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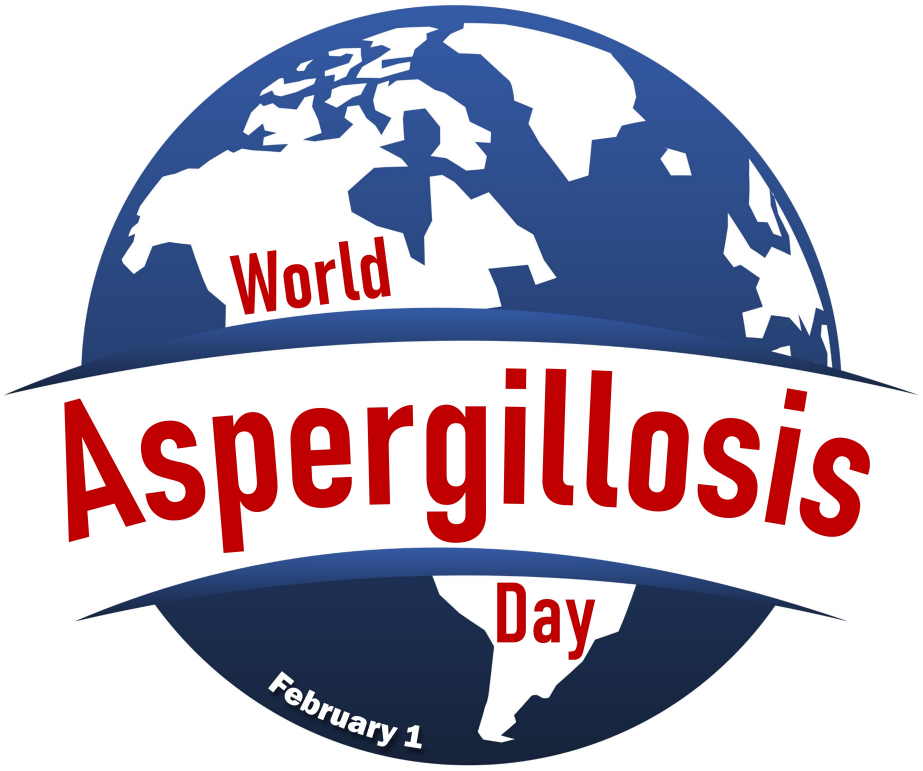

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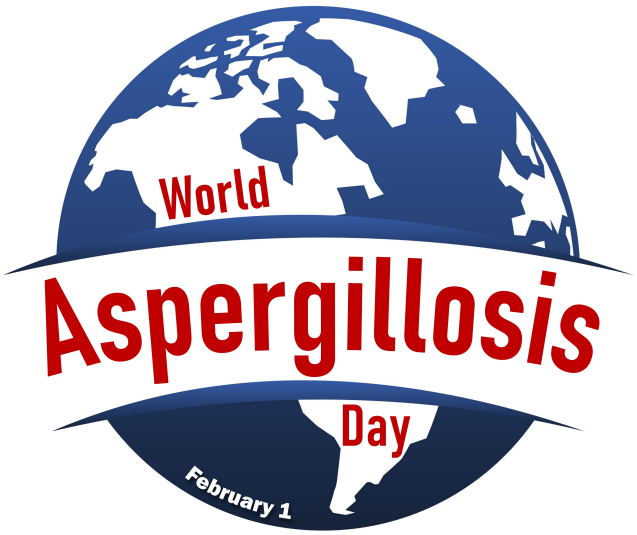

Since February 1, 2018, World Aspergillosis Day has been observed at the initiative of patients from the National Aspergillosis Center in Manchester, to raise awareness of aspergillosis and improve therapeutic strategies for the treatment of this underrecognized infection. Aspergillosis is an opportunistic infection most commonly caused by the fungus *Aspergillus fumigatus*. This infection is gaining increasing importance due to its diverse clinical manifestations and the development of antifungal resistance. Each year, more than 2,113,000 people develop invasive aspergillosis in the context of chronic obstructive pulmonary disease, lung cancer, or hematological malignancies, as well as during treatment in intensive care units, with an annual mortality of 1,801,000 (85.2%). The annual incidence of chronic pulmonary aspergillosis is estimated at 1,837,272 cases, with 340,000 deaths (18.5%).

Na inicijativu pacijenata Nacionalnog centra za aspergilozu u Mančesteru, od 1. februara 2018. godine, obeležava se Svetski dan aspergiloze, sa ciljem podizanja svesti o aspergilozi i unapređenja terapijskih strategija za lečenje ove nedovoljno prepoznate infekcije. Aspergiloza je oportunistička infekcija koju uglavnom izaziva gljivi-ca *Aspergillus fumigatus*. Ova infekcija dobija sve veći značaj zbog različitih obrazaca ispoljavanja, kao i razvoja rezistencije ove gljivice na antimikotike. Svake godine više od 2 113 000 ljudi oboli od invazivne aspergiloze u kontekstu hronične opstruktivne bolesti pluća, karcino-ma pluća ili hematoloških maligniteta, tokom lečenja u jedinicama intenzivne nege, uz godišnju stopu smrtnosti od 1 801 000 (85,2%). Godišnja incidencija hronične plućne aspergiloze iznosi 1 837 272 slučaja, sa 340 000 smrtnih ishoda (18,5%).

M E T A - A N A L Y S I S

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Assessing the impact of nintedanib and pirfenidone on lung function in idiopathic pulmonary fibrosis: a comprehensive meta-analysis

Procena uticaja nintedaniba i pirfenidona na funkciju pluća kod idiopatske plućne fibroze: sveobuhvatna meta-analiza

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Abstract

Background/Aim. The incidence of idiopathic pulmonary fibrosis (IPF) has been increasing each year. Although pirfenidone and nintedanib were approved in 2014, they received only conditional recommendations, and no medication has yet been strongly endorsed for IPF treatment. The aim of the study was to compare the safety and efficacy of pirfenidone and nintedanib. **Methods.** All randomized and non-randomized clinical trials were identified by searching databases for published studies, including Medline, Embase, Scopus, Google Scholar, and ClinicalTrials.gov. A meta-analysis was conducted to evaluate the impact of pirfenidone and nintedanib on clinical outcomes and safety. Patients treated with pirfenidone were compared with those treated with nintedanib. **Results.** This study included twelve papers. Both pirfenidone and nintedanib were found to significantly reduce the decline in mean forced vital capacity (FVC) and mean diffusion capacity of the lungs for carbon monoxide (DLco) at 6 and 12 months. No significant difference was observed between pirfenidone and nintedanib in terms of improvement in FVC or DLco. Similarly, both antifibrotic agents had similar safety profiles. However, patients receiving nintedanib experienced significantly fewer instances of diarrhea ($p < 0.00001$) compared to those receiving pirfenidone, whereas patients receiving pirfenidone experienced significantly fewer instances of skin rash ($p < 0.00001$) compared with those receiving nintedanib. **Conclusion.** Potential differences between pirfenidone and nintedanib can be inferred from the effectiveness ranking derived from this meta-analysis. Further direct comparative studies are necessary to explore this issue, which will help us better understand the potential of combinatorial, sequential, or adjunctive treatment regimens in which both antifibrotic agents might play a crucial role for a specific group of IPF patients.

Keywords:
drug-related side effects and adverse reactions; drug therapy; idiopathic pulmonary fibrosis; nintedanib; pirfenidone; respiratory function test.

Apstrakt

Uvod/Cilj. Učestalost idiopatske plućne fibroze (IPF) je u porastu iz godine u godinu. Iako su pirfenidon i nintedanib odobreni 2014. godine, dobili su samo uslovne preporuke, a nijedan od ta dva leka još uvek nema snažnu preporuku za lečenje IPF. Cilj rada bio je da se uporede bezbednost i efikasnost pirfenidona i nintedaniba. **Metode.** Sve randomizovane i nerandomizovane kliničke studije identifikovane su pretraživanjem baza podataka objavljenih studija, uključujući Medline, Embase, Scopus, Google Scholar i ClinicalTrials.gov. Sprovedena je meta-analiza kako bi se procenio uticaj pirfenidona i nintedaniba na kliničke ishode i bezbednost. Poređeni su bolesnici lečeni pirfenidonom i bolesnici lečeni nintedanibom. **Rezultati.** Studijom je obuhvaćeno 12 radova. Utvrđeno je da su i pirfenidon i nintedanib značajno smanjivali pad srednje vrednosti forsiranog vitalnog kapaciteta (*forced vital capacity* – FVC) pluća i srednje vrednosti difuzionog kapaciteta pluća za ugljen-monoksid (*diffusion capacity of the lungs for carbon monoxide* – DLco) nakon 6 i 12 meseci. Nije uočena statistički značajna razlika između pirfenidona i nintedaniba u pogledu poboljšanja FVC ili DLco. Takođe, oba agensa protiv fibroze pluća imala su slične bezbednosne profile. Međutim, kod bolesnika lečenih nintedanibom zabeleženo je značajno manje epizoda dijareje ($p < 0,00001$) u poređenju sa bolesnicima lečenim pirfenidonom, dok su bolesnici lečeni pirfenidonom imali značajno manje epizoda kožnog osipa ($p < 0,00001$) u poređenju sa

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bolesnicima lečenim nintedanibom. **Zaključak.** O mogućim razlikama između pirfenidona i nintedaniba može se zaključiti na osnovu rangiranja njihove efikasnosti dobijenog ovom meta-analizom. Neophodne su dodatne direktne uporedne studije kako bi se ovo pitanje detaljnije istražilo, što će omogućiti bolje razumevanje potencijala kombinovanih, sekvencijalnih ili dodatnih režima lečenja, u kojima oba agensa protiv fibroze mogu igrati ključnu ulogu za određenu grupu obolelih od IPF.

Ključne reči:
lekovi, neželjeni efekti i neželjene reakcije; lečenje lekovima; pluća, fibroza, idiopatska; nintedanib; pirfenidon; respiratorna funkcija, test.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic lung disease characterized by scar tissue formation in the lungs [1]. It leads to an irreversible decline in lung function. This disease mainly affects older persons, with a higher incidence in males. Progressive lung tissue scarring is responsible for IPF development [2]. Due to discrepancies in reporting and diagnostic criteria, the original rate of prevalence and incidence for IPF is not well-established. However, recent estimates revealed that approximately 3 million people worldwide have been affected by IPF [3, 4]. However, these estimates did not reflect the exact scenario of IPF because there were so many undiagnosed or misdiagnosed cases present worldwide. Some studies also observed a significant delay (median delay of 2.1 years) in IPF diagnosis, resulting in patients often experiencing worsening sign and symptoms and irreversible lung damage [5–7].

Currently, there is no permanent solution regarding the cure for IPF. The available treatment only focuses on symptom management and slowing down disease progression [5]. There are multiple treatments available for IPF. Treatments include medications such as nintedanib and pirfenidone, which inhibit the progression of lung fibrosis and are approved for IPF treatment [8]. Lung transplantation may be the final treatment option for severe IPF patients when other therapies fail. Pulmonary rehabilitation programs can help improve symptoms, enhance endurance during exercise, and upgrade the individual's overall quality of life (QoL) with IPF [9, 10]. Supplemental oxygen therapy was prescribed to alleviate symptoms and improve breathing when the patient's oxygen level was low. Palliative care seems to focus on relieving symptoms and improving the QoL for individuals with IPF [11].

Nintedanib and pirfenidone are medications used in the treatment of IPF. While the exact mechanism of action of nintedanib and pirfenidone is not fully understood, nintedanib primarily acts as a tyrosine kinase inhibitor (TKI). It was noted that in a canine lung infection model, pirfenidone showed antifibrotic activity and reduced fibrosis while improving lung function in a bleomycin-induced hamster lung injury model [12]. The antifibrotic activity might depend on growth factors and cytokines modulation, such as growth factor transformation. Pirfenidone has been approved due to phase 3 studies in mild-to-moderate IPF patients [13], but the safety and efficacy of pirfenidone in advanced IPF patients are still unclear. However, many experiments found that pirfenidone was well accepted and improved the lung function decline in advanced IPF patients [14, 15].

Nintedanib, an intracellular TKI targeting vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) receptors, exhibited antifibrotic and anti-inflammatory effects in a preclinical study. In recent randomized controlled trials (RCTs) involving patients with mild-to-moderate IPF, it was found that nintedanib reduced the rate of decline in forced vital capacity (FVC), and disease progression and adverse events were well tolerated.

Current advancements in understanding the molecular processes in fibrogenesis have opened innovative avenues for targeted therapeutic interventions [1, 16]. One such promising approach is the use of antifibrotic drugs for the treatment of IPF. These drugs act through specific mechanisms involved in preventing or reducing the accumulation of scar tissue in the lungs. Clinical trials have shown that antifibrotic drugs, such as pirfenidone and nintedanib, can reduce the decline in lung function and improve overall QoL in patients with IPF. However, it is important to note that these drugs are not curative and may have side effects. Based on previous qualitative and quantitative reviews, nintedanib and pirfenidone were evaluated for their antifibrotic effects in patients with and without IPF [17, 18]. To gain a deeper understanding of the potential effects of the two antifibrotic agents in patients with IPF, a systematic literature review and meta-analysis was conducted to determine which agent inhibits IPF progression more effectively.

Methods

Literature search

This systematic literature review and meta-analysis was performed using electronic databases such as Medline, Embase, Scopus, Google Scholar, and ClinicalTrials.gov from inception to August 31, 2025. In addition, two independent reviewers conducted an abstract review of all records. For more relevant studies, we used Medical Subject Headings (MeSH) terms "pirfenidone" and "nintedanib" alone and in combination with other terms in the following way: ("pirfenidone" [Supplementary Concept] OR "antifibrotic medication, nintedanib" [MeSH]) AND ("idiopathic pulmonary fibrosis" [MeSH]). The complete search strategy has been provided in the supplementary file (Supplementary Table 1). In addition, a manual bibliographic search was performed for reference lists of published review

articles to collect additional information, and conference abstracts were searched for relevant information.

We have included RCTs and observational studies that reported the pulmonary functions of pirfenidone and nintedanib for IPF patients.

Study selection

Studies were eligible for inclusion if they met the following criteria: the patients in the selected studies were older than 18 years and had IPF; observational studies and RCTs comparing nintedanib and pirfenidone; studies that reported at least one clinical outcome. Full articles were retrieved when titles and/or abstracts met this objective. A manual cross-reference search of relevant articles was conducted. Only English language studies were included. Disagreement about study inclusion between the two reviewers was resolved through discussion with the third reviewer until 100% agreement was reached on the final interpretation of the data.

Studies were excluded if they were published in a language other than English, were animal or *in vitro* studies, were conducted in athletes, children, pregnant or lactating women, or did not have the necessary data, or analyzed the effects of combination therapy involving nintedanib or pirfenidone with other drugs or components. Notably, we did not include papers reporting the mentioned outcomes in formats that could not be converted to mean values and standard deviations (SD).

Quality assessment

The quality of each study included in the analysis was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS) [19] and the Modified Jadad score (MJS) scale [20]. This validated NOS tool consisted of the following three categories: selection, comparability, and outcome assessment. Each category was scored as good, fair, or poor. The MJS scale mainly assesses the randomization, blinding, withdrawals, inclusion-exclusion criteria, adverse events, and statistical analysis. A score of 0 to 3 is considered low-quality, and a score of 4 to 8 is considered high-quality study. Two independent reviewers performed the quality assessment, and disagreements on scores were resolved through discussion.

Data extraction and outcome measure

Two independent reviewers performed data extraction and analysis. Study methodological homogeneity was assessed. In extracting the assessed outcomes, study heterogeneity would not be justified. A customized data-extraction form, as described in the Cochrane Handbook for Systematic Reviews of Interventions, was used to record the duration of the trial, sample size, dropouts, and effect of interventions. The included effectivity in the analysis was as follows: the effect of antifibrotic therapy on diffusing capacity of the lungs for carbon monoxide (DLco) at 6 and 12 months; the effect of antifibrotic therapy on FVC for 6 and 12 months; the effect of antifibrotic therapy on forced expiratory volume in 1 sec (FEV1), 6-min walk test (6-MWT), and total lung capacity; the effect of antifibrotic therapy on all-cause mortality (ACM); the effect of antifibrotic therapy on adverse events, including all adverse events, skin-related adverse events, and diarrhea events.

Sensitivity analysis

The robustness of the pooled estimates was evaluated through sensitivity analyses. We repeated the meta-analyses by excluding studies with a high risk of bias and sequentially removing one study at a time (a process known as "leave-one-out" analysis). These methods examined whether methodological decisions or any single study had an excessive impact on the overall results.

Statistical analysis

Quantitative data were analyzed using the Cochrane Review Manager (RevMan) version 5.2 software and RStudio version 4.3. Summary estimates, including 95% confidence intervals (CIs), were calculated. For continuous outcome data, means and SDs were used to calculate a weighted standardized mean difference (SMD). For studies with different statistical data, the data were converted to mean and SD to calculate and remove missing outcome bias. For dichotomous outcomes, odds ratios (ORs) were calculated. Statistical heterogeneity was assessed using the I^2 test. Random-effect models were used unless significant evidence of statistical heterogeneity or clinical diversity was found. For results showing significant heterogeneity ($I^2 > 50\%$), a p-value < 0.05 was considered statistically significant. For publication bias analysis, we conducted Egger's regression (ER) test and funnel plot. Subgroup and sensitivity analyses were conducted in order to eliminate the heterogeneity from the analysis.

Results

Search results

The detailed database search procedure and study selection are shown in Figure 1 [21–32]. The flow chart of study selection shows the literature search and selection for RCT [28] and non-RCT [21–27, 29–32] studies on the effects of nintedanib and pirfenidone on IPF patients.

Two reviews searched the Medline, Embase, Scopus, Google Scholar, and ClinicalTrials.gov databases independently from inception until April 2025. A total of 1,285 articles were extracted for screening. Articles were excluded due to duplicates, different outcomes and interventions, and lack of data availability. Finally, 12 studies [21–32] were included for extraction and meta-analysis (11 were observational studies [21–27, 29–32], one was an RCT study [28]). A total of 1,631 subjects were administered nintedanib, and 2,218 subjects were in the pirfenidone group [21–32].

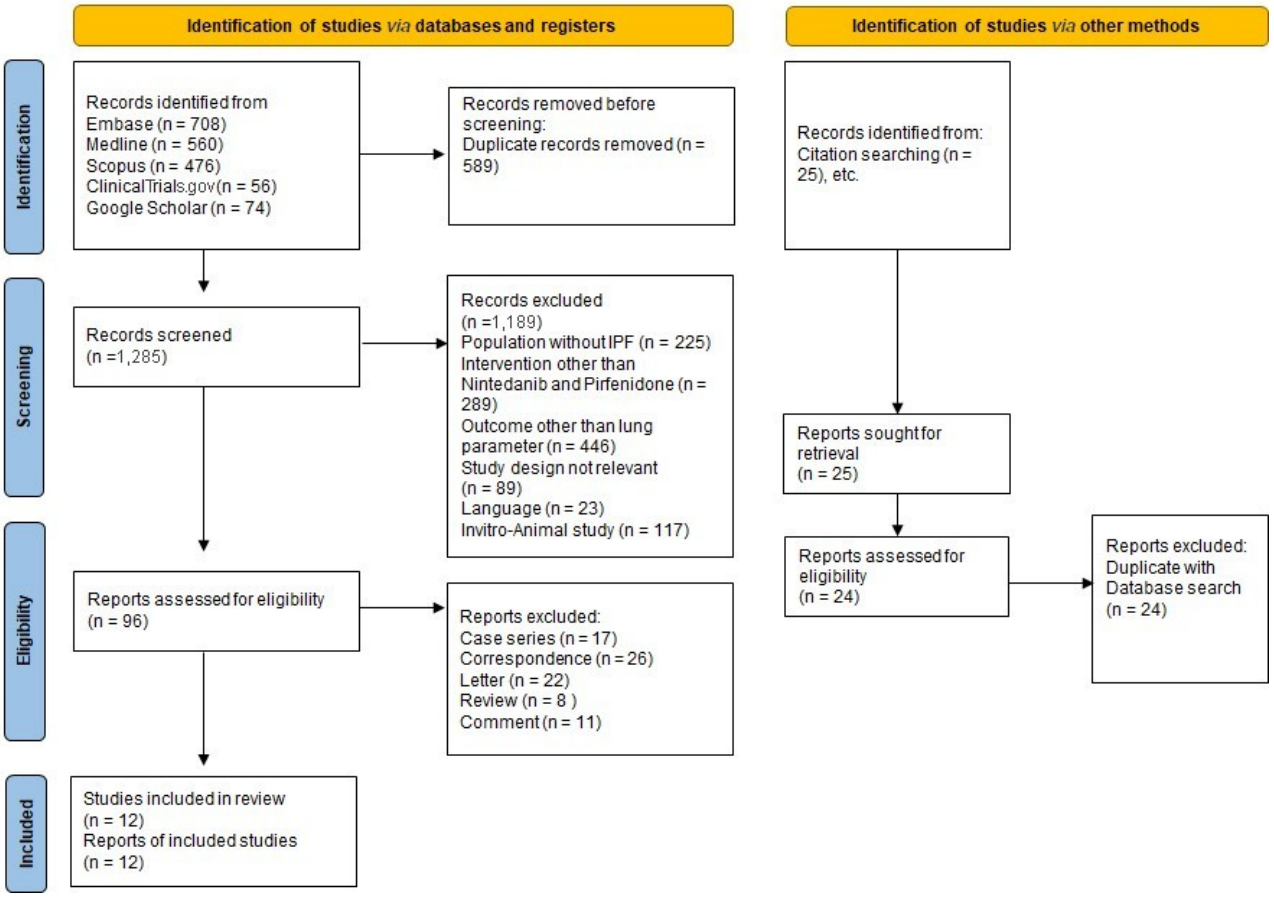

Fig. 1 – PRISMA flow diagram depicting the selection of studies for systematic review and meta-analysis.
PRISMA – Preferred Reporting Items for Systematic reviews and Meta-Analyses.

Study characteristics

The characteristics of the included studies are presented in Table 1 [21–32]. The included participants were aged between 18 and 65 years. Study duration also varied from months to years. Of 12 studies, nine [21, 24–29, 31, 32] reported the DLco, and nine [21, 24–29, 31, 32] reported FVC, four [21, 24, 25, 28] reported FEV1 data, and all studies reported adverse events.

Diffusing capacity of the lungs for carbon monoxide at six and twelve months

The effects of nintedanib and pirfenidone for DLco were documented in one RCT [28], three retrospective observational studies [21, 26, 27], one retrospective cohort study [24], and one prospective cohort study [25]. At 6 and 12 months, pooled data from all investigations were assessed. A total of 645 subjects reported nintedanib and pirfenidone effects. According to the pooled analysis of six studies, nintedanib non-significantly ($p = 0.56$) improved the DLco at 12 and 6 months ($p = 0.10$). A significant ($I^2 = 80\%$) heterogeneity was observed in the 12-and 6-month DLco analysis. The details of the forest plot analysis are described in Figure 2 [21, 24–26, 28, 29] and Table 2. Forest plot of the effect of nintedanib and pirfenidone on DLco in total participants for a 6-month duration was $z = 1.67$, $p = 0.10$, and for 12 months, duration was $z = 0.59$, $p = 0.56$.

Forced vital capacity at six and twelve months

The 12-month FVC with pirfenidone and nintedanib treatment was reported in six trials involving 567 patients [21, 24–26, 28, 29]. A meta-analysis of these six investigations observed there was no significant difference between 12 months of FVC with pirfenidone and nintedanib treatment ($p = 0.40$). In a similar vein, four [21, 24–26] of the six trials, comprising 474 people, reported 6-month FVC following pirfenidone and nintedanib therapy; those receiving nintedanib and pirfenidone therapy demonstrated similar FVC improvement, and no significant ($p = 0.10$) difference was observed. A substantial ($I^2 = 94\%$) heterogeneity was observed in the 12-month FVC analysis. The details of the forest plot analysis are described in Figure 3 [21, 24–26, 28, 29] and Table 2. Forest plot of the effect of nintedanib and pirfenidone on FVC in total participants for a 6-month duration shows $z = 1.63$, $p = 0.10$, and for a 12-month duration, $z = 0.84$, $p = 0.40$.

Table 1

Characteristics of included studies (n = 12)

Author	Design	Intervention and sample size (number)		Study duration (months)	Outcome measure	AE report	Author conclusion
Bargagli et al. 2019 [21]	Re, O	P (52)	N (30)	12	FEV1, FVC, TLC, DLco	yes	A major number of IPF patients showed a good tolerability profile with P. AEs were manageable, but skin reactions and gastrointestinal disorder AEs were observed frequently.
Burgos et al. 2019 [22]	Re, O	P (43)	N (21)	11	FEV1	yes	FVC was improved for those treated with P; however, more AEs were observed with P. On the other hand, the spirometric profile was stabilized with N and better tolerated than P.
Belhassen et al. 2021 [23]	Re, C	P (804)	N (509)	12	ACM, acute respiratory-related hospital admissions, treatment discontinuation	yes	As compared to P, N was associated with a risk of acute respiratory-related hospitalizations, a greater risk of ACM, and a lower risk of treatment discontinuation.
Cameli et al. 2020 [24]	Re, C	P (139)	N (124)	60	FEV1, FVC, TLC, DLco, FEV1/FVC	yes	After 1 year of treatment with N, due to its antiangiogenic properties, it showed a slight decrease in DLco compared with P.
Cerri et al. 2019 [25]	Pr, C	P (78)	N (28)	24	FEV1, FVC, DLco	yes	It was revealed that P and N were equally effective in lowering the DLco and FVC decline vs. non-treated patients after 24 months of treatment.
Feng et al. 2020 [26]	Re, O	P (36)	N (31)	31	FVC, DLco	yes	P decreases the disease progression and improves lung function with very few side effects.
Fournier et al. 2022 [27]	Re, O	P (115)	N (61)	20	FVC, DLco	yes	The occurrence of AEs was higher in the N group compared to the P group.
Kerget et al. 2023 [28]	Ra, Pr	P (15)	N (15)	3	FEV1, FVC, DLco, 6-MWT	yes	It was revealed that P and N were effective in improving radiological scores and PFT parameters. However, it was observed that N had more potential to increase the saturation values and exercise capacity, but it resulted in more AEs than P.
Khan et al. 2023 [29]	Re, C	P (45)	N (36)	12	FVC, DLco	yes	Hospital admission and gastrointestinal disorders were increased in P.
Marijic et al. 2021 [30]	Re, C	P (840)	N (713)	12	ACM, respiratory-related hospitalization	yes	Patient-related outcomes, hospitalization, costs, and mortality rate did not differ between currently available antifibrotic drugs, N and P.
Moor et al. 2020 [31]	Pr, C	P (39)	N (51)	6	FVC, DLco	yes	The incidence of ADR in the N group is higher compared to the P-treated group.
Ntolios et al. 2021 [32]	Pr, C	P (12)	N (12)	12	ΔFVC, ΔDLco, 6-MWT	yes	Patients can continue with the N therapy, those who discontinued P due to ADRs.

AE – adverse events; Re – retrospective study; O – observational study; P – perfenidone; N – nintedanib; FEV1 – forced expiratory volume in 1 sec; FVC – forced vital capacity; TLC – total lung capacity; DLco – diffusing capacity of the lungs for carbon monoxide; IPF – idiopathic pulmonary fibrosis; C – cohort study; ACM – all-cause mortality; Pr – prospective study; Ra – randomized; ACM – all-cause mortality; 6-MWT – 6-minute walk test; PFT – pulmonary function test; ADR – adverse drug reaction.

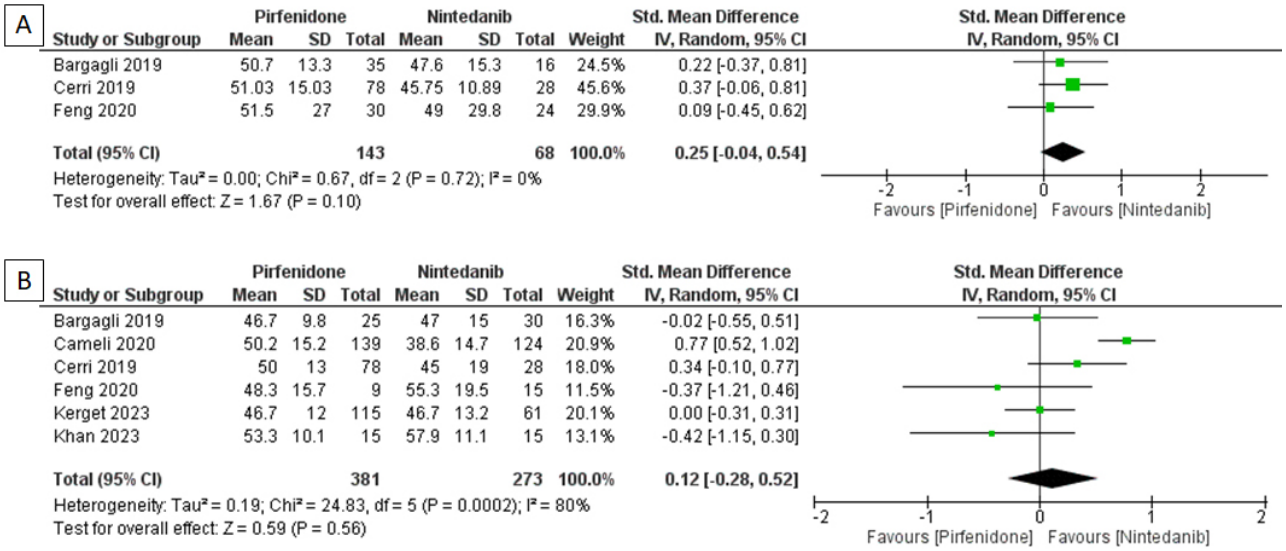

Fig. 2 – Forest plot comparing pirfenidone and nintedanib for DLco: (A) at 6 months; (B) at 12 months.
DLco – diffusion capacity of the lungs for carbon monoxide; SD – standard deviation; Std – standard; CI – confidence interval.

Table 2

Clinical outcomes of the nintedanib group compared to the pirfenidone group

Variables	Study (numbers)	SMD [95% CI]	Value for test (MD = 0)	I^2 (%)	*p*-value for Q test
6-month DLco	3	0.25 [-0.04, 0.54]	0.10	0	0.72
12-month DLco	6	0.12 [-0.28, 0.52]	0.56	80	0.0002
6-month FVC	4	0.16 [-0.03, 0.34]	0.10	0	0.55
12-month FVC	6	0.33 [-0.43, 1.09]	0.40	94	0.00001

SMD – standard mean difference (MD); CI – confidence interval; DLco – diffusing capacity of the lungs for carbon monoxide; FVC – forced vital capacity.

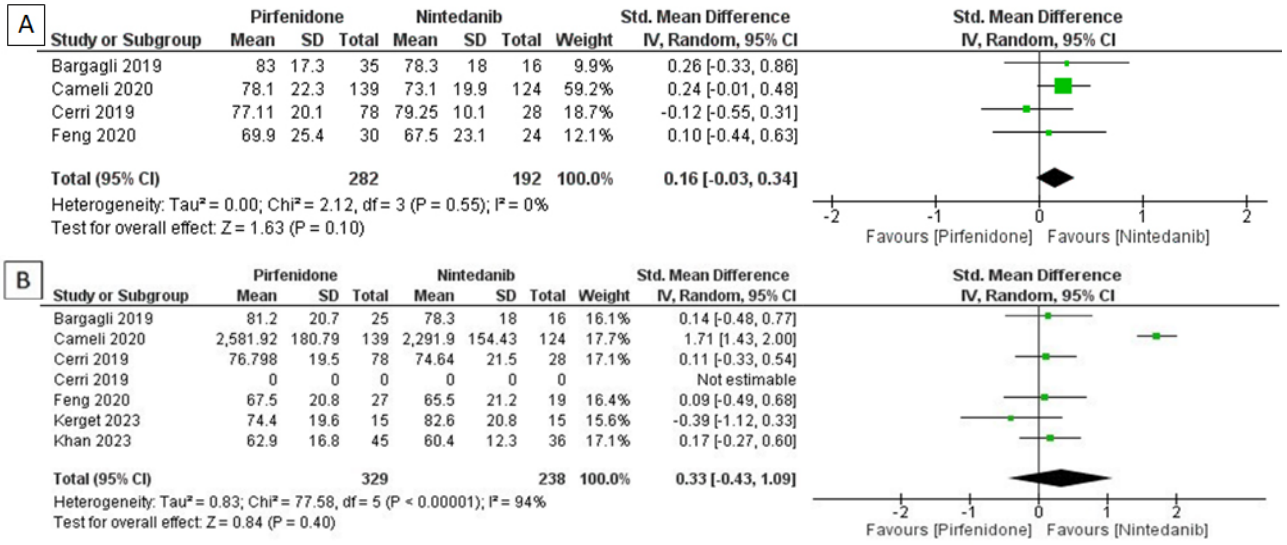

Fig. 3 – Forest plot comparing pirfenidone and nintedanib for forced vital capacity: (A) at 6 months; (B) at 12 months.
SD – standard deviation; Std – standard; CI – confidence interval.

All-cause mortality

A total of three studies [24, 25, 29] reported ACM outcomes. The pooled analysis revealed no significant ($p = 0.38$) difference in mortality between nintedanib and pirfenidone treatment after 12 months (Figure 4 [24, 25, 28, 29] and Table 3). Forest plot of the effect of nintedanib and pirfenidone shows for ACM, $z = 0.88$, $p = 0.38$, and for nausea, $z = 0.05$, $p = 0.96$.

Safety and adverse events

Diarrhea [21, 22, 24, 25, 29], nausea [24, 25, 28, 29], and skin related adverse events [25, 28, 29] were recorded in five, four, and three studies, respectively, involving IPF patients. A pooled analysis revealed that, in comparison to individuals treated with pirfenidone, IPF patients treated with nintedanib experienced considerably (p = 0.006) fewer diarrhea events. On the other hand, skin rash events were considerably (p = 0.002) lower in patients receiving pirfenidone. The OR values are presented in Table 3. The forest plot of the detailed analysis is shown in Figure 5 [21, 22, 24, 25, 28, 29]. Forest plot of the effect of nintedanib and pirfenidone on skin rash shows z = 3.14, p = 0.002, and on diarrhea, z = 2.77, p = 0.006.

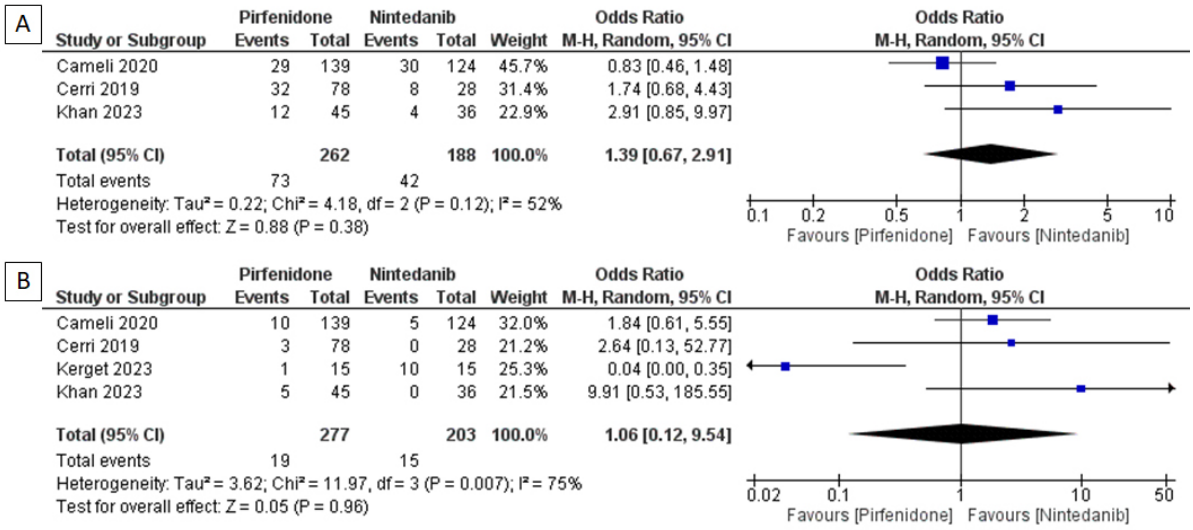

Fig. 4 – Forest plot comparing pirfenidone and nintedanib for: (A) all-cause mortality; (B) nausea. CI – confidence interval.

Table 3

All-cause mortality and safety outcomes of the nintedanib group compared to the pirfenidone group

Variables	No. of study	OR [95% CI]	p-value for test (MD = 0)	I^2 (%)	p-value for Q test
All-cause mortality	3	1.39 [0.67, 2.91]	0.38	52	0.12
Nausea	4	1.06 [0.12, 9.54]	0.96	75	0.007
Skin rash	3	7.22 [2.10, 24.80]	0.002	0	0.77
Diarrhea	5	0.08 [0.01, 0.48]	0.006	78	0.001

No. – number; OR – odds ratio; CI – confidence interval; MD – mean difference.

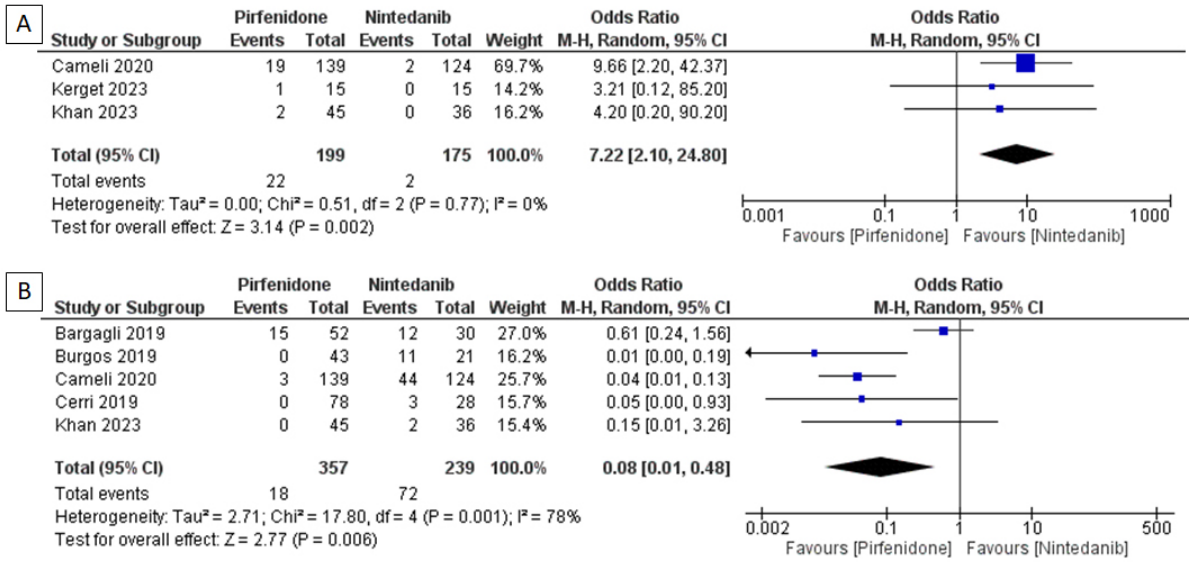

Fig. 5 – Forest plot comparing pirfenidone and nintedanib for: (A) skin rash; (B) diarrhea. CI – confidence interval.

Risk of bias

The quality of each observational study included in the analysis was assessed using the NOS tool [19], and one randomized prospective study was assessed using the MJS scale. Two independent reviewers performed the quality assessment, and disagreements on scores were resolved through discussion. Most studies (n = 9) [21, 23–27, 29, 30, 32] were rated as being of good or fair quality. Only two studies were assessed as poor quality [22, 31]. The quality of one study [28] was assessed using the MJS scale and was rated as good. Detailed quality assessments are presented in Figure 6 [21–27, 29–32] and Supplementary Table 2.

Publication bias

Funnel plots and the ER test were applied to evaluate publication bias for each pulmonary function. The ER test indicated no significant publication bias for DLco at 12 months, DLco at 6 months, FVC at 6 months, nor for ACM, nausea, diarrhea, and skin rash. However, significant publication bias was observed for FVC at 12 months (ER test p = 0.0045). This implies that the underrepresentation of smaller studies with neutral or negative results may inflate the pooled effect size. The results of sensitivity analyses showed that the overall treatment effect was generally robust, even though this finding should be interpreted cautiously. To better understand the actual impact on FVC at 12 months, future research with bigger sample sizes and prospective registration is required. Detailed results are shown in Figure 7 and Supplementary Table 3.

Subgroup analysis

Subgroup analyses based on study design were performed for DLco at 12 months, FVC at 12 months, and nausea to explore potential sources of heterogeneity. Other outcomes included only observational studies and were therefore not subgrouped.

For 12-month DLco, analysis of observational studies showed a small, non-significant improvement in the nintedanib group compared with the pirfenidone group (SMD = 0.14, 95% CI: -0.34 to 0.62; I^2 = 79%). This represented a slight reduction in heterogeneity compared with the overall analysis (SMD = 0.12, 95% CI: -0.28 to 0.52; I^2 = 80%) (Figure 8A [21, 24–26, 29]).

Study ID	Selection	Comparability	Outcome	Quality
Bargagli_2019	4	1	3	GOOD
Burgos_2019	3	1	0	POOR
Belhassen_2021	4	1	2	FAIR
Cameli_2020	4	1	3	GOOD
Cerri_2019	3	1	2	FAIR
Feng_2020	4	1	3	GOOD
Fournier_2022	4	1	3	GOOD
Khan_2023	4	1	1	FAIR
Marijic_2021	4	1	1	FAIR
Moor_2020	3	1	0	POOR
Ntolios_2021	4	1	3	GOOD

Fig. 6 – Risk of bias graph of included trials: risk of bias summary review authors' judgments about each risk of bias item presented as percentages across all included studies. Risk of bias table to assess the quality of the included studies: low risk of bias (green colored), unclear risk of bias (yellow colored), and high risk of bias (red colored).

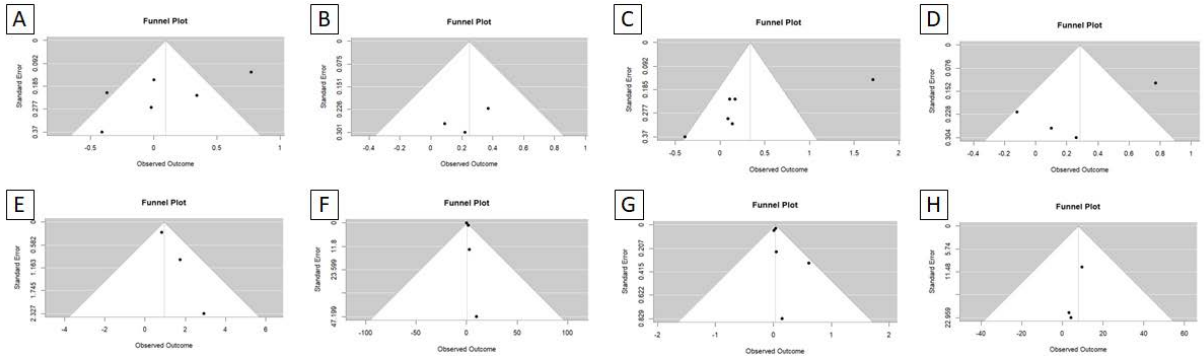

Fig. 7 – Funnel plots assessing publication bias for each endpoint: (A) DLco at 6 months; (B) DLco at 12 months; (C) FVC at 6 months; (D) FVC at 12 months; (E) all-cause mortality; (F) nausea; (G) skin rash; (H) diarrhea. DLco – diffusing capacity of the lungs for carbon monoxide; FVC – forced vital capacity.

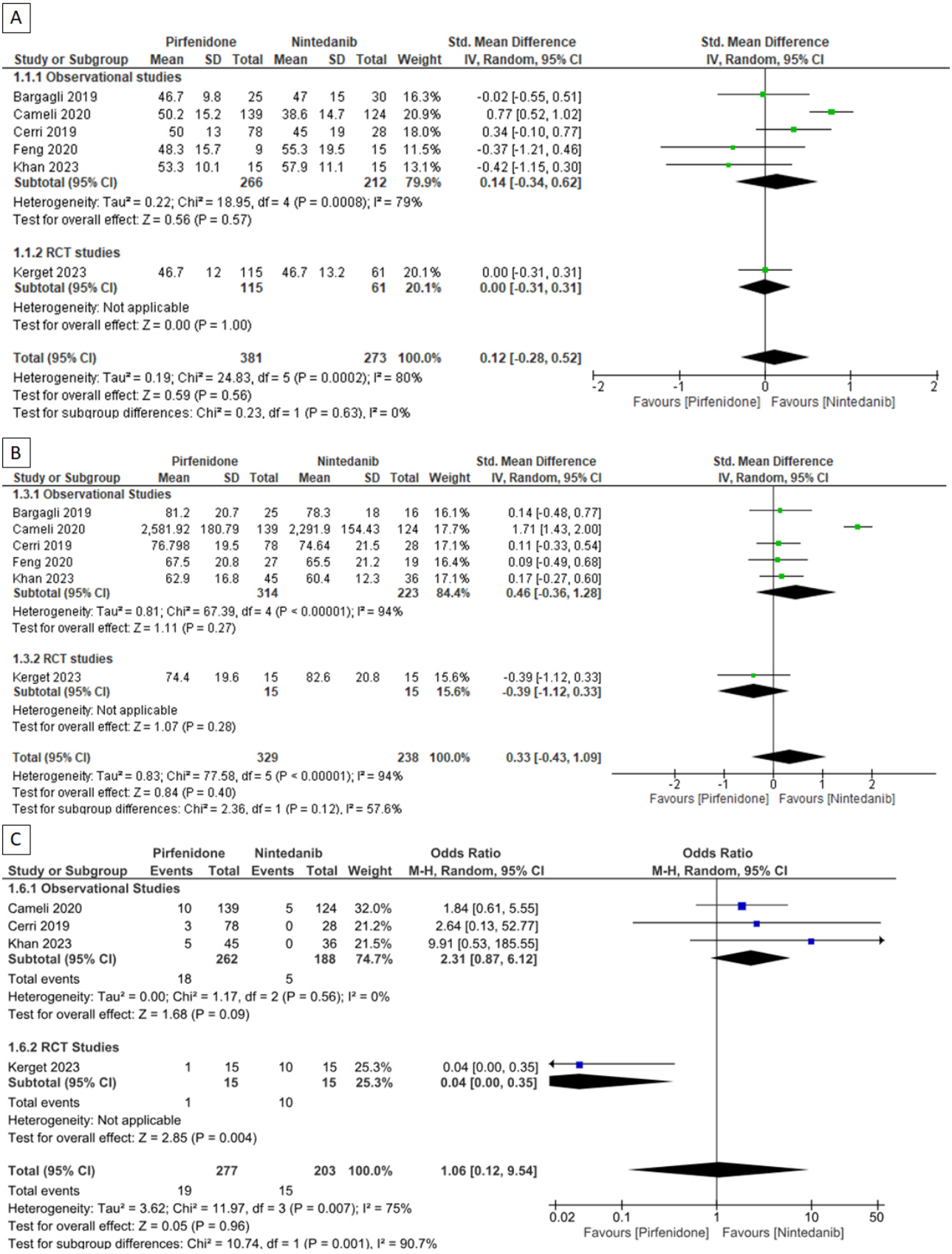

Fig. 8 – Forest plot of subgroup analysis comparing pirfenidone and nintedanib for: (A) DLco at 12 months; (B) FVC at 12 months; (C) nausea.
SD – standard deviation; Std – standard; CI – confidence interval; RCT – randomized controlled trials.

For 12-month FVC, the observational studies subgroup showed no change in heterogeneity compared with the overall analysis (SMD = 0.46, 95% CI: -0.36 to 1.28; I^2 = 94%). The overall effect size was SMD = 0.33 (95% CI: -0.43 to 1.09; I^2 = 94%) (Figure 8B [21, 24–26, 29]).

For nausea, subgroup analysis revealed differences in heterogeneity between study designs. In observational studies, the pooled effect was OR = 2.31 (95% CI: 0.87 to 6.12; I^2 = 0%), whereas in RCTs, the pooled effect was OR = 1.06 (95% CI: 0.12 to 9.54; I^2 = 90.7%) (Figure 8C [24, 25, 29]).

The forest plot analysis of nintedanib and pirfenidone showed the following effects: DLco at 12 months, z = 0.59, p = 0.56; FVC at 12 months, z = 0.84, p = 0.40; nausea, z = 0.05, p = 0.96.

Sensitivity analysis

Sensitivity analysis by sequential exclusion of each study revealed that heterogeneity was primarily driven by Bargagli et al. [21] and Cameli et al. [24] for the individual outcome. For DLco at 12 months and FVC at 12 months, heterogeneity becomes 8% and 0%, respectively, after removing Cameli et al [24]. Similarly, for the diarrhea outcome, removing the Bargagli et al. [21] study reduced the heterogeneity to 0%. Excluding these studies reduced heterogeneity to almost 0%, while the pooled effect estimates for DLco at 12 months and FVC at 12 months remained consistent in direction and magnitude. However, the overall effect size of diarrhea [21, 22, 24, 25, 29] outcome found to be significantly (p < 0.00001) improved with pirfenidone treatment (OR = 0.04, 95% CI (0.01, 0.10) (Figure 9 [21, 22, 24–26, 28, 29]).

The forest plot analysis of nintedanib and pirfenidone showed the following effects: DLco at 12 months, z = 0.10, p = 0.92; FVC at 12 months, z = 0.69, p = 0.49; diarrhea, z = 6.55, p < 0.00001.

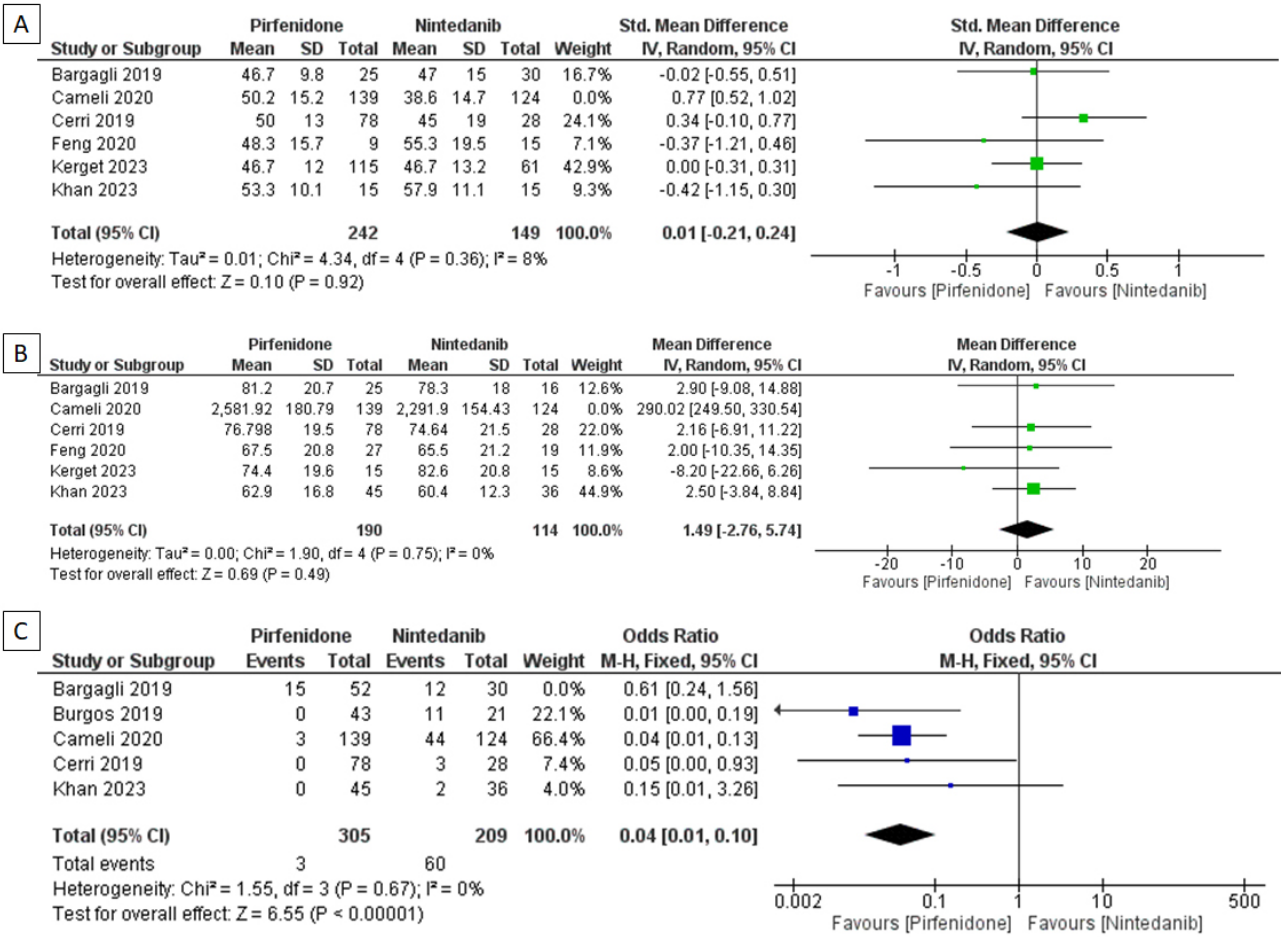

Fig. 9 – Forest plot of subgroup analysis comparing pirfenidone and nintedanib for: (A) DLco at 12 months; (B) FVC at 12 months; (C) diarrhea. FVC – forced vital capacity. For other abbreviations, see Figures 2 and 3.

Discussion

This systematic review and meta-analysis showed an overall reduction in DLco and FVC attributable to nintedanib and pirfenidone.

Although the overall safety profiles of the two antifibrotic agents were similar, they exhibited differences in specific adverse events. Nintedanib was associated with significantly fewer instances of diarrhea, whereas pirfenidone showed significantly fewer cases of skin rash (both p < 0.00001).

The DLco measures how effectively oxygen moves from the lungs into the bloodstream. In IPF, the lungs become scarred and stiff over time, leading to breathing difficulties and reduced lung function [33, 34]. DLco testing is often performed as part of the diagnostic workup for IPF and other respiratory conditions. Due to fibrotic changes in the lungs, DLco is frequently reduced in IPF, impairing gas exchange. The extent of lung tissue scarring and IPF progression can be evaluated through the severity of reduction in DLco [35, 36]. However, it is also important to note that the reduction of DLco in IPF is not always specific to a similar condition; it can be affected by other mechanisms as well, such as pulmonary hypertension, emphysema, or heart failure [37, 38]. Hence, DLco findings are typically interpreted in terms of different clinical and diagnostic findings. Studies that reported the association between DLco and IPF observed that this was a useful tool in monitoring and diagnosing IPF. Moreover, studies have found that DLco correlates with progression and disease severity in IPF, and changes in DLco over time can help predict outcomes and provide guidance for treatment decisions [24, 39, 40]. In general, DLco does not provide a diagnostic picture of IPF, but helps in the evaluation and management of patients with IPF. As a result, clinicians can assess the severity of the disease, monitor its progression, and make informed treatment choices based on this information [41, 42].

DLco improvement in patients with IPF may be explained by several underlying mechanisms. Inhibition of fibrosis: nintedanib and pirfenidone inhibit multiple tyrosine kinases, including FGF receptors, VEGF receptors and PDGF receptors. The receptors are involved in signaling pathways that promote proliferation, fibrosis, differentiation, and migration of fibroblasts, as well as angiogenesis. By inhibiting these pathways, nintedanib and pirfenidone may reduce lung fibrosis progression, lung function prevention, and potentially DLco improvement [43]. Anti-inflammatory effects: nintedanib and pirfenidone exhibit anti-inflammatory properties. Inflammation plays a crucial role in IPF pathogenesis, contributing to fibroblast activation, extracellular matrix deposition, and tissue remodeling. Nintedanib and pirfenidone may ameliorate lung damage and improve DLco by suppressing inflammation [41, 44]. The reduction of excessive extracellular matrix deposition: In IPF, excessive extracellular matrix components, such as collagen, accumulate in the lungs, leading to the formation of scar tissue [45, 46]. The inhibition of PDGF receptors by nintedanib and pirfenidone can result in a decrease in the proliferation and activation of fibroblasts, which are responsible for producing collagen and other extracellular matrix proteins. As a result of reducing the deposition of scar tissue, nintedanib and pirfenidone may improve lung function, including DLco [47]. Preservation of alveolar structure: The alveoli are minute air sacs responsible for gas exchange within the lungs. IPF distorts the alveolar structure due to fibrotic changes, impairing gas exchange and reducing DLco. Due to the antifibrotic properties of both drugs, further deterioration of lung function may be prevented, as well as an improvement in DLco, as a result of their antifibrotic effects [21].

As a whole, nintedanib and pirfenidone exhibit multi-targeted mechanisms of action. This includes inhibition of fibrosis, inflammation, and excessive extracellular matrix deposition, which contribute to its ability to improve DLco in patients with IPF. However, further research is required to fully understand the mechanisms of action of this compound and how it affects DLco in IPF [47]. As a result, in our research, DLco measurement is crucial for assessing antifibrotic agents for IPF. According to the pooled analysis, nintedanib and pirfenidone both revealed the potential to improve the DLco after 6 and 12 months of treatment.

FVC is an important measure of lung function and is the maximum amount of air that a person can forcefully exhale after taking a deep breath. FVC is often used as a diagnostic and monitoring tool in the context of IPF. In IPF, lung tissue becomes stiff, thickened, and scarred over time, which impairs its ability to expand and contract efficiently. This fibrotic process leads to a decrease in lung volume, including vital capacity. Therefore, FVC tends to decrease as IPF progresses, reflecting the decline in lung function. Clinically, monitoring FVC is essential in managing IPF because it is a marker for disease progression. A decline in FVC over time indicates worsening lung function and progression of the disease. Conversely, stabilization or improvement in FVC may suggest a positive response to treatment or a slower disease progression [35]. Moreover, FVC is often used with other pulmonary function tests, FEV1, and imaging studies (such as high-resolution computed tomography) to diagnose and monitor IPF, assess disease severity, and evaluate treatment response [48]. In summary, there is a strong association between FVC and IPF. Monitoring FVC over disease time is essential for assessing disease progression, determining treatment efficacy, and guiding clinical management decisions for patients with IPF.

The mechanism of action of nintedanib and pirfenidone involves targeting multiple tyrosine kinases, enzymes involved in various cellular processes such as cell growth, proliferation, and angiogenesis (formation of new blood vessels) [49]. In IPF, abnormal activation of certain growth factors and signaling pathways leads to excessive collagen deposition and other proteins in the lung tissue, causing fibrosis and impairing lung function, including reduced FVC. Nintedanib and pirfenidone work by inhibiting the activity of several key receptors and signaling pathways involved in fibrosis, particularly those mediated by growth factors such as FGF, PDGF, and VEGF [46, 47, 50]. By blocking these signaling pathways, nintedanib and pirfenidone help suppress fibroblast proliferation, reduce the production of extracellular matrix proteins like collagen, and inhibit the formation of new blood vessels within the fibrotic tissue. These effects collectively contribute to slowing down fibrosis progression and preserving lung function in patients with IPF, including improved FVC [48].

It is important to note that while pirfenidone and nintedanib can help reduce the decline in lung function and improve FVC in some patients with IPF, they may not

completely halt the progression of the disease, and individual responses to treatment can vary. Additionally, the exact mechanisms underlying their effects on FVC improvement may involve complex interactions within lung tissue and other systemic factors beyond their direct antifibrotic actions [48].

One limitation of this meta-analysis is that it included mostly non-RCTs, which limits the strength of causal inference. Most of the included studies were observational, introducing a risk bias. In addition, without any head-to-head trials to better estimate relative effects between interventions, no strong conclusions can be made on comparative effectiveness based on the findings of this meta-analysis. There was a high degree of heterogeneity among the studies, presumably due to differences in characteristics of patients at baseline, disease severity, concomitant therapies, and follow-up time. Any confounding bias could affect our pooled estimates of effect, resulting in either overestimation or underestimation of the treatment effect. The residual heterogeneity that we were unable to explain in the subgroup and sensitivity analyses should also be considered in the interpretability of this systematic review. Therefore, high-quality RCTs, especially head-to-head trials, are required to address the limitations of non-RCTs, strengthen causal inference, and better inform evidence-based clinical decision-making.

Bias may have existed because the search was limited to English-language publications only. In addition, studies were excluded from the meta-analysis if the publication presented results of the mentioned outcomes in forms that could not be converted into means and SDs, which may have influenced the results.

Conclusion

In this evaluation, quantitative evidence suggests that both nintedanib and pirfenidone are effective in slowing the progression of idiopathic pulmonary fibrosis in terms of improvement in diffusion capacity of the lungs for carbon monoxide and forced vital capacity at 6 and 12 months of treatment with similar safety profiles. Therefore, pirfenidone and nintedanib can be considered for managing idiopathic pulmonary fibrosis progression. Consequently, further observational and randomized controlled studies are required to evaluate the robust evidence regarding nintedanib and pirfenidone effects on lung function.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Table 1

Database search strategy for nintedanib and pirfenidone in idiopathic pulmonary fibrosis

Search Strategy (last search date 31 August 2025)

Embase (Ovid):

1. idiopathic pulmonary fibrosis.tw.
2. cryptogenic fibrosing alveolitis.tw.
3. usual interstitial pneumonitis.tw.
4. usual interstitial pneumonia.tw.
5. fibrosing alveolitis.tw.
6. IPF.tw.
7. 1 or 2 or 3 or 4 or 5 or 6
8. pirfenidone.tw.
9. nintedanib.tw.
10. 8 or 9
11. 7 and 10
12. limit 11 to (human)

PubMed:

((((((((idiopathic pulmonary fibrosis[MeSH Terms]) OR (idiopathic pulmonary fibrosis[Text Word])) OR (cryptogenic fibrosing alveolitis[Text Word])) OR (usual interstitial pneumonitis[Text Word])) OR (usual interstitial pneumonia[Text Word])) OR (fibrosing alveolitis[Text Word])) OR (IPF[Text Word])) AND (((pirfenidone [MeSH Terms]) OR (pirfenidone[Text Word])) OR ((nintedanib[MeSH Terms]) OR (nintedanib [Text Word])))) AND Filters: Humans.

Scopus:

((TITLE-ABS-KEY ("PIRFENIDONE") OR TITLE-ABS-KEY ("NINTEDANIB")) AND ((TITLE-ABS-KEY (" IDIOPATHIC PULMONARY FIBROSIS") OR TITLE-ABS-KEY ("CRYPTOGENIC FIBROSING ALVEOLITIS") OR TITLE-ABS-KEY ("USUAL INTERSTITIAL PNEUMONITIS") OR TITLE-ABS-KEY ("USUAL INTERSTITIAL PNEUMONIA") OR TITLE-ABS-KEY ("FIBROSING ALVEOLITIS") OR TITLE-ABS-KEY ("IPF")))

ClinicalTrials.gov

idiopathic pulmonary fibrosis AND nintedanib AND pirfenidone

Google Scholar

idiopathic pulmonary fibrosis AND nintedanib AND pirfenidone

Supplementary Table 2

Study quality assessment trough Modified Jadad scale

Jadad tool	Kerget 2023	
	yes/no	points
Was the study described as randomized?	yes	1
Was the method of randomization appropriate?	yes	1
Was the study described as blinding?	no	0
Was the blinding method appropriate?	no	0
Was there a description of withdrawal and dropouts?	no	0
Was there a clear description of the inclusion/exclusion criteria?	yes	1
Was the method used to assess the adverse effect described?	yes	1
Was the method of statistical analysis described?	yes	1
Total score		5

Supplementary Table 3

Findings of Egger's regression test

Outcomes	Z-value	b (95% CI)	*p*-value	Interpretation
DLco at 6 months	-0.6020	0.9419 (-1.3329, 3.2166)	0.5472	Z < 1.96 indicates $p > 0.05$ No presence of publication bias
DLco at 12 months	-1.6993	0.9129 (-0.0686, 1.8945)	0.0893	Z < 1.96 indicates $p > 0.05$ No presence of publication bias
FVC at 6 months	-1.3631	1.0755 (-0.0974, 2.2485)	0.1729	Z < 1.96 indicates $p > 0.05$ No presence of publication bias
FVC at 12 months	-2.8408	2.3573 (0.9397, 3.7748)	0.0045	Z < 1.96 indicates $p > 0.05$ Presence of publication bias
All-cause mortality	1.2481	0.5441 (-0.2177, 1.3059)	0.2120	Z < 1.96 indicates $p > 0.05$ No presence of publication bias
Nausea	0.9630	0.0385 (-0.5407, 0.6177)	0.3355	Z < 1.96 indicates $p > 0.05$ No presence of publication bias
Skin rash	-0.3155	14.6802 (-30.7697, 60.1301)	0.7524	Z < 1.96 indicates $p > 0.05$ No presence of publication bias
Diarrhea	0.9805	0.0099 (-0.0613, 0.0812)	0.3269	Z < 1.96 indicates $p > 0.05$ No presence of publication bias

CI – confidence interval; DLco – diffusing capacity of the lungs for carbon monoxide; FVC – forced vital capacity.

ORIGINAL ARTICLES

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Invasive pneumococcal and *Haemophilus influenzae* type b disease in the Autonomous Province of Vojvodina, Serbia (2003–2024): trends, vaccination impact, and serotype dynamics

Invazivna oboljenja izazvana pneumokokom i hemofilusom influence tipa b u Autonomnoj Pokrajini Vojvodini, Srbija (2003–2024): trendovi, uticaj vakcinacije i dinamika serotipova pneumokoka

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Abstract

Background/Aim. Invasive pneumococcal disease (IPD) and invasive *Haemophilus influenzae* type b (Hib) disease remain associated with significant morbidity and mortality. The aim of the study was to analyze long-term trends, age-specific and seasonal disease patterns, clinical outcomes, IPD serotype distribution, and the impact of immunization programs on these diseases in the Autonomous Province (AP) of Vojvodina, Serbia, from 2003 to 2024. **Methods.** A descriptive population-based study was conducted using data from the regional communicable disease surveillance system coordinated by the Institute of Public Health of Vojvodina, Serbia. All reported cases of IPD and invasive Hib disease were included in the analysis. **Results.** The Hib immunization program introduced in 2006 led to a sustained reduction in invasive Hib disease, with only sporadic cases reported after 2009. IPD incidence fluctuated over the study period and has increased in recent years despite high coverage (≥ 90%) of the primary pneumococcal conjugate vaccine (PCV) series since 2019. PCV introduction markedly reduced the frequency of serotypes covered by PCV10. Serotype 3 and non-vaccine serotypes persisted, particularly among adults and infants aged < 1 year. Adults aged ≥ 40 years were predominantly affected by IPD, whereas invasive Hib disease mainly occurred in children aged 1–4 years. None of the patients had been previously vaccinated against IPD or invasive Hib disease. Case-fatality rates were significantly higher for IPD (9.6%) than for Hib disease (3.5%) ($p = 0.0361$). In the overall population, PCV10 serotypes declined sharply after vaccine introduction (from 55% to 22%), while PCV13-non-PCV10 serotypes remained stable (~40%), and non-vaccine serotypes increased (from 11% to 28%), with the additional emergence of PCV20-non-PCV15 serotypes. **Conclusion**. The Hib immunization program achieved sustained disease control in the AP of Vojvodina, while IPD remains a public health concern due to persistent serotype 3 and emerging non-vaccine serotypes. Continued surveillance and consideration of higher-valency pneumococcal vaccine (minimum PCV13) or adult-targeted vaccination strategies are crucial to further reduce disease burden.

Key words:
epidemiology; haemophilus influenzae type b; incidence; pneumonia, pneumococcal; serbia; vaccination.

Apstrakt

Uvod/Cilj. Invazivna pneumokokna bolest (*invasive pneumococcal disease* – IPD) i invazivna bolest izazvana bakterijom *Haemophilus influenzae* tipa b (Hib) i dalje su povezane sa značajnim morbiditetom i mortalitetom. Cilj rada bio je da se analiziraju dugoročni trendovi, obrasci specifični za uzrast i sezonski obrasci bolesti, klinički ishodi, distribucija serotipova IPD i uticaj programa imunizacije na ta oboljenja u Autonomnoj Pokrajini (AP) Vojvodini, Srbija, u periodu od 2003. do 2024. godine. **Metode.** Sprovedena je deskriptivna epidemiološka studija korišćenjem podataka iz regionalnog sistema nadzora nad zaraznim bolestima, kojim koordinira Institut za javno zdravlje Vojvodine. U analizu su uključeni svi prijavljeni slučajevi IPD i Hib-izazvane invazivne bolesti. **Rezultati.** Program imunizacije protiv infekcija izazvanih Hib, uveden 2006. godine, doveo je do trajnog sniženja učestalosti Hib-izazvane invazivne bolesti, sa sporadičnim slučajevima oboljenja prijavljenim posle 2009. godine. Incidencija IPD varirala je tokom posmatranog perioda i porasla je u poslednjim godinama, uprkos visokom obuhvatu (≥ 90%) primarnom serijom pneumokokne konjugovane vakcine (*pneumococcal conjugate vaccine* – PCV) od 2019. godine. Uvođenje PCV značajno je smanjilo učestalost serotipova obuhvaćenih PCV10. Serotip 3 i nevakcinalni serotipovi opstali su, naročito među odraslima i odojčadima starosti < 1

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godine. Odrasle osobe starosti ≥ 40 godina pretežno su bile pogođene IPD, dok se Hib-izazvana invazivna bolest javljala uglavnom kod dece uzrasta 1–4 godine. Nijedan od obolelih nije prethodno bio vakcinisan protiv IPD ili Hib-izazvane invazivne bolesti. Stopa smrtnosti bila je značajno viša kod IPD (9,6%) nego kod Hib-izazvane bolesti (3,5%) ($p = 0{,}0361$). U ukupnoj populaciji, učestalost serotipova PCV10 naglo je opala posle uvođenja vakcine (sa 55% na 22%), dok su serotipovi PCV13-ne-PCV10 ostali stabilni (~40%), a prisustvo nevakcinalnih serotipova se povećalo (sa 11% na 28%), uz dodatnu pojavu serotipova PCV20-ne-PCV15. **Zaključak.** Program imunizacije protiv Hib-izazvanih oboljenja obezbedio je zadovoljavajuću kontrolu bolesti u AP Vojvodini, dok, zbog perzistentnog serotipa 3 i novih serotipova koji nisu obuhvaćeni vakcinom, IPD i dalje predstavlja značajan javnozdravstveni problem. Kontinuirani nadzor i razmatranje primene viševalentnih pneumokoknih vakcina (najmanje PCV13) ili strategije vakcinacije usmerene na odrasle osobe su od ključnog značaja za dalje smanjenje tereta bolesti.

Ključne reči:
epidemiologija; hemofilus influence tip b; incidencija; pneumonija, pneumokokna; srbija; vakcinacija.

Introduction

Invasive infections caused by *Streptococcus* (*S.*) *pneumoniae* and *Haemophilus influenzae* type b (Hib) remain major global public health concerns, particularly among infants, young children, and older adults [1–3].

In 2022, 17,700 confirmed cases of invasive pneumococcal disease (IPD) were reported in the European Union/European Economic Area (EU/EEA) with a crude incidence of 5.1 *per* 100,000 population (hereafter expressed *per* 100,000). The highest incidence was recorded among infants < 1 year (13.4 *per* 100,000) and adults aged ≥ 65 years (12.6 *per* 100,000) [4]. In the United States (US), the overall IPD incidence is about 8.3 *per* 100,000, ranging from 10.8 in infants to 27.4 in the oldest adults [5]. Case fatality ratios (CFRs) remain high–around 13% in the EU/EEA and 10–12% in the US, depending on clinical presentation [4, 5]. In developing countries, CFRs may reach 20% for sepsis and up to 50% for meningitis [1].

Before Hib conjugate vaccines, invasive Hib disease was a leading cause of bacterial meningitis in young children, with incidence rates up to 50–60 *per* 100,000 in the US and about 23 *per* 100,000 in Europe among children aged 0–4 years [6, 7]. Following widespread vaccine implementation, Hib incidence in high-coverage regions, including Europe, has declined to about 1 *per* 100,000 or less among children under 5 years. In 2022, the EU/EEA reported a CFR of 9.2% for invasive Hib disease [8]. Before vaccine introduction, Hib meningitis in the US accounted for 50–65% of all cases, with a CFR of 3–6% [9].

Conjugate vaccines against pneumococcal and Hib diseases have markedly reduced incidence and mortality worldwide [1, 2, 10–12]. However, vaccine impact varies by serotype distribution, coverage, age, seasonality, and disruptions to routine immunization during public health crises [1–7, 13, 14].

In Serbia, surveillance of IPD and Hib disease is mandatory but largely passive, resulting in limited epidemiological data [15]. Routine immunization with pneumococcal conjugate vaccine (PCV) was introduced in 2018, with PCV10 administered according to a 3+1 schedule: the first dose at 2 months of age, the second at 4 months, the third at 6 months, and a booster dose one year after the third dose. In 2022, PCV13 replaced PCV10 using the same schedule, which shifted to a 2+1 program in 2023. In 2024, PCV10 was reintroduced using the 2+1 schedule, mainly due to economic rather than evidence-based considerations [16–18]. Mandatory Hib immunization with three doses of the monovalent vaccine during the first year of life (starting at two months) was introduced in Serbia in 2006. Since 2015, both primary and booster doses have been given as part of the combined pentavalent (5-in-1) vaccine that protects against diphtheria, tetanus, pertussis, inactivated polio vaccine, and Hib (DTaP-IPV-Hib) vaccine [16]. Pneumococcal and Hib vaccination is also mandatory for individuals at increased risk, particularly those with underlying conditions [17].

These observations highlight the importance of continuous, comprehensive surveillance to monitor vaccine impact, detect potential serotype replacement, and guide immunization policy. Furthermore, insights into age-specific and seasonal patterns of invasive diseases are crucial for tailoring risk-targeted interventions and optimizing resource allocation [1–4, 8–10, 13, 14], which have not been well described in our territory.

The aim of this study was to analyze incidence trends, clinical outcomes, vaccination coverage, age and seasonal distribution, geographic clustering of IPD and Hib disease, as well as pneumococcal serotype changes before and after PCV introduction in the Autonomous Province (AP) of Vojvodina, Serbia. The findings aim to inform national vaccination strategies and address remaining challenges in controlling invasive bacterial diseases.

Methods

Study design and data sources

This descriptive study was conducted in the AP of Vojvodina, northern Serbia, which has a population of approximately 1.7 million inhabitants, accounting for about 25% of the total population of Serbia [19]. Data were retrieved from the regional communicable disease surveillance system, coordinated by the Institute of Public Health of Vojvodina (IPHV), Novi Sad, Serbia.

All cases (both clinically and laboratory confirmed) of IPD and invasive Hib disease reported between 2003 and 2024 were included in the comprehensive analysis. Hospitals across the AP of Vojvodina submitted notifications of these invasive cases through the national mandatory communicable disease reporting system, as previously described [20].

Case classification followed national surveillance standards and World Health Organization (WHO) guidelines [21, 22]. In addition, the case definition used for IPD was *S. pneumoniae* identified by culture from any normally sterile site (blood, cerebrospinal fluid – CSF, pleural fluid, joint fluid) in a symptomatic person, or *S. pneumoniae* identified in CSF or pleural fluid by antigen detection, immunochromatography, or polymerase chain reaction (PCR) [21]. Similarly, confirmed invasive Hib disease was defined as HiB identified by culture from any normally sterile site (e.g., blood, CSF, pleural fluid, joint fluid) in a symptomatic person, or detected in CSF or pleural fluid by antigen detection, immunochromatography, or PCR [22].

Statistical analysis

Demographic (gender, age, place of residence, district of the AP of Vojvodina), temporal (month, year), and clinical (case status, outcome) variables were analyzed. In order to assess specific characteristics (incidence rate, seasonal pattern, frequency of IPD and invasive Hib disease, and serotype distribution of IPD), patients were divided into six age groups: < 1, 1–4, 5–9, 10–19, 20–39, and ≥ 40 years. Annual and age-specific incidence rates *per* 100,000 were calculated using case counts and population estimates for the whole population and specific age groups in the AP of Vojvodina between 2003 and 2024 [19]. Vaccination coverage against diseases caused by *S. pneumoniae* and Hib was assessed using the administrative method [23], as previously described in detail [24]. Seasonal distribution was assessed by calendar month. Comparisons between the two clinical entities (IPD and invasive Hib disease) regarding gender, age, area of residence, district of the AP of Vojvodina, case status, and outcome were performed using Pearson's Chi-square test or Fisher's exact test, as appropriate. The value of $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using SPSS software, version 21 (IBM Corp., Armonk, NY, USA).

Based on officially reported IPD cases (from communicable disease notifications, excluding laboratory notifications) between 2009 and 2024, the serotype distribution was analyzed for the pre-vaccination and vaccination periods, vaccine and non-vaccine serotypes, and age groups.

All annual data, including official communicable disease notifications, were reported to the Center for Disease Control and Prevention at the IPHV as part of the routine communicable disease surveillance system in the province. As described previously [20, 24–26], these surveillance activities involved continuous collaboration between the IPHV and the district Institutes of Public Health located in the administrative centers of the seven districts of the AP of Vojvodina.

Ethical considerations

Similar to previously used methodology [20, 24–26], the data used in this retrospective study were derived from routine surveillance conducted between 2003 and 2024 at the Center for Disease Control and Prevention, IPHV, Novi Sad. Therefore, approval from an Ethics Committee was not required under Serbian regulations.

Results

Annual incidence rates of IPD and invasive Hib disease from 2003 to 2024 are shown in Figure 1.

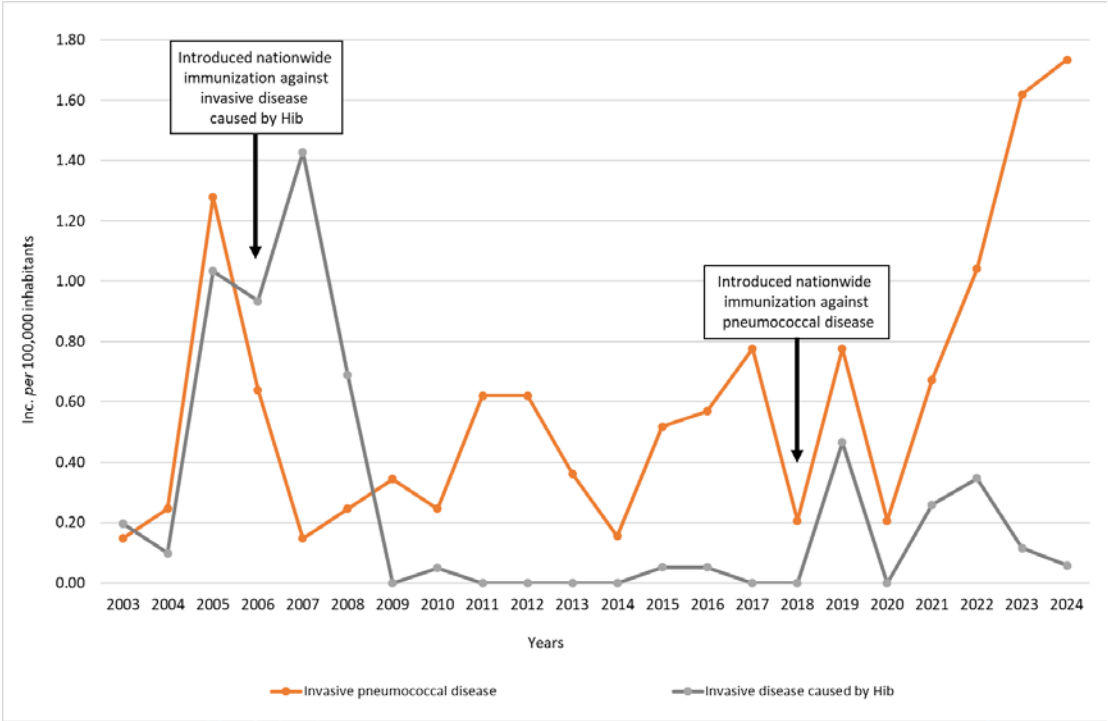

Fig. 1 – Annual incidence rates of invasive pneumococcal disease and invasive *Haemophilus influenzae* type b (Hib) disease in relation to the introduction of nationwide immunization programs in the Autonomous Province of Vojvodina, Serbia (2003–2024).

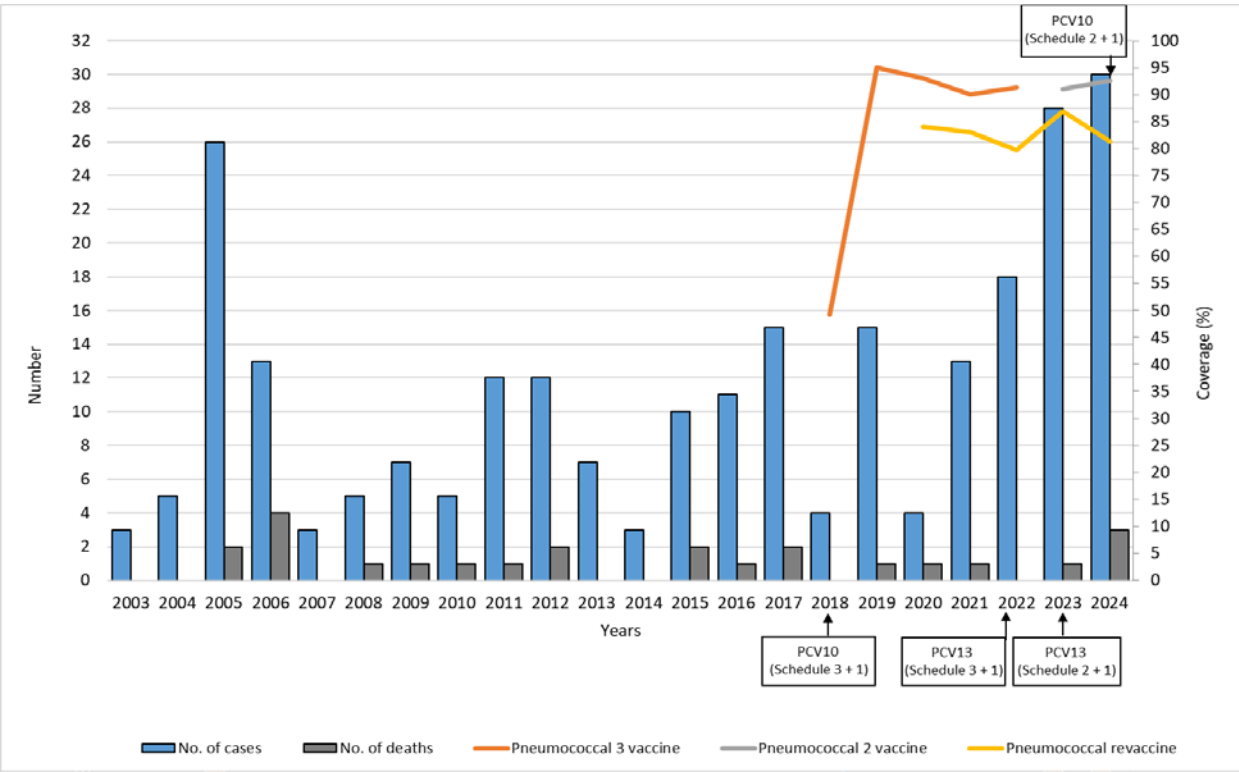

Fig. 2 – Annual number of invasive pneumococcal disease cases and deaths, and vaccination coverage with primary series and booster doses in the Autonomous Province of Vojvodina, Serbia (2003–2024).
PCV – pneumococcal conjugate vaccine; No. – number.
Note: **numbers after PCV (meaning 'valent') refer to the number of specific strains (serotypes) of pneumococcal bacteria that the vaccine targets.**

IPD incidence fluctuated, ranging from 0.15 *per* 100,000 in 2003 and 2007 to a peak of 1.73 in 2024. Nationwide mandatory pneumococcal vaccination for infants began in 2018.

Invasive Hib disease showed a different trend, peaking in 2007 (1.43 *per* 100,000) and rapidly declining after the introduction of mandatory Hib vaccination in 2006. Between 2009 and 2018, incidence was negligible (≤ 0.05 *per* 100,000), with a brief resurgence in 2019 (0.47 *per* 100,000) and moderate levels in 2021–2022, followed by very low rates in 2023–2024.

IPD cases, fatal outcomes, and pneumococcal vaccination coverage in 2003–2024, including vaccine type, schedule, and timeline, are shown in Figure 2.

Reported IPD cases fluctuated, with lows in 2003, 2007, and 2014 (n = 3) and a peak in 2024 (n = 30).

Fatal outcomes ranged from 1 to 4 *per* year, with the highest number in 2006 (n = 4); most years had 1–2 deaths, and there were 3 deaths in 2024.

Mandatory infant pneumococcal vaccination was initiated in 2018, with coverage for the three-dose primary series increasing from 49.3% in 2018 to ≥ 90% from 2019 onwards. Booster coverage ranged from 79.8% to 86.9% between 2020 and 2023, and coverage for the two-dose primary series introduced in 2023 reached 92.6% in 2024.

Invasive Hib cases, fatal outcomes, and Hib vaccination coverage, including vaccine type, schedule, and timeline, from 2003 to 2024, are shown in Figure 3.

Reported cases peaked in 2005 (n = 21), 2006 (n = 19), and 2007 (n = 29). Fatal outcomes were rare, with only four recorded (2004, 2006, 2010, 2021).

Coverage with the three-dose primary Hib series rose steadily after introduction, exceeding 95% from 2008 to 2019, slightly declining to 94% in 2020 and ≤ 93% between 2021 and 2024. Revaccination began in 2015, with coverage ranging from 81.5% (2022) to 93.2% (2019), generally lower than the primary series.

Between 2003 and 2024, the age-specific incidence of IPD and invasive Hib disease in the AP of Vojvodina showed distinct patterns.

IPD incidence was highest in infants (< 1 year), with a peak especially in 2024 (12.69 *per* 100,000). In adults aged ≥ 40 years, incidence was low until the mid-2010s but increased post-PCV introduction, reaching 2.63 *per* 100,000 in 2024.

For invasive Hib disease, incidence was very high among children aged < 5 years before Hib vaccination, peaking between 2005 and 2007 (22.95 *per* 100,000 in infants; 27.95 *per* 100,000 in those aged 1–4 years). After 2006, incidence dropped sharply, with no cases reported in most years from 2009 onwards. Following the introduction of vaccination in 2018, only two peaks occurred–in 2019 (11.56 *per* 100,000) and 2023 (6.35 *per* 100,000)–both in unvaccinated infants aged < 1 year (Figure 4).

Between 2003 and 2024, IPD and invasive Hib disease in the AP of Vojvodina exhibited clear seasonal patterns. IPD peaked in December (n = 34) and October (n = 30),

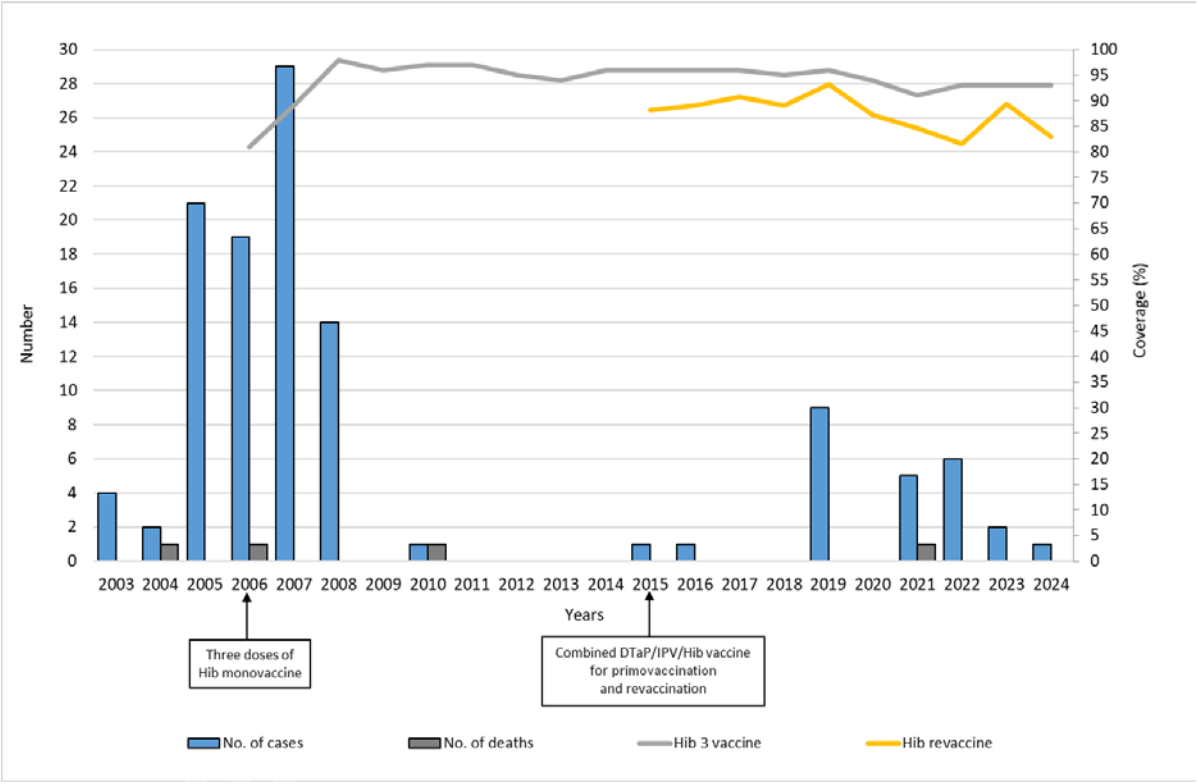

Fig. 3 – Annual number of invasive *Haemophilus influenzae* type b disease cases and deaths, and vaccination coverage with primary series and booster doses in the Autonomous Province of Vojvodina, Serbia (2003–2024).
DTaP-IPV-Hib – vaccine that protects against diphtheria, tetanus, whooping cough, polio, and *Haemophilus influenzae* type B (Hib). No. – number.

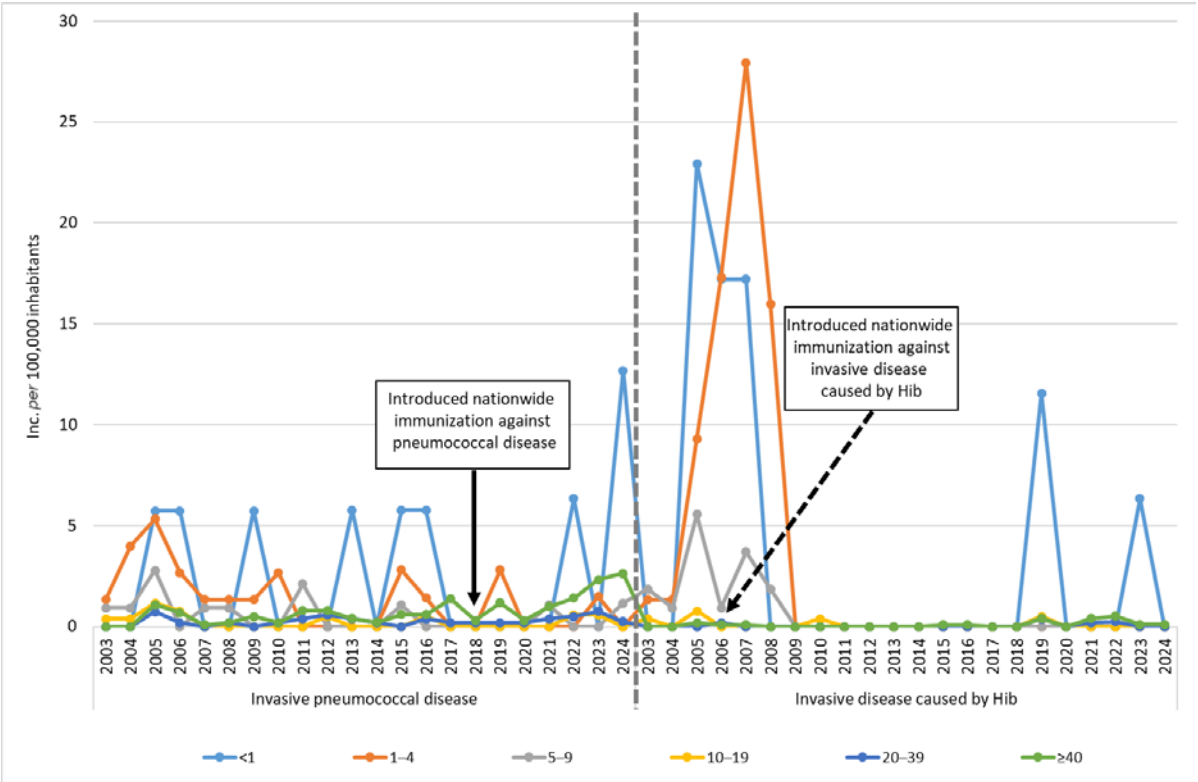

Fig. 4 – Age-specific incidence rates of invasive pneumococcal disease and invasive disease caused by *Haemophilus influenzae* type b (Hib) in the Autonomous Province of Vojvodina, Serbia (2003–2024).
***Note:* the grey dashed line represents the dividing line between the two clinical entities.**

accounting for 25.7% of cases. Invasive Hib disease showed pronounced winter predominance, with December (n = 17), October (n = 15), January (n = 14), and February (n = 15) comprising 53% of cases (Figure 5).

Between 2003 and 2024, IPD in the AP of Vojvodina occurred in all age groups, with the highest counts in adults aged ≥ 40 years, especially in winter. Peaks were in December (n = 27) and January (n = 21), accounting for 28.7% of cases in this group, while children aged < 5 years had few cases year-round. Invasive Hib disease primarily affected young children, particularly those aged 1–4 years, who accounted for most cases nearly every month. Between January and March, 24 cases (43.6% of cases in this age group) were recorded, showing strong winter predominance (Figure 6).

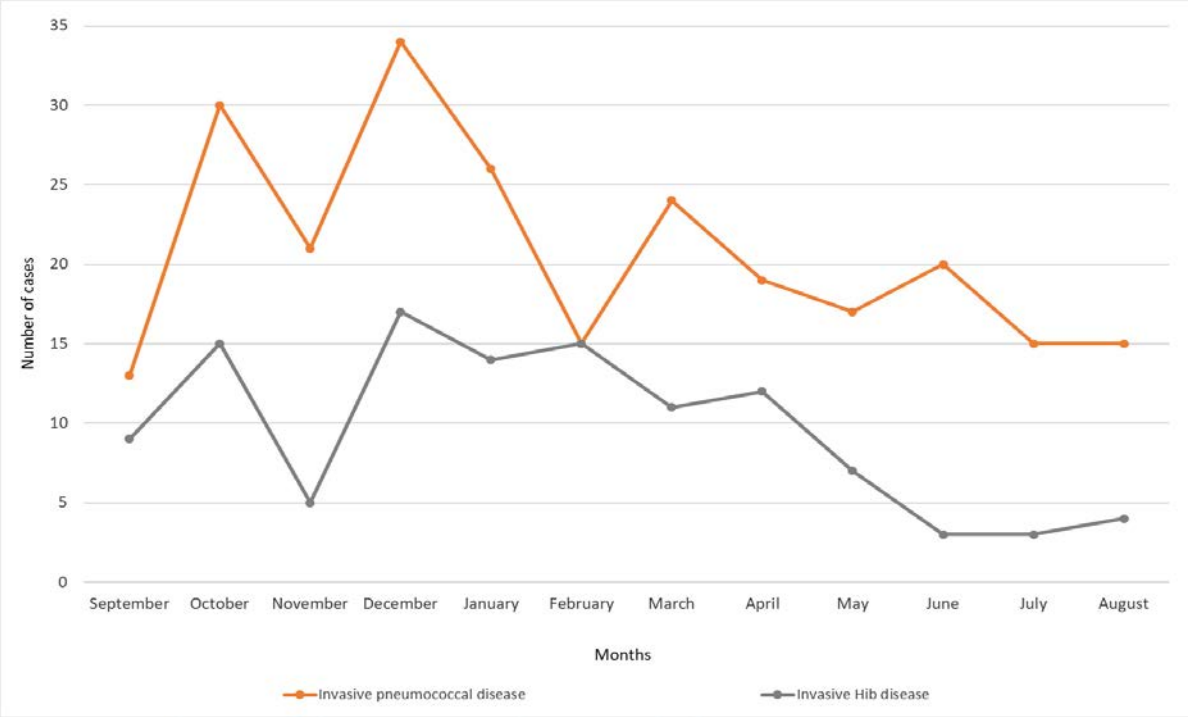

Fig. 5 – Seasonal distribution of invasive pneumococcal disease and invasive disease caused by *Haemophilus influenzae* type b (Hib) cases in the Autonomous Province of Vojvodina, Serbia (2003–2024).

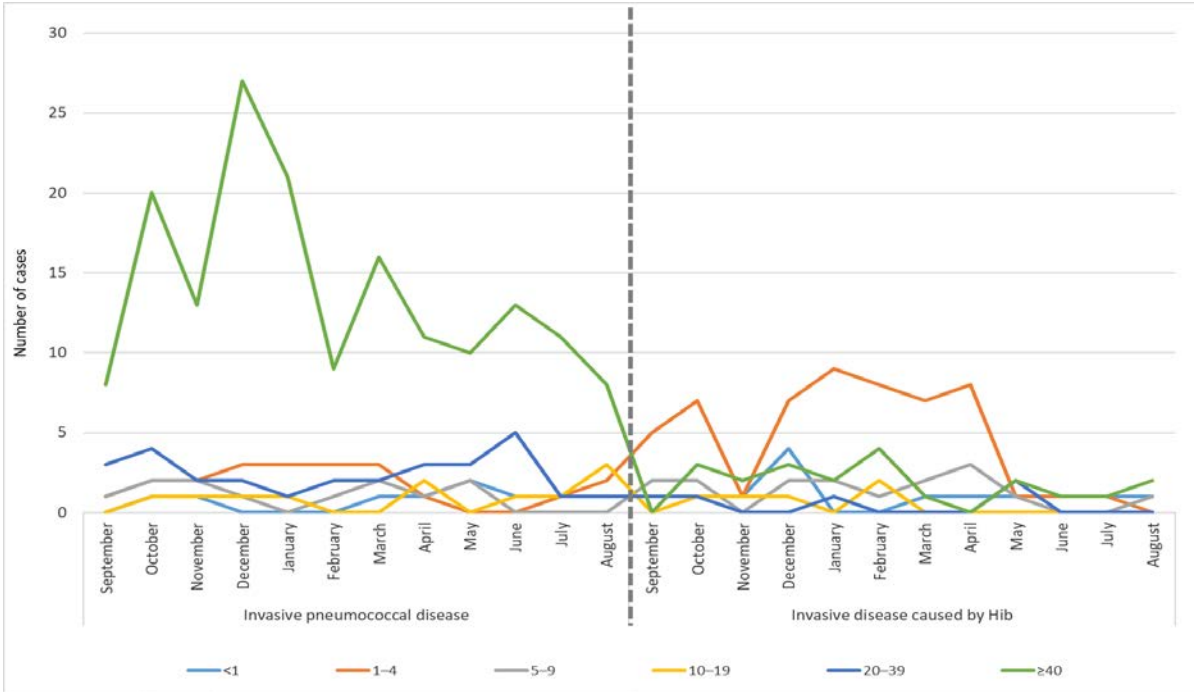

Fig. 6 – Monthly distribution of invasive pneumococcal and invasive cases caused by *Haemophilus influenzae* type b (Hib) in the Autonomous Province of Vojvodina, Serbia, by age groups (2003–2024).
***Note:* the grey dashed line represents the dividing line between the two clinical entities.**

None of the patients had received prior vaccination against IPD or invasive Hib disease. No significant gender differences were observed (IPD 62.65% vs. Hib 55.65% of males; $p = 0.2484$). However, age distribution differed markedly ($p < 0.0001$): IPD mainly affected adults aged ≥ 40 years (67.07%), whereas Hib disease predominated in children aged 1–4 years (47.83%). Both diseases were more common in urban residents (IPD 69.48% vs. Hib 74.78%), but this was not statistically significant ($p = 0.3605$). Pronounced geographic clustering was noted ($p < 0.0001$), with over half of IPD cases, i.e., 53.82%, and 85.22% of Hib cases reported from the South Bačka district. Most cases were laboratory-confirmed, with a higher proportion for IPD than Hib (94.38% vs. 86.09%; $p = 0.0135$). Clinical outcomes also differed ($p = 0.0361$): CFR was 9.64% for IPD (24/249) vs. 3.48% for Hib (4/115) (Table 1).

We analyzed 44 serotypes (18% of all laboratory-confirmed IPD cases) reported between 2009 and 2024, including 26 serotypes pre-vaccination and 18 during the vaccination period.

In the AP of Vojvodina, PCV10 serotype cases declined markedly after vaccine introduction. Serotypes 14 and 19F were the most common PCV10 serotypes in the pre-vaccination period (five and four cases, respectively), while single cases of 4, 6B, 18C, and 23F serotypes were also observed. After vaccination, no cases of 4, 9V, 19F, or 23F were detected. Among additional PCV10 serotypes, 7F persisted with one case in each period; serotypes 1 and 5 were absent. For PCV13 serotypes, serotype 3 remained the most frequent (five cases in the pre-vaccine, and six in the post-vaccine period), 6A disappeared after vaccination, and 19A appeared once during the vaccination period.

Extended PCV15 coverage included one 22F case in the pre-vaccination period; 33F was not detected. PCV20-related serotypes were rare, with single cases of serotype 8 in the pre-vaccine period and 11A and 15B during vaccination. No cases were caused by pneumococcal polysaccharide vaccine (PPV)23-exclusive serotypes (2, 9N, 17F, 20). Non-vaccine serotypes represented a small but notable fraction: 23A (two cases in the pre-vaccine period and one in the post-vaccine period), 15A (one case in the pre-vaccine period), and several others detected only during the vaccination period (15C, 28A, 6C, 7C; one case each) (Figure7).

Table 1

Demographic and clinical characteristics of patients with invasive pneumococcal disease and invasive *Haemophilus influenzae* type b (Hib) disease in the Autonomous Province (AP) of Vojvodina, Serbia (2003–2024).

Variable	Invasive pneumococcal disease (n = 249)	Invasive Hib disease (n = 115)	*p*-value
Gender			
male	156 (62.65)	64 (55.65)	0.2484
female	93 (37.35)	51 (44.35)	
Age group (years)			
< 1	9 (3.61)	13 (11.30)	< 0.0001
1–4	21 (8.43)	55 (47.83)	
5–9	12 (4.82)	16 (13.91)	
10–19	11 (4.42)	5 (4.35)	
20–39	29 (11.65)	5 (4.35)	
≥ 40	167 (67.07)	21 (18.26)	
Area of residence			
urban	173 (69.48)	86 (74.78)	0.3605
rural	76 (30.52)	29 (25.22)	
District of AP of Vojvodina			
North Bačka	20 (8.03)	4 (3.48)	< 0.0001
West Bačka	17 (6.83)	2 (1.74)	
South Bačka	134 (53.82)	98 (85.22)	
North Banat	13 (5.22)	6 (5.22)	
Central Banat	18 (7.23)	2 (1.74)	
South Banat	28 (11.24)	1 (0.87)	
Srem	19 (7.63)	2 (1.74)	
Case status			
laboratory-confirmed	235 (94.38)	99 (86.09)	0.0135
clinically-confirmed	14 (5.62)	16 (13.91)	
Outcome			
survived	225 (90.36)	111 (96.52)	0.0361
fatal cases	24 (9.64)	4 (3.48)	

All values are given as numbers (percentages).

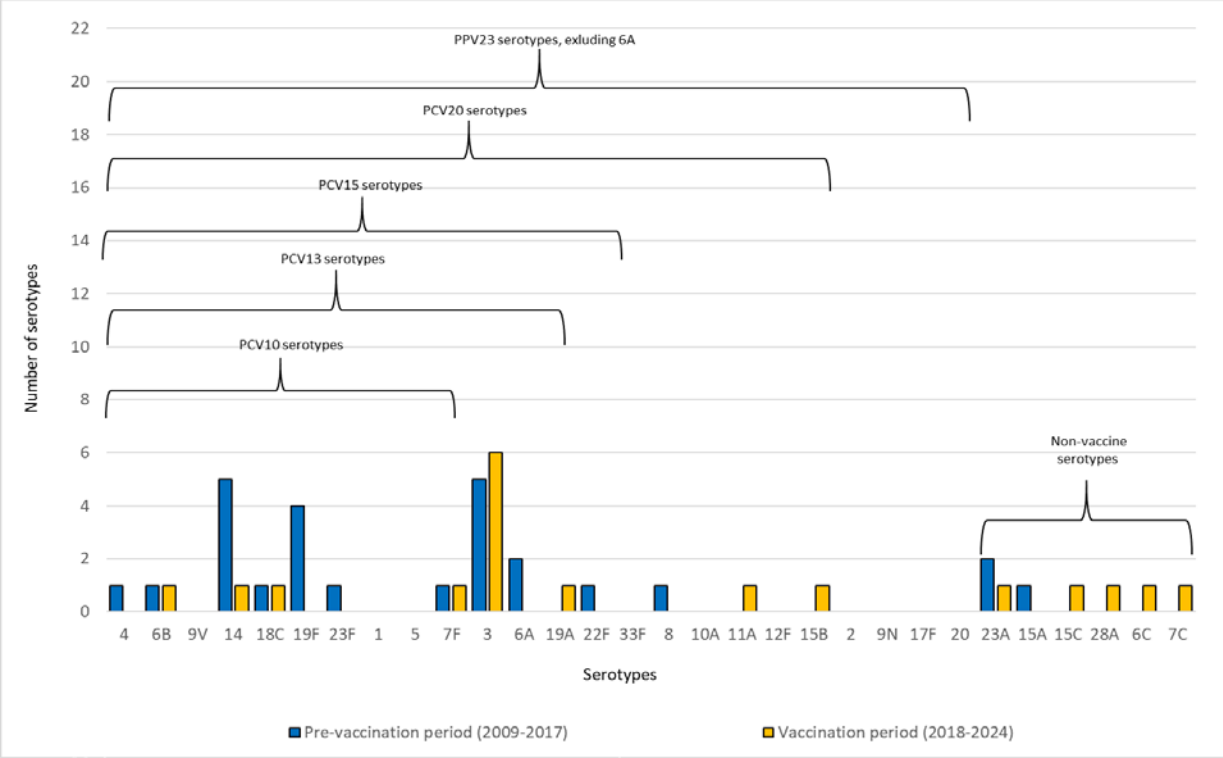

Fig. 7 – Serotype distribution of invasive pneumococcal disease in the Autonomous Province of Vojvodina, Serbia, before and after vaccine introduction (2009–2024).
PCV – pneumococcal conjugate vaccine; PPV– pneumococcal polysaccharide vaccine.
***Note*: numbers after PCV and PPV (meaning 'valent') refer to the number of specific strains (serotypes) of pneumococcal bacteria that the vaccines target.**

In the overall population, prior to vaccine introduction, PCV10 serotypes accounted for about 55% of cases, PCV13-non-PCV10 serotypes for approximately 25%, and non-vaccine serotypes for 11% of cases, with only a minor proportion (around 4%) attributable to PCV20-non-PCV15 and PCV15-non-PCV13 serotypes. During the vaccination period, the proportion of PCV10 serotypes decreased to 22%, while PCV13-non-PCV10 serotypes remained stable at around 40%. Non-vaccine serotypes increased to 28%, with PCV20-non-PCV15 serotypes contributing 11%.

Among children aged ≤ 5 years, PCV10 serotypes dominated in the pre-vaccination period, accounting for approximately 80% of IPD cases, with the remaining 20% caused by non-vaccine serotypes. In the vaccination period, only one 6B serotype from PCV10 was detected in this age group, while PCV13-non-PCV10, PCV20-non-PCV15, and non-vaccine serotypes each accounted for about 20% of cases.

In adults aged ≥ 40 years, PCV10 serotypes accounted for 47% of IPD cases in the pre-vaccination period, with PCV13-non-PCV10 serotypes representing about 35% and non-vaccine serotypes 6%. During the vaccination period, the share of PCV10 serotypes declined to below 20%, while PCV13-non-PCV10 serotypes increased to about 45%, and non-vaccine serotypes rose to 27%. The proportion of PCV15-non-PCV13 and PCV20-non-PCV15 serotypes remained small in both periods (Figure 8).

During the pre-vaccination period (2009–2017), IPD in children aged < 5 years was most frequently associated with PCV10 serotypes, including serotype 6B (one case in a child aged < 1 year) and serotype 14 (two cases in children aged 1–4 years). Serotype 19F was identified in four cases across age groups (1–4 years, 20–39 years, and ≥ 40 years). Serotype 3 predominated among adults, with five cases recorded (one in the 20–39-year age group and four in those aged ≥ 40 years). Additional cases were attributed to non-vaccine serotypes 15A and 23A, each detected only once or twice, among children aged 1–4 years and adults (Figure 9a).

In the vaccination period (2018–2024), the age distribution shifted. Only sporadic cases of PCV10 serotypes persisted: serotype 6B (one case in a child aged 1–4 years), serotype 14 (one case in an adult aged ≥ 40 years), and serotype 18C (one case in a patient aged 20–39 years). Serotype 3 continued to dominate, with six cases in adults (one in the 20–39-year group and five in those ≥ 40 years). Sporadic cases were also attributed to serotypes 7F, 19A, 11A, and 15B, while several non-vaccine serotypes (23A, 15C, 28A, 6C, and 7C) were recorded, predominantly among adults, and sporadically in children aged 5–9 years. Overall, serotypes 14 and 19F were the leading causes of IPD in adults in the pre-vaccination period, whereas serotype 3 predominated in the same age groups in both periods (Figure 9b).

For the six patients with fatal outcomes for whom serotype characterization was available, the majority were male (5/6), with ages ranging from 3 to 77 years. Four cases originated from urban areas, and meningitis was the leading clinical manifestation, identified in four of the fatal

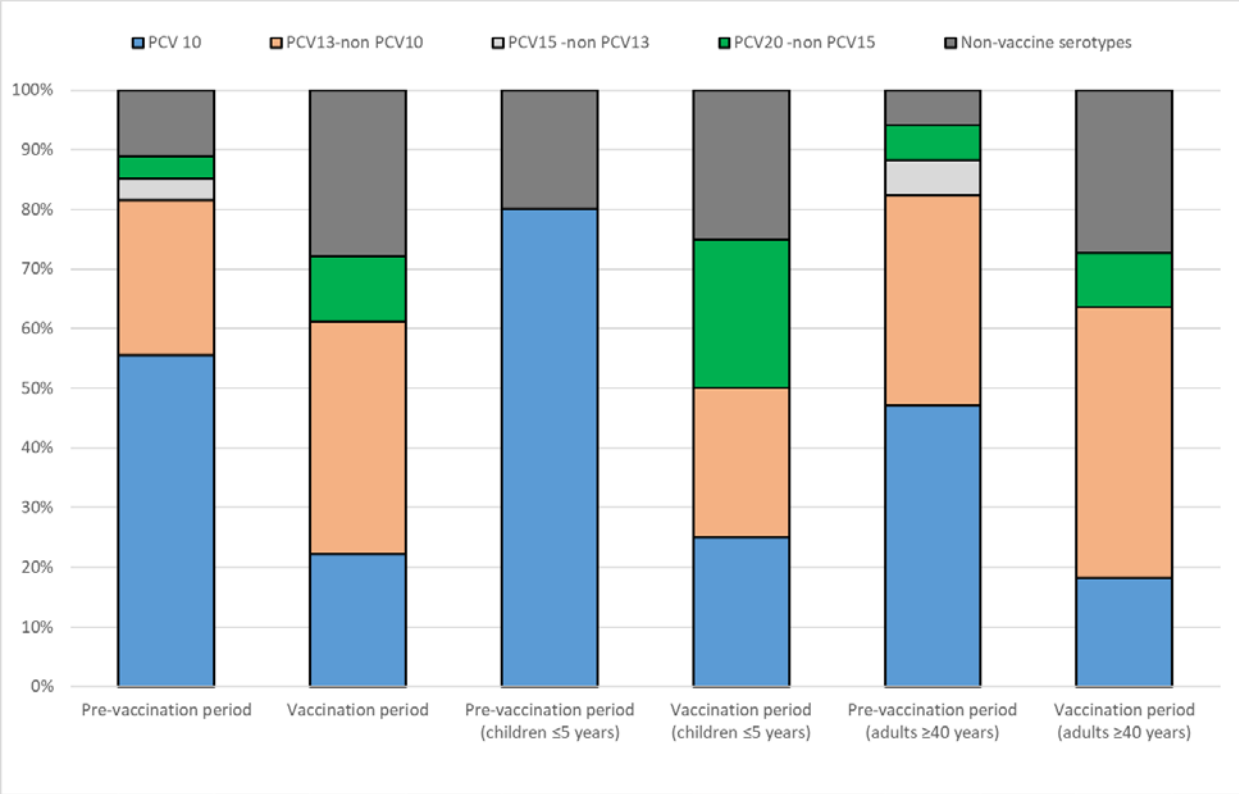

Fig. 8 – Distribution of invasive pneumococcal disease serotypes by pneumococcal conjugate vaccine (PCV) serotypes coverage in children under 5 years and adults ≥ 40 years, during the pre-vaccination (2009–2017) and vaccination (2018–2024) periods.

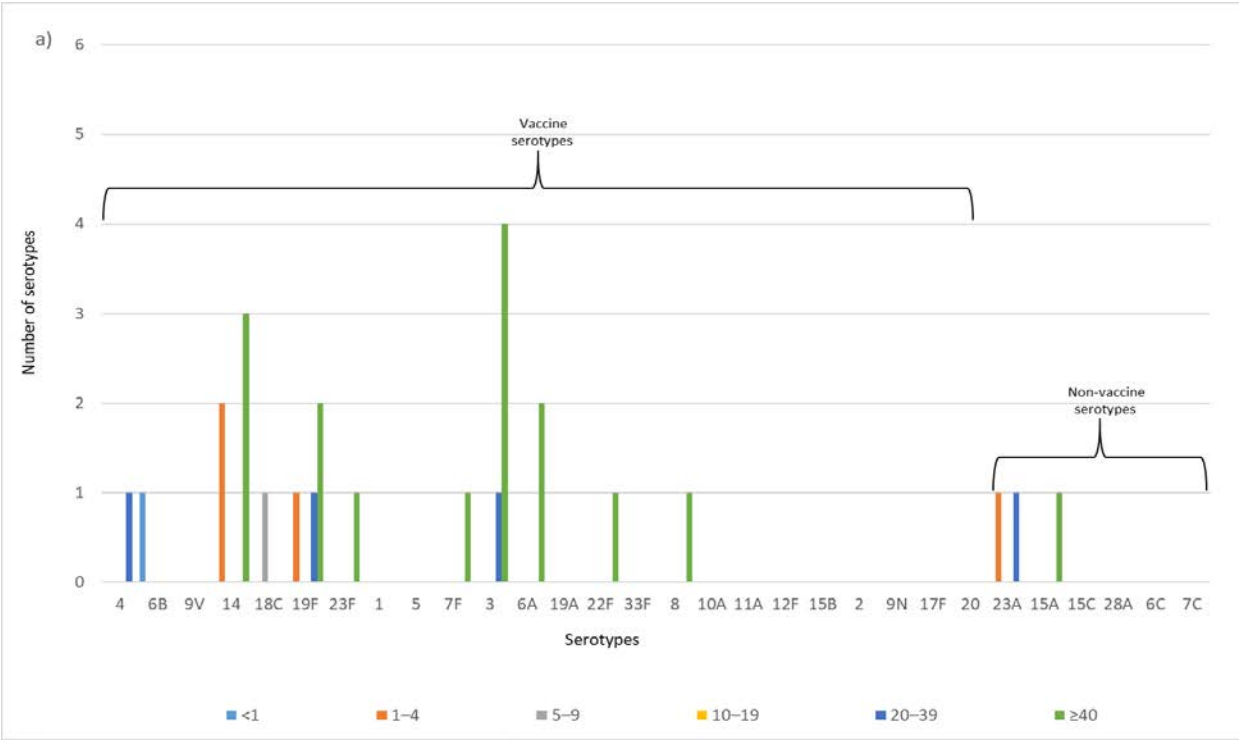

Fig. 9 – Distribution of invasive pneumococcal disease serotypes in the Autonomous Province of Vojvodina, Serbia, by age groups: a) pre-vaccination period (2009–2017) and b) vaccination period (2018–2024).

***Note*: Fig.9 continued on next page.**

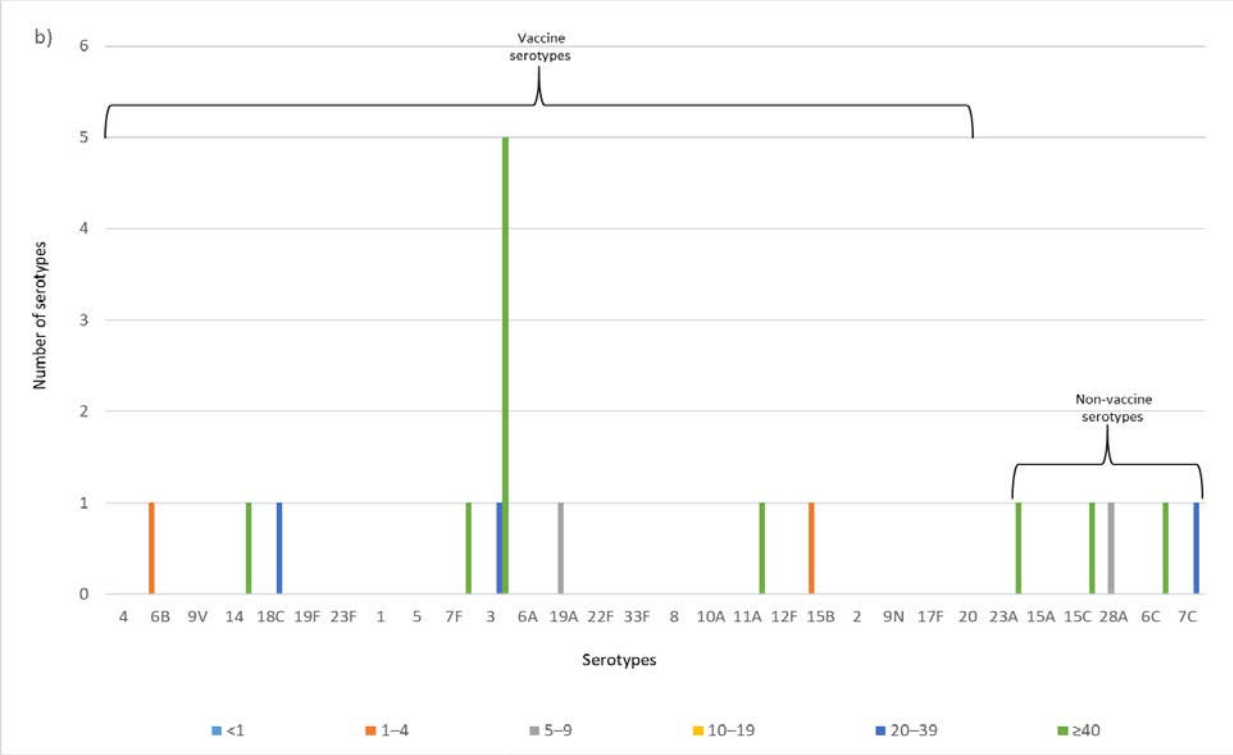

Fig. 9 (Continued) – Distribution of invasive pneumococcal disease serotypes in the Autonomous Province of Vojvodina, Serbia, by age groups: a) pre-vaccination period (2009–2017) and b) vaccination period (2018–2024).

Table 2

Characteristics of patients with total outcomes due to invasive pneumococcal disease in the Autonomous Province of Vojvodina, Serbia (2009–2024).

Gender	Year of death	Age (years)	Area of residence	Diagnosis	Month of death	Serotype
Male	2009	52	Urban	pneumococcal meningitis	March	3
Female	2010	77	Rural	streptococcal pneumonia	June	3
Male	2017	69	Rural	pneumococcal meningitis	March	14
Male	2020	38	Urban	pneumococcal meningitis	November	3
Male	2021	33	Urban	streptococcal pneumonia	February	7C*
Male	2023	3	Urban	pneumococcal meningitis	January	6B

*** Non-vaccine serotype**.

cases. Regarding seasonality, deaths occurred mainly during winter and spring months. Half of the fatal cases were caused by serotype 3. Additional serotypes associated with fatal outcomes included 14, 6B, and 7C, the latter being a non-vaccine serotype recorded during the vaccine period (Table 2). None of the patients with fatal outcomes had a history of vaccination against IPD.

Discussion

To our knowledge, this is the first long-term analysis of IPD and invasive Hib disease in the AP of Vojvodina, Serbia, spanning from 2003 to 2024 and covering pre- and post-vaccination periods. The study demonstrates the sustained success of Hib vaccination and the impact of childhood PCV immunization in reducing vaccine-type IPD, while highlighting persistent disease burden, age-specific vulnerabilities, seasonal patterns, and emerging non-vaccine serotypes. These findings underscore the importance of ongoing surveillance and adaptation of immunization programs [1–4, 18, 27].

According to the most recent Annual Epidemiological Report by the European Center for Disease Prevention and Control (ECDC), IPD incidence in 30 EU/EEA countries declined from 5.6 to 2.7 *per* 100,000 between 2018 and 2021, increasing to 5.1 in 2022 [28]. In the US, where IPD surveillance began in 1998, incidence fell sharply after PCV intro-

duction in 2000, reaching ~5 *per* 100,000 in 2020–2021 and ~8 *per* 100,000 in 2022 [29].

In the EU/EEA, confirmed Hib incidence remained ≤ 0.8 *per* 100,000 between 2018 and 2021, increasing slightly to 0.9 in 2022 [8]. In the US, widespread Hib vaccination in the 1990s reduced incidence in children < 5 years by 99%, to 0.08 *per* 100,000 by 2018 [30]. Globally, WHO reports substantial declines in Hib-related morbidity and mortality [2, 11].

In the AP of Vojvodina, the nationwide Hib immunization program introduced in 2006 led to a sustained decline in invasive Hib disease, with only sporadic cases after 2009. In contrast, pneumococcal vaccination, introduced in 2018, has not yet produced a similar decline in IPD, which has risen over the past five years, peaking in 2023–2024. This difference reflects high coverage and herd immunity for Hib, whereas pneumococcal vaccination is recent, with effects still evolving. The greater diversity of pneumococcal serotypes, including non-vaccine types, variable coverage–especially for revaccination–and improved surveillance likely contributed to the recent increase in IPD cases.

Consistent with the EU/EEA and US data [4, 8, 28–30], our results demonstrate that IPD mainly affected infants aged < 1 year and adults aged ≥ 40 years, while invasive Hib disease was most common in children aged 1–4 years. After Hib vaccination, cases shifted to unvaccinated infants aged < 1 year, demonstrating strong vaccine protection. In contrast, despite PCV introduction in 2018, IPD incidence has not declined consistently. The ongoing burden and recent rise in older adults highlight that high-risk groups remain vulnerable and need targeted prevention.

Both IPD and Hib showed clear seasonality, peaking in the colder months (October–February) and declining in summer. This likely reflects school and preschool attendance among children and increased indoor contact during family gatherings and holiday celebrations among adults, which are particularly frequent during these months in Serbia. Similar seasonal patterns are observed across the EU/EEA, with the lowest incidence in summer and rises in autumn and winter [8, 28]. In Europe, the incidence of both IPD and Hib declined sharply during the COVID-19 pandemic (2020–2021), likely due to public health measures such as physical distancing, mask use, and lockdowns [8]. Following the relaxation of these measures, IPD incidence increased during the winter of 2021–2022, with an unusual rise observed in spring 2022 [28]. Influenza is known to elevate invasive pneumococcal pneumonia incidence and influence seasonal case fatality in the elderly [31]. These observations align with local influenza and acute respiratory infection surveillance, which documented recent influenza resurgence concurrent with rising IPD in this study [32].

Biological differences between Hib and pneumococcus likely contribute to their distinct epidemiology. Although exact basic reproduction ratio (R_0) estimates are unclear, studies suggest Hib has lower transmission potential. Hib carriage is shorter and less likely to spread, consistent with rapid declines after widespread Hib vaccination [33]. In contrast, pneumococcus sustains transmission through prolonged, serotype-dependent carriage and diverse circulating serotypes. Longitudinal and genomic studies show serotype-specific differences in carriage duration and fitness, supporting pneumococcus's higher transmission potential and capacity for serotype replacement under vaccine pressure [34]. Finally, vaccine impact further highlights these differences: Hib conjugate vaccines rapidly and durably reduced carriage and invasive disease, whereas PCVs substantially decreased vaccine-type disease but were followed by variable overall trends due to serotype replacement and heterogeneous herd effects [1, 11, 12]. These findings suggest that Hib has a lower effective reproductive potential than pneumococcus, which explains its faster and more sustained decline compared with IPD after vaccination.

Our results showed no significant gender differences for IPD and invasive Hib disease, consistent with previous surveillance data, although a slightly higher number of male cases was generally observed [4, 5, 8, 9, 28]. In addition, our analysis showed that IPD mainly affected adults aged ≥ 40 years, while invasive Hib disease occurred primarily in children aged 1–4 years. All invasive cases in this study occurred in unvaccinated individuals. These patterns reflect the known epidemiology: Hib historically caused severe disease in young children before vaccination, whereas pneumococcal disease displays a bimodal distribution, affecting both young children and older adults [1–11, 21, 22, 28–30, 35].

Both diseases were more common in urban areas. However, differences from rural areas were not statistically significant, which suggests that population density influences transmission but is not the sole determinant. Case numbers were highest in the South Bačka District, likely reflecting local differences in healthcare access, surveillance, and population susceptibility. As previously noted for invasive meningococcal disease [20], this district hosts the University Clinical Center of Vojvodina–the region's only tertiary care facility–receiving the most severe cases, and it is also the most populous district in the AP of Vojvodina [19]. Laboratory confirmation was more frequent for IPD than Hib, reflecting better diagnostic capabilities for pneumococcus and potential underreporting of Hib due to prior antibiotics or less sensitive methods. Blood cultures are less sensitive for Hib than pneumococcus; for instance, one study found real-time PCR detected Hib DNA in 11 samples, while blood cultures were all negative, highlighting culture limitations for Hib detection [36]. Clinical outcomes differed significantly, with higher case-fatality for IPD (9.6%) than for invasive Hib disease (3.5%). This aligns with previous reports showing that IPD, especially in older adults, carries a greater mortality risk than Hib disease in the post-vaccine era [4, 5, 8, 9].

In a previous study of IPD isolates in the AP of Vojvodina (2009–2016), the most frequent serotypes were 3, 19F, and 14 [37]. A broader Serbian study (2010–2018) also found serotypes 3, 19F, and 14 most common, followed by 6B, 6A, 19A, and 23F [27].

Although not the primary aim, our 2009–2024 analysis in the AP of Vojvodina showed significant shifts in serotype distribution after PCV introduction. PCV10-related serotypes, especially 14 and 19F, declined markedly, consistent with European and global data showing rapid reduction or

near-elimination of vaccine serotypes *via* direct and herd protection [38–41]. Despite routine pneumococcal immunization starting only in 2018, early reductions in specific IPD serotypes are already evident [18].

Serotype 3 persisted across both study periods (pre-vaccination and vaccination), a pattern reported globally [42–44]. In our study, it caused the highest number of adult cases, including half of all fatal outcomes, highlighting its clinical significance and challenges for current vaccines [45, 46]. In the post-PCV period, serotype 3 showed the largest increase, including a sharp rise in children aged < 5 years. The immune response to serotype 3 polysaccharide is known to be suboptimal compared with other vaccine serotypes [18, 47]. Evidence indicates that countries using PCV10 have experienced a linear increase in serotype 3 disease across all ages, whereas PCV13 users saw only a modest decline during the first 3–4 years post-introduction [18, 48]. Given that PCV13 was used in Serbia only from 2022 to 2023, the lack of decline in serotype 3 incidence is expected.

The emergence of non-vaccine serotypes (e.g., 15C, 28A, 6C, 7C) during the vaccine era, though few in number, reflects local [18] and global trends of serotype replacement after PCV introduction [49–51]. Serotype 6C is notable for its high antibiotic resistance [18, 50, 51]. While the overall rise in non-vaccine serotypes was not statistically significant–likely due to the short duration of continuous vaccination–continued surveillance is warranted.

After vaccine introduction, IPD caused by PCV10 serotypes declined from 55% to 22% in the overall population, PCV13-non-PCV10 remained stable (~40%), and non-vaccine serotypes increased from 11% to 28%, with additional PCV20-non-PCV15 emergence. In children aged < 5 years, IPD caused by PCV10 serotypes (80% pre-vaccine) decreased to 20% in the vaccination period, while PCV13-non-PCV10, PCV20-non-PCV15, and non-vaccine serotypes each accounted for ~33%. In adults aged ≥ 40 years, IPD caused by PCV10 fell from 47% to < 20%, PCV13-non-PCV10 rose to ~45%, non-vaccine serotypes to 27%, and PCV15-non-PCV13/PCV20-non-PCV15 remained minor contributors. These findings clearly indicate that the PCV10 immunization program affected not only children but also provided indirect protection to adults.

A previous study from the AP of Vojvodina covering isolates from the pre-vaccine era showed that IPD in children aged < 5 years was dominated by serotypes 19F (44%) and 14 (16%), while serotype 3 predominated in adults aged ≥ 50 years [37]. A broader Serbian study confirmed a higher prevalence of serotype 3 in adults and 19F/14 in young children [27]. In the post-vaccine period (2018–2023), serotype 3 accounted for 33% of IPD cases, followed by 19A, 14, and 6B [18], consistent with our findings showing increased non-vaccine serotypes in adults. Adult fatalities were largely linked to serotype 3, with one death due to a non-vaccine serotype (7C), highlighting the need for continued molecular surveillance. The 2022 ECDC report lists the five most common serotypes as 3, 8, 19A, 22F, and 6C [28].

All 30 EU/EEA countries have introduced pneumococcal vaccination, with childhood immunization mandatory in seven (Bulgaria, Croatia, France, Hungary, Latvia, Poland, Slovakia), similar to Serbia. While schedules vary, Serbia's revaccination – given one year after the second primary PCV10 dose – may benefit from a shorter interval, ideally at 11–12 months based on the EU/EEA schedules [52].

This study has several notable strengths. First, it is based on a comprehensive 22-year dataset (2003–2024), providing one of the longest continuous epidemiological assessments of IPD and invasive Hib disease in the region. Such a long observation period enabled the identification of secular trends, vaccination impact (particularly for Hib invasive disease), and temporal variations, including seasonal patterns. Second, the analysis integrates multiple dimensions of disease dynamics–incidence rates, age-specific distribution, seasonal variation, demographic and clinical characteristics, and serotype distribution–allowing for a holistic evaluation of disease epidemiology and immunization program performance. Third, the assessment of vaccine coverage data alongside incidence trends provides valuable insight into the relationship between immunization program implementation, coverage levels, and disease dynamics, highlighting both the successes of Hib immunization and the ongoing challenges related to IPD. However, due to the relatively short period since the introduction of pneumococcal vaccination, the reintroduction of PCV10 under the 2+1 schedule–driven mainly by economic considerations and persistently low booster-dose coverage (in the second year of life)–the number of IPD cases has not declined during the vaccination period in our setting.

Finally, despite the limited number of cases, by examining serotype distribution in the pre- and post-vaccine periods, this study provides important evidence on serotype replacement and persistence–particularly of serotype 3–and on the emergence of non-vaccine serotypes, which is highly relevant for evaluating the potential need for higher-valency vaccines in the future.

Nevertheless, several limitations should be acknowledged. First, the study relied on passive surveillance data, which may underestimate the true burden of disease due to underreporting or misclassification. Second, although case definitions followed national and WHO standards, diagnostic practices and reporting completeness may have varied across hospitals and over time, potentially affecting case ascertainment. Third, serotype data were available for only 18% of all laboratory-confirmed IPD cases, limiting the representativeness of the serotype distribution analysis. For comparison, among the EU/EEA countries that reported serotyping data in 2022, serotype information was available for 52.3% of cases [28]. However, our dataset included only officially notified communicable diseases (IPD or invasive Hib disease). Moreover, serotyping at the National Reference Center for Streptococci in Serbia is also passive, based on voluntary submission, and covers a limited number of isolates, particularly among children [18]. Fourth, vaccination coverage was assessed using the administrative method, which may overestimate or underestimate true coverage levels. Fifth, potential disruptions of routine immunization and healthcare-seeking behavior during the COVID-19 pandemic could have influ-

enced both disease incidence and vaccine uptake. Finally, as this was a descriptive study, causal inferences between vaccination and observed epidemiological trends cannot be firmly established.

Conclusion

Our study confirms the sustained success of the *Haemophilus influenzae* type b immunization program in substantially reducing disease incidence. Introduction of pneumococcal conjugate vaccines in the Autonomous Province of Vojvodina, Serbia, markedly decreased the circulation of pneumococcal conjugate vaccines10 serotypes; however, serotype 3 and emerging non-vaccine serotypes continue to challenge disease control. Given that none of the patients in this study had received prior vaccination against diseases caused by *Streptococcus pneumoniae* or *Haemophilus influenzae* type b, enhancing physician awareness and education at all levels of healthcare is a critical priority for improving vaccine uptake and disease prevention. Our findings emphasize the need for ongoing surveillance, enhanced laboratory-based serotyping, and regular evaluation of immunization coverage to inform vaccine policy, including the potential use of higher-valency pneumococcal conjugate vaccines (minimum pneumococcal conjugate vaccines 13) or targeted adult vaccination strategies. Maintaining high vaccine uptake, alongside continuous monitoring of vaccine impact, remains essential to further reduce the burden of invasive bacterial diseases in Serbia.

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Simultaneous assessment of TNF-α, TIM-1, and TLR4 plasma levels for predicting the severity of allergic rhinitis

Istovremena procena nivoa TNF-α, TIM-1 i TLR4 u plazmi kao prediktora težine alergijskog rinitisa

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Abstract

Background/Aim. Allergic rhinitis (AR) is a chronic inflammation of the nasal mucosa caused by allergens. To date, some individual biomarkers, such as total immunoglobulin E (IgE), have been shown to be possible factors for the assessment of the severity of AR. The aim of the study was to determine the value of simultaneously measuring the levels of tumor necrosis factor (TNF)-α, T-cell immunoglobulin and mucin domain 1 (TIM-1), and toll-like receptor 4 (TLR4) as predictors of AR severity. **Methods.** The study included two groups of respondents: the AR group – ARG (n = 96), which consisted of patients with AR treated between March 2021 and May 2023, and the control group – CG (n = 60), which consisted of healthy individuals undergoing physical examinations during the same period. Levels of TNF-*a*, TIM-1, and TLR4 were compared between the ARG and CG, and their associations with disease severity were analyzed. **Results.** Logistic regression analysis revealed that elevated eosinophil counts, IgE, TNF-α, TIM-1, and TLR4 levels were independent risk factors for the occurrence of moderate-to-severe AR (odds ratio > 1, $p < 0.05$). The areas under the receiver operating characteristic curves for plasma TNF-α, TIM-1, and TLR4 levels in predicting disease severity were 0.889, 0.831, and 0.842, respectively, while the combined predictive value reached 0.932, indicating excellent diagnostic performance. **Conclusion.** The simultaneous measurement of plasma TNF-α, TIM-1, and TLR4 levels provides a novel and reliable approach for predicting the severity of AR. Their combined assessment demonstrates higher predictive accuracy than that of individual markers, offering potential value for disease stratification and clinical decision-making.

Keywords:
immunoglobulins; prognosis; rhinitis, allergic; severity of illness index; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. Alergijski rinitis (AR) je hronično zapaljenje nosne sluznice izazvano dejstvom alergena. Do sada se pokazalo da su pojedini biomarkeri, kao što je ukupni imunoglobulin E (IgE), mogući pokazatelji za procenu težine AR. Cilj rada bio je da se proceni vrednost istovremenog određivanja nivoa faktora nekroze tumora (*tumor necrosis factor*-TNF)-α, *T-cell immunoglobulin and mucin domain* (TIM)-1 i *toll-like* receptora 4 (TLR4) kao prediktora težine AR. **Metode.** U studiju su uključene dve grupe ispitanika: AR grupa – ARG (n = 96), koju su činili bolesnici lečeni od marta 2021. do maja 2023. godine, i kontrolna grupa – KG (n = 60), koju su činile zdrave osobe koje su tokom istog perioda bile pregledane od strane lekara. Nivoi TNF-*a*, TIM-1 i TLR4 upoređivani su između ARG i KG i analizirana je njihova povezanost sa težinom bolesti. **Rezultati.** Logistička regresiona analiza pokazala je da su povišen broj eozinofila i povišeni nivoi IgE, TNF-α, TIM-1 i TLR4 nezavisni faktori rizika od pojave umerenog do teškog AR (*odds ratio* > 1, $p < 0,05$). Površine ispod *receiver operating characteristic* krive za nivoe TNF-α, TIM-1 i TLR4 u plazmi, u predviđanju težine bolesti, iznosile su 0,889, 0,831 i 0,842, redom, dok je prediktivna vrednost kombinacije markera dostigla 0,932, što ukazuje na odličan dijagnostički učinak.

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Zaključak. Istovremeno merenje nivoa TNF-α, TIM-1 i TLR4 u plazmi pruža nov i pouzdan pristup za predviđanje težine AR. Procena njihove kombinacije pokazuje veću prognostičku tačnost od procene pojedinačnih markera, nudeći potencijalnu vrednost za stratifikaciju bolesti i donošenje kliničkih odluka.

Ključne reči:
immunoglobulini; prognoza; rinitis, alergijski; bolest, indeks težine; faktor nekroze tumora-alfa.

Introduction

Allergic rhinitis (AR) is a common non-infectious immune disorder characterized by variable inflammatory lesions of the nasal mucosa, which arises from immune cell infiltration and the release of various inflammatory cytokines upon exposure to allergens. Its prevalence has been steadily increasing worldwide, and environmental as well as lifestyle factors are believed to contribute to this trend [1]. In mild AR, patients often present with nasal obstruction, itching, sneezing, and rhinorrhea. In moderate-to-severe cases, additional symptoms such as dizziness and impaired memory and cognitive function may occur, which severely compromise both physical and mental health and substantially diminish quality of life [2]. Thoroughly discovering the factors related to the development and progression of AR and accurately assessing the severity of the disease are of great significance for implementing effective treatments and improving patients' quality of life. Tumor necrosis factor (TNF)-α, a systemic inflammatory cytokine, is a key player in immunity and inflammatory responses [3, 4]. T-cell immunoglobulin and mucin domain 1 (TIM-1), linked to atopic diseases, including AR and asthma, has been shown to play a role in regulating T helper (Th) type 2 (Th2) cell responses. Numerous studies have shown that Th type 1 (Th1) cell /Th2 cell immune imbalance acts as a vital immune basis for the development of AR [5, 6]. It is therefore inferred that there is a relationship between TIM-1 and the severity of AR. Toll-like receptor (TLR)4 – TLR4, a member of the TLR family, is a transmembrane receptor in the innate immune system that is involved in the pathogenesis of allergic diseases. Allergic reactions are induced or aggravated by allergen-induced endogenous stimuli that activate target cells through TLR-related signaling pathways [7].

In this study, the differences in the plasma TNF-α, TIM-1, and TLR4 levels were examined in patients with AR, and their associations with disease severity were analyzed to provide a reference for disease assessment and treatment guidance.

Methods

General data

The present study was reviewed and approved by the Hospital Research Ethics Committee of the Zhangjiagang Third People's Hospital (approval from March 5, 2021). A total of 96 patients with AR undergoing treatment in that hospital from March 2021 to May 2023 were selected as a case group (AR group – ARG).

The inclusion criteria were set as follows: patients who met relevant diagnostic criteria for AR according to the Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update and the Chinese Guidelines for the Diagnosis and Treatment of Allergic Rhinitis (2022 edition) [8, 9], which define AR as an immunoglobulin (Ig) E -mediated inflammatory disorder of the nasal mucosa characterized by nasal obstruction, itching, sneezing, and rhinorrhea. In this study, symptoms were required to be triggered by exposure to relevant allergens, including both inhalant allergens (dust mites, pollens, molds, animal dander) and food allergens known to induce IgE-mediated nasal symptoms [10], and to have persisted at least two years. Eligible participants also included those diagnosed with AR for the first time, those who or whose family members had signed the informed consent form, and those with normal cardiac, hepatic, and renal function.

The exclusion criteria involved: patients with a history of trauma surgery or respiratory infection in the last month, those taking anti-allergic drugs or other related drugs within one month before enrollment, those with connective tissue diseases, pregnant or lactating women, those complicated with severe deviation of nasal septum, nasal polyps or sinonasal inflammation, those with atopic dermatitis or asthma, those with respiratory system tumor or other serious diseases, those with systemic acute or chronic infectious diseases, or those with polycythemia, severe anemia, leukocytosis, or other hematologic diseases.

Moreover, 60 healthy people undergoing physical examinations in the same period were incorporated into a control group (CG). The inclusion criteria were set as follows: patients who or whose family members had signed the informed consent form, and those with normal cardiac, hepatic, and renal function. The exclusion criteria involved: patients with a history of trauma surgery or respiratory infection in the last month, those taking anti-allergic drugs or other related drugs within one month before enrollment, those with connective tissue diseases, pregnant or lactating women, those complicated with severe deviation of nasal septum, nasal polyps, or sinonasal inflammation, those with atopic dermatitis or asthma, those with respiratory system tumor or other serious diseases, those with systemic acute or chronic infectious diseases, or those with polycythemia, severe anemia, leukocytosis, or other hematologic diseases.

Measurements

To determine allergen sensitization, all subjects in ARG underwent standardized allergen testing upon enrollment.

Sensitization was evaluated using serum specific IgE (sIgE) testing performed on an automated immunoassay analyzer (ImmunoCAP®, Thermo Fisher Scientific), following the manufacturer's instructions. The allergen panel included the following major inhalant and food allergens: house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*); pollen allergens (grass pollen, tree pollen, and weed pollen mix); food allergens (egg, milk, peanut, shellfish); other allergens (defined as mold spores, animal dander, and miscellaneous food allergens not included in the primary inhalant panel). A sIgE level ≥ 0.35 kU/L was considered positive and indicative of sensitization.

After overnight fasting for 7–8 hr, 4–5 mL of venous blood was collected from the cubital vein of each subject in the morning. The samples were placed into a special anticoagulation tube containing sodium citrate and centrifuged at 3,000 revolutions *per* min (r/min) for 10 min with a centrifugal radius of 10 cm. The plasma was then separated and stored at -70 °C until further analysis. The levels of TNF-*α*, TIM-1, and TLR4 were measured using an enzyme-linked immunosorbent assay (ELISA) in strict accordance with the instructions provided in the corresponding assay kits (Beijing Solarbio Science & Technology Co., Ltd., China).

Eosinophil counts (ECs) were determined from blood samples using an automatic blood cell analyzer (i2000, Sysmex). IgE levels were measured using an automatic protein analyzer (BN™, II Siemens) and corresponding reagents.

Assessment of the severity of allergic rhinitis

AR was classified into mild and moderate-to-severe cases. Mild AR was defined according to the ARIA guidelines as the presence of symptoms such as nasal obstruction, itching, watery rhinorrhea, and paroxysmal sneezing, without sleep disturbance, impairment of daily activities, work, or school performance, and with symptoms not considered troublesome. Moderate-to-severe AR was defined as the presence of one or more of the following: sleep disturbance, impairment of daily activities, leisure, sport, work, or school performance, or symptoms considered troublesome by the patient.

Evaluation of outcomes

Plasma TNF-α, TIM-1, and TLR4 levels were compared between ARG and CG.

According to the severity of AR, ARG was subdivided into a mild group and a moderate-to-severe group. Comparisons were made between the clinical data and plasma TNF-α, TIM-1, and TLR4 levels of the mild and moderate-to-severe groups. Clinical data included gender (male or female), age, family history (yes or no, defined as a documented history of AR, asthma, or atopic dermatitis in first-degree relatives), smoking history (yes or no), drinking history (yes or no), allergen sensitivity (pollen, dust mites, food, or other), EC, and IgE level.

Statistical analysis

Statistical data processing was conducted in the SPSS software, version 23. The measurement data were expressed as mean ± standard deviation (SD) and analyzed using the *t*-test, whereas the count data were presented as number (percentage) – n (%) and analyzed using the χ^2 test. The correlations of plasma TNF-α, TIM-1, and TLR4 levels with the severity of AR were explored using point-biserial correlation analysis. Logistic regression analysis was conducted to discover the factors influencing the severity of AR. The receiver operating characteristic (ROC) curves were plotted to analyze the values of plasma TNF-α, TIM-1, and TLR4 levels for predicting the severity of AR. Differences were considered statistically significant at $p < 0.05$, and highly significant at $p < 0.01$.

Results

The plasma TNF-α, TIM-1, and TLR4 levels were higher in ARG than those in CG ($p < 0.01$) (Table 1, Figure 1).

Among the ARG (n = 96), 37 patients were classified as mild AR (mild group) and 59 patients as moderate-to-severe AR (moderate-to-severe group). Compared with the mild group, the moderate-to-severe group showed significantly higher EC, IgE, TNF-α, TIM-1, and TLR4 levels ($p < 0.01$). No significant differences were observed between the two subgroups in terms of gender distribution, family history, smoking history, drinking history, and allergen sensitivity ($p > 0.05$) (Table 2).

The results of point-biserial correlation analysis showed that the plasma TNF-α, TIM-1, and TLR4 levels were positively related to the severity of AR ($r > 0.5$, $p < 0.01$) (Table 3).

Table 1

Plasma TNF-α, TIM-1, and TLR4 levels in allergic rhinitis and control groups of respondents

Biomarker	Group		*t*	*p*
	allergic rhinitis (n = 96)	control (n = 60)		
TNF-α (ng/L)	24.63 ± 5.81	13.95 ± 2.03	13.711	< 0.001
TIM-1 (ng/mL)	296.53 ± 21.61	98.64 ± 13.75	63.331	< 0.001
TLR4 (μg/L)	64.52 ± 16.34	21.58 ± 10.72	18.060	< 0.001

TNF – tumor necrosis factor; TIM-1 – T-cell immunoglobulin and mucin domain 1; TLR4 – toll-like receptor 4; n– number.

All values are given as mean values ± standard deviation.

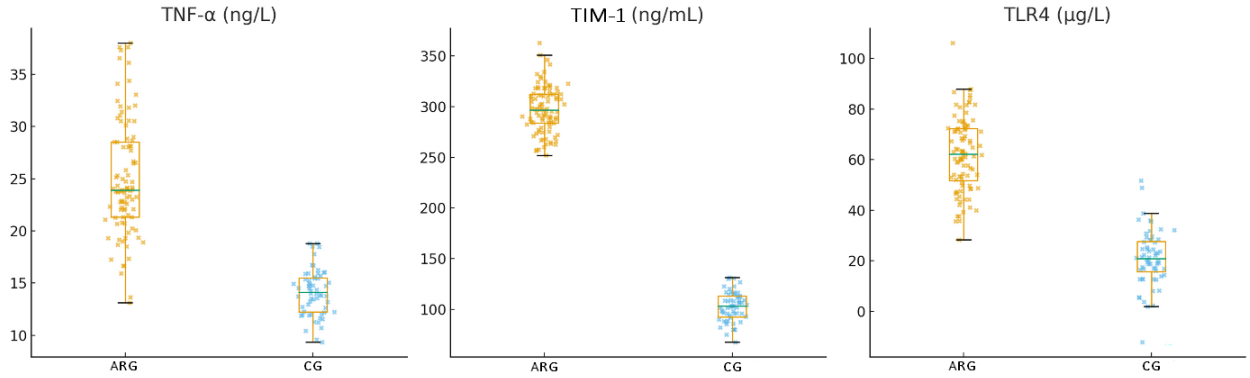

Fig. 1– Plasma TNF-α, TIM-1, and TLR4 levels.
Data are shown as boxplots with overlaid scatter points. The box indicates the interquartile range, the horizontal line within the box marks the median, and whiskers show variability outside the upper and lower quartiles.
TNF– tumor necrosis factor; TIM-1 – T-cell immunoglobulin and mucin domain 1; TLR4 – toll-like receptor 4; ARG – allergic rhinitis group; CG – control group.

Table 2

Clinical data and plasma TNF-α, TIM-1, and TLR4 levels in mild and moderate-to-severe groups of patients with allergic rhinitis

Parameter	Allergic rhinitis group		Statistical value	*p*
	mild (n = 37)	moderate-to-severe (n = 59)		
Gender				
male	19 (51.35)	31 (52.54)	$\chi^2 = 0.013$	0.910
female	18 (48.65)	28 (47.46)		
Age, years	40.79 ± 8.73	39.97 ± 9.54	$t = 0.423$	0.673
Family history				
yes	16 (43.24)	21 (35.59)	$\chi^2 = 0.562$	0.454
no	21 (56.76)	38 (64.41)		
Smoking history				
yes	9 (24.32)	12 (20.34)	$\chi^2 = 0.211$	0.646
no	28 (75.68)	47 (79.66)		
Drinking history				
yes	11 (29.73)	16 (27.12)	$\chi^2 = 0.077$	0.782
no	26 (70.27)	43 (72.88)		
Allergen sensitivity				
pollen	11 (29.73)	20 (33.90)	$\chi^2 = 0.270$	0.966
dust mite	18 (48.65)	28 (47.46)		
food	4 (10.81)	5 (8.47)		
others	4 (10.81)	6 (10.17)		
Eosinophil count ($\times 10^9$/L)	0.12 ± 0.03	0.17 ± 0.04	$t = 6.533$	< 0.001
IgE (IU/mL)	81.56 ± 23.39	116.28 ± 34.59	$t = 5.378$	< 0.001
TNF-α (ng/L)	20.39 ± 4.69	27.28 ± 4.81	$t = 6.896$	< 0.001
TIM-1 (ng/mL)	282.13 ± 15.60	305.55 ± 19.96	$t = 6.065$	< 0.001
TLR4 (μg/L)	52.76 ± 11.57	71.91 ± 14.52	$t = 6.781$	< 0.001

IgE – immunoglobulin E. For other abbreviations, see Table 1.
All values are given as numbers (percentages) or mean values ± standard deviation.

Table 3

Correlations of plasma TNF-α, TIM-1, and TLR4 levels with the severity of allergic rhinitis

Biomarker	*r*	*p*
TNF-α	0.580	< 0.001
TIM-1	0.530	< 0.001
TLR4	0.573	< 0.001

For abbreviations, see Table 1.

Logistic regression analysis was conducted with the indicators presenting significant differences as independent variables and the severity of AR in patients as dependent variables (1 = moderate-to-severe, 0 = mild). It was discovered that high levels of EC [odds ratio (OR): 1.303, 95% confidence interval (CI): 1.021–1.814], IgE (OR: 1.042, 95%

CI: 1.022–1.062), TNF-α (OR: 1.411, 95% CI: 1.146–1.736), TIM-1 (OR: 1.083, 95% CI: 1.021–1.148), and TLR4 (OR: 1.169, 95% CI: 1.076–1.270) were risk factors for moderate-to-severe AR (OR > 1, $p < 0.01$) (Table 4, Figure 2).

The ROC curves were plotted with plasma TNF-α, TIM-1, and TLR4 levels as the test variables and the severity of AR as the state variable (1 = moderate-to-severe, 0 = mild) (Figure 3). It was uncovered that the areas under the ROC curves (AUCs) of plasma TNF-α, TIM-1, and TLR4 levels for predicting the severity of AR were 0.889, 0.831, and 0.842, respectively, and the AUC of the combination of the three indicators reached 0.932, demonstrating certain predictive value (Table 5).

Table 4

Results of multivariate logistic regression analysis on influencing factors for the severity of allergic rhinitis

Independent variable	β	SE	Wald χ^2	*p*	OR	95% CI
Eosinophil count	27.896	6.809	16.787	< 0.001	1.303	1.021–1.814
IgE	0.041	0.010	17.411	< 0.001	1.042	1.022–1.062
TNF-α	0.344	0.106	10.522	0.001	1.411	1.146–1.736
TIM-1	0.079	0.030	6.990	0.008	1.083	1.021–1.148
TLR4	0.156	0.042	13.557	< 0.001	1.169	1.076–1.270
Constant	-40.113	11.465	12.242	< 0.001	< 0.001	-

SE – standard error; OR – odds ratio; CI – confidence interval.
For other abbreviations, see Tables 1 and 2.

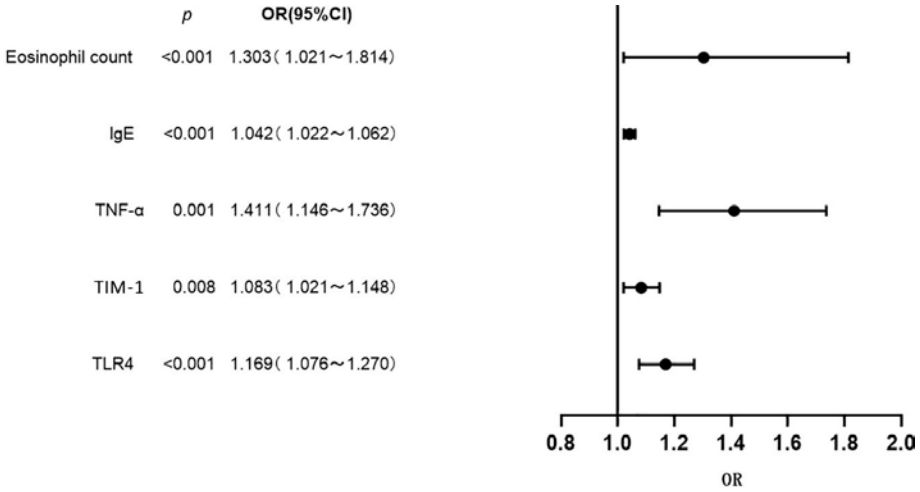

Fig. 2 – Forest plot of clinical characteristics based on multivariate logistic regression analysis.
IgE – immunoglobulin E; OR – odds ratio; CI – confidence interval. For other abbreviations, see Figure 1.

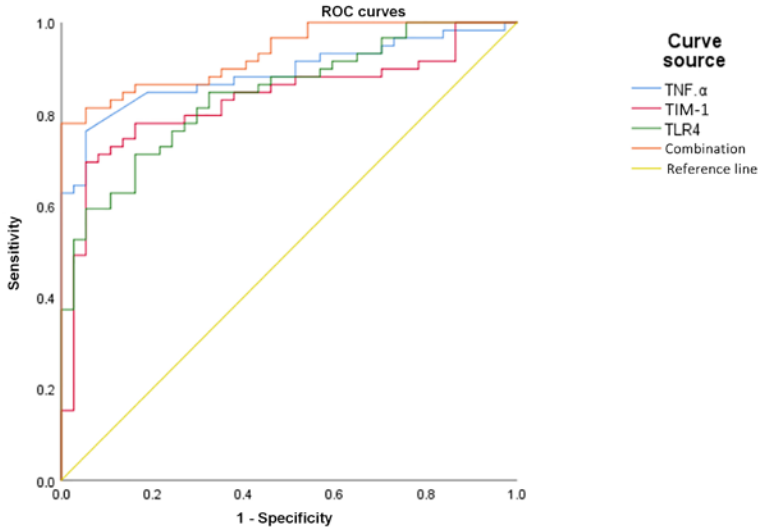

Fig. 3 – ROC curves of plasma TNF-α, TIM-1, and TLR4 levels for predicting the severity of allergic rhinitis.
ROC – receiver operating characteristic. For other abbreviations, see Figure 1.

Table 5

Predictive values of plasma TNF-α, TIM-1, and TLR4 levels for the severity of allergic rhinitis

Variable	AUC	SE	*p*	95% CI	Cut-off value	Sensitivity	Specificity	Youden index
TNF-α	0.889	0.033	< 0.001	0.824–0.955	23.930 ng/L	0.864	0.703	0.567
TIM-1	0.831	0.043	< 0.001	0.747–0.915	286.460 ng/mL	0.847	0.622	0.469
TLR4	0.842	0.039	< 0.001	0.765–0.918	55.705 μg/L	0.847	0.676	0.523
Combination	0.932	0.024	< 0.001	0.885–0.978	-	0.932	0.649	0.581

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

Discussion

Significant progress has been made in understanding the pathogenesis of AR over the past few years. The pathogenesis of AR involves many inflammatory mediators, cytokines, and other related molecules, and the disease is a result of the combined action of multiple factors and pathways [11, 12]. In this study, we demonstrated that plasma TNF-α, TIM-1, and TLR4 levels were significantly elevated in patients with AR compared with healthy controls, and that higher levels of these markers were independently associated with an increased risk of moderate-to-severe disease. Importantly, ROC curve analyses revealed that each biomarker individually had good predictive accuracy, while their combined assessment yielded an excellent discriminative ability (AUC = 0.932), underscoring the value of a multi-marker strategy in stratifying disease severity.

TNF-α is a well-established pro-inflammatory cytokine that mediates multiple aspects of allergic responses, including recruitment of eosinophils and amplification of Th2-driven inflammation [13, 14]. Recent studies have shown that TNF-α is abundantly expressed in mast cells and epithelial cells of the nasal mucosa in AR [15]. Likewise, elevated levels of TNF receptor-related cytokines can be detected in AR patients [16]. *In vivo* experiments further demonstrated that TNF-α inhibition can slow the progression of AR [17]. Collectively, these findings suggest a critical role of TNF-α in the pathogenesis of AR. Consistent with previous evidence, our results revealed that plasma TNF-α levels were significantly elevated in patients with AR, particularly in those with moderate-to-severe disease, suggesting that TNF-α may function not only as a key inflammatory mediator but also as a reliable biomarker indicative of disease severity.

TIM-1 has been recognized as a susceptibility gene in atopic diseases, acting as a modulator of Th2 cell activation and cytokine production [18, 19]. In AR, the Th1/Th2 imbalance, characterized by enhanced Th2 responses, plays a central role in disease pathogenesis [20]. Notably, the discovery of TIM-1 expression in Th2 cells sheds new light on the pathogenic mechanism of allergic diseases [21, 22]. Our data showing elevated TIM-1 levels in moderate-to-severe patients support the hypothesis that TIM-1 contributes to disease exacerbation by promoting Th2 dominance. These results align with previous animal and clinical studies linking the *TIM-1* gene family to airway hyperresponsiveness and IgE production [23, 24], thereby reinforcing its mechanistic involvement in allergic inflammation.

TLR4, a pattern recognition receptor, bridges innate and adaptive immunity by recognizing pathogen- and damage-associated molecular patterns [25, 26]. Recent evidence indicates that TLR4 signaling participates in allergic airway inflammation by activating downstream nuclear factor *kappa* B (NF-*κ*B) and mitogen-activated protein kinase (MAPK) pathways, thereby enhancing cytokine release [27, 28]. The elevated TLR4 levels observed in our cohort, particularly among patients with more severe disease, support the view that innate immune dysregulation contributes to disease progression in AR.

Although each of the three tested markers demonstrated substantial predictive power, their combination significantly enhanced diagnostic performance. This finding emphasizes the multifactorial nature of AR pathogenesis, where both innate and adaptive immune mechanisms converge to drive disease progression. A multi-marker panel may therefore provide a more accurate reflection of the complex immunopathology and enable better risk stratification compared with single biomarkers. Clinically, such a combined assessment could facilitate early identification of patients at risk for severe disease and guide timely initiation of targeted therapies. From a practical perspective, simultaneous measurement of TNF-α, TIM-1, and TLR4 in plasma is minimally invasive and feasible in routine clinical practice. Their combined predictive value could complement traditional clinical assessments, such as symptom scores and eosinophil/IgE levels, providing clinicians with an objective tool for disease monitoring. Moreover, these markers may also serve as potential therapeutic targets. For instance, anti-TNF-α has been evaluated in allergic airway diseases, and modulation of TIM-1 or TLR4 pathways could represent novel strategies for preventing disease progression [29–31].

Limitations of the study

Several limitations of this study should be acknowledged. First, this was a single-center study with a relatively small sample size, which may limit the generalizability of the findings. Second, only baseline biomarker levels were assessed; dynamic changes during disease progression or treatment were not evaluated. Third, mechanistic experiments to directly verify the causal role of these molecules in AR were not conducted. Future multicenter studies with larger cohorts are needed to validate the predictive value of these biomarkers. Additionally, longitudinal investigations and mechanistic studies could

provide further insights into their roles in disease pathogenesis and therapeutic responsiveness.

Conclusion

This study highlights the significant associations of TNF-α, TIM-1, and TLR4 with allergic rhinitis severity. The study also demonstrates the superior predictive accuracy of their combined assessment. These findings provide a novel framework for biomarker-based stratification in allergic rhinitis and may inform both clinical decision-making and the development of targeted therapeutic strategies.

Conflict of interest

The authors declare no conflict of interest.

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Evaluation of the quality of neurosurgical services using the SERVQUAL method

Procena kvaliteta neurohirurških usluga primenom SERVQUAL metode

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Abstract

Background/Aim. In an era of rapid technological innovation and growing patient expectations, healthcare systems worldwide face the need for continuous improvement in service quality. The Service Quality model (SERVQUAL) is a validated instrument for measuring service quality, based on comparing patients' expectations with their perceptions of the service provided. The difference (gap) between these two measures is assessed using a five-point Likert scale across five dimensions: tangibility, reliability, responsiveness, assurance, and empathy. The aim of this study was to measure and compare expectations and perceptions of patients undergoing neurosurgical services, and to examine whether the size of the gap differs between SERVQUAL dimensions. **Methods.** The study included a total of 60 patients who underwent surgery for degenerative lumbar and cervical spine diseases at the Clinic for Neurosurgery, University Clinical Center of Vojvodina, Novi Sad, Serbia, between June and September 2024. A modified SERVQUAL questionnaire was used for data collection. Data were collected on admission and before the patients' discharge. Data were analyzed using descriptive statistics, paired *t*-tests to compare expectations and perceptions, and analysis of variance to examine differences across sociodemographic groups. **Results.** Patients had positive perceptions of the provided services. The results indicated the existence of a gap between patients' expectations and their perceptions of the services received. Mean expectations were high across dimensions (overall 4.85; range 4.79–4.88), as were perceptions (overall 4.90; range 4.85–4.94). All gaps were positive (perceptions > expectations). Dimension-specific gaps were as follows: tangibility 0.06 (p = 0.196), reliability 0.05 (p = 0.132), responsiveness 0.04 (p = 0.249), assurance 0.06 (p = 0.107), empathy 0.06 (p = 0.039). Only the empathy dimension reached statistical significance. No significant differences emerged across sociodemographic subgroups. **Conclusion.** The SERVQUAL method provided clear insights into patients' perceptions of the quality of neurosurgical services. The findings from this study can contribute to improving service quality and increasing patient satisfaction, positively influencing clinical outcomes and the healthcare system as a whole.

Keywords:
neurosurgery; patient satisfaction; quality assurance, health care; spinal diseases; surveys and questionnaires.

Apstrakt

Uvod/Cilj. U eri brzih tehnoloških inovacija i rastućih očekivanja bolesnika, zdravstveni sistemi širom sveta suočavaju se sa potrebom za kontinuiranim unapređenjem kvaliteta usluga. Model *Service Quality* – SERVQUAL je validirani instrument za merenje kvaliteta usluge, koji se zasniva na poređenju očekivanja bolesnika sa njihovom percepcijom pružene usluge. Razlika (jaz) između ove dve mere procenjuje se primenom petostepene Likertove skale u okviru pet dimenzija: opipljivost, pouzdanost, odgovornost, sigurnost i empatija. Cilj rada bio je da se izmere i uporede očekivanja i percepcije bolesnika podvrgnutih neurohirurškim uslugama, kao i da se ispita da li se veličina jaza razlikuje između SERVQUAL dimenzija. **Metode.** Studija je obuhvatila 60 bolesnika koji su operisani zbog degenerativnih bolesti lumbalne i cervikalne kičme na Klinici za neurohirurgiju Univerzitetskog kliničkog centra Vojvodine, Novi Sad, Srbija, u periodu od juna do septembra 2024. godine. Za prikupljanje podataka korišćen je modifikovani upitnik SERVQUAL. Podaci su prikupljani na prijemu i pre otpusta bolesnika. Podaci su analizirani primenom deskriptivne statistike, uparenog *t*-testa za poređenje očekivanja i percepcija, i analize varijanse za ispitivanje razlika između sociodemografskih grupa. **Rezultati.** Bolesnici su imali pozitivne percepcije o

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pruženim uslugama. Rezultati ukazuju na postojanje jaza između očekivanja bolesnika i njihove percepcije primljenih usluga. Prosečna očekivanja bila su visoka u svim dimenzijama (ukupno 4,85; raspon 4,79–4,88), kao i percepcije (ukupno 4,90; raspon 4,85–4,94). U svim dimenzijama utvrđen je pozitivan jaz (percepcije > očekivanja). Jazovi specifični za pojedine dimenzije iznosili su: opipljivost 0.06 (p = 0,196), pouzdanost 0.05 (p = 0,132), odgovornost 0.04 (p = 0,249), sigurnost 0.06 (p = 0,107), empatija 0.06 (p = 0,039). Jedino je dimenzija empatije dostigla statističku značajnost. Nisu utvrđene statistički značajne razlike između sociodemografskih podgrupa. **Zaključak.** Metoda SERVQUAL omogućila je jasan uvid u percepciju bolesnika o kvalitetu neurohirurških usluga. Rezultati ove studije mogu doprineti unapređenju kvaliteta usluga i povećanju zadovoljstva bolesnika, čime se pozitivno utiče na kliničke ishode i zdravstveni sistem u celini.

Ključne reči:
neurohirurgija; bolesnik, zadovoljstvo; zdravstvena zaštita, ocena kvaliteta; kičma, bolesti; ankete i upitnici.

Introduction

The rapidly changing factors driven by globalization, mass customization, digitalization, and the implications of artificial intelligence are transforming the way companies, schools, and healthcare institutions perform their work [1]. These forces have given rise to a new generation of customers who expect the highest level of service quality [2]. In modern healthcare systems, the quality of healthcare services is one of the most critical factors for improving population health and disease prevention [3]. By adopting international healthcare standards, countries and healthcare institutions can ensure a consistent level of healthcare, focus on continuous improvement, and avoid variability in service provision that could compromise patient safety [4]. Healthcare service quality is evaluated through parameters such as patient satisfaction, service safety, resource use efficiency, and achievement of desired health outcomes [5]. Assessing healthcare quality is one of the most important aspects of modern hospital management, directly influencing treatment outcomes, patient satisfaction, and the efficiency of healthcare systems [6]. In modern healthcare, quality is broadly defined as care that is safe, effective, timely, efficient, equitable, and people-centered, as established by the World Health Organization (WHO) [7]. Although clinical outcomes remain essential, numerous studies have documented challenges in service-related dimensions such as waiting times, communication, coordination, and emotional support [8].

Over the past decades, several frameworks have been developed to assess these issues, including the Donabedian structure–process–outcome model and patient experience surveys. A major shift occurred in the second half of the 20th century, when healthcare institutions, inspired by global changes in quality management approaches from industry, began applying methodologies and tools that had proven effective in other sectors [9]. One such tool, developed in the late 1980s in response to the need for more precise measurement and management of service quality, is the Service Quality model (SERVQUAL) [10]. Originally designed to identify and quantify the gap between service users' expectations and their perceptions of delivered services, the SERVQUAL model quickly found application across various industries, including the healthcare sector [11]. Due to its specificity, neurosurgery is an ideal field for applying the SERVQUAL model, which enables detailed analysis and quantification of different service quality dimensions [12].

The SERVQUAL model evaluates five key dimensions of quality: tangibility, reliability, responsiveness, assurance, and empathy [13]. Each dimension reflects a different service aspect, allowing for a comprehensive quality assessment from the patient's perspective [11]. Tangibility relates to the physical aspects of services, including the appearance of hospital facilities, equipment, and hygiene conditions [14]. Reliability refers to the ability of healthcare providers and institutions to consistently and accurately deliver services as promised. Responsiveness denotes the willingness of staff to promptly and effectively respond to patient needs. Assurance includes the staff's competence and their ability to inspire confidence and a sense of safety in patients. Empathy refers to the individual attention and care provided to patients, which is especially important in neurosurgery, where patients often experience high levels of stress and anxiety [15].

The gap in the SERVQUAL method represents the difference between patient expectations before receiving the service and their actual perception afterward [11]. In healthcare, it is particularly important as it directly affects patient satisfaction and treatment outcomes [16]. Understanding this gap helps healthcare management identify improvement areas and develop strategies to reduce the difference between expected and delivered services, thereby increasing patient satisfaction and loyalty [10]. Moreover, in healthcare, most research traditionally focuses on clinical interventions and the technical aspects of service delivery [17]. These elements represent the technical quality that the customer actually receives from a service [18]. However, the functional quality, i.e., the way the service is delivered, especially the patient's perception of the interaction during care, remains relatively underexplored [19]. This interaction is a crucial component of overall service quality, particularly in high-stress, high-stakes fields such as neurosurgery [20].

The SERVQUAL instrument differs from previous methods by measuring both expectations before care and perceptions after care, allowing the identification of specific gaps in service delivery. Previous research has shown that SERVQUAL can support hospital improvement efforts, although cultural adaptation is sometimes required [21]. Evidence in neurosurgery remains limited, particularly for patients undergoing surgeries for degenerative lumbar and cervical spine diseases who face anxiety, pain, and functional limita-

tions that influence their perception of care. Therefore, applying SERVQUAL in this setting can provide useful insights into functional aspects of service quality that cannot be detected through clinical indicators alone.

The aim of this study was to explore patients' expectations and perceptions of neurosurgical services and determine whether there are significant gaps between expectations and perceptions for each SERVQUAL dimension.

Methods

The research was conducted at the Clinic for Neurosurgery of the University Clinical Center of Vojvodina, Novi Sad, Serbia, from June to September 2024. The research was conducted in accordance with the ethical standards, ensuring the anonymity and confidentiality of patient data. The study was approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-290, from August 26, 2024).

Inclusion criteria were as follows: age > 18 years; admission for elective neurosurgical treatment of degenerative diseases of the lumbar or cervical spine; the ability to provide informed consent and complete the questionnaire independently. Exclusion criteria were as follows: surgeries performed for spinal pathology other than degenerative disease (e.g., trauma, tumor, infection); emergency procedures; pediatric patients. All patients who met the inclusion criteria during the study period were consecutively recruited, resulting in a final sample of 60 patients with complete datasets. No eligible patient refused to participate, nor was any excluded due to missing data.

To collect data, a SERVQUAL questionnaire was used, modified to match the specifics of neurosurgical services [22]. Modifications included adapting item phrasing to reflect hospital care, perioperative communication, postoperative monitoring, and staff–patient interaction in neurosurgery, as well as adjusting terminology to the cultural and linguistic context of Serbia, consistent with previous research that recommends local adaptation of SERVQUAL instruments for each country [23]. The original SERVQUAL instrument was linguistically translated and adapted into Serbian using the forward–backward translation method, following the guidelines of the WHO for translation and adaptation of instruments [24]. Following translation, the content validity was reviewed by a panel of healthcare professionals and university researchers to ensure semantic, idiomatic, experiential, and conceptual equivalence with the original items. Furthermore, to confirm the construct validity and internal consistency of the instrument, a set of statistical analyses was conducted. The internal consistency of each SERVQUAL dimension was assessed using Cronbach's alpha coefficient, with all five dimensions exceeding the recommended reliability threshold ($\alpha > 0.85$), indicating a high level of internal consistency. Additionally, an exploratory factor analysis using principal axis factoring with Varimax rotation was performed. The Kaiser-Meyer-Olkin measure was 0.975, and Bartlett's test of sphericity was statistically significant (χ^2 = 16,000, df = 435, $p < 0.001$), confirming the suitability of the data for factor analysis. Three latent factors were extracted, aligning with core SERVQUAL domains while reflecting certain cultural and contextual nuances specific to the Serbian healthcare setting. The complete validation output is stored securely in accordance with institutional data protection procedures. As it contains individual response patterns, it cannot be published or shared in raw form. Nevertheless, the aggregated psychometric results provided in this study confirm strong internal consistency and adequate construct validity of the modified SERVQUAL instrument, fully compliant with current academic and ethical standards for questionnaire-based research.

The SERVQUAL questionnaire included items that cover the five key quality dimensions: tangibility, reliability, responsiveness, assurance, and empathy [19]. Each dimension was evaluated through a set of items relating to specific service aspects. In addition to standard SERVQUAL items, the questionnaire also included demographic questions such as gender, age, and education level [11]. The questionnaire was distributed to patients immediately after hospital admission for surgery and again before discharge to ensure that patients could accurately assess the quality of the services received during their hospital stay. The study's aim and the questionnaire completion process were explained to patients to ensure data accuracy and validity.

Statistical analysis

Data were analyzed using SPSS software for Windows (IBM Corp., Armonk, New York, USA). Descriptive statistics were used to examine basic indicators at the dimension level and for overall expectations and perceptions. Data were presented using means and standard deviations for continuous variables and numbers and proportions for categorical variables. Analysis of variance (ANOVA) was applied to analyze differences in average scores among SERVQUAL dimensions. Average scores were also examined in terms of patients' sociodemographic characteristics. In addition to ANOVA, the *t*-test was used for this purpose. Following the SERVQUAL methodology, differences between patient expectations and perceptions were also examined. The statistical significance of these differences was tested using paired *t*-tests. A *p*-value of 0.05 or less was considered statistically significant.

Results

This cohort study comprised 60 patients with complete data for analysis. Baseline demographic characteristics are presented in Table 1, and Figure 1 displays the income distribution stratification.

Examination waiting times were distributed as follows: < 1 week: 12 (20.0%) patients; 1–2 weeks: 18 (30.0%); 2–3 weeks: 8 (13.3%); 3–4 weeks: 13 (21.7%); 1–2 months: 6 (10.0%); and 3–6 months: 3 (5.0%). No patients reported waiting 2–3 months or > 6 months. Surgical treatment waiting times demonstrated this distribution: < 1 week: 10 (16.7%) patients; 1–2 weeks: 9 (15.0%); 2–3 weeks: 9

Table 1

Demographic and clinical characteristics of patients

Characteristics	Total (n = 60)
Age, years	56.04 ± 12.8
Gender, female	25 (41.7)
Education level	
primary school	11 (18.0)
three-year professional school	14 (23.0)
four-year professional school	21 (35.0)
high school	7 (12.0)
bachelor's degree	5 (9.0)
master's degree	2 (3.0)
Employment sector	
unemployed	10 (17.0)
retired	19 (32.0)
homemaker	2 (3.0)
entrepreneur	4 (7.0)
production sector	8 (13.0)
service sector	4 (7.0)
management	2 (3.0)
education	2 (3.0)
other	9 (15.0)
Marital status	
single	9 (15.0)
married	33 (55.0)
divorced	10 (17.0)
widowed	8 (13.0)

n – number of patients.
All values are given as numbers (percentages) or means ± standard deviations.

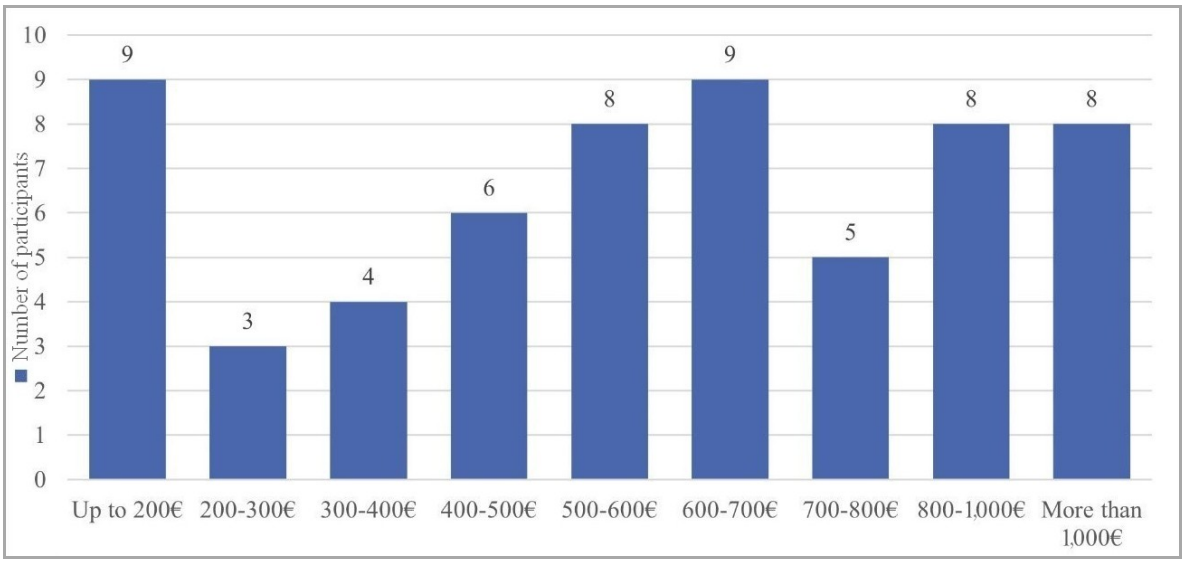

Fig. 1 – Distribution stratification among the patient cohort.

(15.0%); 3–4 weeks: 17 (28.3%); 1–2 months: 4 (6.7%); 2–3 months: 7 (11.7%); 3–6 months: 3 (5.0%); > 6 months: 1 (1.7%). The mean expectation scores across SERVQUAL dimensions are shown in Figure 2.

Reliability demonstrated the highest expectation (4.88), while tangibles yielded the lowest (4.79). The overall expectation mean was 4.85. Although a marginal difference emerged between females (4.89) and males (4.81), independent *t*-test analysis revealed non-significance ($p = 0.091$).

Perception measurements across dimensions are shown in Figure 3.

Mirroring expectation patterns, reliability achieved the highest perception score (4.94), while tangibility remained the lowest (4.85). Gender comparisons again showed non-significant differentials ($p = 0.566$) *via* an independent *t*-test. Statistically significant differences in average scores for expectations and perceptions were not identified concerning the other socio-demographic characteristics of respondents.

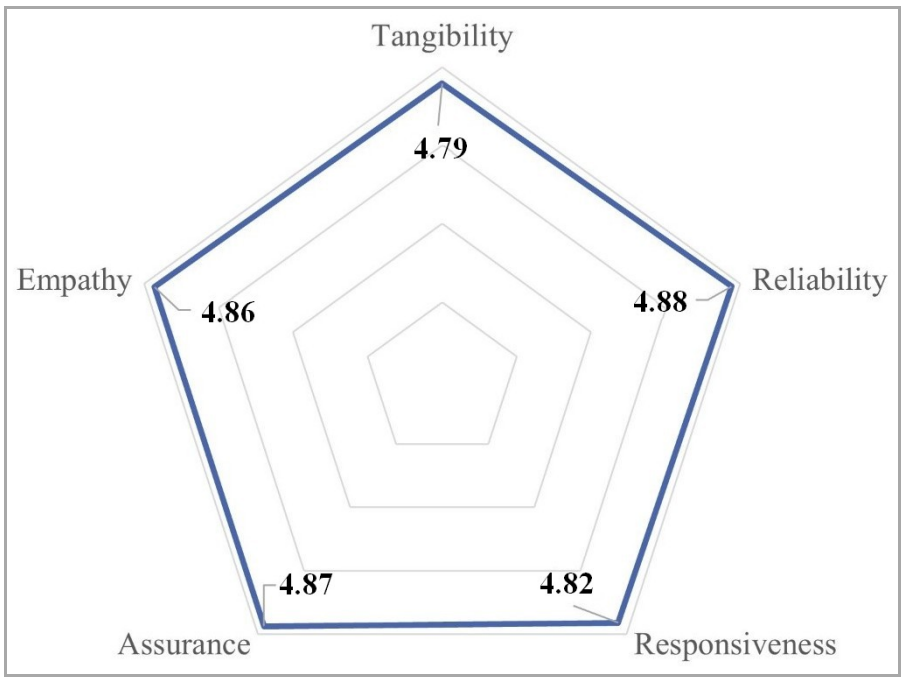

Fig. 2 – The mean expectation scores across SERVQUAL dimensions.

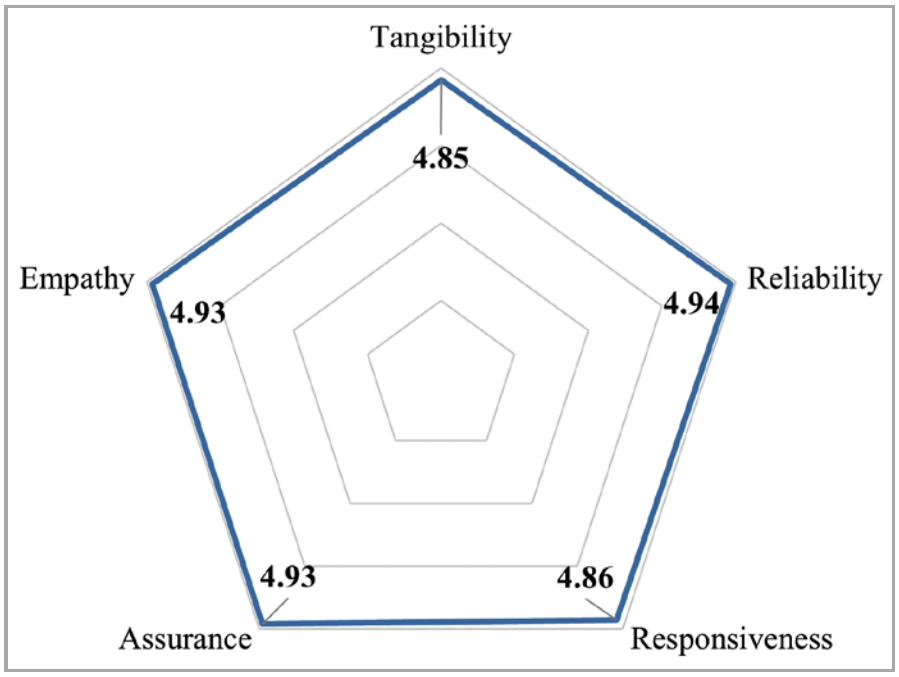

Fig. 3 – The mean perception measurement scores across SERVQUAL dimensions.

Gap analysis

When comparing expectation and perception dimensions, all dimensions showed higher ratings after service delivery (Table 2).

The largest difference, amounting to 0.5833, is observed in the tangibles and empathy dimensions. Before service delivery, patients rated the tangibles dimension at 4.79 and the empathy dimension at 4.86, while these dimensions received ratings of 4.85 (tangibles) and 4.93 (empathy) after service delivery.

Analysis of service quality dimensions revealed consistently positive gap scores (perception minus expectation) across all measured mean scores. As delineated in Table 2, expectations were highest for reliability (4.88 ± 0.32), followed sequentially by assurance (4.87 ± 0.34), empathy (4.86 ± 0.32), responsiveness (4.82 ± 0.37), and tangibility (4.79 ± 0.39). Post-service perceptions exhibited analogous hierarchical ordering, with reliability (4.94 ± 0.25) and assurance (4.93 ± 0.23) maintaining primacy.

Gap magnitudes ranged from 0.04 (responsiveness) to 0.06 (tangibility, reliability, assurance, empathy). Important-

ly, the paired-samples *t*-test demonstrated statistical significance exclusively for the empathy dimension (gap = 0.06, $p = 0.039$). Non-significant differences were observed in tangibility ($p = 0.196$), reliability ($p = 0.132$), responsiveness ($p = 0.249$), and assurance ($p = 0.107$).

To assess whether gender influenced the evaluation of service quality, mean SERVQUAL scores were stratified by gender and compared across all five dimensions. Figure 4 presents the average expectation scores for male and female patients, while Figure 5 shows the corresponding perception scores.

Table 2

Patients' expectations and perceptions of the quality of provided services

Characteristics	Expectations	Perception	Gap scores	*p*-value
Tangibility	4.79 ± 0.39	4.85 ± 0.32	0.06	0.196
Reliability	4.88 ± 0.32	4.94 ± 0.25	0.05	0.132
Responsiveness	4.82 ± 0.37	4.86 ± 0.34	0.04	0.249
Assurance	4.87 ± 0.34	4.93 ± 0.23	0.06	0.107
Empathy	4.86 ± 0.32	4.92 ± 0.20	0.06	**0.039**

All values are given as mean ± standard deviation.
The bold value indicates significant difference ($p < 0.05$).

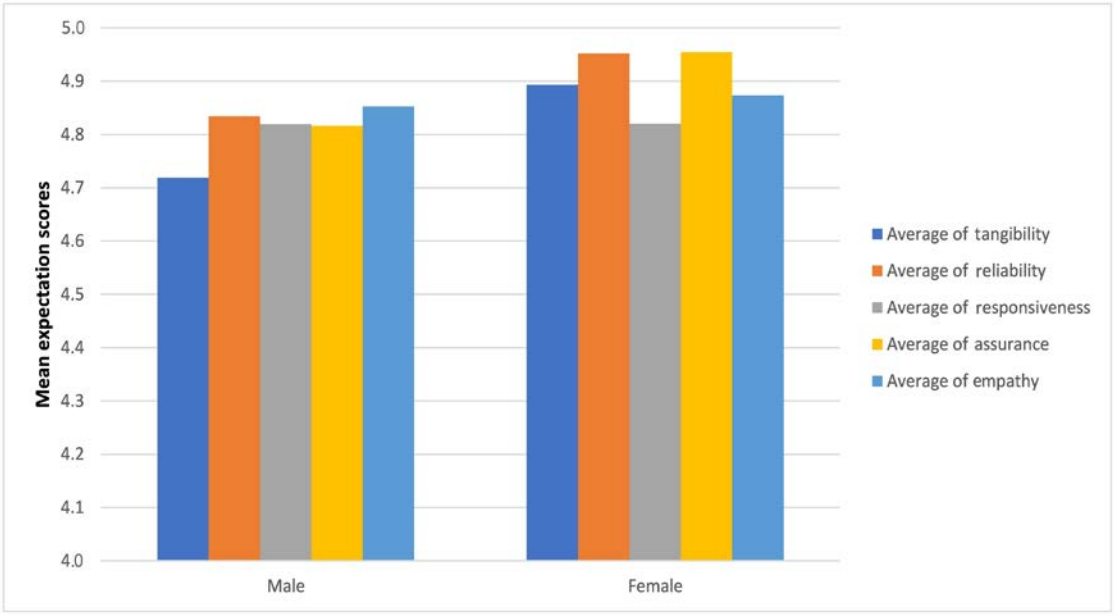

Fig. 4 – Mean expectation scores by gender across the five SERVQUAL dimensions.

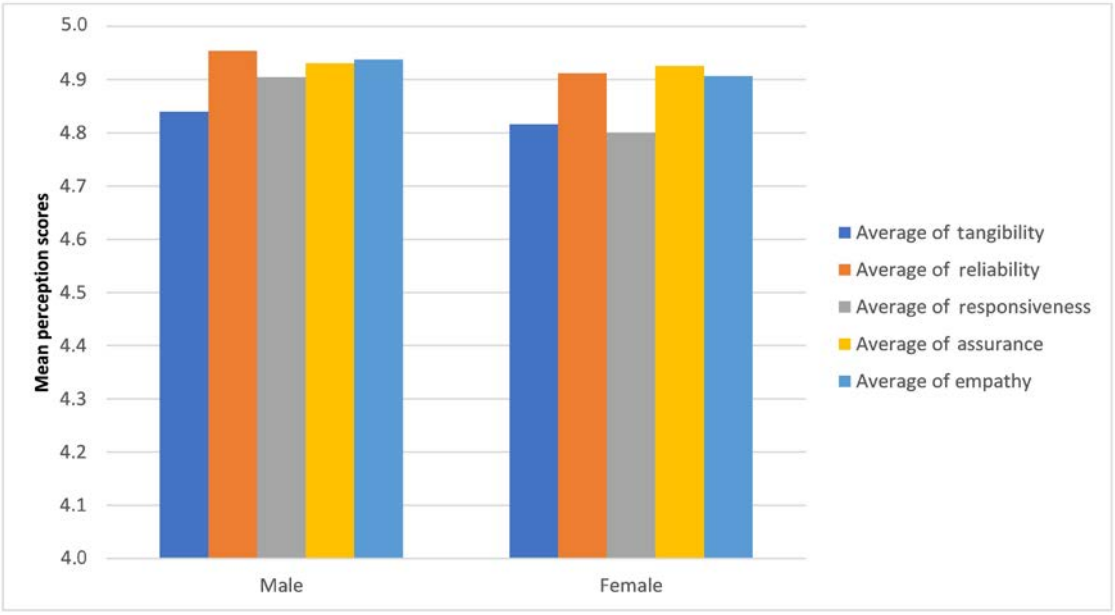

Fig. 5 – Mean perception scores by gender across the five SERVQUAL dimensions.

Both male and female patients reported very high expectations prior to treatment, with grand means ranging from 4.79 to 4.88. Female patients expressed slightly higher expectations in tangibility (4.89 vs. 4.72), reliability (4.95 vs. 4.83), assurance (4.95 vs. 4.82), and empathy (4.87 vs. 4.85), while responsiveness scores were nearly identical across genders. After treatment, perception scores also remained uniformly high for both groups, with grand means ranging from 4.83 to 4.93. In this case, male patients reported marginally higher perception scores across all five dimensions (e.g., responsiveness 4.90 vs. 4.80; empathy 4.94 vs. 4.91). However, in both expectations and perceptions, the observed numerical differences were minimal and did not reach statistical significance ($p > 0.05$), indicating that gender did not meaningfully influence either anticipated or experienced quality of neurosurgical care.

Discussion

The findings of this study provide meaningful insights into how patients perceive the quality of neurosurgical services and how their expectations align with actual experiences. Patients entered the treatment process with high expectations, and these expectations were generally met or even slightly exceeded, supporting a strong match between perceived and expected service quality [15]. All five SERVQUAL dimensions (tangibility, reliability, responsiveness, assurance, and empathy) recorded average scores above 4.5, indicating high levels of perceived service quality across all domains. These results are consistent with findings from Albalas et al. [10], who reported similarly high patient satisfaction levels in Jordanian public hospitals, particularly emphasizing the role of empathy and assurance in shaping perceptions of quality. While perception scores in our study were consistently higher than expectation scores, only the empathy dimension showed a statistically significant difference, suggesting that patients felt they received more emotional support, attentiveness, and personalized care than expected. This aligns with the previous finding, which highlights the importance of psychological and emotional support in neurosurgical care, given the high levels of patient anxiety and stress [25]. The dimension of tangibility also showed a notable, though not statistically significant, gap, underscoring the relevance of physical infrastructure, modern equipment, and environmental comfort to patient satisfaction. These findings are supported by Ulrich et al. [26], who demonstrated that evidence-based hospital design and physical surroundings significantly impact recovery time and patient experience. Furthermore, the consistency of results across demographic groups such as gender, age, and income suggests that patient satisfaction was not significantly influenced by sociodemographic factors. This is in line with the study of Xesfingi and Vozikis [27], who found that equal access to care and consistent delivery reduce variability in perceived service quality across populations. An in-depth comparison between male and female patients confirmed that there were no meaningful differences in either expectations or perceptions across any SERVQUAL dimension. This absence of gender-related variation supports previous findings and suggests that neurosurgical service quality is delivered consistently and equitably, reinforcing the robustness of functional service quality in high-complexity hospital environments [28]. Importantly, our findings reaffirm that the SERVQUAL model, originally developed in Western service sectors, can be reliably applied within healthcare systems in developing countries. In the context of this study, the term “developing countries” refers to lower- and middle-income health systems, including those in Southeastern Europe. Serbia falls within this classification and shares structural and resource characteristics with similar healthcare systems in this category. The successful cultural adaptation and strong psychometric performance of SERVQUAL in our sample confirm its applicability in such settings, thereby expanding evidence from previously studied regions such as Turkey, Jordan, and India [29]. Kilbourne et al. [30] demonstrated the cross-national applicability of SERVQUAL in healthcare environments, especially when culturally adapted and validated for local contexts. This study contributes to the growing body of evidence confirming the model’s flexibility and robustness across diverse healthcare systems, including Serbia’s. The distinction between technical quality, represented by clinical outcomes, and functional quality, defined as the way services are delivered, including communication, empathy, explanation, and respect during care interactions, has been emphasized in prior research [30, 31]. For instance, some previous studies suggest that patients often value the manner in which care is delivered more than its purely clinical success, a finding consistent with our results [10, 15]. From a practical standpoint, the SERVQUAL model proves to be an effective tool for systematically gathering patient feedback, identifying service gaps, and guiding improvements in healthcare delivery. Similar implementations have been documented by Çaha [31], who applied the SERVQUAL model in Turkish hospitals to improve quality management processes. At a broader level, this patient-centered approach can serve as a foundation for evidence-based quality improvement in healthcare. As Flott et al. [32] argue, patient-reported experience measures are not only important for evaluating care but also for driving systemic transformation across healthcare institutions.

Limitations and future research

Despite contributing new findings to a relatively underexplored area in Serbian healthcare, the study has several limitations. The research was conducted in a single clinical center with a sample of 60 patients, which restricts generalizability to wider neurosurgical populations. Additionally, the SERVQUAL instrument relies on subjective self-reporting, which may introduce response bias. A particularly relevant limitation stems from the timing and location of data collection: both expectation and perception questionnaires were completed while patients were still hospitalized. Since patients were in a dependent and vulnerable position, some may have hesitated to report negative experiences despite assurances of anonymity. To minimize this concern, responses were collected anonymously, coded without personal identi-

fiers, and handled independently of clinical staff. However, the possibility of social desirability bias remains and should be acknowledged when interpreting results.

Future investigations should aim to include multiple neurosurgical centers and larger patient cohorts to strengthen external validity and enable cross-institutional comparison. Longitudinal studies could examine whether perceptions change after discharge and whether higher functional quality translates into improved recovery, reduced anxiety, or better clinical outcomes. Since empathy was the only dimension with a statistically significant gap, qualitative research, such as interviews or focus groups, may help identify specific communication practices and staff behaviors that patients value most.

Conclusion

This study evaluated the quality of neurosurgical services in a tertiary hospital setting using the SERVQUAL measurement instrument, which captures both patient expectations and their actual perceptions of care. By applying a structured and validated tool, the research provides a systematic insight into how patients experience key dimensions of healthcare quality, including tangibility, reliability, responsiveness, assurance, and empathy. Across all measured dimensions, patient perceptions of care were high, indicating that patients assessed the delivered services very positively. Although perception scores exceeded expectation scores in every domain, the only statistically significant gap emerged in the dimension of empathy. This finding highlights the prominence of interpersonal and emotional support in shaping patient satisfaction, especially in neurosurgical care, where anxiety, fear, and physical discomfort are common. The practical relevance of these results lies in the emphasis they place on functional quality, the manner in which care is delivered, in addition to technical quality based on clinical outcomes. The SERVQUAL model proved to be a useful method for identifying subtle strengths and shortcomings in patient experience, offering healthcare managers a targeted approach for quality improvement. Interventions aimed at enhancing communication, emotional reassurance, and personalized interactions may yield tangible improvements in patient satisfaction with minimal resource investment. Furthermore, the absence of statistically significant differences across sociodemographic subgroups suggests that the quality of neurosurgical care is consistently delivered, which is an important signal from a health equity perspective.

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Impact of the severity of obstructive sleep apnea-hypopnea syndrome on the quality of life and exercise tolerance in hypertensive patients

Uticaj težine sindroma opstruktivne apneje-hipopneje u snu na kvalitet života i toleranciju napora kod obolelih od hipertenzije

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Abstract

Background/Aim. Obstructive sleep apnea-hypopnea syndrome (OSAHS) is associated with impaired quality of life (QoL) and reduced exercise tolerance. The aim of the study was to determine whether the severity of OSAHS influences the QoL and exercise tolerance in patients with moderate to severe OSAHS and arterial hypertension. **Methods.** The study included 115 consecutive patients with arterial hypertension and either moderate [40 (34.78%)] or severe [75 (65.22%)] OSAHS. Exercise tolerance was assessed using the exercise stress test, while QoL was evaluated with the Short Form-36 (SF-36) questionnaire. **Results.** The groups under study did not differ significantly in terms of age (54.80 ± 8.91 vs. 53.55 ± 9.53, p = 0.494) or sex distribution [females: 11 (27.50%) vs. 13 (17.33%), p = 0.201]. A high prevalence of cardiovascular risk factors was observed in the study population. Patients with severe OSAHS had significantly higher body mass index and neck circumference. Obesity was also more frequent among patients with severe OSAHS. In addition, this group demonstrated significantly higher apnea-hypopnea index, desaturation index, and time spent with oxygen saturation below 90%. There were no statistically significant differences in either exercise tolerance or the SF-36 parameters between the groups. Furthermore, no significant correlations were observed between apnea-hypopnea index, exercise tolerance, and QoL parameters. Multivessel coronary artery disease was detected in two asymptomatic patients. **Conclusion.** Patients with OSAHS overall exhibit multiple cardiovascular risk factors and are characterized by reduced QoL and decreased exercise tolerance. No significant correlation was found between OSAHS severity and exercise tolerance and QoL. The detection of multivessel coronary artery disease in asymptomatic patients in the study underscores the clinical significance of screening for coronary heart disease in patients diagnosed with OSAHS.

Keywords:
coronary artery disease; exercise tolerance; hypertension; quality of life; sleep apnea, obstructive; sleep apnea syndromes.

Apstrakt

Uvod/Cilj. Sindrom opstruktivne apneje-hipopneje u snu (*obstructive sleep apnea-hypopnea syndrome* – OSAHS) povezan je sa smanjenim kvalitetom života (*quality of life* – QoL) i smanjenom tolerancijom fizičkog napora. Cilj rada bio je da se utvrdi da li težina OSAHS-a utiče na QoL i toleranciju fizičkog napora kod bolesnika sa umerenim do teškim OSAHS-om i arterijskom hipertenzijom. **Metode.** Studija je obuhvatila 115 konsekutivnih bolesnika sa arterijskom hipertentijom koji su imali srednje tažak [40 (34,78%)] ili težak [75 (65,22%)] OSAHS. Tolerancija fizičkog napora procenjivana je korišćenjem testa fizičkim opterećenjem, dok je QoL procenjivan korišćenjem upitnika *Short-Form 36* (SF-36). **Rezultati.** Ispitivane grupe se nisu statistički značajno razlikovale prema starosti (54,80 ± 8,91 vs. 53,55 ± 9,53, p = 0,494) i polu [ženska populacija: 11 (27,50%) vs. 13 (17,33%), p = 0,201]. Uočena je visoka prevalencija kardiovaskularnih faktora rizika. Bolesnici sa teškim OSAHS-om imali su značajno višu vrednost indeksa telesne mase i veći obim vrata. Isto tako, kod bolesnika sa teškim OSAHS-om gojaznost je bila zastupljenija. Pored toga, ova grupa bolesnika imala je značajno više vrednosti indeksa apneje-hipopneje, indeksa desaturacije i trajanje perioda u kome je zabeležena sautracija kiseonika ispod 90%. Nije bilo statistički značajne razlike ni u toleranciji fizičkog napora, niti u parametrima upitnika SF-36 između grupa. Takođe, nije utvrđena značajna korelacija između indeksa apneje-

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hipopneje, tolerancije fizičkog napora i parametara QoL. Kod dvoje asimptomatskih bolesnika registrovano je postojanje višesudovne koronarne bolesti. **Zaključak.** Oboleli od OSAHS-a generalno imaju brojne kardiovaskularne faktore rizika, niži QoL i smanjenu toleranciju fizičkog napora. Nije dokazano postojanje značajne korelacije između težine OSAHS-a, tolerancije fizičkog napora i QoL. Otkrivanje višesudovne koronarne bolesti kod asimptomatskih bolesnika u istraživanju naglašava klinički značaj skrininga na koronarnu bolest srca kod obolelih od OSAHS-a.

Ključne reči:
koronarna bolest; vežbanje, tolerancija; hipertenzija; kvalitet života; apneja u snu, opstruktivna; apneja, spavanje poremećaji, sindromi.

Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a breathing disorder characterized by episodes of partial (hypopnea) or complete (apnea) airway collapse during sleep [1]. It represents the most prevalent sleep-breathing disorder [2]. The diagnosis of OSAHS is made by a sleep study, and the severity of OSAHS is usually estimated by the apnea-hypopnea index (AHI). AHI is defined as apneic or hypopnea events that occur during one hour of sleep [3].

The main risk factors for OSAHS are obesity, male gender, age (≥ 50 years), alcohol abuse, smoking, heredity, and craniofacial abnormalities [4]. On the other hand, OSAHS represents an independent risk factor for many cardiovascular diseases (CVDs). Changes in intrathoracic pressure, intermittent episodes of hypoxemia or hypercapnia, and repeated episodes of arousal seen in patients with OSAHS trigger increased sympathetic nervous system activation, oxidative stress, and systemic inflammation [5]. These pathophysiological mechanisms contribute to the increased risk of atrial fibrillation, coronary artery disease (CAD), stroke, heart failure, ventricular tachycardia, and sudden cardiac death [5, 6].

The most common CVD in OSAHS patients is arterial hypertension (HTA) [7]. There appears to be a linear relationship between the severity of OSAHS and the risk of HTA. Furthermore, this relationship seems bidirectional as 44% of HTA patients suffer from moderate to severe OSAHS, and the prevalence even increases in resistant HTA [5, 8]. This is why relevant guidelines suggest screening for OSAHS in patients with resistant or refractory HTA [9]. Likewise, specific HTA phenotypes, such as masked or nocturnal hypertension, are very prevalent in OSAHS patients [10, 11]. In addition, these patients often have an increased blood pressure (BP) variability, an exaggerated morning surge [10, 11], and an impaired circadian rhythm [10–12].

The quality of life (QoL) in patients with OSAHS is significantly impaired. Excessive daytime sleepiness, decreased concentration and memory, irritability, and decreased energy are the reasons why anxiety, depression, and cognitive impairment are very prevalent in OSAHS [13]. Furthermore, patients with OSAHS have lower exercise tolerance compared to healthy individuals, and it is not just because of physical limitations (obesity, decreased energy, etc.), but also due to psychological motivation [14].

The aim of the study was to determine whether the severity of OSAHS influences the QoL and exercise tolerance in patients with moderate to severe OSAHS and HTA.

Methods

Out of 410 consecutive patients with OSAHS, 115 hypertensive patients were included in this prospective study due to strict exclusion criteria. Among them, 40 (34.78%) patients had moderate OSAHS [AHI 15–29 episodes *per* hour (15–29/hr)], while 75 (65.22%) patients had severe OSAHS (AHI ≥ 30/hr). The diagnosis of OSAHS was made based on full-night respiratory polygraphy (RPG) using the Alice NightOne device from Philips Respironics (Eindhoven, Netherlands). Testing was performed at the sleep laboratory of the Clinic for Lung Diseases, University Clinical Center Niš, Niš, Serbia, during the patient's habitual sleep time, following the criteria from the American Academy of Sleep Medicine (AASM) Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea [15]. The parameters of RPG used in the study were AHI, oxygen desaturation index, total sleep time spent with oxygen saturation below 90%, and minimal, average, and maximal oxygen saturation, graded according to AASM recommendations and criteria.

Patients with known CAD, heart failure, severe valvular disease or artificial valve, chronic kidney disease (defined as estimated glomerular rate below 60 mL/min/1.73m^2), patients younger than 40 years or older than 80 years, and patients with impaired physical or mental condition were not included in the study.

After finishing the sleep study, all patients were hospitalized at the Department of Cardiovascular Diseases at the Institute for Treatment and Rehabilitation "Niška Banja", Niš, where clinical assessment and anthropometric measurements were performed. Data on cardiovascular risk (CVR) factors, including HTA, dyslipidemia, stress, smoking, diabetes mellitus (DM), obesity, physical inactivity, and family history of CVD, were also collected. During hospitalization, laboratory assessment, Short-Form 36 (SF-36) Health Status Survey, and exercise stress test (EST) were performed. The ESTs were done on the treadmill (3017 Full Vision Drive, Newton, Kansas, USA) according to the Bruce protocol. Seven patients could not perform EST due to extreme obesity (weight > 130 kg). Tests were limited by submaximal heart rate, symptoms, ischemic changes on electrocardiogram (horizontal or downsloping ST segment depression ≥ 1 mm), complex ventricular arrhythmia (couplets of ventricular premature beats or ventricular tachycardia), hypertensive reaction defined as a sudden increase of systolic BP (values ≥ 220 mmHg), or decrease in systolic BP (values >10 mmHg), or patient request to stop.

Psychological dimensions and the QoL were assessed by the validated SF-36 questionnaire, which has been culturally adapted and validated for the Serbian-speaking population [16]. All patients were divided into two groups: one with moderate OSAHS (AHI 15–29/h) and one with severe OSAHS (AHI ≥ 30/h). All data were analyzed based on the severity of OSAHS.

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), and the Ethics Committee of the Faculty of Medicine, University of Niš, Niš (No. 12-1760-1/2-4, from February 20, 2024).

Statistical analysis

Data were analyzed using the SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as frequencies and percentages, while quantitative data were presented as means ± standard deviations. Data distribution was tested using the Kolmogorov-Smirnov test. Means of normally distributed data were compared using the Student's *t*-test, while the Mann-Whitney *U* test was used for data whose distribution deviates significantly from normal distribution. The Chi-square test was used to compare frequencies. In correlation analysis, Pearson's correlation was used for normally distributed variables, while for data whose distribution deviates significantly from normal distribution, Spearman's rank correlation was used. Statistical significance was accepted for $p < 0.05$.

Results

The study included 115 patients (age 53.98 ± 9.3 years) with HTA and moderate or severe OSAHS. Patients were divided into two groups according to the severity of OSAHS: 40 (34.78%) patients with moderate OSAHS and 75 (65.22%) patients with severe OSAHS. All parameters were compared between the groups.

All sleep study parameters were significantly better in patients with moderate OSAHS than in those with severe OSAHS (Table 1). Patients with severe OSAHS had significantly higher values of AHI (51.84 ± 16.97 vs. 23.08 ± 4.63, $p < 0.001$), oxygen desaturation index (53.15 ± 22.17 vs. 24.98 ± 10.22, $p < 0.001$), and total sleep time spent with oxygen saturation below 90% (36.38 ± 27.09 vs. 18.56 ± 27.86, $p < 0.001$) compared to moderate OSAHS. On the other hand, patients with moderate OSAHS had significantly higher values of minimum (92.72 ± 11.11 vs. 67.41 ± 13.28, $p < 0.001$) and average oxygen saturation (92.3 ± 3.29 vs. 89.56 ± 4.18, $p < 0.001$) compared to those with severe OSAHS.

We then compared CVR factors and anthropometric parameters between the groups (Table 2). Patients with severe OSAHS had significantly higher neck circumference (46.23 ± 3.58 vs. 43.97 ± 4.20, $p = 0.004$) and body mass index (BMI) values (36.79 ± 5.72 vs. 34.25 ± 5.88,

Table 1

Sleep study parameters

Parameters	OSAHS		Total	*Z*	*p*-value
	moderate	severe			
Apnea-hypopnea index *per* hour	23.08 ± 4.63	51.84 ± 16.97	41.83 ± 19.59	-8.808	< 0.001
Oxygen desaturation index *per* hour (%)	24.98 ± 10.22	53.15 ± 22.17	43.35 ± 23.16	-6.404	< 0.001
Time spent with oxygen saturation below 90% (%)	18.56 ± 27.86	36.38 ± 27.09	30.32 ± 28.51	-3.659	< 0.001
Minimal oxygen saturation (%)	92.72 ± 11.11	67.41 ± 13.28	76.22 ± 66.96	-3.770	< 0.001
Average oxygen saturation (%)	92.3 ± 3.29	89.56 ± 4.18	90.48 ± 4.1	-3.951	< 0.001

OSAHS – obstructive sleep apnea-hypopnea syndrome. All values are given as mean ± standard deviation.

Table 2

Cardiovascular risk factors and anthropometric measurements

Parameters	OSAHS		Total	$t/Z/\chi^2$	*p*-value
	moderate	severe			
Gender, female	11 (27.50)	13 (17.33)	24 (20.87)	1.633[a]	0.201
Smoking	23 (57.50)	39 (52.00)	62 (53.91)	0.318[a]	0.573
Physical inactivity	14 (35.00)	35 (46.67)	49 (42.61)	1.452[a]	0.228
Obesity	31 (77.50)	71 (94.67)	102 (88.70)	7.667[a]	**0.006**
Stress	13 (32.50)	15 (20.00)	28 (24.35)	2.213[a]	0.137
Diabetes mellitus	6 (15.00)	25 (33.33)	31 (26.96)	4.453[a]	0.035
Heredity	25 (62.50)	42 (56.00)	67 (58.26)	0.453[a]	0.501
Dyslipidaemia	30 (75.00)	50 (66.67)	80 (69.57)	0.856[a]	0.355
Age, years	54.8 ± 8.91	53.55 ± 9.53	53.98 ± 9.30	0.687	0.494
Waist circumference (cm)	118.33 ± 15.11	123.42 ± 12.36	121.65 ± 13.53	-1.919	0.058
Neck circumference (cm)	43.97 ± 4.20	46.23 ± 3.58	45.46 ± 3.94	-2.970	**0.004**
Body mass index (kg/m^2)	34.25 ± 5.88	36.79 ± 5.72	35.90 ± 5.88	-2.242	**0.027**
Number of cardiovascular risk factors	4.60 ± 1.55	4.75 ± 1.23	4.70 ± 1.35	-0.638	0.524

OSAHS – obstructive sleep apnea-hypopnea syndrome. All values are given as numbers (percentages) or mean ± standard deviation. The bold values indicate a significance level of $p < 0.05$.
***Note*: [a] Student's *t*-test was used.**

Table 3

Laboratory measurements

Parameters	OSAHS		Total	*Z*	*p*-value
	moderate	severe			
Cholesterol, mmol/L	5.27 ± 1.08	5.08 ± 1.17	5.15 ± 1.14	-0.781	0.435
LDL, mmol/L	2.96 ± 1.12	3.07 ± 1.06	3.03 ± 1.08	-0.209	0.835
HDL, mmol/L	1.18 ± 0.31	1.07 ± 0.27	1.11 ± 0.29	-1.647	0.099
Triglyceride, mmol/L	2.33 ± 1.44	2.03 ± 0.94	2.13 ± 1.14	-0.523	0.601
Glucose, mmol/L	5.73 ± 1.20	5.84 ± 0.81	5.8 ± 0.96	-1.204	0.229
AST, U/L	21.65 ± 6.92	21.35 ± 8.40	21.45 ± 7.89	-0.650	0.516
ALT, U/L	29.25 ± 14.9	29.29 ± 15.45	29.28 ± 15.19	-0.015	0.988
Creatinine, μmol/L	91.30 ± 15.99	93.97 ± 15.93	93.04 ± 15.93	-0.341	0.733
Urea, mmol/L	5.08 ± 1.28	5.40 ± 1.43	5.29 ± 1.38	-1.087	0.277
Uric acid	354.88 ± 77.71	379.31 ± 85.60	370.81 ± 83.42	-1.577	0.115
eGFR	114.05 ± 35.16	129.53 ± 3 2.54	124.15 ± 34.13	-2.646	**0.008**
Modified eGFR*	91.64 ± 25.05	100.19 ± 22.75	97.24 ± 23.81	-1.969	**0.049**
Sedimentation	20.55 ± 18.92	19.56 ± 13.88	19.90 ± 15.74	-0.062	0.951
Leukocyte	7.16 ± 1.65	7.46 ± 1.58	7.36 ± 1.60	-0.722	0.470
Erythrocyte	4.85 ± 0.45	4.87 ± 0.44	4.86 ± 0.44	-0.279	0.780
Hematocrit	0.43 ± 0.04	0.43 ± 0.03	0.43 ± 0.04	-0.553	0.580
Hemoglobin	143.13 ± 14.95	144.45 ± 12.40	143.99 ± 13.29	-0.264	0.791
Thrombocyte	262.30 ± 52.48	254.91 ± 55.20	257.48 ± 54.15	-0.810	0.418

LDL – low-density lipoprotein; HDL – high-density lipoprotein; AST – aspartate aminotransaminase; ALT – alanine aminotransaminase; eGFR – estimated glomerular filtration rate. For other abbreviations, see Table 1.
All values are given as mean ± standard deviation. The bold values indicate a significance level of $p < 0.05$.
***Note*: * used for an overweight patient.**

Table 4

Exercise stress test

Parameters	OSAHS		Total	χ^2/Z	*p*-value
	moderate	severe			
Submaximal heart rate achieved	26 (68.42)	59 (84.29)	85 (78.70)	3.698[a]	0.054
Ischemia	3 (7.50)	3 (4.00)	6 (5.22)	0.646[a]	0.421
Arrhythmias	2 (5.26)	7 (10.00)	9 (8.33)	0.723[a]	0.395
Level	2.71 ± 1.14	2.61 ± 1.04	2.65 ± 1.07	-0.259	0.796
Duration (min)	6.53 ± 3.25	6.14 ± 2.95	6.28 ± 3.05	-0.384	0.701
Double product before test	9,541.05 ± 1,718.26	9,876.86 ± 1,877.82	9,758.7 ± 1,822.31	-1.017	0.309
Double product after test	21,409.5 ± 5,804.29	22, 177.73 ± 6,896.41	21,910.52 ± 6,521.74	-1.398	0.162

OSAHS – obstructive sleep apnea-hypopnea syndrome; min – minutes.
All values are given as numbers (percentages) or mean ± standard deviation.
***Note*: [a] Chi-square test was used.**

$p = 0.027$) compared to those with moderate OSAHS. In addition, a significantly higher proportion of patients with severe OSAHS suffered from obesity (94.67% vs. 77.5%, $p = 0.006$).

No significant differences were observed in the investigated laboratory parameters between the groups except for creatinine clearance (129.53 ± 32.54 vs. 114.05 ± 35.16, $p = 0.008$) and creatinine clearance adjusted for overweight patients (100.19 ± 22.75 vs. 91.64 ± 25.05, $p = 0.049$). Both parameters were statistically higher in patients with severe OSAHS (Table 3).

The ESTs were performed in 108 patients (38 with moderate, and 70 with severe OSAHS). The differences in exercise tolerance among the groups were shown in Table 4. There were no significant differences in the examined parameters among the groups.

A total of 113 (39 with moderate and 74 with severe OSAHS) patients completed the SF-36 questionnaire. Two patients did not complete the questionnaire, and their data were not included in the study. The physical component summary (PCS) was higher in the moderate OSAHS group, while the mental component summary (MCS) was higher in the severe OSAHS group (Table 5), but without statistical significance.

After excluding DM, obesity, and stress, a correlation analysis was performed between AHI and EST level, PCS, and MCS (Table 6). No significant correlation was observed between AHI and EST level, PCS, or MCS.

Table 5

The quality of life assessed by the SF-36 questionnaire

Parameters	OSAHS		Total	*Z*	*p*-value
	moderate	severe			
Physical functioning	51.88 ± 26.33	53.69 ± 25.90	53.05 ± 25.95	-0.494	0.621
Limitations due to physical health	44.23 ± 43.48	45.61 ± 40.83	45.13 ± 41.57	-0.339	0.735
Limitations due to emotional problems	52.99 ± 43.07	60.81 ± 39.16	58.11 ± 40.53	-0.870	0.384
Energy/fatigue	49.10 ± 19.60	51.15 ± 19.65	50.44 ± 19.57	-0.585	0.559
Emotional well-being	66.26 ± 18.28	67.46 ± 18.11	67.04 ± 18.10	-0.348	0.728
Social functioning	66.35 ± 27.23	66.05 ± 25.04	66.15 ± 25.70	-0.150	0.881
Pain	52.31 ± 29.28	55.47 ± 25.86	54.38 ± 27.00	-0.647	0.518
General health	50.00 ± 18.25	48.92 ± 17.79	49.29 ± 17.88	-0.219	0.827
Health change	40.38 ± 24.07	49.32 ± 28.66	46.24 ± 27.39	-1.519	0.129
Physical component summary	65.59 ± 13.29	63.77 ± 13.69	64.40 ± 13.52	-0.531	0.595
Mental component summary	76.83 ± 16.16	79.16 ± 14.55	78.35 ± 15.1	-0.901	0.367

SF-36 – Short-Form 36. OSAHS – obstructive sleep apnea-hypopnea syndrome.
All values are given as mean ± standard deviation.

Table 6

Correlation between AHI and EST, MCS, and PCS

Index		EST level	PCS	MCS
AHI	*R*	-0.0120	0.086	0.098
	p	0.902	0.387	0.324

AHI – apnea-hypopnea index; EST – exercise stress test; PCS – physical component summary; MCS – mental component summary.

Discussion

The study evaluated 115 patients with moderate or severe OSAHS and HTA, focusing on disease severity, CVR profile, QoL, and exercise tolerance. As expected, patients with severe OSAHS had higher BMI and neck circumference, in line with previous research linking obesity and anthropometric markers to OSAHS severity [17–21]. A high prevalence of CVR factors was observed, underscoring the substantial cardiovascular burden in this population. However, no significant differences were detected between moderate and severe OSAHS in QoL measures or exercise tolerance. These findings suggest that disease severity may not reliably predict exercise tolerance or perceived health status. Finally, detection of ischemic changes and confirmed CAD in some patients further highlights the necessity of cardiovascular screening in this population.

The study population comprised predominantly males [91 (79.13%)], with only 24 (20.87%) female participants. Our results are in concordance with the results of previous trial [22]. The higher prevalence of OSAHS in males can be explained by several factors. Women less frequently report typical OSAHS symptoms like snoring or witnessed apneas [23]. On the other hand, the male respiratory tract is longer and more prone to collapse compared to the female respiratory tract [24]. Nevertheless, the most important explanation for sex-related differences in OSAHS prevalence is the protective role of progesterone and estrogen, which enhance upper airway dilator muscle function and reduce the likelihood of developing OSAHS [25]. As anticipated, all assessed sleep study parameters showed significantly better values in moderate OSAHS than in severe OSAHS.

Patients with severe OSAHS had significantly higher neck circumference and BMI values, and a greater proportion of them were obese compared to those with moderate OSAHS. This is in concordance with previous research [17–21]. The obesity and high BMI values significantly increase the chance of OSAHS development [17], and a positive correlation between BMI and OSAHS severity has been found—the higher the BMI, the higher the AHI [18]. Moreover, it has been shown that neck circumference can predict both the presence and severity of OSAHS in snoring patients [19], and that neck circumference positively correlates with OSAHS severity [20]. Some evidence suggests that this correlation is even stronger than that observed between OSAHS and general obesity [21].

A high prevalence of CVR factors was noticed in OSAHS patients, including obesity (88.70%), dyslipidaemia (69.57%), smoking (53.91%), heredity (58.26%), physical inactivity (42.61%), and DM (26.96%). These findings align with previous research, which highlights high CVR in patients with OSAHS [26, 27]. However, standard CVR assessment tools may underestimate or inadequately represent CVR associated with OSAHS [28].

Several factors contribute to impaired QoL in patients with OSAHS [29, 30]. Depression [29], poor sleep quality [30], comorbidities, excessive daytime sleepiness [30], and cognitive dysfunction [31] are frequently observed in this population and are associated with reduced QoL. Surprisingly, the severity of OSAHS does not influence the QoL [32]. Our study confirmed these findings as all investigated parameters obtained by the SF-36 questionnaire showed no significant differences between the groups. Furthermore, there were no statistically

significant differences in PCS and MCS between the groups. These findings may be attributed to several factors, including potential adaptation mechanisms in patients with more severe OSAHS, the subjective nature of QoL assessments, and the limited sample size of the study.

Patients with OSAHS have lower levels of physical activity compared to individuals without OSAHS, and regular physical activity has been shown to reduce the risk of OSAHS development [33]. Obesity, daytime sleepiness, fatigue, and decreased energy contribute to reduced exercise tolerance in OSAHS patients. It might be hypothesized that exercise tolerance decreases with increasing OSAHS severity. Exercise tolerance was assessed using EST rather than cardiopulmonary exercise testing (CPET), the latter being a more sensitive method for evaluating exercise tolerance in patients with OSAHS [34]. In the present study, there were no statistically significant differences in exercise tolerance on EST between the investigated groups. Moreover, the anticipated negative correlation between AHI and exercise tolerance was not observed in our analysis. These findings are consistent with previous studies reporting weak or absent associations between AHI and objective measures of exercise tolerance, particularly in men [35], suggesting that OSAHS severity is not always a reliable predictor of exercise tolerance [36]. Finally, it should be noted that we used EST rather than CPET, which is considered a more sensitive method for assessing exercise tolerance in patients with OSAHS [37].

Six (5.22%) patients of the 109 who underwent EST exhibited ischemic changes on the electrocardiogram. Three of these patients were referred for stress echocardiography, which revealed no exercise-induced wall motion abnormalities. Conversely, three patients underwent coronary angiography, with one demonstrating multivessel CAD. Moreover, one patient was directly referred for coronary angiography due to high CVR. Multivessel CAD was also identified in this patient. These findings underscore the importance of screening for CAD in patients with OSAHS [38].

The RPG was performed instead of respiratory polysomnography, which remains the gold standard for OSAHS diagnosis. This decision was necessitated by the lack of readily available respiratory polysomnography facilities within our medical center. Although it has certain limitations, RPG remains a valuable and widely used tool for assessing respiratory events during sleep, particularly in resource-constrained settings [39]. In addition, exercise tolerance was assessed using EST instead of CPET, although CPET represents a more sensitive and comprehensive method for evaluating exercise tolerance in patients with OSAHS. The predominance of male (79.13%) participants reduces the generalizability of our results to female patients.

Conclusion

Patients with obstructive sleep apnea-hypopnea syndrome overall exhibit multiple cardiovascular risk factors and are characterized by reduced quality of life and decreased exercise tolerance. Nevertheless, in our sample, increasing severity of obstructive sleep apnea-hypopnea syndrome did not correspond to significant differences in quality-of-life domains or exercise tolerance. Importantly, we detected multivessel coronary artery disease in asymptomatic patients, which underscores the clinical importance of coronary artery disease screening in patients diagnosed with obstructive sleep apnea-hypopnea syndrome.

Conflict of interest

The authors declare no conflict of interest.

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Nikolai Sergeyevich Korotkov: 120 years of his arterial blood pressure measurement method

Nikolaj Sergejevič Korotkov: 120 godina njegove metode merenja arterijskog krvnog pritiska

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Abstract

Nikolai Sergeyevich Korotkov (1874–1920) was a Russian military doctor and surgeon who discovered the technique of non-invasive measurement of arterial blood pressure. Measuring arterial blood pressure is possible based on the tones recorded with a stethoscope, now known as Korotkov sounds, using a sphygmomanometer. The tones disappear when the air pressure in the cuff placed around the upper arm exceeds the systolic pressure. The appearance of the first of a total of five tones occurs after the gradual deflation of the cuff and indicates the systolic pressure. It is several millimeters of mercury higher than that obtained by palpation of the radial artery, as was determined until then. The disappearance of the last, fifth tone, with a further reduction of air pressure in the cuff, indicates the diastolic pressure. Until that moment, measuring diastolic pressure in such a painless and simple manner had not been possible. Exactly 120 years have passed since Korotkov, at only 31 years of age, first announced his discovery in 1905 in Petrograd. The method was recognized by the professional community in 1939. Since then, Korotkov's sounds have been accepted worldwide for measuring arterial blood pressure. This method has remained unsurpassed to this day.

Keywords:
blood pressure determination; history, 20th century; history of medicine; russia; surgeons.

Apstrakt

Nikolaj Sergejevič Korotkov (1874–1920) bio je ruski vojni lekar, hirurg, koji je otkrio tehniku neinvazivnog merenja arterijskog krvnog pritiska. Merenje arterijskog krvnog pritiska je moguće na osnovu tonova registrovanih stetoskopom, danas poznatih kao Korotkovljevi, uz korišenje sfingomanometra. Tonovi nestaju kada je pritisak vazduha u manžetni postavljenoj oko nadlaktice viši od sistolnog pritiska. Pojava prvog od ukupno pet tonova javlja se posle postepenog ispuštanja vazduha i označava sistolni pritisak. On je za nekoliko milimetara živinog stuba viši od onog dobijenog palpacijom radijalne arterije, kako se do tada određivalo. Nestanak poslednjeg, petog, tona uz dalje smanjenje vazdušnog pritiska u manžetni, označava dijastolni pritisak. Njega do tog momenta nije bilo moguće meriti ovako bezbolnim i jednostavim načinom. Upravo se navršilo 120 godina od kada je Korotkov, sa svega 31 godinom života, svoje otkriće prvi put saopštio 1905. godine u Petrogradu. Metoda je prepoznata od stručne javnosti 1939. godine. Od tada su u svetu prihvaćeni Korotkovljevi tonovi za merenje arterijskog krvnog pritiska. Ova metoda je do danas ostala neprevaziđena.

Ključne reči:
krvni pritisak, merenje; istorija, 20. vek; istorija medicine; rusija; hirurzi.

Introduction

High arterial blood pressure (BP) is a common and widespread disease worldwide. It is estimated that almost a third of humanity has this health problem, which has numerous, sometimes life-threatening complications [1]. In order to properly treat and control it, simple, reliable, accurate, and frequent measurements are necessary. Owing to the discovery of a young Russian military surgeon, Nikolai Sergeyevich Korotkov (Figure 1) [2], this has been done for more than a century.

Harold Segall was the first to conduct an intensive study of Korotkov in 1939. The first photograph of Korotkov was published quite late, in 1970. The second one was published in 1976 by Segall. He himself was involved in BP research and was very interested in the life and work of

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Korotkov. In 1940, Segall wrote: "After Korotkov introduced the auscultatory method in 1905, its use became almost universal, and interest in measuring diastolic BP by any other method diminished considerably", [3].

Fig. 1 – Nikolai Sergeyevich Korotkov [2]. (https://www.wikidata.org/wiki/Q918543)

In 1939, the Joint Committee of the American Heart Association and the Cardiac Society of Great Britain and Ireland officially recognized and accepted worldwide Korotkov's method for BP determination. Some have published translations of his original work, with the intention of reducing the great ignorance surrounding his work, despite its enormous importance in the discovery of BP measurement [4].

Nikolai Sergeyevich Korotkov

It should be noted that the biographical data of Nikolai Sergeyevich Korotkov is not unique nor uniform, but differs from author to author. There are variations in the translation of the name itself: Nikolay Sergeevich Korotkov, Nicolai Sergeyevich Korotkoff, and Nikolai Sergeyevich Korotkov. In this paper, we used the third version.

Nikolai Sergeyevich Korotkov was born on February 13, 1874, in Kursk, into an Orthodox merchant family. There, he completed primary and secondary school education with excellent results. He enrolled in the Faculty of Medicine in Kharkov (now Kharkiv, Ukraine) in 1893, where he finished one semester according to some sources [5], while other sources mention one year [4] or even longer. After that, he transferred to Moscow University in 1895, where he graduated with excellent grades in 1898. He was 24 years old at the time. Following this, he was appointed a resident intern at the Surgical Clinic of Moscow University under Prof. Alexander A. Bobrov, one of the preeminent surgeons. He worked there for two years, without pay. He compensated for this by working in private practice.

As a young doctor, he served voluntarily in several wars. In 1900, he was in the Far East during the Boxer Rebellion in China. He was sent there by the University, as part of the Red Cross under the leadership of Dr. Ivan Pavlovich Aleksinsky, a former student of Prof. Bobrov. He traveled to the battlefield by the Trans-Siberian Railway and returned to Moscow *via* Japan, Singapore, Ceylon, the Suez Canal, the Mediterranean, and the Black Sea. For his "exceptionally zealous efforts in helping the sick and wounded soldiers," he was awarded the Order of St. Anna 3rd class [4]. After returning from the war, Korotkov translated Eduard Albert's monograph "Diagnosis in Surgery" from German into Russian.

From 1901 to 1903, he was again at Bobrov's Clinic in Moscow as the "chief ordinator" (a senior hospital physician) [5]. In the subsequent period, from 1903, he went to Saint Petersburg, Russia, at the invitation of his colleague Sergei P. Fedorov, a surgeon, former assistant of Prof. Bobrov, who worked as a professor at the Imperial Military Medical Academy. Dr. Korotkov was appointed an instructor at the Women's Department of the Surgical Clinic, headed by Dr. Fedorov. He worked as an unpaid doctor. Shortly thereafter, he was allowed to take his first (theoretical and practical in 1903) and second (theoretical in 1904) doctoral exams. "It should be noted that the professor who proctored Korotkov's examination in physiology was Ivan Petrovich Pavlov, the 1904 Nobel laureate in Physiology or Medicine for studies on digestion" [6]. After these exams, he temporarily stopped working on the thesis to take care of his health, which was already deteriorating, possibly due to tuberculosis.

During the Russo-Japanese War (1904–1905), he voluntarily went to Manchuria, to the city of Harbin. There he worked as a senior surgeon for the Second Hospital Unit of the Red Cross of St. George, and then as a surgeon in the First (sometimes reported as Second) Main Hospital in Harbin, China [5, 6]. His wife, Elena Aleseevna Grigoryeva, accompanied him on that journey as a Red Cross nurse. By the spring of 1905, they were expecting their first child.

He returned from the war to Saint Petersburg in April 1905 and continued to work on his doctoral thesis. In a brief statement in the Report of the Imperial Military Medical Academy in Saint Petersburg dated November 8, it was described how Korotkov measured the arterial BP of the wounded soldiers. The text was 281 words long [7]. In it, Dr. Korotkov described his technique for determining systolic and diastolic BP. It was entitled "On the issue of the methods for measuring blood pressure". At the age of only 31, he presented his technique for measuring arterial BP. He himself was not aware of the significance of his discovery. He is remembered worldwide for this achievement. Exactly 120 years have passed since this highly significant discovery was made.

The following month, he published another short statement in order to experimentally support his theory. In a series of canine experiments, he demonstrated that the occurrence of tones and murmurs was of arterial, not cardiac, origin. He reported the results of these experiments at a scientific seminar on December 13 [8].

Dr. Korotkov was, first and foremost, a war surgeon and a practicing physician, not primarily a researcher. While treating wounded soldiers with arm and leg injuries, his main concern, as a wartime vascular surgeon, was to assess the adequacy of the collateral arterial circulation. He needed to find reliable clinical signs that could predict "whether the collateral blood supply was undiminished, so that the injured artery could be safely ligated when amputation was likely" [9]. Dr. Korotkov wrote: "The unpredictable results of operations are unpleasant for the physician and even more unpleasant for the patient. Therefore, it would be advisable to seek the basic signs by which it is possible to know whether the patient would be dead or alive after the ligation of the artery." [5]. Searching for a solution to this problem, he discovered auscultatory measurement of BP. This unique method was new. Until then, the accepted method was palpation of the radial artery, which determined only systolic BP.

Both wars enabled him to collect material for his doctoral dissertation. He obtained valuable data on the diagnosis and treatment of traumatic aneurysms, collecting 44 cases of arterial aneurysms and 41 cases of arteriovenous aneurysms [4, 6]. All cases of vascular injury were thoroughly investigated, analyzed, and registered. "Korotkov's spiritual mentor was Nikolai Ivanovich Pirogov (1810–1881), the greatest surgeon in the history of medicine and the founder of scientific war surgery", as stated in the original text quoted here [6].

In 1910, he defended his 150-page dissertation, entitled *Experience in determining the strength of arterial collaterals* (in Russian: *Опыт определения силы артериальных коллатералей*), in which he also briefly described BP measurement [5].

Korotkov's sound

In his work, Korotkov used previous discoveries such as the Riva-Rocci sphygmomanometer and a children's stethoscope, which he placed directly below the cuff [4, 6, 9].

In 1886, the Italian physician Scipione Riva-Rocci (1863–1937) discovered, for that time, a new non-invasive method for measuring BP [10]. The method, still used to this day, includes a cuff placed around the middle third of the upper arm, which, when inflated, occludes blood flow in the brachial artery, accompanied by the disappearance of the pulse in the radial artery. With gradual deflation, the pulse returns, indicating the systolic BP. The cuff is connected to a mercury manometer.

Korotkov considered the tones and murmurs in the vessel to be due to compression of the brachial artery and their potential relationship with BP values. Normally, blood in the brachial artery moves in a laminar (smooth) flow, which is not audible with a stethoscope. When the air is gradually released from the cuff, due to the reduction in compression of the brachial artery, the flow becomes turbulent, which allows these tones to be heard with a stethoscope. When the brachial artery begins to decompress, after inflating to the point of complete disappearance of sounds (complete obliteration), a pulsating blood flow is established, which generates sounds named after Korotkov, known as "Korotkov sounds" [7].

Korotkov sounds are divided into five phases: (I) clear knock sounds are heard, at least two consecutive knocks (emergence); (II) attenuation of the knock sounds (softening); (III) return of the knock sounds, with increased sharpness and intensity (sharpening); (IV) sudden attenuation of the sounds (damping); (V) complete disappearance of all sounds [7].

Systolic BP is when the first knock sound is heard in the stethoscope (phase I), and diastolic BP is when there is no more sound (phase V). These two phases, I and V, are therefore the determinants of systolic and diastolic BP. The systolic pressure measured this way was a few millimeters higher than the one obtained by palpation of the radial artery. Measuring the diastolic BP at that time was imprecise and challenging for researchers [7].

This non-invasive method of measuring BP is the "gold standard", even today. It may be at risk of being replaced by the increasing use of automated devices. This is, however, unlikely, because they must compare their algorithms, through international protocols, with arterial BP measured by the Korotkov method [11].

The Korotkov method is one of the most useful in diagnosing, treating, monitoring, and preventing cardiovascular diseases [12].

The rest of the biography

In 1908–1909, before defending his dissertation, Korotkov worked as a research physician in the Vitimsko-Olekminsky mining district in Siberia, where dry and very cold winters were thought to alleviate tuberculosis to some extent. He was able to return to Saint Petersburg. After defending his dissertation, he returned to Siberia as a surgeon to the workers of the Lensk gold mines (named after the Lena River, not the city of Lensk). Here, he witnessed some violence by Tsarist authorities and was deeply affected by the murder of unarmed striking miners. After this, he returned to Saint Petersburg [4, 6]. During the First World War, he served as a military surgeon at the Charitable House for disabled soldiers in Tsarskoe Selo [4] (now the town of Pushkin), Russia. Later, after the October Revolution, he became physician-in-chief of the Mechnikov Hospital in Petrograd (renamed from Saint Petersburg). After that, he was a senior physician at the Petrograd Hospital on Zagorodny Avenue [8]. From 1913 until his death, he was in a more favorable financial situation, so he was able to devote himself to reading and painting, and even return to scientific research work.

He died prematurely on March 14, 1920, at the age of 46. It is believed that he succumbed to tuberculosis. He was buried at the Academy site in Bogoslovskoe Cemetery, Saint Petersburg. One of the streets within the Military Medical Academy, where Korotkov worked, is named after him [6].

His wife died during the siege of Leningrad (renamed from Petrograd) in 1941. His son Sergey also followed in his

father's medical footsteps. He was a doctor, a specialist in sports medicine and rehabilitation. He died (around) 1978 [5].

There is little information available about the family of Nikolai Korotkov. This applies not only to Nikolai Sergeyevich Korotkov himself, but also to his wife, son, and his son's family, whom his wife outlived [5].

Some biographical information was published in English by Harold Segall in 1965 [6]. However, due to differences between the Russian and American education systems, Segall misinterpreted certain facts of Korotkov's life [8].

Conclusion

Nikolai Sergeyevich Korotkov discovered a non-invasive method of measuring blood pressure that remains the "gold standard" to this day. Although the reasons for the interruption of his promising academic career remain unclear, his achievement at the age of 31 was truly epochal, demonstrating both the simplicity and remarkable accuracy of auscultatory measurement of systolic and diastolic blood pressure.

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C O R R I G E N D U M

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I. The article “Clinical and laboratory status in Parkinson’s disease patients with and without polyneuropathy“ published in the October 2024 print issue of the *Vojnosanitetski pregled* (Vojnosanit Pregl 2024; 81(10): 613–8; https://doi.org/10.2298/VSP240528061P) by Sanela Popović, Nemanja Popović, Dragica Hajder, Smiljana Kostić, Aleksandra Lučić Prokin, contains error with respect to the e-mail address of the corresponding author Sanela Popović. Instead of the existing e-mail address sanela_bozic@yahoo.com, it should be sanela.popovic@mf.uns.ac.rs

This article was corrected Online [1].

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

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

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

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

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

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

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

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

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

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ETHICAL APPROVAL

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

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

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

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ORIGINAL ARTICLE

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

PRELIMINARY REPORT

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

SHORT REPORT

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

REVIEW ARTICLES

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

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

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

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

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

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

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

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

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

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

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

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

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BOOK REVIEW

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

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

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

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

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The first page of the manuscript should include the following:

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

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

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

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

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QUESTIONNAIRES

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

TABLES AND FIGURES

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

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

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ACKNOWLEDGEMENTS

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

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Article with more than 6 authors

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

Volume with a Supplement

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

Issue with a Supplement

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

Volume with Part (Pt)

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

Issue with Part (Pt)

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

Issue with no Volume

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

No Volume or Issue

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

Pagination with Roman numerals

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

Book

Printed Book

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

Book in electronic format

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

Chapter

In an edited book

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

In an edited electronic (online) book

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

Website

Homepage

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

Part of a website

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

Conference Proceedings

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

Article from Conference Proceedings

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

Dissertation

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

Other published articles

News article

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

Holy Bible

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

Dictionaries and similar references

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

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

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УПУТСТВО ЗА АУТОРЕ

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

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

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

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

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

СЛАЊЕ РУКОПИСА

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

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

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

ОПШТА УПУТСТВА

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ОБАВЕЗНА ПРАТЕЋА ДОКУМЕНТА

ИЗЈАВА АУТОРА И АУТОРСТВО

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

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

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

ЕТИЧКА САГЛАСНОСТ

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

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

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

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

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

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

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

ПЛАГИЈАРИЗАМ

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

КОРИШЋЕЊЕ AI

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

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

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

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

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

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

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

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

ТИПОВИ РУКОПИСА

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

ОРИГИНАЛНИ ЧЛАНАК

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

ПРЕТХОДНО САОПШТЕЊЕ

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

КРАТКО САОПШТЕЊЕ

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

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

Преглед, критичка анализа и синтеза постојећих научних сазнања о изабраној теми. Аутори обухватају сву доступну припадајућу литературу за одређени временски период, приказују резултате релевантних истраживања, идентификују недостатке, ограничења или контроверзе и указују на правце будућих истраживања, дајући своје виђење проблема у виду закључног става. Аутори чланка ове категорије могу бити они који су објавили минимално пет радова публикованих у часописима с рецензијом (М20) из области прегледног рада.

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МЕТА-АНАЛИЗА

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

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

АКТУЕЛНА ТЕМА

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

У ФОКУСУ

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

КАЗУИСТИКА

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

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

УВОДНИК

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

ПИСМО УРЕДНИКУ

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

ИСТРАЖИВАЧКО ПИСМО

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

ИСТОРИЈА МЕДИЦИНЕ/СТОМАТОЛОГИЈЕ/ФАРМАЦИЈЕ

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

КЛИНИЧКО ИСТРАЖИВАЊЕ

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

ПРИКАЗ КЊИГЕ

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

ИЗВЕШТАЈ СА НАУЧНОГ ИЛИ СТРУЧНОГ СКУПА

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

ОБИМ РУКОПИСА

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

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

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

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

ПРИПРЕМА РАДА

НАСЛОВНА СТРАНА

На првој страници рукописа треба навести следеће:

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

АПСТРАКТ

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

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

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

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

КЉУЧНЕ РЕЧИ

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

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

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

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

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

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

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

ПРИЛОЗИ

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

Табеле

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

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

Слике (илустрације)

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

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

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

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

СКРАЋЕНИЦЕ

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

ДЕЦИМАЛНИ БРОЈЕВИ

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

ЈЕДИНИЦЕ МЕРА

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

ЗАХВАЛНИЦА

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

СТАТИСТИЧКА АНАЛИЗА

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

ЛИТЕРАТУРА

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

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

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

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

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

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НАКНАДЕ ЗА ОБЈАВЉИВАЊЕ ЧЛАНКА

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

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