of responses. Frequencies and percentages were calculated for categorical variables. To assess the interrelationships between key constructs, Pearson's correlation coefficient was applied. The interpretation of correlation strength followed Cohen's guidelines (1988), with significance levels set at $p < 0.05$ and $p < 0.01$. Multiple linear regression analysis was performed to evaluate the predictive power of selected constructs, including "cultural capital", "physical culture", "quality of military medical education", "career", and "status" on cadets' enrollment decisions. The assessment of the regression model included the evaluation of standardized regression coefficients to determine the relative influence of predictors, the coefficient of determination (r^2) to quantify the explained

Table 1

Cronbach's alpha value for scales

Scales	n	Cronbach's alpha	Reliability assessment
Factor 1: Reputation	3	0.738	acceptable
Factor 2: Benefits	3	0.720	acceptable
Factor 3: Cultural capital	4	0.745	acceptable
Factor 4: Career	3	0.716	acceptable
Factor 5: Personal development	3	0.725	acceptable
Factor 6: Quality of military medical education	4	0.748	acceptable
Factor 7: Status	3	0.786	acceptable
Factor 8: Physical culture	3	0.762	acceptable
Factor 9: Influence of close people	5	0.756	acceptable
Factor 10: Self-perception	3	0.712	acceptable
Construct scale	34	0.870	good
Decision scale	5	0.765	acceptable
Full scale	39	0.929	excellent

n – number of questions.

variance, and the analysis of variance (ANOVA) test (F-statistic) to verify the overall significance of the model. Statistical significance for individual predictors was determined using a threshold of $p < 0.05$. The reliability of the measurement scales was assessed using Cronbach's alpha coefficient, with values above 0.70 deemed acceptable, above 0.80 considered good, and above 0.90 regarded as excellent. The assumptions of normality, homoscedasticity, and multicollinearity were examined to ensure the validity of statistical inferences. The applied methodology provided a robust framework for analyzing the factors influencing cadets' enrollment decisions, ensuring the reliability of the study findings.

Results

The reliability of the scales was verified using Cronbach's alpha coefficient, which was acceptable for the construct scales (α ranging from 0.712 to 0.786) and the "decision" scale ($\alpha = 0.765$), good for the construct scale ($\alpha = 0.870$), and excellent for the entire measurement scale ($\alpha = 0.929$), which confirms the required level of reliability and internal consistency of the scales (Table 1). Descriptive statistics of the constructs and decisions are presented in Table 2. The results of the Pearson correlation analysis, presented in Table 3, showed a moderate to strong correlation (r ranging from 0.347 to 0.677) and a highly significant correlation between the construct "decision" and all other constructs ($p < 0.01$). The inter-construct correlations are moderate and also highly significant in most cases.

The results of the multiple regression analysis (Table 4) indicate that the regression model is significant, as evidenced by a significant F-statistic in the ANOVA test ($p < 0.001$). The predictor set of constructs explains 63.4% of the variability of the dependent variable ($r^2 = 0.634$). The standardized regression coefficients β show that the strongest influence on the decision is exerted by the constructs: "cultural capital" ($\beta = 0.260$; $p < 0.01$), "physical culture" ($\beta = 0.210$; $p < 0.05$), "quality of military medical education" ($\beta = 0.191$; $p < 0.05$), "career" ($\beta = 0.176$; $p < 0.05$), and "status" ($\beta = 0.171$; $p < 0.05$). Other constructs with weak positive or negative influence on the "decision" variable do not have a statistically significant impact.

Table 2

Descriptive statistics of constructs/factors and decisions

Variable	Mean ± SD	Min–Max
Factor 1: Reputation	4.36 ± 0.67	2.33–5.00
Factor 2: Benefits	4.19 ± 0.60	2.33–5.00
Factor 3: Cultural capital	4.08 ± 0.81	1.00–5.00
Factor 4: Career	3.93 ± 0.81	1.67–5.00
Factor 5: Personal development	4.32 ± 0.66	2.00–5.00
Factor 6: Quality of military medical education	4.42 ± 0.62	2.25–5.00
Factor 7: Status	4.28 ± 0.79	1.00–5.00
Factor 8. Physical culture	4.20 ± 0.78	1.67–5.00
Factor 9: Influence of close people	3.64 ± 0.86	1.60–5.00
Factor 10: Self-perception	4.36 ± 0.64	2.33–5.00
Decision	4.23 ± 0.79	1.60–5.00

SD – standard deviation; min – minimum; max – maximum.

Table 3

Correlation matrix between the construct "decision" and all other constructs

Construct	Decision	1	2	3	4	5	6	7	8	9
Factor 1: Reputation	0.490**									
Factor 2: Benefits	0.363**	0.324**								
Factor 3: Cultural capital	0.677**	0.496**	0.217*							
Factor 4: Career	0.508**	0.246**	0.271**	0.500**						
Factor 5: Personal development	0.470**	0.394**	0.343**	0.433**	0.398**					
Factor 6: Quality of military medical education	0.627**	0.624**	0.376**	0.547**	0.315**	0.476**				
Factor 7: Status	0.584**	0.439**	0.290**	0.578**	0.311**	0.458**	0.505**			
Factor 8: Physical culture	0.647**	0.553**	0.380**	0.581**	0.379**	0.559**	0.671**	0.476**		
Factor 9: Influence of close people	0.347**	0.274**	0.217*	0.363**	0.271**	0.180*	0.439**	0.293**	0.394**	
Factor 10: Self-perception	0.550**	0.401**	0.133	0.580**	0.392**	0.341**	0.550**	0.633**	0.497**	0.225*

Pearson correlation analysis (r) was used.

***Note*: * Significance at the 0.05 level; **Significance at the 0.01 level.**

Table 4

The connection and influence of constructs/factors on the decision

Predictor variables	Non-standard coefficients	Standard coefficients (β)	*t*	*p*-value
Constant	-0.422	-	-0.907	0.367
Factor 1: Reputation	-0.008	-0.007	-0.091	0.928
Factor 2: Benefits	0.093	0.070	1.052	0.295
Factor 3: Cultural capital	0.254	0.260	2.974	**0.004**
Factor 4: Career	0.172	0.176	2.469	**0.015**
Factor 5: Personal development	-0.027	-0.023	-0.303	0.762
Factor 6: Quality of military medical education	0.242	0.191	2.027	**0.045**
Factor 7: Status	0.171	0.171	2.028	**0.045**
Factor 8: Physical culture	0.212	0.210	2.327	**0.022**
Factor 9: Influence of close people	-0.023	-0.025	-0.375	0.708
Factor 10: Self-perception	0.023	0.019	0.220	0.826

Bold values indicate significance level of $p < 0.01$ and $p < 0.05$.

***Note*: model statistics ($F = 19.067$; $p < 0.001$, $r^2 = 0.634$, adjusted $r^2 = 0.601$).**

Discussion

"Cultural capital" ($\beta = 0.260$; $p < 0.01$) turned out to be the most important predictor in the cadets' decision to enroll in MF MMA. When commenting on statements related to patriotism, readiness to defend the country, and inclination towards discipline and military culture, the respondents indicated that their personal beliefs, aligned with the values of the military organization, largely influenced their decision to enroll in the MF MMA. This result confirmed the previous research findings showing that the personal preferences and values passed on to the individual by the social environment in which they grow up significantly influence the choice of the prospect HEI, and thus the future profession [5, 12, 13, 27].

The second most influential factor, "physical culture" ($\beta = 0.210$; $p < 0.05$), has not yet been identified in the literature related to the choice of HEIs. However, this factor is specific to the MHEI-related study. It groups the statements reflecting the respondents' perception of the MF MMA as an MHEI where "one can develop the mind and body equally" highlighting personal physical development as a direct benefit of studying there. In addition, respondents evaluated the statement on the attractiveness of MF MMA for future cadets based on the availability of high-quality sports facilities. The mentioned factor can be related to findings from previous literature indicating that HEIs with high-quality infrastructure, including sports facilities, tend to be more appealing to future students [22].

The decision to enroll in MF MMA is significantly positively influenced by the perception of the "quality of military medical education" ($\beta = 0.191$; $p < 0.05$). Within this construct, the respondents evaluated statements regarding whether MF MMA truly educates its students (cadets) for their future profession, whether the curriculum comprises a unity of theory and practice, and whether modern teaching aids and methods are used. Additionally, they acknowledged that MHEIs have an advantage over their civilian counterparts because they provide additional specific skills (e.g., obtaining a driver's license, learning to ski, earning a foreign language certificate) and free educational resources (e.g., textbooks, access to the latest scientific and professional literature). This finding is consistent with previous reports highlighting that the "content of the study program" of HEI selection is particularly important for students who consider the quality and relevance of the curriculum in relation to their academic and professional goals [26]. Some studies indicate that a significant number of young people, when choosing a faculty, prioritize acquiring specific knowledge and skills for their future profession and favor these institutional factors over calculating potential costs and benefits [29]. The strong and very significant correlation between this factor and the factor "physical culture" ($r = 0.671$; $p < 0.01$) supports the conclusion that the study program of the MF MMA, enriched with content not present at other similar civilian faculties, gives an advantage when deciding on enrollment.

The influence of the construct "career" ($\beta = 0.176$; $p < 0.05$) explains respondents' attitude towards opportunities for a predictable career, professional development, education, and vertical advancement within the military organization. This result can be explained by the fact that the MMA, a renowned tertiary healthcare institution, is seen as an institution where young officers (doctors) can successfully specialize in their desired branches of medicine. The results mentioned above are consistent with the findings of a study of the factors influencing the selection of Turkish MA, including professional development and advancement opportunities in the "Career Opportunities" factor [5]. In the literature related to research on the choice of civilian HEIs, the factor of career prospects refers to the possibilities of professional practice during education and the opportunity to develop, advance, and earn well in a particular profession [21, 30]. This construct can be partially interpreted through the prism of the only research conducted in Serbia related to the criteria for selecting a HEI. It emphasizes that the possibility of employment after graduation is the most important factor in choosing a HEI [6], and the Ministry of Defence guarantees secure employment as one of the key benefits to graduates of the MF MMA.

The perception of the social status of an officer/a military doctor and the belief that this title brings recognition from the immediate environment and prestige in society is expressed in the construct "status" ($\beta = 0.171$; $p < 0.05$). To this should be added the possibility of choosing a specialization for the most successful graduates in the generation. It can be concluded that the choice of this MHEI is considerably motivated by a

positive perception of the specific characteristics of the military organization and the institutional reputation of the MF MMA, the Ministry of Defence, and the SAF as a socially valuable resource. The moderately strong correlation of high significance between the constructs "status" and "cultural capital" ($r = 0.578$; $p < 0.01$) indicates that, in the profession of a military doctor and an officer of the SAF, the respondents recognized a mechanism for rising on the social ladder that is in line with their personal beliefs about the military profession and the defense system as an institution. The factor influencing the choice of the MA in Turkey, referred to as "elitism", complements the identified influential factor of "status" [5].

Even though it was expected that the construct "influence of close people" would have a positive and significant impact on the decision to enroll, in relation to the construct "cultural capital", this is not the case in the given model. This result could be partially explained by the assumption that cultural capital is an intrinsic motivator for choosing the MF MMA, while the influence of close people, however, represents an external force whose impact may vary with the strength and consistency of the messages that close people send. It was also observed that respondents valued statements related to the influence of parents and family significantly more than those of relatives and friends on the decision to enroll. Although numerous studies emphasize the significant and positive influence of parents and the immediate environment, some suggest that this influence is context-dependent and can, in certain circumstances, be negative [14, 15].

The influence of the construct "reputation" on the decision in the given model is extremely low and without statistical significance ($\beta = - 0.007$; $p = 0.928$). The high mean scores for the statements comprising the "reputation" construct (all above 4) indicate that this perception is already well established in the respondents' minds. Most of them expressed a homogeneously positive opinion about the MF MMA as an exceptional educational institution, the professors' quality, and the diploma's "value", which reduces variability and makes this factor less relevant for explaining differences in decision-making. Therefore, it can be assumed that the reputation of the MF MMA is implied and, as such, represents a necessary but insufficient factor influencing the enrollment decision.

The specificity of the results of this research lies in the fact that the construct "benefits" does not have a significant impact ($\beta = 0.070$; $p < 0.295$) on the decision to enroll, which is not in line with the results of the research on the choice of HEIs in Serbia, where economic criteria prevail, and among them, the possibility of employment after graduation is the first in rank. Although the statement "It is important to me that after completing my studies I have secured employment" within the "benefits" construct was rated highly on average, which corresponds to the results in the previously mentioned research [6], it did not show statistical significance concerning the decision to enroll.

Even though the statements that form the constructs "self-perception" and "personal development" were clearly distinguished in the qualitative research, it was shown that in the regression model, they have a weak and statistically insignificant influence on the decision.

The research results presented in this paper are, to some extent, consistent with those of two recent studies on factors influencing the selection of civilian medical faculties in China and Pakistan [31, 32]. Personal interests, family influence, and the social status of the medical profession support the conclusion that factors linked to cultural capital and the social environment in which a prospective student is raised influence the choice of medical faculty. On the other hand, direct material benefits appear to play a more important role in the faculty selection process for future civilian doctors than for their military counterparts. The research results above [31, 32] indicate that, in addition to the already mentioned, personal interest in medicine is a key motivator for choosing a civilian medical faculty. The absence of that factor in the presented model indicates that cadets of the MF MMA view interest in the medical profession as hierarchically superior to other considerations, which influenced their decision to apply for enrollment at the MF MMA [32].

The main limitation of this research lies in the sample composition, as it included only cadets of the MF MMA and candidates who had already expressed their intention to enroll in this HEI. Although the survey was conducted anonymously, full verification of respondents' identities was not possible due to the use of online forms (Google Forms). The presence of cadets at different educational stages in the surveyed group can also be viewed as a limitation. Furthermore, the research is retrospective, which could have led to distorted attitudes, especially among respondents in their later years of study. Due to the relatively small sample size in relation to the number of items in the instrument, exploratory or confirmatory factor analysis was not performed. To ensure adequate statistical power for such an analysis, a minimum of 5 to 10 participants *per* item is generally recommended. Given the total of 39 items in the instrument, a significantly larger sample would be required, which should be addressed in future research.

Besides the contribution to the expansion of scientific knowledge related to the selection of MHEIs, the results of this research are practically applicable in creating promotional strategies for the MF MMA aimed at attracting an adequate number of motivated candidates for enrollment.

Future research should be directed towards understanding the differences in the factors that influence the choice of MF MMA and civilian medical faculties, as well as the influence of the sources of information that students (cadets) use when deciding to enroll in a particular HEI. Future studies should also include high school students in order to examine the factors influencing the choice of a HEI by those who have not yet made a final decision about their choice of enrollment.

Conclusion

The results of the research presented in this paper indicate that cadets' decisions to enroll at the MF MMA are shaped by a combination of personal and institutional factors,

predominantly of a social nature. In addition to their desire to practice medicine and become doctors, young men and women who choose the MF MMA are also socially predisposed to military service, primarily due to personal values, family influence, or cultural capital. They perceive the profession of an officer/military doctor as a potential for gaining social status, professional growth, career development, and advancement within the service. The specificities of military education, its focus on acquiring practical knowledge relevant to the future profession, and additional content make the MF MMA attractive for future cadets. As a particular advantage of military education, cadets see its focus on developing physical abilities, which is a factor that has not been identified in scientific literature so far.

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Received on April 2, 2025
Revised on May 25, 2025
Revised on June 2, 2025
Accepted on June 11, 2025
Online First July 2025

Appendix: Questionnaire

Please answer the following questions by selecting only one response *per* item.
Note: Responses are given using a five-point Likert scale:
a) Strongly disagree
b) Mostly disagree
c) Neutral/Not sure
d) Mostly agree
e) Strongly agree

FACTOR 1: REPUTATION

1. The school I enrolled in (or am applying for) is an exceptional educational institution.
2. A diploma from the Medical Faculty of the Military Medical Academy (MF MMA) is more valuable than one from a civilian university.
3. Top-tier professors teach at the MF MMA.

FACTOR 2: BENEFITS

4. It is important to me to have secured employment after graduation.
5. As an officer/military doctor of the Serbian Armed Forces (SAF), I will have a good salary.
6. The fact that cadets receive monthly pay in addition to free education is a major advantage over civilian faculty.

FACTOR 3: CULTURAL CAPITAL

7. I consider myself a patriot.
8. Every person should be ready to defend their country with arms in case of war.
9. I like order and discipline.
10. I have always enjoyed military-themed movies.

FACTOR 4: CAREER

11. I enrolled in a military school because I knew I could advance and attain a high rank.
12. Predictability in career progression is important when choosing a profession.
13. I would like to acquire high-level professional qualifications and competencies throughout my career.

FACTOR 5: PERSONAL DEVELOPMENT

14. Graduates of the MF MMA are more mature and better prepared for life than their civilian peers.
15. Military schools help individuals develop skills and abilities to better cope with both professional and personal challenges.
16. I enrolled in a military school because I wanted to become independent.

FACTOR 6: QUALITY OF MILITARY MEDICAL EDUCATION

17. The MF MMA truly prepares students for their future profession.
18. Military schools have an advantage over civilian ones due to specific skills acquired – driving license, foreign language certification, skiing, etc.
19. One of my reasons for choosing a military school was the integration of theory and practice.
20. I believe that modern teaching tools and methods are used at the Military Academy/MF MMA.

FACTOR 7: STATUS

21. I feel empowered in uniform.
22. I believe that officers deserve a high social status.
23. As an officer of the SAF, I would be respected by those around me.

FACTOR 8: PHYSICAL CULTURE

24. At a military school, you develop both mind and body equally.
25. Higher education institutions with good sports facilities are more attractive to future students.
26. I knew that after completing military school, I would be in excellent physical shape.

FACTOR 9: INFLUENCE OF CLOSE PEOPLE

27. The military has always been highly respected in my family.
28. My parents supported my decision to enroll in the Military Academy/MF MMA.
29. Since childhood, I have heard stories about the military from my parents and close relatives.
30. My friends had a positive view of the military, which influenced my decision.
31. People around me have a high opinion of the military.

FACTOR 10: SELF-PERCEPTION

32. I believe I am capable of meeting the challenges of studying at the MF MMA.
33. I was born to be an officer/military doctor.
34. I am talented in leadership and command.

ENROLLMENT DECISION

35. The MF MMA was my first choice for higher education.
36. When the time came to apply, I had no doubts about enrolling at the MF MMA.
37. I would recommend a close friend or family member to enroll in a military school.
38. I have no regrets about enrolling at the MF MMA.
39. I am proud that I will one day become an officer/military doctor in the SAF.

ORIGINAL ARTICLE

UDC: 615.282:[615.03/.038:615.015.3
DOI: https://doi.org/10.2298/VSP250320054M

Two-compartment pharmacokinetic model of itraconazole after single oral dose administration – gender differences

Dvoprostorni farmakokinetički model itrakonazola nakon oralne primene jedne doze leka – razlike među polovima

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Abstract

Background/Aim. Itraconazole (ICZ) is a widely used antifungal drug with hypervariable pharmacokinetics (PK), which is the result of the molecule's nature itself, as well as the influence of multiple factors. One of the factors is gender, but its importance is not yet substantiated. The aim of the study was to examine the effect of gender on ICZ PK using a two-compartment model, obtained after a single oral dose of the drug, under fed conditions, in healthy participants of both genders. **Methods.** A previously conducted bioequivalence study of two pharmaceutical formulations of a 100 mg oral dose of ICZ in 38 healthy participants (22 men and 16 women) yielded 114 sets of ICZ plasma concentrations. Of these, 64 sets (40 from men and 24 from women) were analyzed in this study using Kinetica software as they fit the two-compartment model. ICZ plasma concentrations were determined by a previously validated liquid chromatographic method with mass spectrometric detection. Statistical analyses in SPSS included Mann-Whitney *U* and Fisher's exact tests for group comparisons, along with Spearman's correlation for parameter relationships. **Results.** Poorer ICZ absorption was observed in females compared to males, accompanied by differences in the drug's distribution process between the central and peripheral compartments and *vice versa.* What's more, there are also differences in ICZ elimination between genders, with it being more effective in women. This isn't solely a result of a more prominent first-pass effect, but is also connected to the terminal phase of elimination after oral administration of the drug. **Conclusion.** The application of a two-compartment model for ICZ after its single oral dose administration under fed conditions in healthy research participants provided a more detailed insight into the variable PK of this drug, as well as into the existing gender-based differences.

Key words:
administration, oral; dose-response relationship, drug; itraconazole; pharmacokinetics; sex factors.

Apstrakt

Uvod/Cilj. Itrakonazol (ITZ) je široko korišćen antimikotik koji ima veoma varijabilnu farmakokinetiku (FK), što je rezultat same prirode molekula leka, kao i uticaja više faktora. Jedan od faktora uticaja je pol, ali njegov značaj još uvek nije potvrđen. Cilj rada bio je da se ispita uticaj pola na FK ITZ-a primenom dvoprostornog modela, koji je dobijen nakon primene leka *per os*, u jednoj dozi, na pun želudac kod zdravih ispitanika oba pola. **Metode.** Prethodno sprovedena studija bioekvivalencije dve oralne farmaceutske formulacije ITZ-a od 100 mg kod 38 zdravih učesnika (22 muškarca i 16 žena) rezultirala je sa 114 setova plazma koncentracija ITZ-a. Od toga je u ovoj studiji analizirano 64 seta (40 od muškaraca i 24 od žena) korišćenjem softvera *Kinetica*, jer je njihovom primenom dobijen dvoprostorni model. Koncentracije ITZ-a u plazmi bile su određene prethodno validiranom metodom tečne hromatografije sa masenom spektrometrijskom detekcijom. Statističke analize u SPSS-u obuhvatale su Mann-Whitney *U* i Fisher-ov egzaktni test za poređenje grupa, kao i Spearman-ovu korelacionu analizu odnosa parametara. **Rezultati.** Slabija resorpcija ITZ-a otkrivena je kod ženskog pola u odnosu na muškarce, a praćena je i razlikama koje su se pojavile u procesu distribucije leka iz centralnog u periferni prostor i obrnuto. Šta više, postoje razlike i u eliminaciji ITZ-a među polovima, koja je efektivnija kod žena. Ovo nije samo rezultat izraženijeg efekta prvog prolaza, već je povezano i sa terminalnom fazom eliminacije nakon oralne primene leka. **Zaključak.** Primena dvoprostornog modela ITZ-a nakon njegove primene u jednoj dozi *per os* kod zdravih učesnika u istraživanju omogućila je detaljniji uvid u varijabilnu FK ovog leka, kao i u razlike koje postoje među polovima.

Ključne reči:
oralna primena; lekovi, odnos doza-odgovor; itrakonazol; farmakokinetika; pol, faktor.

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Introduction

Itraconazole (ICZ), an orally active triazole, acts as an antifungal agent with a broad spectrum of activity [1–4]. However, due to its unpredictable oral bioavailability, its pharmacokinetics (PK) is variable and non-linear, characterized by prolonged clearance and slow accumulation [5–7]. The variability in oral bioavailability is attributed to the ICZ molecule itself, which is a weak base with very high lipophilicity, resulting in poor absorption from the gastrointestinal tract [8]. The extent and rate of the release of the active substance into the bloodstream significantly depend on the pharmaceutical formulation of the drug, particularly in the case of ICZ capsules [9–11]. Conventional capsules require an acidic environment in the stomach for optimal solubility, which affects drug absorption and optimal bioavailability [12]. Additionally, ICZ is extensively metabolized by cytochrome P450 3A (CYP3A) 4 – CYP3A4 in the liver, producing numerous metabolites, with hydroxy-itraconazole (OH-ICZ) being the most significant active one [13–15]. Due to its variable PK, particularly oral bioavailability, ICZ is classified as a highly variable drug. This classification indicates that intra-subject variability for parameters such as maximum drug concentration (C_{max}) and the area under the concentration-time curve (AUC) exceeds 30% [11, 16]. Furthermore, bioequivalence studies have shown that the within-subject coefficient of variation for C_{max} ranged from 44.95% to 69.1% [11, 16, 17]. ICZ volume of distribution (V_d) is about 70 L, and it is excreted mostly as inactive metabolites, approximately 35% in the urine and 54% in the feces [18, 19].

Numerous factors influence the absorption, V_d, biotransformation, and/or clearance (Cl) of drugs [20–22]. This is particularly significant for hypervariable drugs like ICZ. While the impact of different pharmaceutical formulations is well established, factors such as patient ethnicity, age, body mass index, gender, and interactions with other drugs or food have not been sufficiently investigated as sources of ICZ PK variability [10–12, 23–34]. The effect of patient age on ICZ PK remains unclear, as previous studies have reported contradictory results, even in an examination ranging from infants to adolescents [28, 31]. In our previous study [33], the tested PK parameter (AUC_∞) of both ICZ and OH-ICZ was included in the multiple linear regression analysis, and age was not a significant variable affecting their PK. This was not surprising, given that the ages of healthy participants ranged from 23 to 55 years. Similar results were observed when this analysis tested the influence of body weight on the selected PK parameter of ICZ and OH-ICZ, which was not corrected according to the body weight of the subjects. Additionally, since the participants were healthy individuals enrolled in a clinical trial – with no concomitant drug use, a standardized meal prior to drug administration, and shared ethnicity – their gender should be analyzed in more detail. In accordance with this, our previous research highlighted the importance of gender as a potential factor influencing the PK of ICZ in healthy subjects [33]. This was expedient since some authors, using a population PK model obtained after administering a single dose of the drug to healthy individuals, showed that gender did not affect ICZ PK, while others showed the opposite results [11, 28, 29].

The PK of ICZ has been assessed in numerous studies following intravenous and oral administration [17, 28, 33–38]. Non-compartment analysis has frequently been used, alongside the one-compartment PK open model, although ICZ follows multicompartment kinetics [8, 37].

The aim of this study was to further investigate the influence of gender on the ICZ PK following a single oral dose administered to healthy subjects under fed conditions, using a two-compartment open PK model.

Methods

Investigational drug

The 100 mg ICZ capsules used in the previous clinical PK study were sourced from two different manufacturers, whose bioequivalence had been established in an earlier study [17].

Participants

A total of 38 healthy participants (22 men and 16 women) were selected based on predefined inclusion criteria and participated in the study after providing informed consent and receiving comprehensive information about the study. The average age and body mass index of male participants were 38 ± 6.8 years (range 26–55 years) and 24.87 ± 2.80 (range 19.93–29.94), respectively. The average age and body mass index of female participants were 38 ± 6.7 years (range 23–50 years) and 24.82 ± 2.86 (range 19.49–28.69), respectively. All procedures related to the participants have already been explained [17]. However, considering that the participants in the study were healthy subjects, the exclusion criteria are additionally stated. These were primarily clinically relevant abnormalities in the medical history, medical examination, hematology and biochemistry tests, and urinalysis. Moreover, exclusion criteria included: use of any drugs within 14 days before the start of the study, except oral contraceptives; known drug allergy to ICZ; smoking; a recent history of drug or alcohol abuse, or a positive urine screening test for psychoactive substances; participation in other clinical studies within three months prior to the study initiation; positive test results for hepatitis B surface antigen, anti-hepatitis C virus, and/or anti-human immunodeficiency virus antibodies; unwillingness to conform to the study protocol.

Investigational study design

A randomized three-sequence, three-period, two-treatment, partially replicated crossover study in which two pharmaceutical formulations of ICZ were compared was performed [17]. The clinical protocol was approved by the Ethics Committees of the Military Medical Academy (No. 103/2024) and Medical Faculty of the Military Medical Academy (No. 2/11/2024) and approved by the Medicines and Medical Devices Agency of Serbia (No. 515-04-01565-14-1 from December 24, 2014).

Sample collection and analytical method

Since 38 subjects were enrolled, with 16 blood samples *per* subject during one period, and the protocol demanded three treatment periods, there were a total of 114 sets of ICZ plasma concentrations for analysis [17]. The previously established liquid chromatography method with mass spectrometric detection was used [17, 39].

Pharmacokinetic parameters and two-compartment model

Individual plasma concentrations of ICZ were analyzed, and PK parameters for the two-compartment model were calculated using 64 sets of ICZ plasma concentrations (40 sets from male and 24 from female participants). PK parameters were calculated using Kinetica software, version 5.0 (Thermo Fisher Scientific Inc., United States). The remaining 50 sets of ICZ concentrations (26 from women and 24 from men) could not fit the two-compartment open PK model.

PK parameters used in the two-compartment analysis included: k_a – the absorption rate constant calculated according to the equation: $k_a = \ln(2)/t_{1/2ka}$, where $t_{1/2ka}$ represents an absorption half-life; $C_{maxcalc}$ – maximum (peak) plasma drug concentration; $t_{maxcalc}$ – the time where t = C_{max}; $C_{maxcalc\ corr}$ and AUC_{corr} were obtained by dividing calculated values $C_{maxcalc}$ and AUC with the dose-to-body weight ratio; V1/F – volume of the central compartment in the two-compartment model; k_e – central compartment elimination rate constant; k_{12} – constant rate of transition from the central to peripheral compartment; k_{21} – constant rate of transition from the peripheral to central compartment; V_z/F – volume of distribution during the terminal phase after extravascular administration; α and β – exponents; A – intercept of the linear equation on log transformed data; B – slope of the linear equation on log transformed data; Cl/F – apparent total body clearance of the drug from plasma after oral administration.

Statistical analysis

Statistical analysis was performed using the SPSS software version 26.0 (IBM, USA, 2019). Comparison between genders for continuous variables was conducted using the Mann-Whitney *U* test. Fisher's exact test was used to examine the interrelation of PK parameters ($k_a < k_e$ and $k_a > k_e$) in men and women. Spearman's correlation analysis was used to assess relationships between PK parameters. The value of $p < 0.05$ was considered statistically significant.

Results

After *per os* ICZ administration, a two-compartment PK model was obtained (Tables 1 and 2). Further exploration of the defined model after the application of orally administered immediate-release formulations of ICZ (capsule) included correlations between k_a median values and other ICZ PK parameters that reflect the absorption properties of the drug *in vivo*. A statistically significant moderate correlation between k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$ parameters of ICZ, respectively, was shown (Figure 1A, B). There was no correlation between k_a and t_{max} and k_a and $C_{maxcalc\ corr}$, respectively. Evaluation of the influence of gender on ICZ absorption following single-dose oral administration, using a two-compartment model, indicated that there was no significant difference in the median values of k_a between men and women. However, a significant difference between genders was observed comparing the calculated values of the ICZ parameter C_{max} corrected by the ratio of the received drug dose and body weight ($C_{maxcalc\ corr}$). Its value was significantly lower in women (Table 1). Moreover, statistically significant moderate correlations between ICZ parameters k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively, were observed in the male gender. No such correlations were detected in women (Figure 1A, B). Furthermore, we examined correlations between ICZ parameters of absorption and distribution. We showed statistically significant moderate positive correlations between k_a and k_{12} and k_a and k_{21}, respectively, considering the total number of sets of ICZ plasma concentrations, as well as male sets of plasma concentrations. This correlation was not found in women (result not shown).

Statistically significant, very strong negative correlations between $C_{maxcalc\ corr}$ and V1/F parameters of ICZ were found when the calculation of all examined 64 sets of concentrations was performed, as well as both for male and female sets of ICZ plasma concentrations (Figure 2A). Moderate negative correlations between the parameters $C_{maxcalc\ corr}$ and V_z/F were

Table 1

Pharmacokinetic parameters of itraconazole absorption calculated from 64 sets of plasma concentrations after administration of a single oral dose of 100 mg of itraconazole obtained by the two-compartment open model

Parameters	Gender		*p*-value
	men (n = 40)	women (n = 24)	
k_a (h^{-1})	0.45 (0.08–1.26)	0.49 (0.12–1.09)	0.840
$t_{maxcalc}$ (h)	4.60 (2.79–7.44)	5.09 (1.44–6.93)	0.149
$C_{maxcalc}$ (ng/mL)	52.40 (13.25–200.65)	37.07 (14.22–172.92)	0.111
$C_{maxcalc\ corr}$ (ng/mL/mg/kg)	45.14 (8.75–190.61)	24.28 (8.53–117.59)	0.012
AUC ((h)*(ng/mL))	854.17 (241.74–7,847.21)	792.24 (202.01–2,105.82)	0.318
AUC_{corr} ((h)*(ng/mL)/mg/kg)	763.10 (137.79–6,277.77)	507.03 (169.69–1,684.66)	0.061

k_a – absorption rate constant; $t_{maxcalc}$ – time at which maximum concentration of a drug is achieved in plasma; $C_{maxcalc}$ – maximum plasma drug concentration; AUC – area under the concentration-time curve; $C_{maxcalc\ corr}$ and AUC_{corr} – values obtained by dividing calculated values of $C_{maxcalc}$ and AUC by dose-to-body weight ratio; n – number. Values are presented as median (minimum–maximum).

***Note*: *The value of $p < 0.05$ was considered significant according to the Mann-Whitney *U* test.**

Table 2

Pharmacokinetic parameters of itraconazole distribution and elimination calculated from 64 sets of plasma concentrations after administration of a single oral dose of 100 mg of itraconazole obtained by the two-compartment open model

Parameters	Gender		*p*-value
	men (n = 40)	women (n = 24)	
V_1/F (L/kg)	16.66 (0.24–92.89)	30.90 (0.50–106.84)	0.057
V_z/F (L/kg)	182.71 (9.68–2,543.09)	358.31 (7.32–6,013.46)	0.016
k_e (L/h)	0.13 (0.01–0.27)	0.11 (0.03–1.08)	0.305
k_{12} (L/h)	0.26 (0.01–16.23)	0.26 (0.15–6.53)	0.688
k_{21} (L/h)	0.04 (0.01–10.21)	0.04 (0.002–3.11)	0.560
A	116.63 (4.76–440.80)	75.28 (13.99–463.46)	0.197
α	0.42 (0.20–16.28)	0.44 (0.27–6.53)	0.739
B	8.34 (0.34–65.50)	4.69 (0.004–23.63)	0.029
β	0.01 (0.00–0.21)	0.008 (0.00–1.08)	0.228
Cl/F (L/h)	212.86 (23.17–752.12)	226.75 (0.20–900.04)	0.318

V_1/F – volume of the central compartment in two-compartment model; V_z/F – volume of distribution during the terminal phase after extravascular administration; k_e – central compartment elimination rate constant; k_{12} – constant rate of transition from the central to peripheal compartment; k_{21} – constant rate of transition from the peripheral to central compartment; A – intercept of the linear equation on log transformed data; B – shape of the linear equation on log transformed data; α (alpha) and β (beta) – exponents; Cl/F – apparent total body clearance of the drug from plasma after oral administration.

Values are presented as median (minimum–maximum). The value of $p < 0.05$ was considered significant according to the Mann-Whitney *U* test.

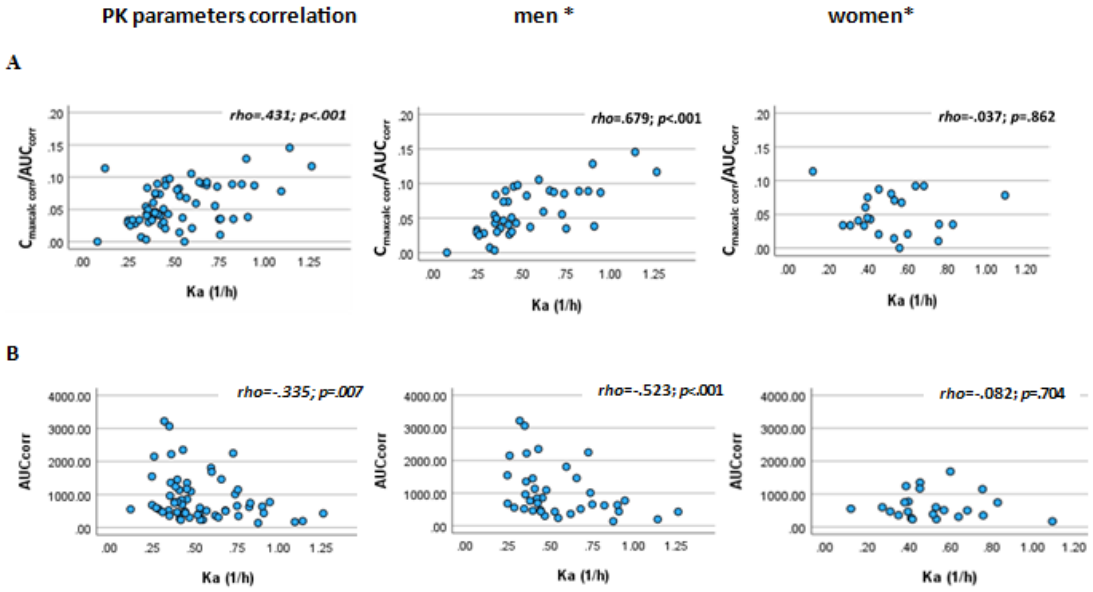

Fig. 1 – The correlations between ICZ PK parameters for the assessment of the absorption process: A) k_a and $C_{maxcalc\ corr}/AUC_{corr}$; B) k_a and AUC_{corr} of ICZ

ICZ – itraconazole; PK – pharmacokinetic; rho – Spearman's rank correlation coefficient. For other abbreviations, see Table 1.

Correlations were performed by using Spearman's correlation analysis ($p < 0.05$; $p < 0.001$ indicates significant correlation).

***Note*: *Sets of ICZ plasma concentrations obtained from men and women.**

also highly statistically significant when all examined sets of plasma concentrations were considered. This was also the case related to men, but not to women (Figure 2B). On the other hand, it was shown that correlations between parameters AUC_{corr} and V1/F were moderate and statistically significant for all 64 examined series, which was also related to the male gender, but not to the female (Figure 2C). The examined correlation of the $C_{maxcalc\ corr}/AUC_{corr}$ with the V1/F parameter showed a moderate negative correlation that was statistically significant for all examined sets of ICZ plasma concentrations, as well as for women, but not for men (Figure 2D).

In contrast, a moderate negative significant correlation between the parameters $C_{maxcalc\ corr}/AUC_{corr}$ and V_z/F was obtained, which was significant for the overall examined sets of concentrations, as well as for the male gender, but not for the females (Figure 2E). In addition, it was shown that the value of the ICZ parameter k_a was higher than the k_{12} value, and both

of these parameters had higher values than that of k_{21} ($k_a > k_{12} > k_{21}$) in 90% of men and 82% of women sets of ICZ concentrations, respectively.

Regarding parameters of the distribution, the median value of V_z/F was significantly higher for women than men, 182.71 (9.68–2,543.09) vs. 358.31 (7.32–6,013.46), respectively. Moreover, parameter B, defined as the intercept of the extrapolation of the β-phase to time zero in the two-compartment open model, was significantly lower in women than in men (Table 2).

The correlation of ICZ PK parameters of distribution in male and female genders indicated a statistically significant positive correlation between k_{12} and α parameters in both genders (Figure 3A). The correlation between the parameters k_{12} and β was not significant in men, in contrast to women, in whom a moderate negative statistically significant correlation was observed (Figure 3B). The situation was similar concerning the correlation between the ICZ parameters V1/F and k_{21}, which was positive and statistically significant in women but not in men (Figure 3C).

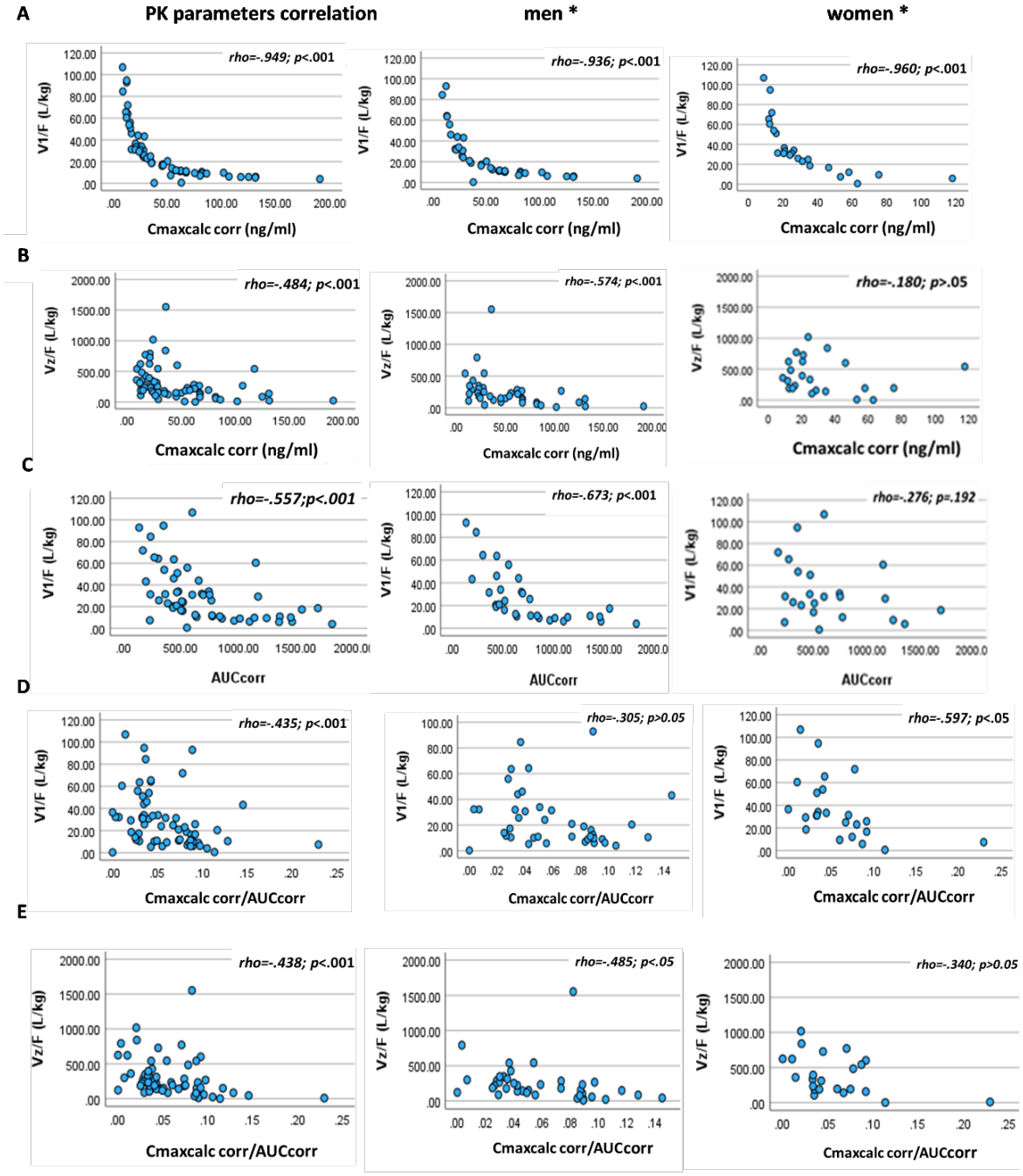

Fig. 2 – The correlations between ICZ PK parameters of absorption and distribution, respectively: A) $C_{maxcalc\ corr}$ and V_1/F; B) $C_{maxcalc\ corr}$ and V_z/F; C) AUC_{corr} and V_1/F; D) $C_{maxcalc\ corr}/AUC_{corr}$ and V_1/F; E) $C_{maxcalc\ corr}/AUC_{corr}$ and V_z/F
For abbreviations, see Figure 1 and Tables 1 and 2.
Correlations were performed by using Spearman's correlation analysis ($p < 0.05$; $p < 0.001$ indicates significant correlation).
***Note*: *Sets of ICZ plasma concentrations obtained from men and women.**

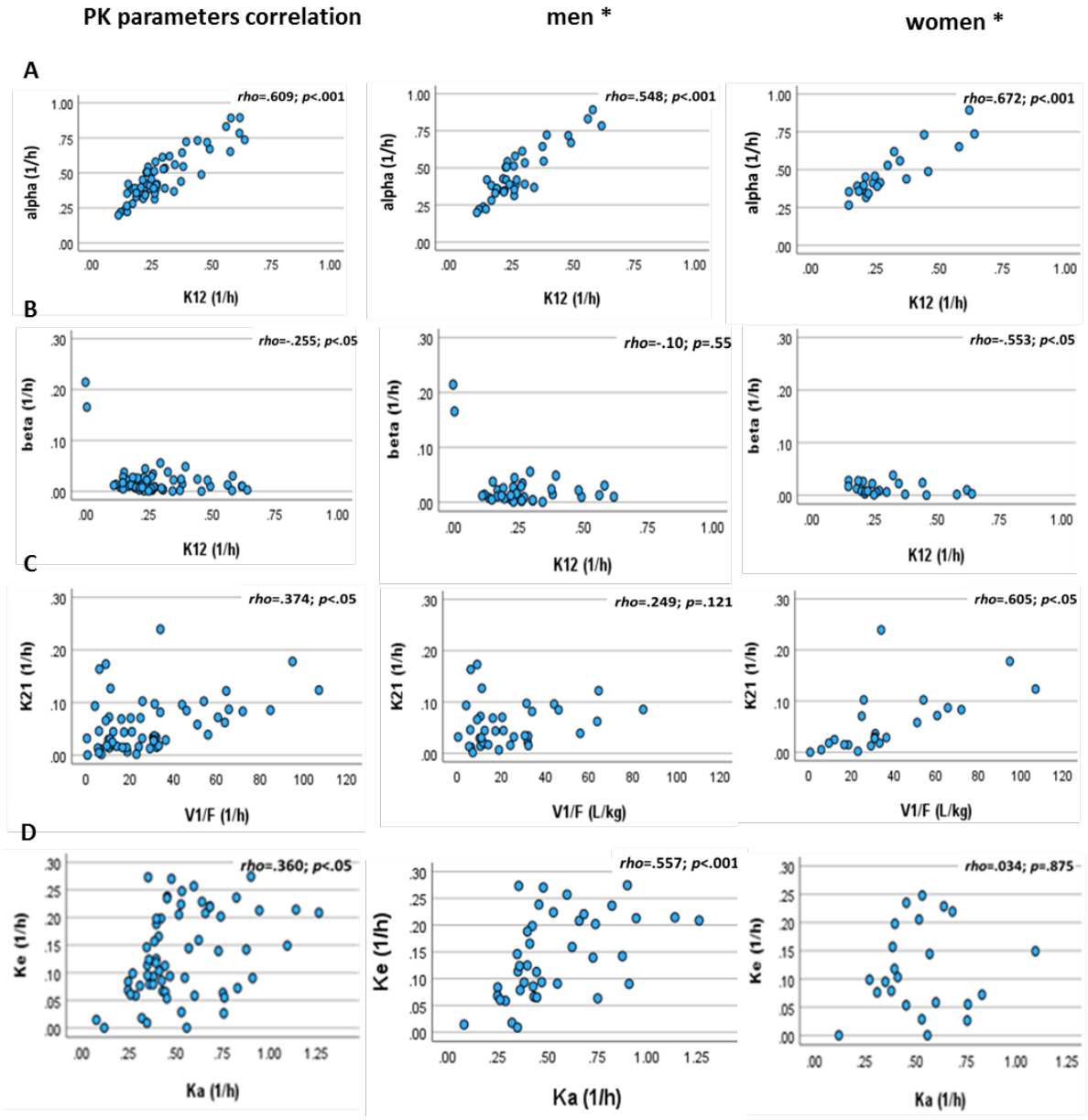

Fig. 3 – The correlations between ICZ PK parameters: k_{12} and alpha (A), k_{12} and beta (B), V1/F and k_{21} (C), k_a and k_e (D)

For abbreviations, see Table 2 and Figure 1.

Correlations were performed by using Spearman's correlation analysis ($p < 0.05$; $p < 0.001$ indicates significant correlation).

***Note*: *Sets of ICZ plasma concentrations obtained from men and women.**

On the other hand, a statistically significant positive correlation between V_1/F and V_z/F was found in men, whereas no such correlation was found in females (data not shown).

When the parameters of elimination, k_e and Cl/F, were considered, no significant differences were identified between males and females (Table 2).

The correlation between ICZ PK parameters related to absorption and elimination in male and female genders was also investigated. The correlation between ICZ parameters k_a and k_e was moderately positive and statistically significant only in the male gender (Figure 3D). On the other hand, the relation of ICZ parameters such as $k_a > k_e$ was present in 100% of male sets of drug concentrations and 87.5% of female sets ($p < 0.05$ according to Fisher's exact test). Therefore, the opposite relation, $k_a < k_e$, was not present in the male gender, while it accounted for 12.5% of female sets of ICZ concentrations ($p = 0.016$ according to Fisher's exact test).

Discussion

Our previous study included 38 healthy participants, each of whom provided 16 blood samples during a single investigation period, with the overall study designed to include three such periods [17, 33]. As a result, 114 sets of plasma concentrations of the drug itself, as well as its metabolite

OH-ICZ, were obtained and included in the PK analyses. In the present study, our results indicated that following oral administration of a 100 mg capsule in the fed state, two-compartment PK model parameters could be calculated by using 64 ICZ sets of plasma concentrations, 40 sets obtained from men and 24 from women. Additionally, in order to confirm the obtained two-compartment model, we used a novel method called the direct model [40] and presented good correlations between the following parameters: k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$.

Differences in ICZ PK parameters between genders have been observed using non-compartmental analysis and a one-compartment open model [33]. The present study employed a two-compartment model to further elucidate these differences. No significant differences were found between men and women in the median values of the parameter k_a. However, statistically significant moderate correlations were observed in male gender between ICZ parameters k_a and AUC_{corr}, as well as between k_a and $C_{maxcalc\ corr}/AUC_{corr}$. No such correlations were detected in women. Moreover, a significant difference between genders was found in the comparison of the $C_{maxcalc\ corr}$, with values being significantly lower in women. This finding is particularly noteworthy, as $C_{maxcalc\ corr}$ represents the parameter $C_{maxcalc}$ corrected according to the body weight of the subjects. This substantiates our previous findings that gender, and not the body mass index, significantly influenced the ICZ PK. Accordingly, when all 114 ICZ concentration sets were analyzed using non-compartmental analysis, women showed significantly lower median values for $C_{maxcalc\ corr}$, $AUC_{72hcorr}$, and $AUC_{\infty corr}$ compared to men. Moreover, when the results of the open one-compartment model parameters were analyzed, values of $C_{maxcalc\ corr}$ and AUC_{corr} were significantly lower in women than in men [33]. In accordance with that, a strong positive correlation was observed between parameters AUC and AUC_{corr}, as well as C_{max} and $C_{maxcalc\ corr}$ when considering one-compartment and two-compartment models, respectively [33]. All this indicates a poorer ICZ absorption in women compared to men. It is already known that certain parameters influencing the absorption process differ between genders. These differences can be attributed to variations in gastric acid secretion levels, with some studies indicating that it is lower in women [8, 22, 29]. This follows from the fact that ICZ is a very lipophilic drug, ionizing only at low pH, so the greater acid secretion in the stomach, the better solubility in water, which is evidently the case to a greater extent in men. Moreover, the speed of emptying the contents from the stomach and intestinal motility are higher in men, so this further favors greater absorption of the drug in men [22]. In accordance with this, authors Fagiolino et al. [11] analyzed the data on the ICZ bioequivalence study and concluded that women have less oral bioavailability and a more variable AUC than men. This may also explain the lack of correlation in our two-compartment model in women between ICZ parameter k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively.

When we considered ICZ distribution in a two-compartment open model, it was found that the parameter V_z/F was significantly higher in women than in men. Corresponding parameter V_d/F obtained from non-compartment analysis and one-compartment model was also significantly higher in the female gender in our previous study [33]. It can be explained by the fact that ICZ has very high lipophilicity and an extremely high volume of distribution [18, 19]. Since the amount of fat in the body does not account for lean body mass and muscles, and when the same body mass index in both genders exists, the female gender, on average, has at least 10% more body fat compared to men [41–44], these are in favor of significantly higher ICZ volume of distribution in women. In addition, our previous study showed that weight did not influence ICZ PK after single-dose oral administration, as selected ICZ PK parameters were corrected with the dose-to-body weight ratio [33]. The parameters corrected in the same way were included in the present investigation, which was performed by using a two-compartment open model. This further indicated that gender, rather than body mass, influences the ICZ PK. Furthermore, a statistically significant negative correlation was found between V_1/F and V_z/F in men, which was not the case in women. Taking into account that the parameter V_1/F shows the apparent volume of the central or plasma compartment, and V_z/F indicates the apparent volume of distribution during the terminal phase after non-intravenous drug administration in a two-compartment model, all this supports the different distribution of ICZ in males and females. Moreover, while no significant correlation between the parameters k_{12} and β was observed in men, a moderate, statistically significant negative correlation was identified in women. In contrast, the correlation between ICZ parameters V_1/F and k_{21} was significantly positive in women but not in men. These findings highlight the differences observed in the process of drug distribution between the central and peripheral compartments between genders.

Since all PK processes occur simultaneously in the body [44] results concerning values of ICZ volume of distribution are in accordance with the findings that the parameter $C_{maxcalc}$ value, corrected for body weight ($C_{maxcalc\ corr}$), is significantly lower in females. Moreover, the differences found in the correlations between gender, which refer to the relationship between absorption and distribution parameters in this study, also support different PK of ICZ in women and men (negative correlations between parameters $C_{maxcalc\ corr}$ and V_z/F; $C_{maxcalc\ corr}/AUC_{corr}$ and V_1/F, and $C_{maxcalc\ corr}/AUC_{corr}$ and V_z/F were highly statistically significant in men, but not in female gender). According to the mentioned direct model [40], the relationships among the constants $k_a > k_{12} > k_{21}$ were satisfied in 90% of men and 82% of women sets of ICZ concentrations, respectively, providing more rationale for setting the two-compartment model for this drug. Again, there were no significant correlations between parameters k_a and k_{12} and k_a and k_{21}, respectively, in the female gender, which was the case in men.

When the β exponent, also referred to as the post-distribution or terminal phase in the two-compartment model, was considered, the results indicated that the parameter B is significantly lower in women. Namely, the β hybrid constant

is related to the elimination of the parent drug from the systemic circulation through metabolism and/or excretion, including the effects of overlapping the processes of elimination and distribution, which is not yet finished [44]. ICZ is extensively metabolized by the liver *via* the CYP3A4 enzyme, as the major enzyme involved, resulting in various metabolites. However, the main metabolite, OH-ICZ, exhibits trough plasma concentrations about twice as high as those of ICZ [13–15]. In our previous investigation, not only were the values of C_{max} and AUC significantly lower for both ICZ and OH-ICZ in the female gender, but women also exhibited significantly lower medians of plasma concentrations of both the parent drug and metabolite in comparison to males 72 hrs after administration of ICZ [33]. Since it was related to the metabolite to a greater extent, it pointed out less exposure to OH-ICZ in the female gender compared with males. Therefore, gender differences related to the CYP3A4 enzyme that metabolizes ICZ predominantly in the liver could be one of the causes [45, 46]. Namely, women are thought to have approximately 1.4 times higher CYP3A4 activity than men. Moreover, Wolbold et al. [47] examined 39 human liver tissue samples and found that expression of this enzyme is twice as high in women as in men. Sakuma et al. [48] presented two possible mechanisms for the more dominant expression of the CYP3A enzyme in women. The first mechanism is that activation of the pregnane X receptor by female sex hormones plays an important role in the dominant expression of the CYP3A enzyme in women. The second one is related to the influence of growth hormone (GH) [49, 50]. In a person with GH-deficient secretion, a different expression of CYP3A enzyme exists, depending on the way of substitution therapy administration [50]. When this hormone was given continuously (imitating the way GH is secreted in the female gender), the activity of the CYP3A4 group of enzymes was increased, while when it was given in pulses (which is the way GH is secreted in the male gender), its activities were decreased. Therefore, the elimination of the ICZ is more effective in women as a result not only of the more prominent first-pass effect but also related to the terminal phase of elimination after oral administration. These differences are also substantiated by the findings that the correlation of ICZ parameters k_a and k_e was moderately positive and statistically significant only in the male gender. Furthermore, in contrast to all ICZ concentration series obtained from males in whom the parameter k_a was greater than k_e, the flip-flop model was present in 12.5% of female sets of ICZ concentrations ($p = 0.016$). This phenomenon is defined when the PK parameter k_a is less than the k_e for some drugs [44]. Related to ICZ in women, it seems that it is related to the "flip" scenario, in which limited absorption is more prominent, resulting in a slower rise in plasma concentrations after oral administration [51].

In addition to the physical and chemical properties of the drug, other factors that contribute to this are the already mentioned physiological factors, specifically related to gender, such as variations in gastrointestinal tract physiology, including gastric pH, transit time, and enzyme activity, which can affect absorption speed. As already mentioned, women have less oral bioavailability and a more variable AUC than men [11], in accordance with our findings of the lack of correlation between absorption parameters in women in two-compartment analysis.

Moreover, the application of the ICZ two-compartment PK model after its oral administration was possible by using 64 sets of ICZ plasma concentrations (40 from men and 24 from healthy female participants), out of 114 total obtained in our previous study [33]. For the remaining 50 sets of ICZ plasma concentrations, the two-compartment model analysis could not be calculated by Kinetica software version 5.0. This can be attributed to the slowed and delayed distribution phase, as it was mentioned [51]. According to the literature, when the initial distribution phase is small compared to the total AUC, the two-compartment model "falls" to the one-compartment model [9, 52–55]. This is actually in accordance with our previous results [33], since when all 114 sets of ICZ plasma concentrations were taken into analysis by a one-compartment model, all PK parameters of the drug could be calculated and used for further analysis. Moreover, this is also related to the hypervariability of ICZ during the absorption process, meaning that variability for the parameters C_{max} and AUC is larger than 30%, as was already shown in our ICZ bioequivalence study [17]. In the presented paper, it was additionally considered in the context of PK differences between genders. We strongly support the introduction of therapeutic monitoring of ICZ in everyday clinical practice, which would allow individualization of the ICZ dose by checking plasma or serum drug concentrations and adjusting its dose, especially in patients with serious fungal infections [30].

Study limitations

A limitation of the study could be the relatively small number of sets of ICZ concentrations enabling the formation of a two-compartment PK model of ICZ compared to the total number of sets of concentrations obtained after oral administration of ICZ in all study participants. However, in our previous work, positive strong correlations between one-compartment and two-compartment models for parameters AUC and AUC_{corr}, as well as $C_{maxcalc}$ and $C_{maxcalc\ corr}$, respectively, were shown [33]. Moreover, the obtained two-compartment model after extravascular administration of the drug was verified using a direct model, which was substantiated by good correlations between the parameters ka and AUC_{corr}, as well as k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively, obtained using our data.

Conclusion

Our results indicated that following oral administration of a 100 mg capsule in the fed state, two-compartment pharmacokinetic model parameters could be calculated by using 64 itraconazole sets of plasma concentrations, 40 sets obtained from men and 24 from women. Itraconazole parameter C_{max} corrected by the dose-to-body weight ratio, i.e., $C_{maxcalc\ corr}$, was significantly lower in women than in men, while statistically significant correlations between parameters k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively, were observed in men,

but not in women. The median value of parameter V_z/F was significantly higher in women than in men, while parameter B, the intercept of the extrapolation of the β-phase to time zero, was significantly lower in women than in men. Additionally, the correlation of itraconazole parameters k_a and k_e was positive and statistically significant only in the male gender, while the relation of itraconazole parameters such as $k_a < k_e$ was not present in the male gender, but it accounted for 12.5% of female sets of itraconazole concentrations, and the difference was statistically significant. Therefore, the two-compartment open model of itraconazole following a single oral dose under fed conditions in healthy participants provided a detailed insight into its variable pharmacokinetics and gender-based differences.

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Received on March 20, 2025
Revised on April 22, 2025
Accepted on April 29, 2025
Online First July 2025

ORIGINAL ARTICLE

UDC: 355/359:61]::[616.28-008.14::355.54/.55
DOI: https://doi.org/10.2298/VSP250402051J

The impact of gender and risk factors for hearing impairment on audiogram changes in military cadets during shooting exercises

Uticaj pola i faktora rizika za oštećenje sluha na promene u audiogramu kod vojnih kadeta u toku gađanja

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Abstract

Background/Aim. Military personnel are frequently exposed to high levels of noise, where hearing loss can affect their combat performance. The aim of this study was to assess the impact of impulse noise produced by firearms during shooting exercises, analyze the effect of noise through pure-tone audiometry, and correlate it with sex differences and risk factors for hearing impairment. **Methods.** The study included 105 cadets, 75 men and 30 women, aged 19 to 25 years, who underwent regular firearm shooting as part of their military training. We observed the results of audiometric examinations conducted at three time points: 24 hrs before noise exposure, immediately after the noise exposure [shooting with a "*Crvena Zastava*" 99 pistol, i.e., CZ99], and 24 hrs after the shooting. Data on risk factors were obtained from a questionnaire with anamnestic data of the cadets. **Results.** Immediately after noise exposure, there was significant sensorineural hearing impairment at 2,000 Hz and 4,000 Hz, with complete recovery after 24 hrs. Our study revealed that male participants are more susceptible to this type of noise exposure compared to female participants. Additionally, we found that 22.9% of the respondents had risk factors for post-shooting hearing impairment, which included smoking, noise exposure within the last 7 days, field work within the past 72 hrs, and hereditary hearing loss in the family. **Conclusion.** Despite the regular use of hearing protection, hearing impairment after acute acoustic trauma caused by shooting in military personnel remains inevitable and is more pronounced in men than in women. Our results suggest that the presence of risk factors for hearing impairment contributes to an increase in the temporary threshold shift, i.e., a decrease in hearing.

Key words:
audiometry; hearing loss; hearing loss, noise-induced; military medicine; risk factors; sex factors.

Apstrakt

Uvod/Cilj. Vojno osoblje je često izloženo visokim nivoima buke, gde gubitak sluha može uticati na njihove borbene sposobnosti. Cilj rada bio je da se proceni uticaj impulsne buke koju proizvodi pucanje vatrenim oružjem tokom vežbi gađanja, analizira uticaj buke tonskom audiometrijom i poveže sa polnim razlikama i faktorima rizika za oštećenje sluha. **Metode.** U studiju je bilo uključeno 105 kadeta, 75 muškaraca i 30 žena, uzrasta od 19 do 25 godina, koji u sklopu svog školovanja obavljaju redovna gađanja iz vatrenog oružja u toku vojne obuke. Posmatrali smo rezultate audiometrijskog ispitivanja sprovedenog u tri termina: 24 h pre izlaganja buci, neposredno nakon izlaganja buci [gađanje iz pištolja „Crvena Zastava" 99, tj., CZ99] i 24 h nakon izvršenog gađanja. Podaci o faktorima rizika dobijeni su iz upitnika sa anamnestičkim podacima kadeta. **Rezultati.** Neposredno nakon izlaganja pucnju, dolazilo je do značajnog senzorineuralnog oštećenja sluha na 2.000 Hz i 4.000 Hz, uz kompletni oporavak nakon 24 h. Naša studija je pokazala da su ispitanici muškog pola osetljiviji na ovakvo izlaganje buci u odnosu na ispitanice ženskog pola. Takođe, ustanovili smo da je 22,9% ispitanika imalo faktore rizika za oštećenje sluha nakon pucnja, što uključuje pušenje, izloženost buci u poslednjih 7 dana, rad na terenu u poslednja 72 h i nasledni gubitak sluha u porodici. **Zaključak.** Uprkos regularnom korišćenju zaštitnih sredstava za sluh, oštećenje sluha nakon akutne akustične traume izazvane pucanjem kod pripadnika vojne populacije ostaje neizbežno i izraženije je kod muškaraca u odnosu na žene. Naši rezultati ukazuju da postojanje faktora rizika za oštećenje sluha doprinosi privremenom povećanju praga sluha tj. smanjenju sluha.

Ključne reči:
audiometrija; sluh, gubitak; sluh, gubitak izazvan bukom; vojna medicina; faktori rizika; pol, faktor.

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Introduction

Hearing plays a crucial role in a soldier's performance and is essential for effective communication. Noise-induced hearing loss (NIHL) is a significant impairment in the military and can affect combat performance. Unlike civilians, military personnel are frequently exposed to intense noise levels and often have no choice but to remain in noisy environments in order to complete specific tasks and missions [1]. The harmful effects of noise depend on its characteristics (type, intensity, constancy or intermittence, impact) and the duration of exposure, directionality, and individual sensitivity of the person exposed to the noise [2]. Occupational NIHL is the most prevalent work-related illness globally [3], particularly affecting professions exposed to high levels of noise, such as military personnel [4–7]. Gunshot noise ranks as a fourth-degree noise, with measured sound pressure levels ranging from 140 A-weighted decibels [dB(A)] to 180 dB(A) upon using firearms [8]. Audiometry has shown the greatest hearing loss at 4,000 Hz following high-intensity impulse noise in occupationally exposed individuals, occurring in 86.7% of cases, even at lower noise levels measured during shooting from pistols and revolvers, with a maximum measured value of 113.1 dB for a 0.40 calibre pistol and 116.8 dB for a 0.38 calibre revolver [9].

Inner ear injuries caused by acute acoustic trauma can be classified as temporary threshold shift (TTS) or permanent threshold shift (PTS) [10], depending on the duration and intensity of noise exposure. Generally, hearing recovers after TTS within 24–48 hrs [10–13], hence it is often not recognized as a potential issue. Nevertheless, if the noise exposure that induces TTS is repeated, it may evolve to PTS. This is a common mechanism of hearing impairment in occupational exposure to noise [14]. Mechanisms by which intense noise induces hearing loss include physical injury of structures in the inner ear, inflammation, ischaemia, oxidative stress, and excessive activity of nerve endings in the cochlea, resulting in functional hearing impairment [6]. Brief exposure to high-level impulse noise induces physical disorganization and damage of stereocilia as well as direct injury of both supporting cells and sensory neurons [15], resulting in functional hearing impairment. For such an injury, the major factor is the maximum sound pressure, while the duration of exposure is less important [16]. The most vulnerable structure to noise is the basal cochlea. This finding is responsible for pronounced sensitivity for noise-induced hearing impairment at high frequencies (both TTS and PTS). The individual's audiometric tests may vary; hence, the smallest measurable values of TTS and PTS are not well defined. This led to the development of various standards for defining significant hearing impairment, commonly referred to as "standard threshold shift" (STS). According to the Occupational Safety and Health Administration (OSHA), the accepted STS in the given ear is established at an average value of 10 dB increase in threshold compared to the individual's baseline audiogram (or recently obtained) measured at 2,000, 3,000, and 4,000 Hz [17]. Ryan et al. [14] in their review of basic and clinical observations of noise-induced TTS and PTS also stated that STS for work-related injury should be set at least at 25 dB when hearing impairment is confirmed on repeated tests within 30 days.

The aim of this study was to examine whether the presence of risk factors for the occurrence of hearing damage in the subjects, such as smoking, previous exposure to noise, and genetic predisposition, affects the recovery of the hearing threshold following exposure to gunfire from a "*Crvena Zastava*" 99 pistol, i.e., CZ99. Additionally, the study examined whether gender could have an influence on hearing impairment after acute acoustic trauma, as well as a difference in hearing recovery after shooting, measured by audiometry.

Methods

The study was conducted at the Military Academy and the Military Medical Academy, the Institute of Occupational Medicine, and the Institute of Medical Research, Belgrade, Serbia, from November 2022 to April 2023. Approval for this study was obtained from the Ethics Committee of the Faculty of Medicine of the Military Medical Academy, University of Defence (No. 1/2022). All participants signed informed consent.

In this interventional prospective study, 105 cadets of both genders were enrolled (75 males and 30 females). The participants performed regular shooting exercises with the CZ99 pistol at a semi-enclosed shooting range, using earplugs for hearing protection. The intervention in this study is related to exposure to noise and diagnostic testing through audiometry [18]. The criteria for exclusion, shooting conditions, and terms of audiometric investigation are described in detail in our previous study [19].

Pure-tone audiometry was performed using the Bell Plus audiometer (Inventis, Padova, Italy). The device is suitable for field use and determines both air and bone conduction sound transmission. Calibration on an audiometer was done every 12 months. For assessment of air conduction, the pure-tone thresholds were determined at the following frequencies: 125, 250, 500, 1,000, 2,000, 4,000, 6,000, and 8,000 Hz, and for bone conduction at 250, 500, 1,000, 2,000, and 4,000 Hz [18].

For data analysis, we used SPSS version 26.0 software (IBM, USA, 2019). Continuous variables are presented as mean ± standard deviation or as median with interquartile range (25th and 75th percentiles), while categorical variables are presented as frequencies of individual categories. For between-group comparison, we used the Mann-Whitney *U* test, and for paired comparison within a group, we used the Wilcoxon test. For continuous variables, comparisons within groups at three different time points were performed using analysis of variance (ANOVA) for data with a parametric distribution. The correlation between variables of interest was tested using Spearman's correlation. The value of $p < 0.05$ was considered significant throughout all analyses. Results are presented as mean ± standard deviation.

Results

A total of 105 participants were included in the study, of whom 72.4% were men and 27.6% were women. Among

them, 22.9% had risk factors for hearing impairment after shooting. These risk factors included smoking, noise exposure in the past 7 days, field work in the past 72 hrs, and a family history of hearing loss. Additional participant characteristics are shown in Table 1.

Average audiometric test values of the right and left ear before shooting at 2,000 and 4,000 Hz showed no significant difference (2,000 Hz: 10.38 ± 1.50 dB right vs. 10.57 ± 1.74 dB left, $p = 0.348$; 4,000 Hz: 10.95 ± 2.51 dB right vs. 10.81 ± 2.61 dB left, $p = 0.604$) (Figure 1).

Immediately after shooting, audiogram analysis at 2,000 Hz demonstrated significant average hearing loss both in the right (10.38 ± 1.50 dB vs. 12.14 ± 3.46 dB, $p < 0.001$) and left ear (10.57 ± 1.74 dB vs. 12.81 ± 3.73 dB, $p < 0.001$) (Figure 2).

Table 1

Basic characteristics of examinees

Parameters	Yes	No
Smoking	20 (19.0)	84 (81.0)
Alcohol consumption	10 (9.5)	95 (90.5)
Previous noise exposure	2 (1.9)	103 (98.1)
Hay fever	5 (4.8)	100 (95.2)
Infection	2 (1.9)	103 (98.1)
Hereditary	2 (1.9)	103 (98.1)
Total risk factors	24 (22.9)	81 (77.1)

All values are given as numbers (percentages).

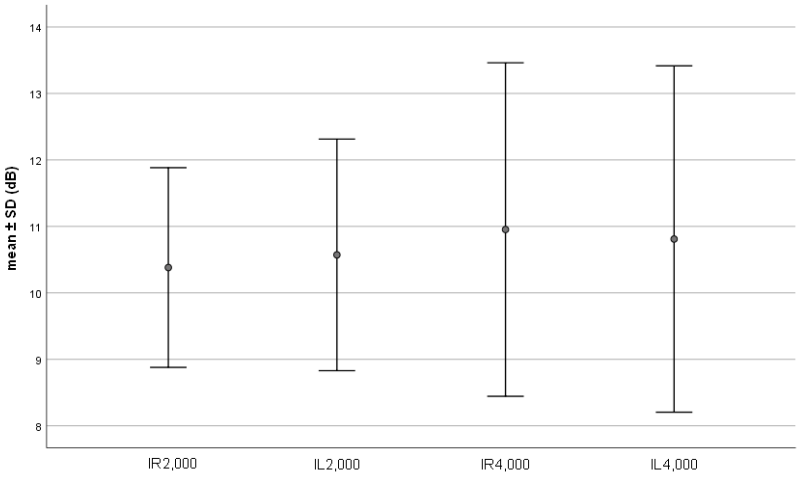

Fig. 1 – Audiometric test of the right and left ear before shooting

R – right ear; L – left ear; I – first time point at 2,000 Hz and 4,000 Hz; SD – standard deviation.
***Note*: The y-axis shows the level of audibility, i.e., hearing before shooting, as well as the shift in hearing threshold after shooting.**

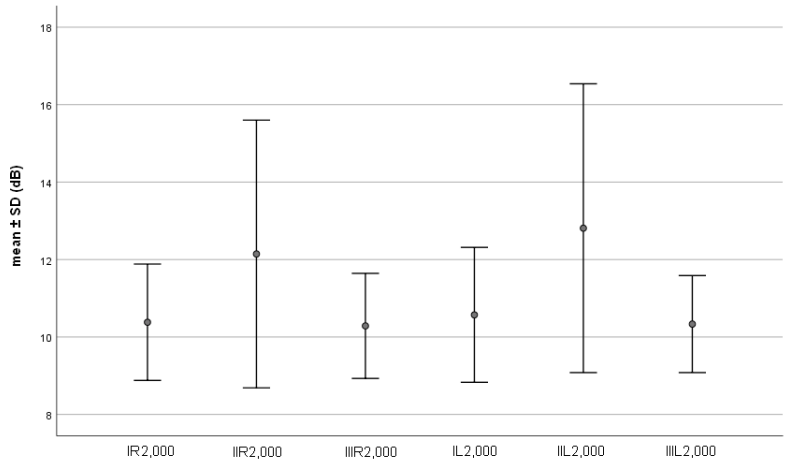

Fig. 2 – Comparison of average audiometric values of the left and right ear before shooting, immediately after shooting, and 24 hrs after shooting at 2,000 Hz.

R – right ear; L – left ear; I, II, III – first, second, and third time points at 2,000 Hz; SD – standard deviation.
***Note*: The y-axis shows the level of audibility, i.e., hearing before shooting, as well as the shift in hearing threshold after shooting.**

Comparison of values obtained 24 hrs after shooting with pre-shooting levels revealed no significant difference in either ear.

Analysis of hearing at 4,000 Hz demonstrated significant difference of average values again both on the right ear (10.95 ± 2.51 dB vs. 12.52 ± 4.45 dB, $p < 0.001$) and on the left ear (10.81 ± 2.61 dB vs. 13.19 ± 5.15 dB, $p < 0.001$) before/immediately after shooting (Figure 3).

Analysis of audiogram differences by gender revealed significantly increased values only in male participants on the right ear at 4,000 Hz immediately after shooting (Table 2).

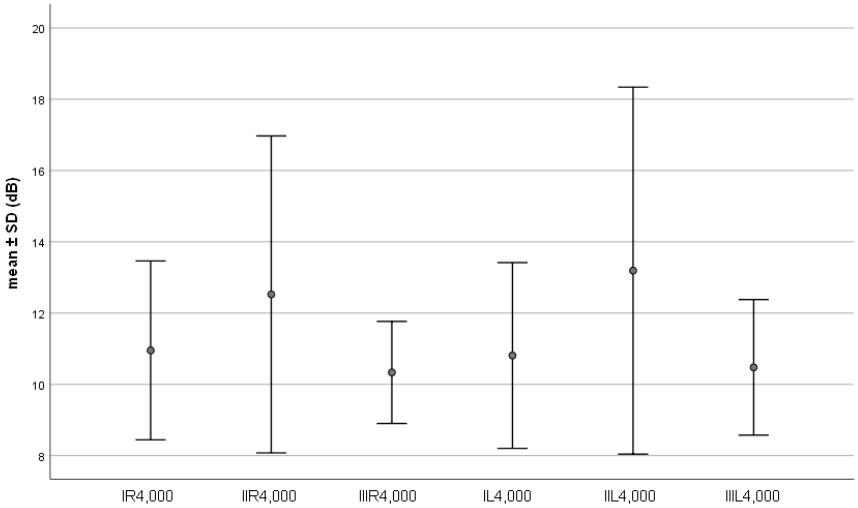

Fig. 3 – Comparison of average audiometric values of the left and right ear before shooting, immediately after shooting, and 24 hrs after shooting at 4,000 Hz
R – right ear; L – left ear; I, II, III – first, second, and third time points at 4,000 Hz; SD – standard deviation.
***Note*: The y-axis shows the level of audibility, i.e., hearing before shooting, as well as the shift in hearing threshold after shooting.**

Table 2

Gender difference analysis and average audiogram values according to time of acute acoustic trauma exposure at 2,000 and 4,000 Hz

Frequency	Ear	Time	Gender	Mean ± SD (dB)	*p*-value
2,000 Hz	Right	I	male	10.26 ± 1.12	0.195
		I	female	10.69 ± 2.21	
		II	male	12.43 ± 3.70	0.163
		II	female	11.38 ± 2.64	
		III	male	10.33 ± 1.49	0.599
		III	female	10.17 ± 0.93	
2,000 Hz	Left	I	male	10.59 ± 1.82	0.845
		I	female	10.52 ± 1.55	
		II	male	13.03 ± 3.92	0.337
		II	female	12.24 ± 3.16	
		III	male	10.33 ± 1.25	0.954
		III	female	10.34 ± 1.29	
4,000 Hz	Right	I	male	11.12 ± 2.78	0.274
		I	female	10.52 ± 1.55	
		II	male	13.29 ± 4.94	**0.004**
		II	female	10.52 ± 1.55	
		III	male	10.46 ± 1.67	0.141
		III	female	10.00 ± 0.86	
4,000 Hz	Left	I	male	11.05 ± 2.98	0.122
		I	female	10.17 ± 0.93	
		II	male	13.68 ± 5.68	0.112
		II	female	11.90 ± 3.11	
		III	male	10.59 ± 2.15	0.314
		III	female	10.17 ± 0.93	

All values are given as mean ± standard deviation (SD).
The bold value indicates the significance level of $p < 0.05$.

Participants with identified risk factors demonstrated a significant increase in the average audiogram on the right ear at 2,000 Hz before shooting (Table 3).

Examination of the correlation between cumulative risk factors in males and females and audiometric findings before shooting, right after shooting, and 24 hrs after shooting (measured at 2,000 Hz) showed statistical significance in females. A strong statistical correlation was observed between the cumulative number of risk factors and audiometric results for the right ear at all three measurement points: before, immediately after, and 24 hrs after the shooting (Table 4).

Discussion

Despite the use of personal hearing protection, a large number of professional soldiers and officers experience disability and hearing loss, both gradual and sudden [20, 21].

In our investigation, when we observed all participants together, we found statistically significant hearing impairment measured at 2,000 Hz and 4,000 Hz in both ears immediately after noise exposure. The hearing of our subjects has completely recovered 24 hrs after the shooting, i.e., we found no statistically significant differences between the results before

Table 3

Average audiogram values of the right and left ear before, immediately after, and 24 hrs after shooting at 2,000 and 4,000 Hz according to the presence of risk factors

Frequency	Ear	Time	Risk Factors	Mean ± SD (dB)	*p*-value
2,000 Hz	Right	I	no	10.19 ± 0.95	**0.013**
		I	yes	11.04 ± 2.54	
		II	no	12.16 ± 3.53	0.924
		II	yes	12.08 ± 3.27	
		III	no	10.31 ± 1.45	0.752
		III	yes	10.21 ± 1.02	
2,000 Hz	Left	I	no	10.56 ± 1.77	0.865
		I	yes	10.63 ± 1.69	
		II	no	12.72 ± 3.80	0.639
		II	yes	13.13 ± 3.55	
		III	no	10.37 ± 1.32	0.580
		III	yes	10.21 ± 1.02	
4,000 Hz	Right	I	no	10.86 ± 2.34	0.511
		I	yes	11.25 ± 3.04	
		II	no	12.16 ± 4.54	0.125
		II	yes	13.75 ± 3.97	
		III	no	10.37 ± 1.54	0.629
		III	yes	10.21 ± 1.02	
4,000 Hz	Left	I	no	10.80 ± 2.68	0.960
		I	yes	10.83 ± 2.41	
		II	no	13.15 ± 5.50	0.878
		II	yes	13.33 ± 3.81	
		III	no	10.49 ± 1.87	0.862
		III	yes	10.42 ± 2.04	

All values are given as mean ± standard deviation (SD).
The bold value indicates the significance level of $p < 0.05$.

Table 4

Correlation of audiogram values to cumulative risk factors in all participants (males and females)

Frequency	Ear	Time	Female		Male	
			rs	*p*-value	*rs*	*p*-value
2,000 Hz	Right	I	**+0.640**	**0.000**	+0.118	0.310
		II	**+0.381**	**0.041**	-0.110	0.344
		III	**+0.556**	**0.002**	-0.146	0.209
2,000 Hz	Left	I	+0.256	0.179	-0.023	0.846
		II	+0.166	0.389	+0.032	0.787
		III	+0.354	0.059	-0.164	0.157
4,000 Hz	Right	I	-0.115	0.551	+0.044	0.705
		II	+0.256	0.179	+0.182	0.115
		III	/	/	-0.073	0.530
4,000 Hz	Left	I	-0.064	0.741	+0.014	0.906
		II	+0.000	1.000	+0.084	0.471
		III	-0.064	0.741	-0.067	0.563

Spearman's correlation (*rs*) was used.
Bold values indicate the statistical significance ($p < 0.05$).

and 24 hrs after the shooting. This can be explained by the fact that all the respondents were completely healthy and belonged to the young population. The frequency of hearing impairment increases with age [22–24].

When we investigated the gender impact, we found a trend of higher sensitivity to noise in males compared to females, with statistical significance at 4,000 Hz in the right ear. Consistent with our findings, Villavisanis et al. [25] reported in their research that gender is a significant biological variable influencing hearing sensitivity; it significantly affects hearing loss on both sides, more in men compared to women.

Several studies have pointed out the specific gender-dependent organization of the cochlea, as well as other components of both classical and non-classical auditory pathways in the brain [26–28]. Therefore, it is believed that the auditory system in males and females is not equally sensitive to every type and frequency of sound and that the auditory regions in females are more resistant to damage caused by prolonged noise compared to males [29]. Considering this, the American National Institute of Health recommended the inclusion of gender as a biological variable in future research regarding hearing assessment [30, 31].

In addition, Villavisanis et al. [25] confirmed the impact of sexual hormones on hearing, emphasizing the protective effect of estrogen. In addition to its role in promoting female reproductive functions, estrogen, with its most potent endogenous form, estradiol, plays a protective role in auditory function [32, 33]. Estrogen synthesis is mainly located in the ovaries. However, several other tissues and organs, including adipose tissue, brain, skin, and bone, also produce estrogen [32]. Regardless of gender, those tissues convert androgen hormones into estrogens through the enzyme aromatase. The role of these local estrogens is to improve cardiovascular or neurological functions and to protect the local environment against degeneration and injuries [34]. Among tissues that express the enzyme aromatase and hence are able to produce estrogen locally is the auditory cortex. This production enables the better processing of auditory stimuli in the central nervous system [35]. In accordance with the previously reported influence of estrogen on hearing function, Delhez et al. [33] reported the relation between fluctuation in estrogen concentrations during the menstrual cycle and hearing sensitivity in women.

In our study, we hypothesize that the non-genomic pathway of estrogen effect in female cadets may be activated, given their brief exposure to intense noise. The non-genomic mechanism acts by binding to estrogen receptors (ER) in the cell membrane [36, 37], activating ionic channels and cytoplasmic kinases within seconds or minutes. Other authors established that in the inner ear of human adults, there are two types of ER – ERα in the spiral ganglion and ERβ in the stria vascularis [38], which highlights the importance of gender.

In addition to the hypothesis of better adaptation to acute exposure to noise in women, some authors found similar resilience in long-term exposure. A Norwegian study that investigated the influence of gender on hearing loss showed that men's hearing improved after cessation of occupational noise exposure, while that was not so significant in women [39, 40].

While no significant differences were observed between the left and right ear before the shooting, a significantly greater hearing impairment was found in men compared to women immediately after the shooting, specifically in the right ear at 4,000 Hz. The position of the firearm could not be associated with this finding, since the gun was at an equal distance from both ears while shooting (cadets fired while standing with arms outstretched). These findings are in accordance with a study that included as many as 95,000 industrial workers professionally exposed to noise, where it was determined that there is a more pronounced hearing loss in men, with greater sensitivity of the right ear [41]. Although our study focused on acute noise exposure, contrary to the study mentioned above, our results are partially in agreement with theirs, considering that in males we also found more expressed hearing impairment in the right ear. These results can be explained by the fact that the majority of our subjects were right-handed. Despite the pistol being positioned at an equal distance from both ears, military training involves various shooting exercises with different types of weapons, in diverse body positions, using stationary and mobile targets. This could serve as an explanation for the higher sensitivity of the right ear.

Regarding risk factors, although data remain limited, existing studies indicate that smoking is associated with an elevated risk of hearing loss, and that the risk increases the longer someone continues with this habit. It has been concluded that the risk may decrease over time after quitting smoking [42]. In our investigation, smoking was the most prevalent risk factor, reported by 19 male participants and 1 female. Alcohol consumption was the second most frequent, observed in 10 male participants, 6 of whom also reported smoking. Other risk factors, including prior noise exposure, infection, and heredity predispositions, were rarely recorded. In the entire study group (males and females together), we found a statistically significant difference in baseline levels of audiometry at 2,000 Hz between the group with risk factors and the group without. The presence of risk factors increases sensitivity to noise.

Other authors also found a similar correlation. In their research, Rocha et al. [43] analyzed audiometry values in two groups of military personnel: those with a history of noise exposure as a risk factor and a control group without prior exposure. They observed that the noise-exposed groups, regardless of age, showed changes in their high-frequency thresholds when compared to the control group.

Recent studies have identified a group of genes associated with susceptibility to NIHL, suggesting that genetic predisposition plays a role in its development, along with environmental factors. These genes influence processes such as oxidative stress progression, deoxyribonucleic acid damage repair, apoptosis, as well as the structure of the cilia themselves [44]. However, in our study, only two participants reported a positive family history of hearing loss, so our data are insufficient for research in that direction.

Among the female participants, risk factors were identified in only three individuals, each presenting with one of the following: smoking, hay fever, or previous noise exposure. However, results of audiometry at 2,000 Hz showed statistically significant correlation with the presence of risk factors

across all three time points. Such a result is somewhat unexpected and certainly deserves further research.

In this sense, it is important to monitor hearing at high frequencies using the conventional audiometry method for the early detection of noise-induced hearing loss.

Limitations of the study

In this study, we had to look at all risk factors cumulatively rather than individually, as we are dealing with a young, healthy population where the frequency of risk factors is low. Therefore, a significantly larger number of respondents should be included in subsequent research, so that we can evaluate the individual impact of risk factors. In addition, audiometry was performed immediately after shooting nearby the shooting theatre in a silent room. That is why the conditions were somewhat different compared to the first and third measurements.

Conclusion

The results of our research suggest that, in a population of young and healthy military personnel, acute noise exposure from gunfire leads to hearing impairment at 2,000 and 4,000 Hz, with full recovery observed after 24 hrs. Audiometric data indicated a higher hearing sensitivity to impulse noise in male subjects. They also showed that the presence of risk factors for hearing damage was associated with higher baseline hearing levels. Such results support the idea that the harmful effects of high-intensity acute impulse noise cannot be completely avoided, despite the regular use of earplugs, and that hearing protection in military service should be improved in the future. In this way, the cumulative harmful effects of noise during mandatory periodic shooting exercises should be minimized, both during cadet training and later during military service.

Acknowledgements

This study was supported by a Grant from the Ministry of Defence of the Republic of Serbia (Project No. MFVMA 01/20-22).

Conflict of interest

The authors declare no conflict of interest.

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Received on April 2, 2025
Revised on April 29, 2025
Revised on May 26, 2025
Accepted on May 28, 2025
Online First July 2025

ORIGINAL ARTICLE

UDC: 004:61]:616.348/.349-002-07-037-08
DOI: https://doi.org/10.2298/VSP250131049S

Observational study on the potential mechanism of SiNi powder in the treatment of ulcerative colitis based on network pharmacology and machine learning

Opservaciona studija o potencijalnom mehanizmu delovanja SiNi praha u lečenju ulceroznog kolitisa zasnovana na farmakologiji mreže i mašinskom učenju

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Abstract

Background/ Aim. Chronic idiopathic ulcerative colitis (UC) damages and disrupts the intestinal mucosa. Diagnosing UC and differential diagnosis is tough. Its anti-inflammatory and immunosuppressive properties make SiNi powder (SNP) a popular treatment for inflammatory illnesses. The multi-target mechanism of SNP on UC is unknown. The aim of this study was to examine the potential mechanisms of SNP in UC treatment through network pharmacology and machine learning approaches, identify novel diagnostic biomarkers, and develop a predictive model for UC diagnosis. **Methods.** The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) assessed active constituents and target proteins. Using two public datasets (GSE87473 and GSE75214), differential analysis was conducted on the gene expression matrix of UC to find the intersection of differentially expressed genes and SNP-related targets. Hub genes were assessed using several machine-learning algorithms to create a prediction model. Single-cell analysis studies were used to diagnose genes and immune cells. NetworkAnalyst predicted upstream transcription factors, micro-ribonucleic acids, and the protein-compound network. **Results.** According to the TCMSP database, the SNP included 95 active constituents and 795 associated targets against UC. After identifying 79 overlapping genes, machine learning discovered five hub genes: *TRPV1*, *ABCG2*, *BACE2*, *MMP3*, and *LIPC*. Diagnostics were verified using external datasets. These genes were used to create a predictive model with a large area under the curve (AUC = 1,000) and an external validation dataset with 1,000 AUCs, demonstrating excellent accuracy of the predictive model and the hub genes. **Conclusion.** SNP and UC are associated, and hub genes were found to evaluate UC risk. This computational technique opens new avenues for UC biomarker and therapeutic target research, although further experimental validation is required to confirm and validate these results.

Key words:
colitis, ulcerative; medicine, chinese traditional; gene expression; network pharmacology; treatment outcome.

Apstrakt

Uvod/Cilj. Hronični idiopatski ulcerozni kolitis (UK) oštećuje i remeti crevnu mukozu. Dijagnostikovanje UK-a i uspostavljanje diferencijalne dijagnoze je teško. Antiinflamacijska i imunosupresivna svojstva SiNi praha (SNP) čine ga popularnim tretmanom za inflamacijske bolesti. Višestruki mehanizam delovanja SNP-a u lečenju UK-a nije poznat. Cilj rada bio je da se ispitaju potencijalni mehanizmi delovanja SNP-a u lečenju UK-a primenom pristupa farmakologije mreže i mašinskog učenja, identifikuju novi dijagnostički biomarkeri i razvije prediktivni model za dijagnozu UK-a. **Metode.** Baza podataka i platforma za analizu sistemske tradicionalne kineske medicine (*Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform* – TCMSP) procenila je aktivne sastojke i ciljne proteine. Korišćenjem dva javno dostupna skupa podataka (GSE87473 i GSE75214), sprovedena je diferencijalna analiza matrice ekspresije gena kod UK-a u cilju pronalaženja preklapanja diferencijalno ispoljenih gena i meta delovanja povezanih sa SNP-om. *Hub* geni procenjeni su korišćenjem nekoliko algoritama mašinskog učenja da bi se kreirao model predikcije. Studije analize na nivou pojedinačnih ćelija korišćene su za identifikaciju gena i imunskih ćelija. *NetworkAnalyst* je predvideo ushodne transkripcione faktore,

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mikro-ribonukleinske kiseline i mrežu protein-jedinjenja. **Rezultati.** Prema TCMSP bazi podataka, SNP je uključivao 95 aktivnih sastojaka i 795 povezanih meta delovanja protiv UK-a. Nakon identifikovanja 79 gena koji se preklapaju, mašinsko učenje je otkrilo pet *hub* gena: *TRPV1*, *ABCG2*, *BACE2*, *MMP3* i *LIPC*. Provera dijagnostike je izvršena korišćenjem eksternih skupova podataka. Ovi geni su korišćeni za kreiranje prediktivnog modela sa velikom površinom ispod krive [*area under the curve* (AUC) = 1.000] i skupa podataka za eksternu validaciju sa 1.000 AUC, demonstrirajući odličnu tačnost prediktivnog modela i *hub* gena. **Zaključak.** SNP i UK su povezani, a utvrđeni su *hub* geni za procenu rizika od UK-a. Ova računarska tehnika otvara nove puteve za istraživanje biomarkera UK-a i ciljeva terapijskog delovanja, iako je potrebna dalja eksperimentalna provera da bi se potvrdili i validirali ovi rezultati.

Ključne reči:
kolitis, ulcerozni; medicina, kineska, tradicionalna; geni, ekspresija; farmakologija, mrežna; lečenje, ishod.

Introduction

Inflammatory bowel disease (IBD) is a persistent and relapsing inflammatory disorder of unknown etiology that impacts the gastrointestinal system. Ulcerative colitis (UC), a subtype of IBD, is defined by persistent inflammation of the colonic mucosa. Prevalent symptoms include diarrhea, stomach discomfort, the excretion of mucus and blood, and weight loss [1]. In addition to intestinal symptoms, UC often presents with extraintestinal manifestations that impact the liver and bile ducts, such as fatty liver and primary sclerosing cholangitis [2, 3]. The pathogenesis of UC remains incompletely elucidated; however, it is acknowledged as a complex IBD. The worldwide incidence and prevalence of UC are increasing rapidly, presenting a considerable threat to global public health. Despite advances in treatment options, effectively managing UC remains a significant challenge, particularly in achieving consistent clinical remission and preventing disease progression [4].

Traditional Chinese medicine (TCM) has promising advantages in UC treatment [5, 6]. Conventional Chinese medicine primarily modulates inflammatory cytokines, intestinal microbiota, and the immune mechanism while also protecting the intestinal mucosa [7]. Hence, it can play a role in treating UC. SiNi powder (SNP), a classical Chinese medicine formula, has demonstrated effectiveness and is reported to cause fewer adverse reactions in the treatment of inflammatory and bowel diseases [5, 8, 9]. Each component of SNP – *Radix Bupleuri, Aurantii Fructus Immaturus, Radix Paeoniae Alba,* and *Licorice* – may contribute distinctly to its therapeutic effects. Nonetheless, the exact mechanism by which SNP and its constituent components exert therapeutic benefits in UC has yet to be completely clarified. Network pharmacology, using public databases and accessible data, is an innovative, promising, and cost-effective method for identifying bioactive constituents, predicting drug action targets, and examining drug action processes through the lens of biological network equilibrium [10]. Moreover, in contrast to experimental medication approaches, network pharmacology prioritizes the multifaceted control of signaling pathways, making it particularly suitable for elucidating the mechanisms of conventional Chinese medicine, which has several chemical constituents and molecular targets [11].

In this study, we aimed to identify active constituents and their metabolites from selected medications using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), based on oral bioavailability (OB) and drug similarity index. Then, the SNP-related differentially expressed genes (DEGs) of UC were screened by differential expression analyses using carefully selected public datasets. Subsequently, various machine learning algorithms were applied to identify key genes and develop a prediction model. The performance of the prediction model was validated using a nomogram and an external dataset. Finally, regulatory networks for transcription factor (TF) and micro-ribonucleic acid (RNA) – miRNA were built, along with a protein-compound network of hub genes. This will help future research on how these hub genes are controlled. While this computational approach provides valuable insights, experimental validation was still required to confirm the findings and assess their therapeutic relevance.

The aim of this study was to examine the potential mechanisms of SNP in UC treatment through a comprehensive bioinformatics approach that combines network pharmacology and machine learning, to identify and validate novel diagnostic biomarkers for UC, and to develop a robust prediction model for UC diagnosis.

Methods

Assessment of active constituents and target proteins

Chai Hu (CH, *Radix Bupleuri*), Zhishi (ZS, *Aurantii Fructus Immaturus*), Bai Shao (BS, *Paeoniae Radix Alba*), and Gan Cao (GC, *Licorice*) are included in SNP. The active constituents of these Chinese medications were identified using TCMSP (http://tcmspw.com/tcmsp) [10]. OB denotes the rate and extent of a drug's absorption into the systemic circulation. Drug-like (DL) features refer to the characteristics of a drug that has a certain functional category or exhibits analogous physical attributes. The drug half-life indicates the concentration of the medication in the bloodstream or body and is a crucial measure for determining the dosing interval, the delivered dosage, and drug accumulation [5]. The compounds exhibiting elevated action were further evaluated under the criteria of OB > 30%, DL > 0.18, Caco-2 permeability > -0.4, and half-life > 3 hrs, as previously documented [11]. The targeted proteins for each molecule were retrieved from the TCMSP database and standardized to a uniform gene nomenclature *via* the Universal Protein Resource (UniProt) protein database (http://www.uniprot.org/uploadlists/).

Collection and analysis of ulcerative colitis-related targets

To identify disease-specific targets, the GeneCards® database (https://www.genecards.org/) was used. It is a comprehensive platform that integrates information about all known human genes, including their roles in genome organization, protein expression, transcriptional regulation, inheritance patterns, and biological functions [12]. The database was queried specifically for UC-related targets using a systematic search strategy that incorporated multiple UC-associated keywords and synonyms to ensure comprehensive coverage of disease-relevant genes. The selection of UC-related targets followed a stringent filtering process based on relevance scores provided by the GeneCards® database. To increase the reliability of these results, emphasis should be placed on targets that have strong experimental evidence linking them to the development of UC. This approach allowed us to establish a high-confidence set of disease-specific targets for subsequent analysis. To identify potentially therapeutic targets of SNP in UC treatment, a systematic intersection analysis was performed between the UC-related targets and the previously identified SNP targets. This analysis was conducted using Venny 2.1.0, a precise tool for comparing multiple gene sets and identifying overlapping elements. The intersection analysis revealed targets that are both disease-relevant and potentially modulated by SNP components, suggesting possible therapeutic mechanisms. To visualize and analyze the complex relationships between the overlapping targets and their corresponding bioactive compounds, we employed Cytoscape 3.7.1. This powerful network analysis tool enabled us to construct and analyze interaction networks that illuminate the potential mechanisms of SNP in UC treatment. The visualization included both direct interactions and secondary connections, providing a comprehensive view of the potential therapeutic pathways.

Gene expression profiles and dataset selection

Our study utilized carefully selected public datasets from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). After a comprehensive review of available UC-related datasets, two bulk gene expression datasets were selected based on their sample size, data quality, and clinical annotation completeness: GSE87473 (106 UC, 21 normal) and GSE75214 (97 UC, 11 normal). These datasets were chosen specifically because they represent diverse patient populations and provide sufficient statistical power for robust analysis. The GSE87473 dataset was generated using the GPL13158 platform (HT_HG-U133_Plus_PM) Affymetrix HT HG-U133+ PM Array Plate, which offers comprehensive coverage of the human transcriptome. The GSE75214 dataset, based on the GPL6244 platform (HuGene-1_0-st) Affymetrix Human Gene 1.0 ST Array [transcript (gene) version], provides complementary data with different technical specifications, allowing for cross-platform validation of these findings.

Data preprocessing followed a rigorous protocol using the R package "GEOquery". The quality control process included several critical steps, as follows: a) probes successfully annotated with gene symbols; b) probes removed due to lacking gene symbols; c) probes removed because they matched multiple symbols; d) the number of unique gene symbols remains after resolving duplicates by selecting the maximum expression value.

To enhance the understanding of cellular heterogeneity in UC, single-cell RNA sequencing data were incorporated from GSE214695, which includes detailed transcriptional profiles from six UC rectum samples. This dataset provides crucial insights into cell-type-specific gene expression patterns and their potential roles in disease progression.

The integration of both bulk and single-cell RNA sequencing data provides complementary perspectives on UC pathogenesis, allowing for a more comprehensive analysis of disease mechanisms and potential therapeutic targets. While these publicly available datasets have inherent limitations, careful selection and preparation techniques improve the reliability of the results.

Differentially expressed gene analysis and functional analysis

Differential gene expression analysis was performed using the R Package Linear Models for Microarray Data (LIMMA), which employs robust linear modeling and empirical Bayes methods particularly suited for microarray data analysis. To ensure statistical rigor while maintaining biological relevance, a dual-threshold approach was implemented for identifying DEGs between UC and healthy controls in the GSE87473 dataset. Genes were considered significantly differentially expressed when meeting both statistical and biological significance criteria: FDR < 0.05 to control for multiple testing and an absolute $\log_2$ fold change ($|\log_2 FC| > 1$ to ensure biological meaningfulness of the expression differences. For a comprehensive functional interpretation of SNP-related DEGs, pathway and functional enrichment analyses were conducted using the Metascape database. This platform was selected for its integration of multiple authoritative databases, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and BioCarta. The enrichment analysis encompassed three major GO categories: biological process (BP), cellular component, and molecular function, alongside KEGG pathway analysis. Statistical significance was determined using an adjusted *p*-value threshold of 0.05, with correction for multiple testing using the Benjamini-Hochberg method. To ensure the robustness of the findings, functional categories and pathways were focused on at least three genes and required a minimum overlap of 10% between the gene set and each functional category. This comprehensive analytical approach allowed us to identify both statistically significant and biologically relevant gene expression changes while providing insights into the

functional implications of SNP-related genes in UC pathogenesis.

Screening and validation of diagnostic markers

A multi-algorithm machine learning approach was employed to identify robust diagnostic biomarkers for UC, incorporating three complementary methodologies to minimize algorithm-specific biases and enhance the reliability of the findings. The first approach utilized Random Forests, implemented through the "randomForest" R package, which can capture non-linear relationships to achieve optimal performance. Key parameters were tuned, including the number of trees (ntree) and the number of variables sampled at each split (mtry), allowing for the modeling of complex interactions between genes. The importance of each feature was assessed using both the mean decrease in accuracy and the mean decrease in the Gini index. The second method employed the Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression, implemented using the "glmnet" R package. This approach is particularly effective for handling high-dimensional data while preventing overfitting through L1 regularization. The optimal regularization parameter lambda (λ) was determined through 10-fold cross-validation, selecting the value that minimized partial likelihood deviance. The minimal λ value was specifically chosen to balance between model parsimony and predictive accuracy. The third approach utilized Support Vector Machine-Recursive Feature Elimination (SVM-RFE), which iteratively removes features based on their contribution to the classification boundary. The algorithm was implemented with a linear kernel and performed recursive feature elimination within a nested cross-validation framework to prevent selection bias. To ensure robust biomarker selection, attention was focused on genes that were consistently identified as significant across all three algorithms. This conservative approach helps mitigate algorithm-specific biases and increases the likelihood of identifying genuinely important biological signals rather than technical artifacts. For external validation, the independent dataset GSE75214 was utilized, which represents a different patient cohort and technical platform. The performance of the selected biomarkers was evaluated through multiple metrics: a) receiver operating characteristic (ROC) curves and corresponding area under the curve (AUC) values to assess discriminative ability; b) calibration curves to evaluate the agreement between predicted and observed probabilities; c) decision curve analysis to assess clinical utility across different threshold probabilities. Additionally, sensitivity analyses were performed to evaluate the robustness of the findings to various analytical choices, including different cross-validation schemes and parameter settings. The final nomogram was constructed based on the validated biomarkers and underwent rigorous calibration assessment. This comprehensive validation strategy addresses potential overfitting concerns and provides strong evidence for the generalizability of the findings across different patient populations and technical platforms.

Single-cell RNA sequencing analysis

To investigate cell-type-specific gene expression patterns in UC, comprehensive single-cell RNA sequencing analyses were performed using the Seurat R package version 4.0.2 (PMID: 33835452). The analysis pipeline incorporated stringent quality control measures to ensure data reliability while maximizing biological signal retention. Quality control thresholds were established based on the distribution of key technical metrics across all cells. Three-tier filtering approach was implemented: a) cell filtering based on RNA content (200–5,000 total RNAs) to exclude potential doublets (high RNA count) and empty droplets or dying cells (low RNA count); b) mitochondrial content filtering [excluding cells with > 10% mitochondrial unique molecular identifier (UMI) rate] to remove dying or stressed cells; c) gene filtering to remove mitochondrial genes, which can introduce technical artifacts due to variable cell stress levels.

Following quality control, data normalization was performed using the "NormalizeData" function in Seurat, employing a global-scaling normalization method that normalizes gene expression measurements for each cell by total expression and log-transforms the result. This approach accounts for differences in sequencing depth between cells while preserving biological variation. For dimensionality reduction, highly variable genes were identified using a variance-stabilizing transformation approach, selecting the top 2,000 genes that exhibited high cell-to-cell variation. Principal Component Analysis (PCA) was then performed on the scaled data of these variable genes. The optimal number of principal components (top 15) for downstream analysis was determined through multiple methods, including elbow plots and jackstraw analysis. Cell clustering was performed using the Uniform Manifold Approximation and Projection algorithm, which maintains both the local and global structure of the high-dimensional data. To ensure robust cluster identification, multiple resolution parameters and assessed cluster stability were employed using the Silhouette coefficient. Cell type annotation was conducted using the SingleR R package (PMID: 30643263), which leverages reference transcriptomic datasets to automatically annotate cell types. Manual curation of established cell-type-specific markers validated these annotations. Gene expression patterns were visualized using the "featureplot" function, with expression levels normalized and scaled for optimal visualization. This comprehensive single-cell analysis approach provides detailed insights into the cellular heterogeneity of UC tissue and the cell-type-specific expression patterns of the identified biomarkers.

Regulatory network construction and analysis

To elucidate the complex regulatory mechanisms underlying UC pathogenesis and the therapeutic effects of SNP, multiple regulatory networks were constructed using NetworkAnalyst 3.0 [(https://www.networkanalyst.ca/), (PMID:30931480)]. This comprehensive analysis encompassed three distinct but interconnected regulatory layers, each explored in detail below.

First, TF interactions with the characteristic genes were analyzed. TFs, as key regulators of gene expression, can bind to specific DNA sequences and modulate transcription. The analysis incorporated both direct TF-gene interactions and indirect regulatory relationships through intermediate molecules. To ensure biological relevance, interactions were filtered based on experimental evidence from curated databases. Second, miRNA-mediated regulation was investigated by constructing a diagnostic marker – miRNA coregulatory network. The miRNAs, as endogenous short non-coding RNAs, play crucial roles in post-transcriptional regulation through mRNA degradation or translation inhibition. Experimentally validated miRNA-target interactions were integrated from multiple databases to build a comprehensive regulatory network. Third, protein-drug interactions were examined to understand potential therapeutic mechanisms. The characteristic genes were analyzed in the context of known drug-target interactions, generating a diagnostic marker – drug/chemical coregulatory network. This analysis helps identify potential therapeutic compounds and predict drug sensitivity based on structural features.

Statistical analysis and data processing

All computational analyses were performed using the R programming language, version 4.1.1 (https://www.r-project.org/). A comprehensive statistical framework was implemented to ensure robust and reproducible results.

Group comparisons were conducted using non-parametric Wilcoxon tests, following an assessment of data distributions for normality. Results are presented as mean ± standard deviation, with statistical significance interpreted through multiple thresholds: not significant ($p \geq 0.05$); significant (*) ($p < 0.05$); highly significant (**) ($p < 0.01$); very highly significant (***) ($p < 0.001$); extremely significant (****) ($p < 0.0001$). For correlation analyses, Pearson correlation coefficients were calculated to assess relationships between continuous variables. The choice of Pearson correlation was based on preliminary assessments of linear relationships and data distributions. Statistical significance for correlations was set at $p < 0.05$. To control multiple testing where applicable, the Benjamini-Hochberg false discovery rate (FDR) was employed for correction. Effect sizes were calculated alongside *p*-values to provide a complete picture of the biological significance of the findings. All statistical tests were two-sided, and confidence intervals were set at 95%. Data visualization was performed using ggplot2 and other specialized R packages, with consistency in color schemes and plotting parameters maintained throughout the analysis.

Results

Identification and characterization of SNP active constituents and their targets

Through systematic analysis of the TCMSP database using multiple pharmacological parameters (OB, DL, Caco-2 permeability, and half-life), 95 active ingredients were identified in SNP that met the stringent selection criteria. These active ingredients were associated with 795 potential molecular targets, providing a comprehensive framework for understanding the therapeutic mechanisms of SNP. To identify disease-relevant targets, differential expression analysis was performed between UC and normal samples using the GSE87473 dataset. This analysis revealed 79 significantly DEGs that overlapped with SNP targets, suggesting potential therapeutic mechanisms. The differential expression patterns of these genes are visualized in a volcano plot (Figure 1A) and heatmap (Figure 1B), demonstrating a clear separation between UC and normal samples. To understand the contribution of individual components, the relationships were mapped between the four primary constituents of SNP (*Radix Bupleuri*, *Aurantii Fructus Immaturus*, *Radix Alba*, and *Licorice*) and their respective targets using Cytoscape visualization (Figure 1C). The expression profiles of all 79 SNP-related DEGs in GSE87473 are detailed in Figure 1D, revealing distinct patterns of regulation in UC compared to normal tissue. To elucidate the functional relationships among these targets, a protein-protein interaction network was constructed (Figure 1E). This network analysis revealed several highly connected nodes, suggesting key regulatory hubs in SNP's mechanism of action. The network structure indicates potential synergistic effects between different components of SNP in modulating UC-related pathways. Functional enrichment analysis using GO and KEGG pathways provided insights into the BPs affected by SNP-related DEGs. Key enriched pathways included regulation of inflammatory response, suggesting direct relevance to UC pathogenesis. Additional enriched processes encompassed circulatory system regulation, hormone response pathways, nutrient sensing, and smooth muscle cell proliferation (Figure 1F–G). These findings indicate that SNP may act through multiple complementary mechanisms to ameliorate UC symptoms.

This comprehensive analysis reveals that SNP's therapeutic effects likely arise from the coordinated action of multiple active ingredients targeting various disease-relevant pathways. The identification of specific molecular targets and pathways provides a foundation for understanding SNP's mechanism of action in UC treatment.

To identify robust diagnostic biomarkers for UC, a comprehensive machine-learning strategy was implemented, incorporating complementary algorithms. The SVM-RFE algorithm initially identified 50 candidate genes with potential diagnostic values (Figure 2A). In parallel, LASSO regression analysis, which excels at handling high-dimensional data while preventing overfitting, identified seven genes with strong predictive capabilities (Figure 2B–C).

Through intersection analysis of these independently identified gene sets, five robust core biomarkers were discovered: *TRPV1*, *ABCG2*, *BACE2*, *MMP3*, and *LIPC* (Figure 2D). Each of these genes demonstrated strong biological relevance to UC pathogenesis.

The diagnostic potential of these biomarkers was rigorously evaluated using ROC curve analysis. Each biomarker demonstrated strong discriminative ability with AUC values

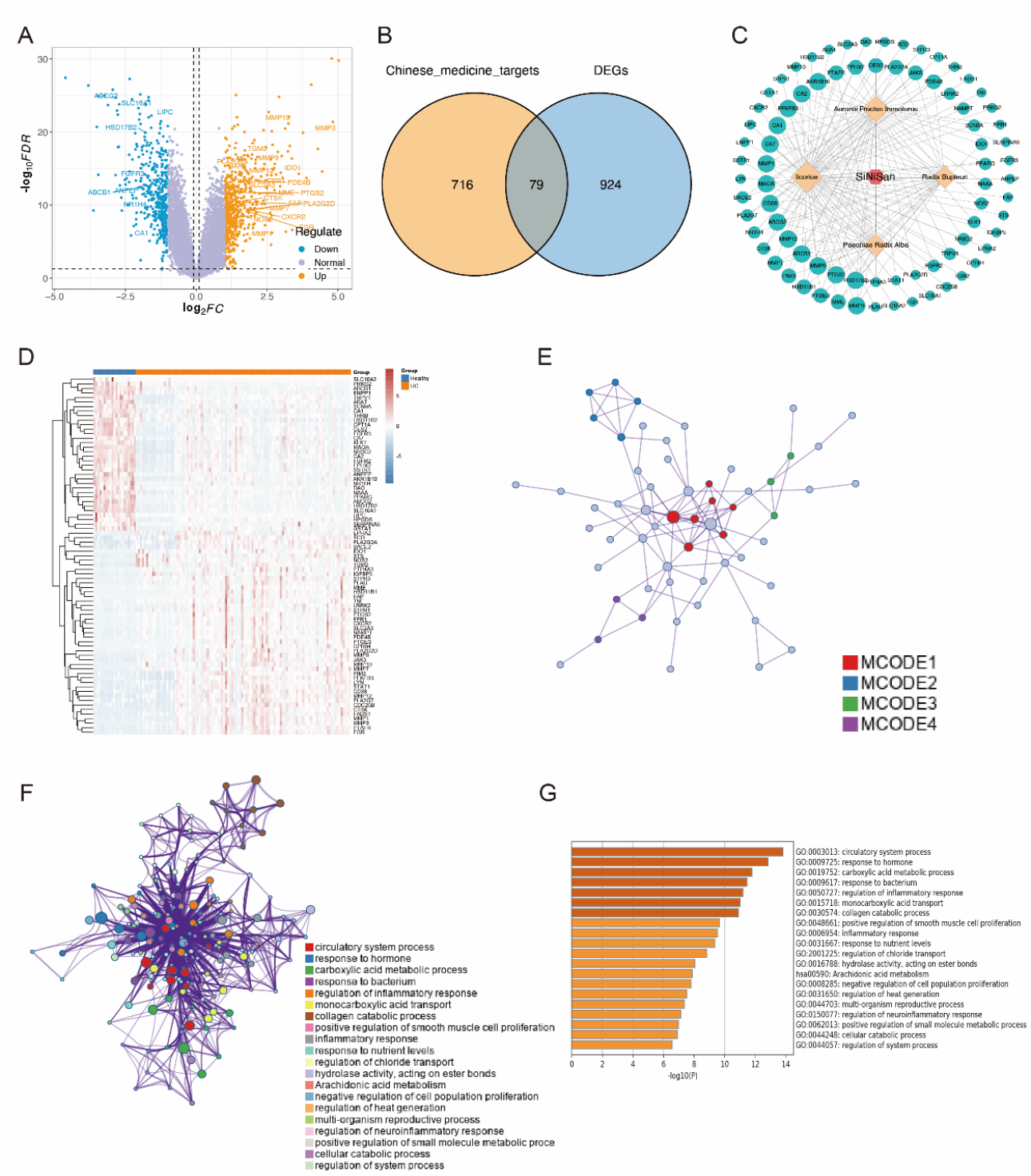

Fig. 1 – Identification and validation of hub genes using machine learning approaches: A) volcano plot displaying differentially expressed genes (DEGs) identified between ulcerative colitis (UC) and normal colon samples within the training dataset (GSE87473); B) intersection of Chinese medicine targets and DEGs; C) compound-target network illustrates the relationship between the four constituent herbs of SNP and the 79 overlapping genes network constructed using Cytoscape 3.7.1.; D) heatmap displaying the expression profiles of all 79 overlapping SNP-related DEGs across UC and normal samples from the GSE87473 dataset; E) protein-protein interaction network constructed for the 79 overlapping genes using the STRING database *via* Metascape and visualized in Cytoscape; F) bar chart illustrating the results of Gene Ontology Biological Process (BP) enrichment analysis for the 79 overlapping genes, performed using Metascape; G) bar chart illustrates the results of Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for the 79 overlapping genes, performed using Metascape.
-log$_{10}$ FDR – negative logarithm base 10 of the false discovery rate; log$_2$ FC – logarithm base 2 fold change; MCODE – Molecular Complex Desection; log$_{10}$ (*p*) – negative base-10 logarithm of a *p*-value; STRING – Search Tool for the Retrieval of Interacting Genes/Proteins.

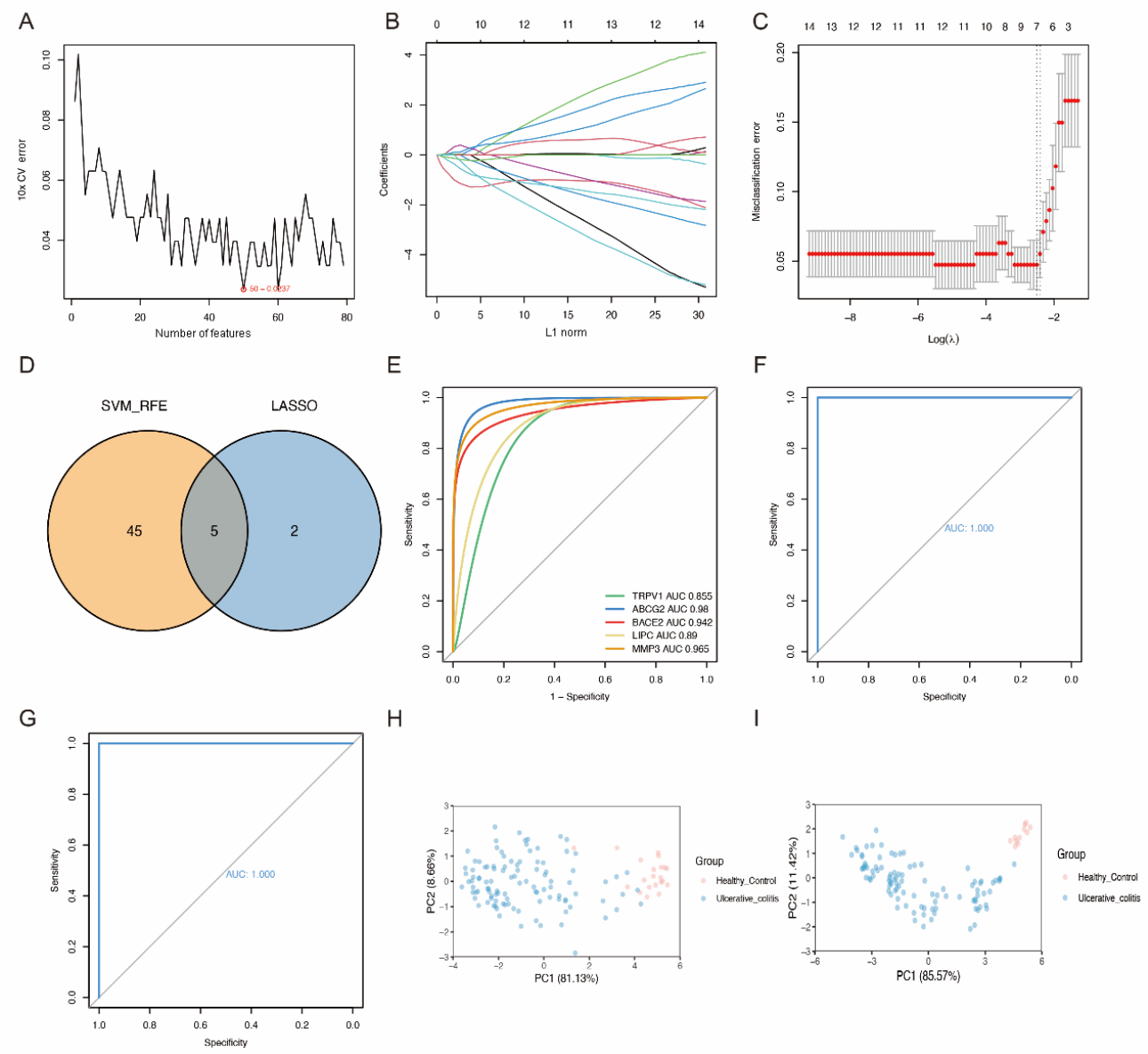

Fig. 2 – Identification and interaction network analysis of candidate diagnostic biomarkers: A) plot showing the cross-validation error curve as a function of the number of features considered during Support Vector Machine-Recursive Feature Elimination (SVM-RFE) analysis applied to the 79 overlapping genes in the training dataset (GSE87473); B) Least Absolute Shrinkage and Selection Operator (LASSO) regression coefficient profiles for the 79 overlapping genes in the training dataset (GSE87473). Each colored line represents the coefficient path for a single gene as the regularization parameter lambda (λ) changes; C) plot depicting the selection of the optimal tuning parameter in the LASSO model using 10-fold cross-validation. The λ value corresponding to the minimum partial likelihood deviances typically chosen; D) Venn diagram illustrates the intersection of candidate genes identified by LASSO regression (seven genes) and SVM-RFE (refined list). The overlap yields the final set of five robust core hub genes: *TRPV1*, *ABCG2*, *BACE2*, *MMP3*, and *LIPC*; E) ROC curves evaluating the diagnostic performance of each of the five individual hub genes in distinguishing UC from normal samples in the training dataset (GSE87473). The AUC value is provided for each gene, indicating its individual discriminative ability; F) ROC curve evaluating the performance of the diagnostic model built using the combined expression of the five hub genes in the training dataset; G) ROC curve evaluating the performance of the five-gene diagnostic model in the independent external validation dataset (GSE75214); H) Principal Component Analysis (PCA) plot based on the expression levels of the five hub genes in the training dataset (GSE87473). Blue dots represent normal samples, and red dots represent UC samples, demonstrating clear separation between the groups based on these genes; I) PCA plot based on the expression levels of the five hub genes in the validation dataset (GSE75214).

10 × CV error – 10-fold cross-validation error; L1 norm – LASSO regression normal; log – logarithm; AUC – area under the curve; ROC – receiver operating characteristic; PC – principal component; UC – ulcerative colitis.

exceeding 0.85 (Figure 2E), suggesting high sensitivity and specificity for UC detection. To enhance clinical applicability, a diagnostic nomogram was developed using the Rms package, which integrates the expression patterns of all five biomarkers.

Crucially, these findings were validated using an independent dataset. The diagnostic model maintained exceptional performance in both the training cohort (GSE87473) and the validation cohort (GSE75214), achieving perfect discrimination with AUC values of 1,000 in both datasets (Figures 2F–G). This remarkable consistency across different patient populations supports the robustness of the biomarker selection.

PCA further confirmed the diagnostic value of these biomarkers, revealing distinct clustering patterns between UC and normal samples in both GSE87473 and GSE75214 datasets (Figure 2H–I). This clear separation in gene expression patterns provides additional evidence for the biological relevance and diagnostic utility of the identified biomarkers.

These results demonstrate not only the statistical robustness of the machine-learning approach but also the biological significance of the identified biomarkers. The consistent performance across multiple validation steps suggests potential clinical utility for UC diagnosis and monitoring. However, further experimental validation was recognized as valuable for confirming the mechanistic roles of these biomarkers in UC pathogenesis.

To elucidate the complex regulatory mechanisms governing the candidate diagnostic biomarkers, comprehensive molecular interaction networks were constructed and analyzed. This multi-layer analysis encompassed miRNA regulation, TF control, and drug-target interactions, providing insights into potential therapeutic interventions.

Analysis of miRNA-mediated regulation revealed a complex regulatory network comprising 193 miRNAs interacting with the five potential biomarkers (Figure 3A). Of particular interest, three miRNAs emerged as potential master regulators: hsa-mir-1-3p, hsa-let-7b-5p, and hsa-mir-124-3p, each demonstrating the capacity to modulate multiple candidate diagnostic genes simultaneously. These findings suggest a coordinated post-transcriptional regulatory mechanism that may be crucial in UC pathogenesis.

TF analysis identified 32 TFs involved in regulating the candidate diagnostic genes (Figure 3B). Among these, GATA2 emerged as a particularly significant regulator, demonstrating potential simultaneous control over four of the five biomarkers: MMP3, TRPV1, ABCG2, and BACE2. This finding suggests that GATA2 might serve as a master regulator in the transcriptional control of UC-related genes and could represent a potential therapeutic target.

To explore potential therapeutic implications, drug-gene interactions were analyzed using the NetworkAnalyst database. This analysis revealed a comprehensive interaction network involving 369 potential target drugs/compounds and the five biomarkers (Figure 3C). Several compounds demonstrate notable interaction patterns with multiple biomarkers, including: a) benzo(a)pyrene, known for its effects on inflammatory pathways; b) cyclosporine, an established immunomodulator; c) estradiol, important in inflammatory response regulation; d) quercetin, a flavonoid with anti-inflammatory properties.

The interactions of these compounds with multiple biomarkers suggest potential therapeutic mechanisms, possibly explaining some of the observed clinical effects in UC treatment. The extensive network of drug-gene interactions provides a valuable resource for drug repurposing efforts and the development of novel therapeutic strategies.

The identification of these regulatory networks and potential therapeutic compounds offers new insights into UC pathogenesis and treatment. However, experimental validation would be necessary to confirm the functional significance of these regulatory relationships and the therapeutic potential of the identified compounds.

To understand the cellular context of the identified biomarkers, a comprehensive single-cell RNA sequencing analysis was performed using the UC dataset GSE214695.

Following quality control and data integration, 28,262 cells were analyzed from UC tissue samples. Stringent quality control measures were applied to ensure data reliability, including the removal of cells with aberrant RNA content (< 200 or > 5,000 total RNAs) and high mitochondrial content (> 10% UMI rate), which could indicate cellular stress or technical artifacts. Dimensionality reduction and clustering analysis, based on the top 2,000 highly variable genes and first 15 principal components, revealed 10 distinct major cell populations in the UC microenvironment (Figure 4A): a) adaptive immune system cells (B cells, $CD4^+$ T cells, $CD8^+$ T cells); b) innate immune system cells (monocytes, natural killer cells, dendritic cells, neutrophils, macrophages); c) structural cells (fibroblasts, epithelial cells).

Cell-cell interaction analysis revealed complex communication networks within the UC microenvironment (Figure 4B–C). B cells and epithelial cells emerged as major cellular hubs, suggesting their central role in disease pathogenesis. The interaction strength analysis highlighted particularly strong connections between immune system cells and epithelial cells, indicating active immune-epithelial crosstalk in UC tissue. Expression analysis of these diagnostic biomarkers revealed distinct cell-type-specific patterns (Figure 4D): a) *ABCG2* and *BACE2* showed the highest expression in epithelial cells, suggesting their involvement in epithelial barrier function and homeostasis; b) *MMP3* was predominantly expressed in fibroblasts, consistent with its role in tissue remodeling and extracellular matrix modification.

The distribution of diagnostic biomarkers across various cell types (Figure 4E) provides crucial insights into their functional roles in UC pathogenesis. This cell-type-specific expression pattern suggests that the identified biomarkers may be involved in multiple aspects of UC pathophysiology, from epithelial barrier dysfunction to immune response regulation.

These single-cell findings not only validate the biomarker selection but also provide important context for understanding their roles in UC pathogenesis. The cell-type-specific expression patterns suggest potential cellular mechanisms through which SNP might exert its therapeutic effects.

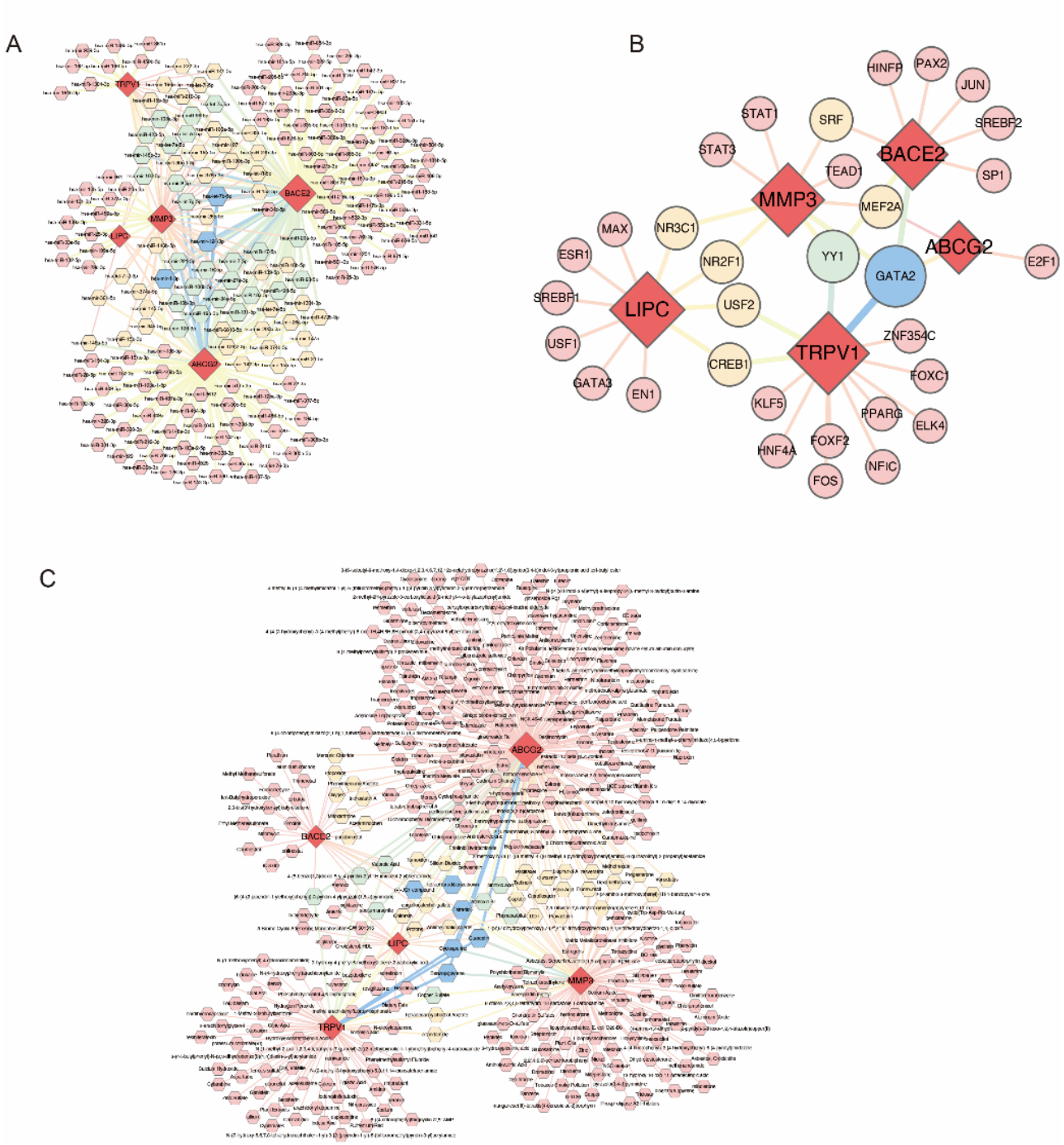

Fig. 3 – Cell-type-specific expression analysis using single-cell transcriptomics: A) predicted micro-ribonucleic acid (miRNA)-hub gene regulatory network. Larger nodes represent the five hub genes, while smaller nodes represent the 193 potentially interacting miRNAs identified from databases. Edges indicate predicted miRNA-target gene interactions; B) predicted transcription factor (TF)-hub gene regulatory network. Larger nodes represent the five hub genes, while smaller nodes represent the 32 potentially interacting TFs identified from databases such as JASPAR. Edges indicate predicted regulatory relationships (e.g., TF binding to promoter regions); C) predicted drug/compound-hub gene interaction network. Larger nodes represent the five hub genes, while smaller nodes represent 369 potentially interacting drugs or chemical compounds identified from databases such as DrugBank. Edges indicate known or predicted interactions between the compounds and the hub gene proteins.

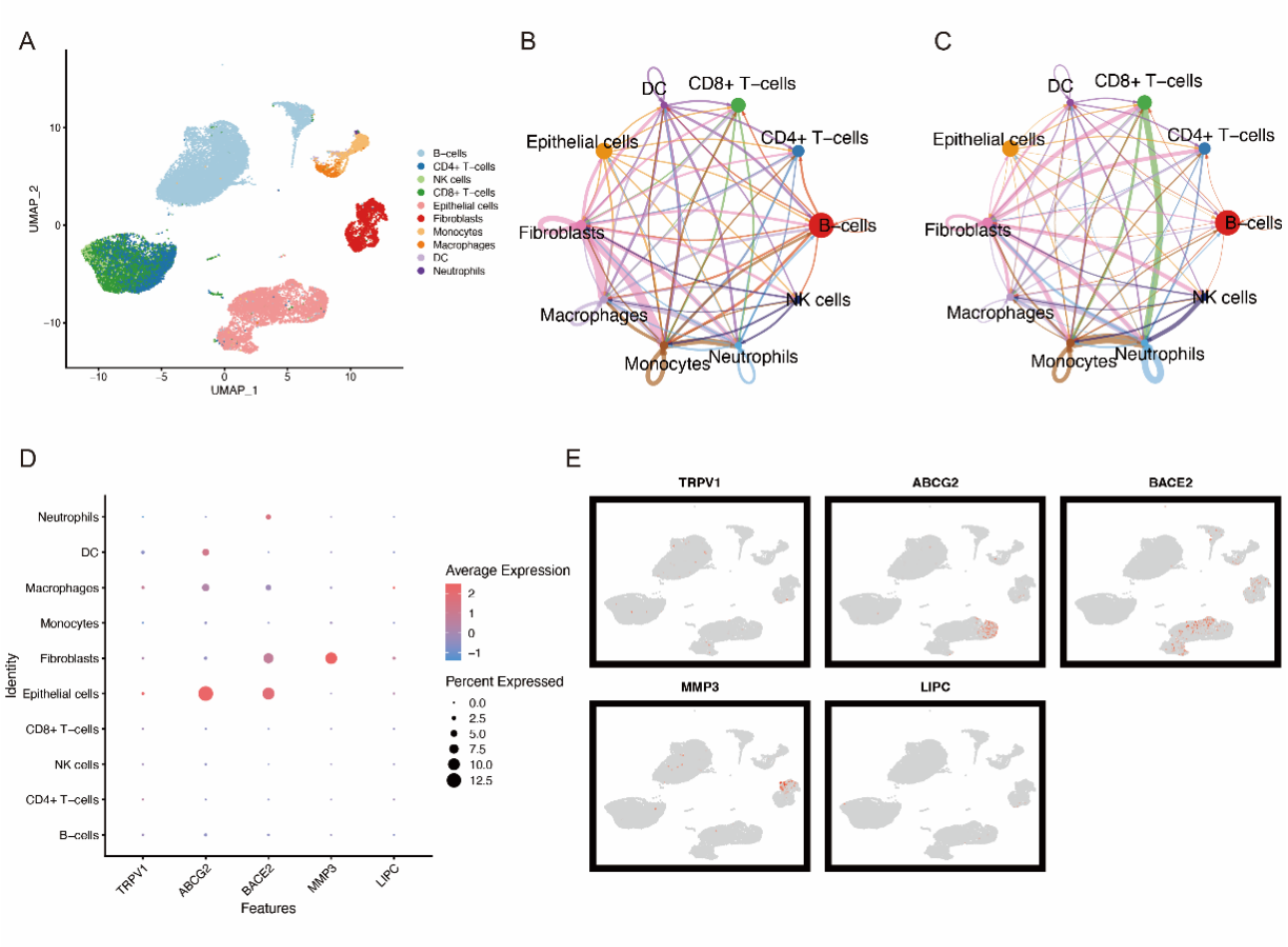

Fig. 4 – Single-cell transcriptome analysis of ulcerative colitis (UC)-related diagnostic biomarkers and cell-type interactions: A) Uniform Manifold Approximation and Projection (UMAP) plot visualizing the cellular landscape of UC rectum tissue. Each point represents a single cell, colored according to its assigned cell type identity based on marker gene expression; B) circle plots summarize the overall predicted cell-cell communication network among the identified cell types. The thickness or color intensity of lines connecting cell types indicates the relative strength or number of inferred interactions; C) network plot detailing the strength of predicted interactions for specific signaling pathways or ligand-receptor pairs between different cell types; D) dot plots show the expression levels of individual hub genes; E) UMAP plots summarize the normalized expression distribution of all five hub genes across the major annotated cell types.
NK – natural killer; DC – dendritic cells.

Discussion

UC represents a significant challenge in gastroenterology, primarily affecting adults aged 30–40 years with substantial impacts on quality of life and work productivity [12, 13]. While considerable progress has been made in understanding UC, both diagnosis and management remain challenging. Currently, diagnosis relies heavily on invasive colonoscopy procedures, highlighting the urgent need for reliable, non-invasive diagnostic biomarkers [14]. Moreover, existing treatment options often show variable efficacy and may be associated with significant side effects, creating a clear need for alternative therapeutic approaches.

In this context, the study investigated SNP, a TCM formulation with documented anti-inflammatory properties and effectiveness in treating inflammatory and bowel diseases [15]. Combining network pharmacology with machine learning approaches aimed to address three critical gaps in current knowledge: the identification of novel diagnostic biomarkers, the elucidation of SNP's mechanism of action, and the potential for personalized treatment approaches based on molecular profiles.

However, several important limitations of this approach were acknowledged. First, these findings are primarily based on computational analyses of public datasets, and while statistically robust, they require experimental validation. Second, the gene expression datasets used, though carefully selected, may not fully represent the diversity of UC patient populations. Third, while the machine learning approach identified promising biomarkers, their clinical utility needs to be validated in prospective studies.

The network pharmacology analysis revealed important insights into the mechanism of action of SNP. A detailed interaction network was created by carefully studying active

parts and the possible targets they could interact with. This network shows how SNP parts are connected and how they affect biology. This analysis identified 79 genes that were both targeted by SNP and differentially expressed between healthy and UC samples in the GEO database, suggesting potential therapeutic mechanisms.

The functional enrichment analysis through GO and KEGG pathways revealed several key BPs affected by these 79 SNP-related genes. Of particular significance was the enrichment of inflammatory response pathways, which aligns with the known pathophysiology of UC [16]. The enriched pathways included: regulation of inflammatory response, immune system modulation, cellular stress response, tissue repair and regeneration, and mucosal barrier function.

However, these computational findings are promising and acknowledge that they represent predictions that require experimental validation. The complex nature of both UC pathogenesis and TCM formulations means that additional mechanisms may exist beyond those identified in the analysis. Future studies should focus on experimental verification of these predicted pathways, particularly the specific roles of individual SNP components in modulating inflammatory responses.

Additionally, the interaction between different components of SNP (*Radix Bupleuri*, *Aurantii Fructus Immaturus*, *Radix Alba*, and *Licorice*) may produce synergistic effects that are not fully captured by the current analysis. Understanding these synergistic interactions could be crucial for optimizing therapeutic approaches.

Through the application of complementary machine learning approaches (SVM-RFE and LASSO) to the set of 79 DEGs, five robust core biomarkers were identified with potential diagnostic significance: *TRPV1*, *ABCG2*, *BACE2*, *MMP3*, and *LIPC* [17–21]. Each of these biomarkers demonstrates distinct biological roles and potential mechanistic connections to UC pathogenesis.

TRPV1 functions as a polymodal sensory transducer, responding to various stimuli, including heat, acidic pH, and both endogenous and exogenous inflammatory mediators [17]. Its role in UC is particularly interesting, as evidenced by research demonstrating its involvement in the protective effects of propolis on colonic tissue in UC patients [18]. The sensitivity of TRPV1 to multiple inflammatory signals makes it a potentially valuable diagnostic indicator of disease activity.

ABCG2, an important membrane transporter, has emerged as a key player in various cellular stress responses, including inflammation, tissue damage, and hypoxia [19, 20]. While its precise inhibitory mechanism in UC remains to be fully elucidated, studies have demonstrated reduced *ABCG2* expression in UC patients, with this reduction showing a negative correlation with specific functional miRNAs [21]. This relationship suggests a potential regulatory network that could be therapeutically targeted.

BACE2's significance in UC pathogenesis is highlighted by its regulation through the JAK2/STAT5 signaling pathway [22]. Its influence on interleukin (IL)-1R2 activity provides a mechanistic link to UC development, suggesting potential therapeutic applications [23]. The connection to established inflammatory pathways strengthens its potential as both a diagnostic marker and therapeutic target.

MMP3, a member of the zinc-dependent endopeptidase family, demonstrates particularly promising clinical utility. Its expression spans multiple cell types relevant to UC pathogenesis, including immune cells, connective tissues, and endothelial cells. The direct correlation between serum MMP3 levels and disease activity in pediatric UC patients, as demonstrated by Kofla-Dłubacz et al. [21], suggests its potential as a practical biomarker for monitoring disease progression.

The *LIPC* gene, encoding a 65-kilodalton (kD) glycoprotein, presents an intriguing connection between metabolic regulation and UC pathogenesis. Its association with metabolic and circulatory system disorders suggests potential mechanisms linking systemic metabolism to intestinal inflammation [24, 25]. The gene's structure, spanning 35 kilobases (kb) with nine exons and eight introns, provides multiple potential regulatory points that could be targeted therapeutically.

This multi-biomarker panel represents distinct but potentially interconnected aspects of UC pathophysiology, from inflammatory signaling (TRPV1) to tissue remodeling (MMP3) and metabolic regulation (LIPC). However, these experimental validations acknowledged that the utility of these biomarkers in clinical settings is essential. Future studies should focus on validating these markers in larger, diverse patient cohorts, investigating potential interactions between these biomarkers, developing practical diagnostic assays, and exploring their potential as therapeutic targets.

The current landscape of UC diagnosis presents significant challenges, primarily due to the absence of reliable, non-invasive biomarkers and robust predictive models [26, 27]. Although traditional diagnostic methods such as colonoscopy remain the gold standard, they are invasive and may fail to capture the molecular complexity of the disease. Recent advances in machine learning approaches have opened new avenues for improving UC diagnosis and prediction [28], with techniques such as logistic regression demonstrating particular promise in analyzing complex biological datasets [29].

In this study, a sophisticated dual-algorithm approach was employed, combining LASSO and SVM-RFE methodologies to develop a diagnostic model based on the identified key genes. The model's performance was remarkable, achieving perfect discrimination with AUC values of 1,000 in both the training and validation cohorts across different algorithms. While these results are encouraging, they must be interpreted within the context of several important considerations.

The performance metrics, while statistically impressive, highlight the need for broader validation. The analysis, based on publicly available datasets, may not fully capture the heterogeneity of UC presentations across different patient populations. Furthermore, the perfect discrimination observed in the model, while mathematically sound, suggests the need for testing in larger, more diverse cohorts to ensure generalizability in real-world clinical settings.

A critical limitation of the current study is the absence of experimental validation. The molecular mechanisms predicted by computational analyses require verification through carefully designed cell-based experiments and animal models. Such validation would not only confirm the biological relevance of the identified biomarkers but also provide insights into their potential as therapeutic targets. Although supported by computational analysis, the relationship between SNP components and these molecular targets likewise requires experimental confirmation.

The translation of these findings into clinical practice presents additional challenges. The relationship between gene expression patterns and protein levels needs to be established, and the stability of these markers across different technical platforms must be verified. Moreover, the development of practical, cost-effective diagnostic assays based on these markers requires significant additional research and validation.

The findings also raise important questions about the mechanism of action of SNP in UC treatment. While the computational approach suggests specific molecular targets and pathways, the complex interactions between different components of this traditional medicine warrant further investigation. Understanding these interactions could provide valuable insights for optimizing treatment strategies and potentially developing more targeted therapeutic approaches.

While our integrated approach provided insights, particularly the single-cell analysis, which offered valuable cellular context for the identified biomarkers (e.g., ABCG2/BACE2 in epithelial cells, MMP3 in fibroblasts), we recognize its current scope has limitations. Further in-depth single-cell analyses, such as trajectory inference, detailed cell-cell communication focusing specifically on the hub genes, and the construction of cell-type-specific regulatory networks, are warranted in future studies to fully elucidate the mechanistic roles of these genes within distinct UC cell populations. Our current findings provide a solid foundation for these necessary future investigations. Similarly, the diagnostic model built using the five hub genes requires careful interpretation. While achieving perfect AUC scores of 1.000 in both training and validation datasets mathematically indicates strong separation within these specific cohorts, we absolutely agree that such performance necessitates extreme caution. Perfect scores in complex biological systems raise significant concerns about potential overfitting, even with external validation, or suggest the model might be highly specific to characteristics shared by samples within these particular datasets (GSE87473, GSE75214). Consequently, the selected genes, while highly discriminatory here, may not generalize perfectly to the broader spectrum of UC heterogeneity found in larger, more diverse real-world populations. Although methodological diligence was applied, including careful control to prevent data leakage and appropriate use of cross-validation during feature selection (e.g., for LASSO lambda tuning), the possibility of subtle biases persisting in retrospective data analysis cannot be entirely ruled out. Therefore, as acknowledged throughout our discussion and limitations, these findings, particularly the remarkable AUC values, demand rigorous external confirmation. We strongly emphasize the critical need for validation in larger, prospective, multi-center clinical cohorts encompassing diverse patient characteristics before any conclusions about the model's real-world clinical utility can be drawn. Furthermore, regarding comparison with other state-of-the-art methods, we acknowledge that this study did not perform a direct quantitative benchmarking of our five-gene signature against other previously published UC diagnostic models, representing another limitation and an avenue for future comparative studies.

Moving forward, these limitations and considerations point to several crucial directions for future research. Large-scale validation studies in diverse patient populations will be essential for confirming the clinical utility of the identified biomarkers. Experimental studies focusing on molecular mechanisms will help establish the biological basis for computational predictions. Finally, the development of practical diagnostic tools based on these findings will require careful consideration of both technical and clinical implementation challenges.

The study has identified a signature of five hub genes (*TRPV1*, *ABCG2*, *BACE2*, *MMP3*, and *LIPC*) that demonstrate a significant correlation with SNP's therapeutic effects in UC. Not only do these genes serve as potential diagnostic biomarkers, but they also provide insights into the molecular mechanisms underlying both UC pathogenesis and SNP's therapeutic action.

These hub genes represent diverse BPs relevant to UC pathophysiology. *TRPV1*'s role in inflammatory signaling and pain sensation suggests its involvement in both symptom manifestation and disease progression [17, 18]. *ABCG2*'s function in cellular stress response and barrier protection points to its potential role in maintaining intestinal homeostasis [19, 20]. *BACE2*'s connection to the JAK2/STAT5 pathway highlights the involvement of key inflammatory signaling cascades [22, 23]. *MMP3*'s role in tissue remodeling and its correlation with disease activity make it particularly promising as a monitoring biomarker [21]. *LIPC*'s involvement in metabolic regulation suggests a previously underappreciated connection between metabolic processes and UC pathogenesis [24, 25].

The integration of these markers into a diagnostic model represents a step toward more precise and personalized approaches to UC management. However, the translation of these findings into clinical practice will require careful validation through experimental studies and clinical trials. Understanding the specific mechanisms by which SNP modulates these genes could lead to optimized treatment strategies and potentially the development of new therapeutic approaches.

Furthermore, these findings lay the groundwork for future investigations into both traditional medicine mechanisms and novel therapeutic strategies for UC. The identification of these molecular targets may facilitate the development of more targeted treatments and provide a scientific basis for understanding the multi-component, multi-target nature of TCM formulations such as SNP.

This study thus bridges traditional medicine and modern molecular biology, offering new perspectives on UC pathogenesis and treatment while highlighting the potential for integrating traditional and modern therapeutic approaches.

Limitations of the study

This study possesses several significant limitations inherent to its computational design and reliance on publicly available data. Foremost among these is the complete lack of experimental validation, which is the most critical constraint. All findings regarding SNP's potential mechanisms, the identified hub genes, and the diagnostic model's performance are derived solely from bioinformatics analyses and predictions. Confirmation through dedicated *in vitro*, *in vivo*, and prospective clinical studies is essential to establish the biological relevance, functional roles, diagnostic accuracy, and therapeutic potential of these findings. Furthermore, the study's conclusions depend heavily on the accuracy and completeness of the public databases utilized (e.g., TCMSP, GeneCards®, NetworkAnalyst resources) and the representativeness of the specific GEO datasets chosen. These datasets may possess inherent limitations, including data quality issues, technical variations, limited clinical annotation depth, and they may not fully capture the broad heterogeneity of the global UC patient population, thereby potentially limiting the generalizability of our results. While the achievement of perfect AUC scores (1,000) is mathematically noteworthy, it must be interpreted with caution, as it raises concerns about potential overfitting or model specificity, highlighting the urgent need for validation in diverse and independent cohorts. Additionally, the inherent complexity of SNP as a multi-component formula makes it challenging to computationally dissect synergistic effects or attribute outcomes definitively, and real-world variability is not captured. The depth of the single-cell analysis was also limited, providing cellular context but lacking deeper functional investigation. Finally, it is crucial to reiterate that this study identifies correlations and associations, not causal relationships, which can only be established through experimental work. These limitations collectively underscore that the study provides hypotheses and potential leads rather than confirmed mechanisms or clinically actionable tools.

Conclusion

This study bridges TCM and modern molecular biology, offering new perspectives on UC pathogenesis and SNP's therapeutic mechanisms. The identified biomarkers and regulatory networks provide valuable foundations for future experimental research and potential clinical applications. However, translation of these findings into clinical practice will require rigorous experimental validation, prospective clinical studies, and development of practical diagnostic assays.

Acknowledgement

We wish to express our gratitude to all research contributors who were not listed as co-authors for their valuable contributions to this study.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This study was supported by the Liaoning Provincial Talent Project (No. XLYC2002002) and explores the mechanism of brain-gut interaction by UC-mediated 5-HT signaling system based on microbial metabolomics.

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Received on January 31, 2025
Revised on May 5, 2025
Revised on June 4, 2025
Accepted on June 11, 2025
Online First July 2025

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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