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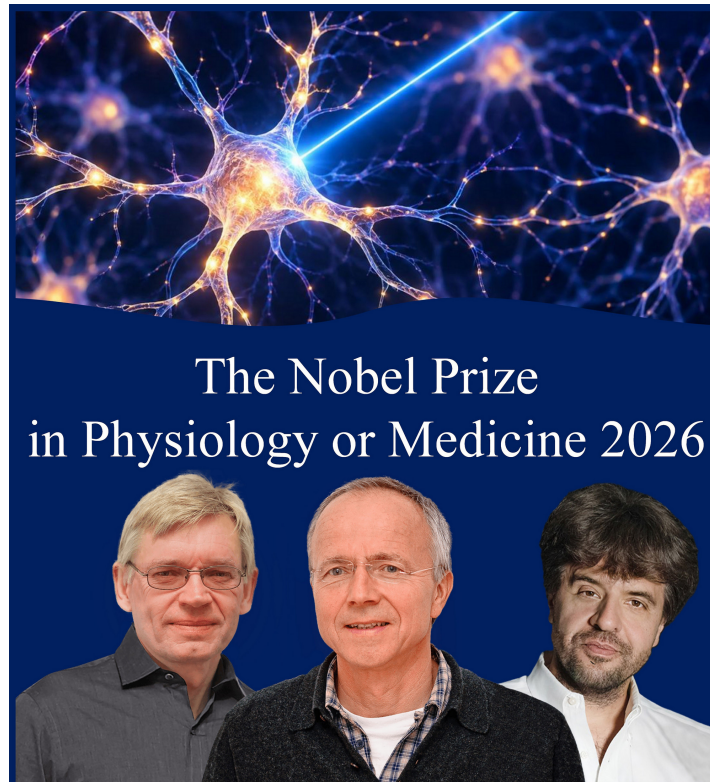


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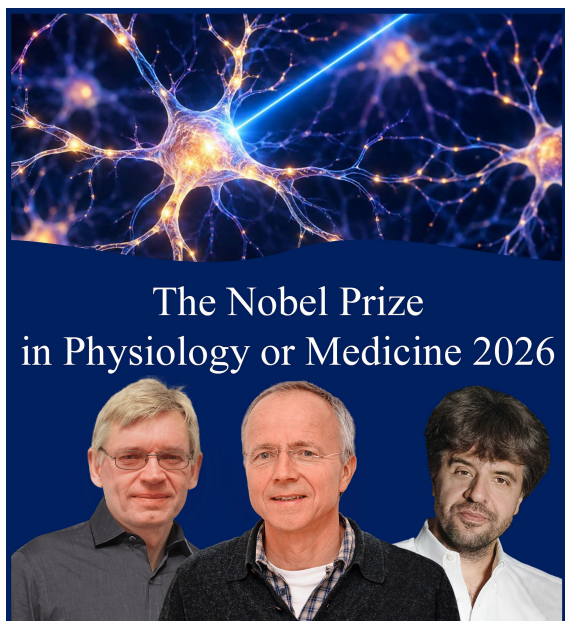
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The 2026 Nobel Prize in Physiology or Medicine is shared equally by Peter Hegemann, Georg Nagel, and Karl Deisseroth for their discoveries concerning light-gated ion channels and optogenetics. Peter Hegemann and Georg Nagel discovered channelrhodopsin, an algal protein with unique properties. When blue light is shone on this protein, a channel opens, allowing charged ions to enter the cell and generate an electrical impulse. Karl Deisseroth introduced the gene for channelrhodopsin into rat neurons and successfully triggered a nerve signal by directing blue light onto the cells. The method of controlling neural signals using light is called optogenetics, and it has fundamentally transformed our understanding of one of humanity's greatest mysteries: how our wondrous brain works.

Nobelovu nagradu za fiziologiju ili medicinu za 2026. godinu ravnopravno dele Piter Hegeman, Džordž Nejgel i Karl Dajserot za otkrića u vezi sa jonskim kanalima čije je otvaranje i zatvaranje kontrolisano svetlošću i optogenetikom. Piter Hegeman i Džordž Nejgel otkrili su kanalrodopsin, protein algi sa jedinstvenim svojstvima. Kada se na ovaj protein usmeri plava svetlost kroz njega se otvara kanal, putem koga naelektrisani joni ulaze u ćeliju, stvarajući električni impuls. Karl Dajserot je uneo gen za kanalrodopsin u nervne ćelije pacova i usmeravanjem plave svetlosti na ćelije uspeo da izazove nervni signal. Metoda kontrole nervnih signala pomoću svetlosti se naziva optogenetika i iz temelja je promenila naše razumevanje jedne od velikih misterija čovečanstva: kako funkcioniše naš čudesni mozak.



Impact of a continuous multidisciplinary rehabilitation pathway on clinical outcomes in patients with acute ischemic stroke after mechanical thrombectomy

Uticaj kontinuiranog multidisciplinarnog programa rehabilitacije na kliničke ishode kod bolesnika sa akutnim ishemijskim moždanim udarom nakon mehaničke trombektomije

Bingmeng Chen*, Chenyi Zhou†, Qinqye Jin*

*The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China; †Yueqing Hospital of Traditional Chinese Medicine, Yueqing, Zhejiang, China

Abstract

Background/Aim. Acute ischemic stroke remains a major cause of long-term disability, and optimal rehabilitation following mechanical thrombectomy is still being researched. The aim of this prospective randomized controlled study was to evaluate whether the continuous multidisciplinary rehabilitation (CMR) program, initiated within 24–72 hrs after mechanical thrombectomy and maintained for 3 months, was associated with improved neurological and functional outcomes. **Methods.** A total of 100 patients with acute ischemic stroke who underwent mechanical thrombectomy between October 2021 and October 2024 were randomly assigned (1 : 1) to either the CMR group or the control group (CG) (50 patients each). Patients in CG received routine post-procedural medical treatment and nursing care, whereas the patients in the CMR group received a structured multidisciplinary rehabilitation program in addition to routine care. The primary outcome was the National Institutes of Health Stroke Scale (NIHSS) score at 3 months. Secondary outcomes included Fugl-Meyer Assessment (FMA), Barthel Index (BI), Montreal Cognitive Assessment (MoCA), Motor

Assessment Scale (MAS), patient satisfaction, complication incidence, and favorable prognosis. Longitudinal changes were analyzed using mixed-design repeated-measures analysis of variance – ANOVA. **Results.** Baseline demographic and clinical characteristics were comparable between groups (all $p > 0.05$). Compared with CG, the CMR group demonstrated significantly greater improvements in MoCA, MAS, FMA, BI, and NIHSS scores at 1 and 3 months post-intervention (all $p < 0.05$). Furthermore, the CMR group demonstrated a significantly higher patient satisfaction rate and favorable prognosis rate, as well as a lower incidence of complications ($p < 0.05$). **Conclusion.** A CMR program initiated within 24–72 hrs after mechanical thrombectomy and maintained for 3 months was associated with improved short-term neurological, cognitive, motor, and functional recovery. The observed benefits should be interpreted as the cumulative effects of sustained rehabilitation rather than early initiation alone.

Keywords:

endovascular procedures; ischemic stroke; neurology; physical and rehabilitation medicine; rehabilitation.

Apstrakt

Uvod/Cilj. Akutni ishemijski moždani udar ostaje značajan uzrok dugotrajne onesposobljenosti, a optimalna rehabilitacija nakon mehaničke trombektomije je i dalje predmet istraživanja. Cilj ove prospektivne randomizovane kontrolisane studije bio je da proceni da li je kontinuirani multidisciplinarni program rehabilitacije (*continuous multidisciplinary rehabilitation* – CMR program), započet u roku od 24–72 sata nakon mehaničke trombektomije i nastavljen tokom 3 meseca, povezan sa poboljšanim neurološkim i

funkcionalnim ishodima. **Metode.** Ukupno 100 bolesnika sa akutnim ishemijskim moždanim udarom, koji su podvrgnuti mehaničkoj trombektomiji između oktobra 2021. i oktobra 2024. godine, nasumično je raspoređeno (1 : 1) ili u CMR grupu ili u kontrolnu grupu (*control group* – CG) (obe po 50 bolesnika). Bolesnici u CG primali su rutinski postproceduralni tretman i medicinsku negu, dok su bolesnici u CMR grupi, pored rutinske nege, primali i strukturirani multidisciplinarni program rehabilitacije. Primarni ishod bio je rezultat na skali *National Institutes of Health Stroke Scale* – NIHSS nakon 3 meseca. Sekundarni

ishodi uključivali su Fugl-Majerovu procenu (*Fugl-Meyer Assessment* – FMA), Bartelov indeks (*Barthel Index* – BI), Montrealsku kognitivnu procenu (*Montreal Cognitive Assessment* – MoCA), Skalu za procenu motorike (*Motor Assessment Scale* – MAS), zadovoljstvo bolesnika, učestalost komplikacija i povoljnu prognozu. Longitudinalne promene analizirane su korišćenjem analize varijanse (*analysis of variance* – ANOVA) mešovito dizajna sa ponovljenim merenjima. **Rezultati.** Demografske i kliničke karakteristike na početku istraživanja bile su ujednačene između grupa (sve $p > 0,05$). U poređenju sa CG, CMR grupa je pokazala značajno veća poboljšanja u MoCA, MAS, FMA, BI i NIHSS rezultatima nakon 1 i 3 meseca od intervencije (sve $p < 0,05$). Pored toga, u CMR

grupi zabeležena je značajno veća stopa zadovoljstva bolesnika i povoljne prognoze, kao i značajno niža incidencija komplikacija ($p < 0,05$). **Zaključak.** CMR program započet u roku od 24–72 sata nakon mehaničke trombektomije i održavan tokom 3 meseca bio je povezan sa poboljšanim kratkoročnim neurološkim, kognitivnim, motoričkim i funkcionalnim oporavkom. Uočena poboljšanja treba tumačiti kao kumulativne efekte kontinuirane rehabilitacije, a ne samo kao posledicu njenog ranog započinjanja.

Ključne reči:

endovaskularne procedure; moždani udar, ishemijski; neurologija; medicina, fizikalna; rehabilitacija.

Introduction

Acute ischemic stroke (AIS) is a major ischemic cerebrovascular disorder characterized by sudden onset, rapid progression, and high rates of neurological disability. AIS occurs due to occlusion of cerebral arteries, resulting in cerebral ischemia, hypoxia, and subsequent neuronal necrosis¹. These pathological changes frequently lead to cognitive impairment, motor dysfunction, and other neurological deficits that substantially impair activities of daily living (ADLs) and quality of life². Mechanical thrombectomy (MT) has become the standard minimally invasive intervention for selected patients with AIS caused by large vessel occlusion, as it can rapidly restore cerebral perfusion, reduce infarct progression, and improve clinical outcomes³. However, despite successful recanalization, many patients continue to experience persistent neurological and functional impairments. Without timely post-procedural rehabilitation, patients remain at risk for muscle atrophy, joint stiffness, cognitive decline, and long-term disability⁴.

Continuous multidisciplinary rehabilitation (CMR) initiated during the early post-thrombectomy period, guided by the principle of neuroplasticity, is designed to support neurological recovery throughout both the acute and subacute phases after stroke⁵. Comprehensive rehabilitation strategies, including cognitive training, motor rehabilitation, and ADLs training, may promote recovery of neurological function and improve patient independence⁶. Although several studies have investigated rehabilitation following MT, available evidence remains methodologically heterogeneous, with variations in rehabilitation protocols, outcome measures, and follow-up assessments⁷. Furthermore, comprehensive evaluations encompassing neurological, cognitive, motor, and daily living domains remain scarce in the current literature.

To date, evidence specifically evaluating CMR initiated within 24–72 hrs after MT is exceptionally sparse. In addition, most previous studies have primarily focused on single functional domains rather than comprehensive multidimensional recovery outcomes. Therefore, the aim of this study was to evaluate whether a CMR pathway initiated within 24–72 hrs after MT and maintained over a 3-month

period was associated with improved neurological and functional recovery. Given the multicomponent nature of rehabilitation interventions and the methodological limitations of existing evidence, the findings of this study should be interpreted as exploratory and association-based rather than definitive evidence of causal therapeutic efficacy.

Methods

Study population

This single-center prospective randomized controlled study was conducted at the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, China, between October 2021 and October 2024. The study aimed to evaluate the association between a CMR pathway initiated early after MT and short-term neurological and functional recovery.

The study protocol, including the routine-care control condition and the structured multidisciplinary rehabilitation intervention, was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China (No. 243/CI/08/2020) before participant enrollment. Written informed consent was obtained from all participants or their legal representatives prior to study enrollment. All study procedures were conducted in accordance with the Declaration of Helsinki and institutional ethical standards. The study was not prospectively registered in a public clinical trial registry.

A total of 100 patients with AIS who underwent MT during the study period were prospectively enrolled according to predefined eligibility criteria. No formal *a priori* sample size calculation was performed before study initiation. The sample size was determined pragmatically based on the number of eligible patients treated during the study period and the feasibility of completing the planned 3-month follow-up assessments. Consequently, this study should be considered an exploratory randomized clinical study. Patients were randomly assigned in a 1 : 1 ratio to either the CMR group or the control group (CG) (50 *per* group) using a computer-generated random allocation

sequence generated by the principal investigator before study initiation (Figure 1). Eligible participants were enrolled by the treating neurologist, rehabilitation physician, research nurse, or principal investigator. Allocation concealment was ensured through a centralized procedure. After confirming participant eligibility and obtaining written informed consent, treatment assignment was performed through the centralized allocation system according to the predetermined computer-generated randomization sequence. The assigned treatment group was communicated to the clinical team responsible for implementing the intervention. Investigators

responsible for participant enrollment did not have access to the randomization sequence before allocation, thereby minimizing selection bias.

Due to the nature of the rehabilitation intervention, blinding of participants and therapists was not feasible. However, outcome assessments were performed by trained rehabilitation personnel who were not involved in delivering the intervention and were blinded to treatment allocation throughout the study. Outcome assessors did not have access to participants' group assignments, minimizing the risk of assessment bias.

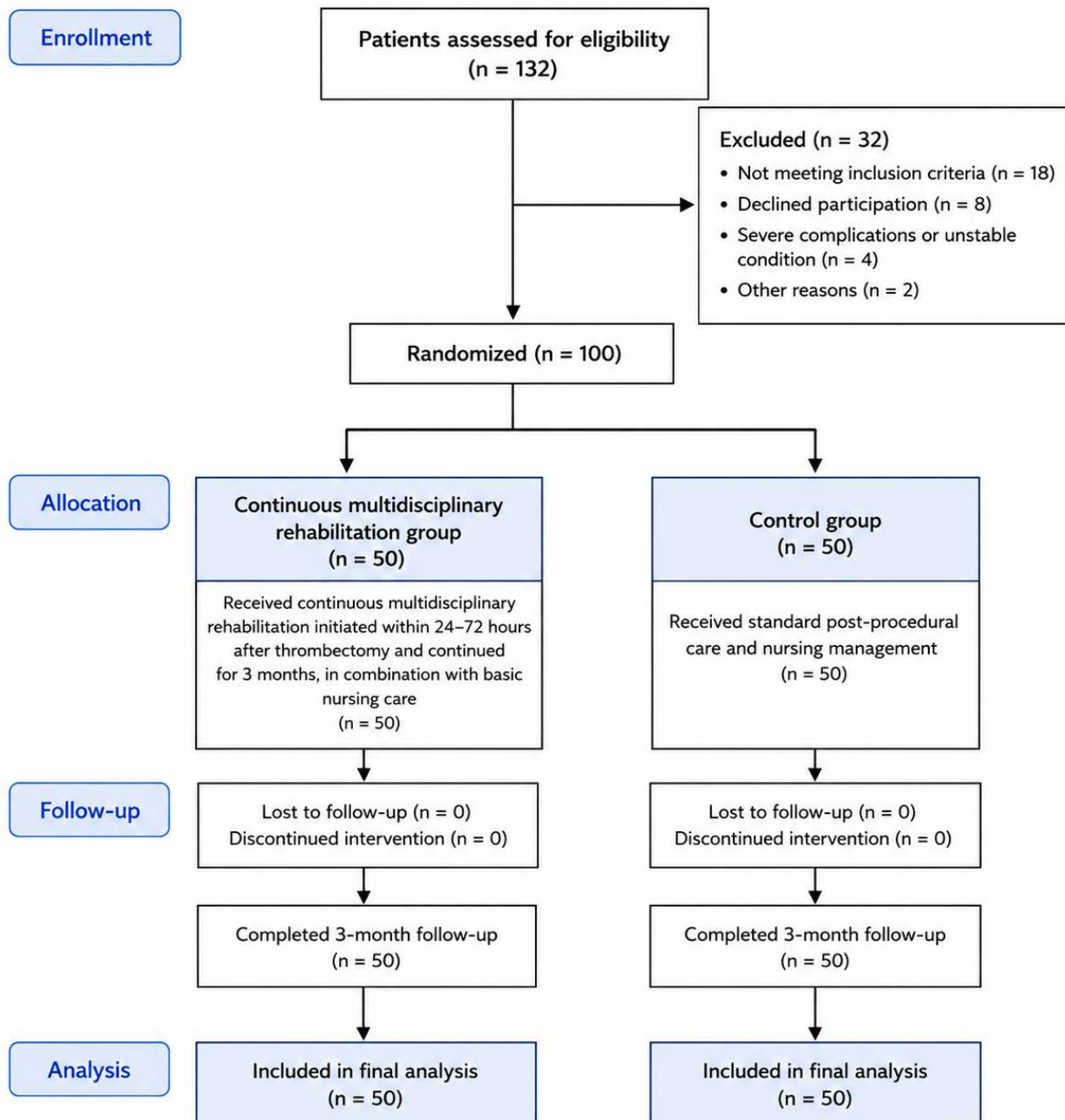


Fig. 1 – CONSORT-style flow diagram illustrating patient screening, randomization, intervention allocation, follow-up, and final analysis.
n – number; CONSORT – Consolidated Standards of Reporting Trials.

All randomized participants completed the 3-month follow-up and underwent all scheduled outcome assessments. There were no participants who withdrew consent, discontinued the intervention, or were lost to follow-up. Consequently, no outcome data were missing, and all randomized participants were included in the final analyses, which were performed according to the complete-case principle.

Inclusion criteria were as follows: patients diagnosed with AIS according to the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke (2023 Edition)⁸, confirmed by cranial computer tomography – CT or magnetic resonance imaging – MRI examination; successful completion of MT, with stable vital signs and clear consciousness after the procedure; onset-to-puncture time < 8 hrs; written informed consent obtained from all patients or their legal representatives prior to enrolment; ability to cooperate with the full course of rehabilitation intervention and outcome assessments.

Exclusion criteria included the following: presence of severe dysfunction of major organs, including the heart, liver, kidneys, or lungs; presence of other neurological disorders, including intracerebral hemorrhage, brain tumors, or Parkinson's disease; severe cognitive impairment, psychiatric illness, or communication disorders preventing participation in rehabilitation training or assessments; limb deformities, fractures, severe arthritis, or other conditions affecting motor rehabilitation; development of severe post-procedural complications, including intracranial hemorrhage or severe infection, requiring termination of rehabilitation intervention; loss to follow-up or withdrawal during the study period.

Of the 100 enrolled patients, 57 were male and 43 were female, with ages ranging from 45–78 years and a mean age of 62.35 ± 8.72 years. The sites of arterial occlusion included the middle cerebral artery in 68 cases, the anterior cerebral artery in 17 cases, and the vertebrobasilar artery in 15 cases. The onset-to-puncture time ranged from 2.5 to 7.8 hrs, with a mean duration of 5.12 ± 1.36 hrs. The interval between thrombectomy and initiation of rehabilitation ranged from 1 to 3 days, with a mean duration of 1.85 ± 0.42 days. Intravenous thrombolysis prior to MT was administered according to institutional stroke management protocols when clinically indicated.

Patients in CG received routine post-procedural treatment and standard nursing care following MT. Routine medical management included antiplatelet therapy, treatment to improve cerebral circulation, neurotrophic therapy, blood pressure and blood glucose control, infection prevention, and other supportive therapies according to institutional stroke management protocols. Standard nursing care included clinical condition monitoring, positional care, oral care, skin care, nutritional support, and general health education.

During the 3-month follow-up period, patients in CG did not participate in the structured CMR program evaluated in this study. Accordingly, they did not receive the scheduled rehabilitation sessions incorporated into the intervention protocol, including structured cognitive rehabilitation, motor

rehabilitation, ADLs training, low-frequency electrical stimulation, acupuncture, massage therapy, or other supervised rehabilitation sessions delivered by the multidisciplinary rehabilitation team.

At the time this study was conducted, a structured multidisciplinary rehabilitation pathway following MT had not been routinely implemented as part of standard post-procedural care at our institution. Therefore, CG reflected the prevailing institutional standard of care rather than an intentional withholding of rehabilitation. All participants in CG received the routine medical management and standard nursing care that were routinely available at our institution throughout the study period but did not receive the additional structured multidisciplinary rehabilitation program evaluated in this trial.

In addition to standard medical management and routine nursing care identical to that received by CG, patients in the CMR group underwent a structured CMR program initiated within 24–72 hrs after MT following clinical stabilization and subsequently continued throughout the 3-month study period.

The CMR program was delivered over 3 months under the supervision of trained rehabilitation personnel. Rehabilitation was provided 5 days *per* week and consisted of multiple supervised sessions scheduled according to each patient's neurological status, functional tolerance, and clinical stability. The total daily rehabilitation time was approximately 90–120 min and included cognitive rehabilitation, motor rehabilitation, ADLs training, and adjunctive rehabilitation therapies. The duration and intensity of each component were individualized according to patient tolerance and recovery progression to ensure patient safety and maximize adherence. All participants allocated to the CMR group completed the scheduled multidisciplinary rehabilitation program during the study period, and no participant discontinued the intervention because of rehabilitation-related adverse events.

The rehabilitation program was initiated during hospitalization and continued on an outpatient basis following discharge under the supervision of trained rehabilitation personnel.

Cognitive function rehabilitation training

Targeted cognitive rehabilitation training was performed under the supervision of trained personnel and individualized according to patient tolerance and neurological status. The intervention included multiple structured components aimed at improving attention, memory, executive function, language ability, and visuospatial perception.

Attention training consisted of number cancellation tasks and auditory attention exercises, in which patients identified designated numbers or responded to specific auditory stimuli.

For memory training, patients engaged in short-term and long-term memory rehabilitation exercises, including recall of past events, word repetition, and picture memorization.

Executive function training utilized graphic sequencing, simple calculations, and object classification exercises to enhance logical thinking and executive control.

Language rehabilitation training targeted speech impairment through pronunciation exercises, verbal expression training, and reading comprehension activities, which progressed gradually from simple syllables and words to complex sentence-level tasks.

Visuospatial ability training utilized jigsaw puzzles, block assembly tasks, and clock-drawing exercises to improve visuospatial perception and constructional coordination.

Each of these given sessions lasted approximately 15–20 min and were delivered as part of the scheduled daily multidisciplinary rehabilitation program. Training intensity and progression were adjusted according to individual patient tolerance to minimize excessive fatigue and ensure rehabilitation safety.

Motor function and limb motor function rehabilitation training

Motor rehabilitation was implemented progressively according to the principle of gradual advancement from low-intensity passive training to active functional rehabilitation. The rehabilitation protocol was individualized based on patient recovery status and tolerance.

During the acute phase, spanning 1 to 2 weeks post-MT, passive rehabilitation exercises were primarily performed to reduce the risk of joint stiffness and muscle atrophy. Rehabilitation therapists assisted patients with passive limb movements, including flexion, extension, rotation, adduction, and abduction exercises. Each movement was maintained for approximately 10–15 sec and repeated 10–15 times *per* joint during scheduled rehabilitation sessions, with progression according to individual recovery status and patient tolerance. Deep breathing and coughing exercises were additionally encouraged to reduce the risk of pulmonary complications.

During the recovery phase, spanning 3 to 6 weeks post-MT, rehabilitation was gradually transitioned to active functional training. Patients underwent transfer training from supine-to-sitting and sitting-to-standing positions to improve balance and limb control. Active limb flexion, extension, and elevation exercises were also performed to enhance motor recovery. For patients capable of standing independently, wall-standing exercises and supervised walking training were conducted, with progressive increases in walking duration and distance. Each session lasted approximately 15–20 min and was performed during scheduled rehabilitation sessions, with progression according to each individual's recovery status and patient tolerance.

During the late rehabilitation phase, spanning 7 to 12 weeks post-MT, rehabilitation intensity was further increased according to patient tolerance and functional recovery. Stair-climbing exercises and obstacle-crossing activities were performed to improve coordination, balance, and exercise endurance. Fine motor rehabilitation, including object grasping

and bead-stringing exercises, was conducted to improve hand motor function and dexterity. Each session lasted approximately 20–30 min and was performed during scheduled rehabilitation sessions, with progression based on individual recovery status and patient tolerance.

The rehabilitation protocol was adjusted according to individual patient tolerance and neurological recovery to ensure safety and avoid excessive physical fatigue.

Rehabilitation training for activities of daily living

Structured ADLs rehabilitation training was performed to improve patient independence and functional self-care ability. Training protocols were individualized according to each patient's neurological status and recovery progression.

For eating training, patients were instructed to use spoons and chopsticks during feeding activities. Training initially focused on slow eating with small food portions and gradually progressed according to swallowing safety and coordination to reduce the risk of choking.

For personal hygiene training, patients underwent supervised training in washing, hair combing, shaving, and dressing activities. Rehabilitation initially involved assisted performance and progressively transitioned toward independent completion as functional ability improved.

For bowel and bladder training, patients were guided to establish regular bowel and urination habits and received training aimed at improving voluntary bowel and bladder control.

For walking and stair-climbing training, progressive walking and stair-climbing training were conducted in combination with motor rehabilitation exercises to enhance mobility, balance, and independent living capacity.

Each rehabilitation session lasted approximately 20–30 min and was delivered as part of the scheduled multidisciplinary rehabilitation program. Training intensity and rehabilitation content were individualized according to each patient's recovery status, functional tolerance, and safety considerations.

Adjuvant rehabilitation for neurological function

In addition to the primary rehabilitation program, adjunctive physical rehabilitation therapies were administered to support neurological recovery and functional improvement. These interventions included low-frequency pulsed electrical stimulation, acupuncture, and rehabilitation massage.

Low-frequency electrical stimulation was applied to selected muscle groups and affected limbs to facilitate neuromuscular activation and improve motor responsiveness. Trained personnel also performed acupuncture and rehabilitation massage to alleviate limb stiffness, muscle spasticity, and sensory discomfort, while potentially promoting neurological recovery and improved circulation.

Each adjunctive rehabilitation session lasted approximately 20 min and was administered as part of the scheduled multidisciplinary rehabilitation program according to the patient's clinical condition and rehabilitation needs.

These adjunctive therapies were incorporated as supportive components of the comprehensive multidisciplinary rehabilitation program and were not evaluated as isolated therapeutic modalities.

Outcome measures

All outcome assessments were performed by professionally trained rehabilitation therapists and medical staff using standards-based evaluation procedures to improve assessment consistency and reliability. Outcome measurements were obtained at baseline, 1 month, and 3 months after intervention unless otherwise specified.

The primary outcome was the change in National Institutes of Health Stroke Scale (NIHSS) score at 3 months after intervention.

Outcomes

The primary outcome was neurological impairment evaluated using NIHSS⁸, which assesses 11 domains with total scores ranging from 0 to 42 points. Higher scores indicate more severe neurological deficits.

Secondary outcomes comprised assessments of cognitive function, motor recovery, ADLs, patient satisfaction, complication incidence, and favorable prognosis. Cognitive function was evaluated using the Montreal Cognitive Assessment (MoCA)⁹ at baseline, 1 month, and 3 months post-intervention. The MoCA assesses multiple cognitive domains including attention, memory, executive function, language, and visuospatial ability, with total scores ranging from 0 to 30 points. Higher scores indicate better cognitive performance, and scores < 26 points indicate cognitive impairment.

Motor recovery was measured using the Motor Assessment Scale (MAS), a validated functional assessment instrument designed to evaluate motor performance after stroke. The MAS assesses eight functional motor tasks, including supine-to-side lying, supine-to-sitting, balanced sitting, sitting-to-standing, walking, upper-arm function, hand movements, and advanced hand activities. Higher scores indicate better motor function and recovery¹⁰.

Limb motor function was evaluated using the Fugl-Meyer Assessment (FMA)¹¹ at baseline, 1 month, and 3 months after intervention. The FMA includes upper- and lower-extremity motor function assessments with total scores ranging from 0 to 100 points. Higher scores indicate better limb motor recovery.

Functional independence in ADLs was assessed using the Barthel Index (BI)¹² at baseline, 1 month, and 3 months after intervention. The BI covers 10 functional domains, including feeding, dressing, personal hygiene, walking, and stair climbing, with total scores ranging from 0 to 100 points. Higher scores indicate greater independence in daily living activities.

Patient satisfaction was evaluated at 3 months after intervention using a self-designed institutional satisfaction questionnaire consisting of 8 items related to rehabilitation

effectiveness, therapist service quality, rehabilitation protocol satisfaction, and nursing care quality. Each item was scored on a 5-point Likert scale, with total scores ranging from 8 to 40. Satisfaction rates were calculated as the proportion of patients classified as “extremely satisfied” or “satisfied”. The questionnaire demonstrated acceptable internal consistency reliability (Cronbach $\alpha = 0.86$)¹³. Because the questionnaire was developed for institutional use and has not undergone external validation, patient satisfaction was considered a secondary exploratory outcome and was interpreted with appropriate caution.

Complications occurring during hospitalization were prospectively recorded, including pressure ulcers, pulmonary infection, deep vein thrombosis, and joint stiffness. Pressure ulcers were diagnosed according to the National Pressure Injury Advisory Panel – NPIAP criteria during routine skin examinations. Pulmonary infection was diagnosed by the treating physician based on compatible clinical symptoms, laboratory findings, and chest imaging. Deep vein thrombosis was confirmed using compression Doppler ultrasonography when clinically suspected. Joint stiffness was diagnosed by rehabilitation physicians based on reduced passive joint range of motion during standardized physical examinations. Complications were assessed throughout hospitalization by treating physicians and rehabilitation staff who were not directly involved in delivering the rehabilitation intervention¹⁴. Outcome assessors responsible for data collection were blinded to treatment allocation whenever feasible. All complications were identified through routine clinical monitoring and standardized daily assessments rather than being recorded only when clinically apparent.

Favorable prognosis at 3 months was defined as an exploratory composite functional outcome based on predefined thresholds of cognitive and motor recovery, including a MoCA score ≥ 26 points, MAS score ≥ 30 points, and FMA score ≥ 85 points¹⁵. Patients meeting all three predefined criteria were classified as having a favorable prognosis. Because no universally accepted composite endpoint incorporating these functional assessments has been established for patients undergoing MT, this outcome was considered exploratory and was used to provide an integrated assessment of short-term neurological and functional recovery. It was not intended to replace the standard 90-day modified Rankin Scale (mRS), which should be incorporated in future prospective studies to facilitate comparison with the broader stroke literature¹⁶.

Because multiple functional domains were evaluated, repeated measurements were analyzed longitudinally to assess changes over time and differences between treatment groups. The exploratory composite favorable prognosis endpoint was analyzed separately to provide an overall assessment of multidimensional functional recovery.

Statistical analysis

Statistical analyses were performed using the SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were initially assessed for normality using

the Shapiro–Wilk test. Normally distributed continuous variables are presented as mean $\pm$ standard deviation – SD, whereas non-normally distributed variables are presented as median and interquartile range – IQR. Repeated outcome measurements obtained at baseline, 1 month, and 3 months were analyzed using two-way repeated-measures analysis of variance (ANOVA), with time as the within-subject factor and treatment group as the between-subject factor. Main effects of time and group, as well as time $\times$ group interaction effects, were evaluated to assess differential longitudinal treatment responses between groups. When significant main effects or interaction effects were identified, Bonferroni-adjusted *post hoc* pairwise comparisons were performed to compare the three assessment time points (baseline vs. 1 month, baseline vs. 3 months, and 1 month vs. 3 months) within each predefined outcome measure (MoCA, MAS, FMA, BI, and NIHSS). The reported *p*-values for the repeated-measures ANOVA represent the overall model effects, whereas Bonferroni-adjusted *p*-values were used for the *post hoc* pairwise comparisons. Categorical variables are presented as frequencies and percentages. Comparisons between groups were performed using the chi-square test or Fisher's exact test, as appropriate. Effect sizes were additionally evaluated using partial eta squared (η^2) for repeated-measures analyses where applicable. A two-sided *p*-value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

As shown in Figure 1, 132 patients were assessed for eligibility, of whom 100 met the eligibility criteria and

were randomly assigned in a 1 : 1 ratio to the CMR group ($n = 50$) or CG ($n = 50$). All randomized participants completed the 3-month follow-up, and no participants were lost to follow-up, discontinued the intervention, or had missing outcome data. Consequently, all randomized participants were included in the final analysis. The participant enrollment, randomization, follow-up, and analysis process is illustrated in Figure 1.

Baseline demographic and clinical characteristics are summarized in Table 1. No statistically significant differences were observed between the groups with respect to age, sex distribution, body mass index, vascular risk factors, baseline NIHSS score, occlusion location, intravenous thrombolysis rate, onset-to-puncture time, or time from thrombectomy to rehabilitation initiation (all $p > 0.05$). Effect size analyses demonstrated negligible-to-small between-group differences (Cohen $d \leq 0.09$; Cramér $V \leq 0.051$), confirming excellent baseline comparability between the two groups (Table 1).

Longitudinal functional outcomes

Baseline scores for all functional assessments (MoCA, MAS, FMA, BI, and NIHSS) were comparable between the two groups (all $p > 0.05$). During the 3-month follow-up, both groups demonstrated progressive improvements in neurological and functional recovery; however, improvements were consistently greater in the CMR group. Compared with CG, patients in the CMR group achieved significantly higher MoCA scores at both follow-up assessments (all $p < 0.001$) (Table 2). Similarly, MAS scores were significantly higher in the CMR group throughout the follow-up (all $p < 0.001$) (Table 3). Limb motor recovery, assessed using FMA, also

Table 1

Baseline demographic and clinical characteristics of the study participants

Variable	Group		Test statistic (<i>df</i>)	<i>p</i> -value	Effect size
	CMR	control			
Age, years	62.18 $\pm$ 8.65	62.52 $\pm$ 8.81	$t(98) = 0.194$	0.847	Cohen $d = 0.04$
Male/female	29/21	28/22	$\chi^2(1) = 0.041$	0.839	Cramér $V = 0.020$
Body mass index, kg/m ²	24.85 $\pm$ 3.12	25.13 $\pm$ 3.26	$t(98) = 0.438$	0.662	Cohen $d = 0.09$
Hypertension	31 (62.0)	29 (58.0)	$\chi^2(1) = 0.167$	0.683	Cramér $V = 0.041$
Diabetes mellitus	18 (36.0)	20 (40.0)	$\chi^2(1) = 0.170$	0.680	Cramér $V = 0.042$
Hyperlipidemia	16 (32.0)	18 (36.0)	$\chi^2(1) = 0.178$	0.673	Cramér $V = 0.043$
Smoking history	22 (44.0)	24 (48.0)	$\chi^2(1) = 0.161$	0.688	Cramér $V = 0.041$
Previous stroke history	9 (18.0)	11 (22.0)	$\chi^2(1) = 0.251$	0.616	Cramér $V = 0.051$
Intravenous thrombolysis before thrombectomy	14 (28.0)	16 (32.0)	$\chi^2(1) = 0.190$	0.663	Cramér $V = 0.044$
Baseline NIHSS score	18.75 $\pm$ 3.26	18.96 $\pm$ 3.18	$t(98) = 0.305$	0.761	Cohen $d = 0.07$
Middle cerebral artery occlusion	34 (68.0)	34 (68.0)	$\chi^2(1) = 0.000$	1.000	Cramér $V = 0.000$
Anterior cerebral artery occlusion	8 (16.0)	9 (18.0)	$\chi^2(1) = 0.071$	0.790	Cramér $V = 0.027$
Vertebrobasilar artery occlusion	8 (16.0)	7 (14.0)	$\chi^2(1) = 0.078$	0.780	Cramér $V = 0.028$
Onset-to-puncture time, hrs	5.12 $\pm$ 1.36	5.08 $\pm$ 1.29	$t(98) = 0.151$	0.880	Cohen $d = 0.04$
Time from thrombectomy to rehabilitation initiation, days	1.86 $\pm$ 0.43	1.83 $\pm$ 0.41	$t(98) = 0.356$	0.722	Cohen $d = 0.08$

CMR – continuous multidisciplinary rehabilitation; *df* – degrees of freedom; NIHSS – National Institutes of Health Stroke Scale. Note: Data are presented as mean $\pm$ standard deviation or numbers (percentages). Continuous variables were compared using the independent-samples *t*-test, and categorical variables were compared using the chi-square test. Effect sizes are reported as Cohen d for continuous variables and Cramér V for categorical variables.

Table 2

**Longitudinal changes in Montreal Cognitive Assessment (MoCA)
scores between the continuous multidisciplinary rehabilitation (CMR) and control groups**

Group/effect	Baseline MoCA	1 month after intervention	3 months after intervention	<i>F</i> (<i>df</i> 1, <i>df</i> 2)	<i>p</i> -value	Partial η^2
CMR group	20.15 $\pm$ 2.36 (19.36–20.94)	24.32 $\pm$ 2.18 (23.62–25.02)	27.56 $\pm$ 1.89 (26.94–28.18)	–	–	–
Control group	20.32 $\pm$ 2.28 (19.55–21.09)	22.15 $\pm$ 2.05 (21.47–22.83)	24.23 $\pm$ 2.01 (23.53–24.93)	–	–	–
Time effect	–	–	–	<i>F</i> (2, 196) = 56.82	< 0.001	0.290
Group effect	–	–	–	<i>F</i> (1, 98) = 24.19	< 0.001	0.200
Time $\times$ group interaction	–	–	–	<i>F</i> (2, 196) = 18.35	< 0.001	0.160

df – degrees of freedom.

Note: Data are presented as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CIs). Longitudinal changes were analyzed using a mixed-design repeated-measures analysis of variance (ANOVA). *F* values, *df*, exact *p*-values, and partial eta squared (η^2) are reported for the main effects of time, group, and the time $\times$ group interaction. MoCA scores are given as mean $\pm$ SD (95% CI).

Table 3

**Longitudinal changes in Motor Assessment Scale (MAS)
scores between the continuous multidisciplinary rehabilitation (CMR) and control groups**

Group/effect	Baseline MAS	1 month	3 months	<i>F</i> (<i>df</i> 1, <i>df</i> 2)	<i>p</i> -value	Partial η^2
CMR group	18.23 $\pm$ 3.15 (17.34–19.12)	25.46 $\pm$ 3.02 (24.60–26.32)	32.15 $\pm$ 2.78 (31.36–32.94)	–	–	–
Control group	18.45 $\pm$ 3.08 (17.58–19.32)	21.32 $\pm$ 2.89 (20.50–22.14)	27.56 $\pm$ 2.91 (26.73–28.39)	–	–	–
Time effect	–	–	–	<i>F</i> (2, 196) = 56.42	< 0.001	0.365
Group effect	–	–	–	<i>F</i> (1, 98) = 40.78	< 0.001	0.294
Time $\times$ group interaction	–	–	–	<i>F</i> (2, 196) = 14.53	< 0.001	0.129

df – degrees of freedom.

Note: Data are presented as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CIs). Longitudinal changes were analyzed using a mixed-design repeated-measures analysis of variance (ANOVA). *F* values, *df*, *p*-values, and partial eta squared (η^2) are reported for the main effects of time, group, and the time $\times$ group interaction. MAS scores are given as mean $\pm$ SD (95% CI).

Table 4

**Longitudinal changes in Fugl-Meyer Assessment (FMA)
scores between the continuous multidisciplinary rehabilitation (CMR) and control groups**

Group/effect	Baseline FMA	1 month after intervention	3 months after intervention	<i>F</i> (<i>df</i> 1, <i>df</i> 2)	<i>p</i> -value	Partial η^2
CMR group	48.65 $\pm$ 8.72 (46.17–51.13)	69.32 $\pm$ 7.85 (67.09–71.55)	88.75 $\pm$ 6.54 (86.89–90.61)	–	–	–
Control group	49.12 $\pm$ 8.56 (46.69–51.55)	60.15 $\pm$ 7.62 (57.99–62.31)	76.32 $\pm$ 7.18 (74.28–78.36)	–	–	–
Time effect	–	–	–	<i>F</i> (2, 196) = 153.32	< 0.001	0.623
Group effect	–	–	–	<i>F</i> (1, 98) = 81.34	< 0.001	0.452
Time $\times$ group interaction	–	–	–	<i>F</i> (2, 196) = 110.34	< 0.001	0.521

df – degrees of freedom.

Note: Data are presented as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CIs). Longitudinal changes were analyzed using a mixed-design repeated-measures analysis of variance (ANOVA). *F* values, *df*, *p*-values, and partial eta squared (η^2) are reported for the main effects of time, group, and the time $\times$ group interaction. FMA scores are given as mean $\pm$ SD (95% CI).

showed significantly greater improvement in the CMR group than in CG (all $p < 0.001$) (Table 4). ADLs, measured using BI, improved significantly in both groups but were consistently higher in the CMR group at the 1- and 3-month follow-up assessments (all $p < 0.001$) (Table 5). Furthermore, neurological impairment assessed using NIHSS decreased significantly in both groups, with significantly lower NIHSS scores ob-

served in the CMR group throughout follow-up (all $p < 0.001$) (Table 6, Figure 2). Mixed-design repeated-measures ANOVA demonstrated significant main effects of time and group, together with significant time $\times$ group interaction effects for all longitudinal outcome measures (all $p < 0.001$), with moderate-to-large effect sizes, indicating significantly greater recovery trajectories in the CMR group than in CG (Tables 2–6).

Table 5

**Longitudinal changes in Barthel Index (BI) scores between
the continuous multidisciplinary rehabilitation (CMR) and control groups**

Group/effect	Baseline BI	1 month after intervention	3 months after intervention	<i>F</i> (<i>df</i> ₁ , <i>df</i> ₂)	<i>p</i> -value	Partial η^2
CMR group	38.25 ± 7.62 (36.09–40.41)	59.68 ± 7.35 (57.59–61.77)	86.75 ± 6.89 (84.79–88.71)	–	–	–
Control group	38.76 ± 7.45 (36.64–40.88)	50.12 ± 7.18 (48.08–52.16)	72.35 ± 7.02 (70.36–74.34)	–	–	–
Time effect	–	–	–	<i>F</i> (2, 196) = 45.12	< 0.001	0.316
Group effect	–	–	–	<i>F</i> (1, 98) = 38.55	< 0.001	0.280
Time × group interaction	–	–	–	<i>F</i> (2, 196) = 22.79	< 0.001	0.190

df – degrees of freedom.

Note: Data are presented as mean ± standard deviation (SD) with 95% confidence intervals (CIs). Longitudinal changes were analyzed using a mixed-design repeated-measures analysis of variance (ANOVA). *F* values, *df*, *p*-values, and partial eta squared (η^2) are reported for the main effects of time, group, and the time × group interaction. BI scores are given as mean ± SD (95% CI).

Table 6

**Longitudinal changes in the National Institutes of Health Stroke Scale (NIHSS)
scores between the continuous multidisciplinary rehabilitation (CMR) and control groups**

Group/effect	Baseline NIHSS	1 month after intervention	3 months after intervention	<i>F</i> (<i>df</i> ₁ , <i>df</i> ₂)	<i>p</i> -value	Partial η^2
CMR group	18.75 ± 3.26 (17.82–19.68)	10.23 ± 2.89 (9.41–11.05)	5.12 ± 2.15 (4.51–5.73)	–	–	–
Control group	18.96 ± 3.18 (18.06–19.86)	13.65 ± 2.76 (12.87–14.43)	9.87 ± 2.36 (9.20–10.54)	–	–	–
Time effect	–	–	–	<i>F</i> (2, 196) = 112.35	< 0.001	0.53
Group effect	–	–	–	<i>F</i> (1, 98) = 34.67	< 0.001	0.26
Time × group interaction	–	–	–	<i>F</i> (2, 196) = 23.14	< 0.001	0.19

df – degrees of freedom.

Note: Data are presented as mean ± standard deviation (SD) with 95% confidence intervals (CIs). Longitudinal changes were analyzed using a mixed-design repeated-measures analysis of variance (ANOVA). *F* values, *df*, *p*-values, and partial eta squared (η^2) are reported for the main effects of time, group, and the time × group interaction. NIHSS scores are given as mean ± SD (95% CI).

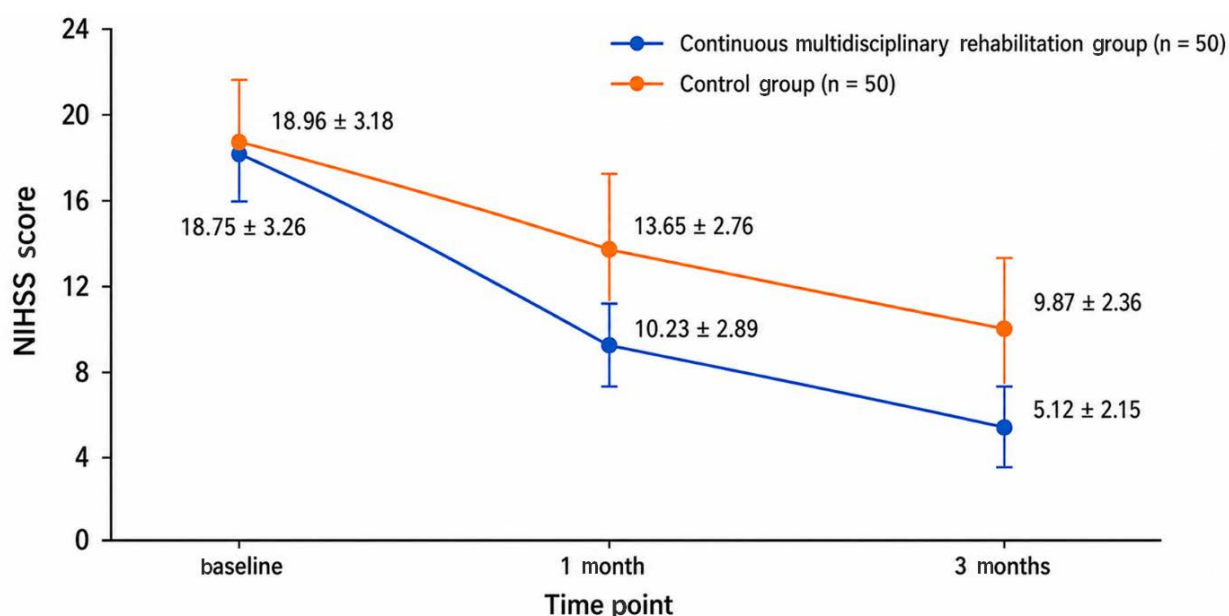


Fig. 2 – Longitudinal changes in neurological recovery between the continuous multidisciplinary rehabilitation group and the control group following mechanical thrombectomy.

Data are presented as mean ± standard deviation (SD). Repeated-measures ANOVA was used to evaluate longitudinal changes over time. Significant time × group interaction effects indicated different recovery trajectories between groups during follow-up.

NIHSS – National Institutes of Health Stroke Scale; n – number; ANOVA – analysis of variance.

Patient satisfaction and complication incidence

At the 3-month follow-up, the patient satisfaction rate, assessed using the institutional questionnaire, was significantly higher in the CMR group than in CG (94.0% vs. 74.0%, $\chi^2(1) = 6.353$, $p = 0.012$). As this represented an exploratory secondary outcome based on a non-externally validated questionnaire, the findings should be interpreted cautiously (Table 7).

Similarly, the incidence of complications during the intervention period was significantly lower in the CMR group than in CG (8.0% vs. 24.0%, $\chi^2(1) = 5.004$, $p = 0.025$). Recorded complications included pressure ulcers, pulmonary infection, deep vein thrombosis, and joint stiffness, which were prospectively identified through routine clinical monitoring and standardized daily assessments throughout the intervention period. The corresponding effect size and odds ratio further supported the clinical significance of the reduction in complication incidence (Table 7).

Favorable prognosis outcomes

Using the predefined exploratory composite functional outcome, the favorable prognosis rate was significantly higher in the CMR group than in CG (76.0% vs. 48.0%, $\chi^2(1) = 7.111$, $p = 0.008$) (Table 8). The corresponding effect size analysis demonstrated a clinically meaningful between-group difference, further supporting the beneficial effect of the CMR pathway on short-term functional recovery.

Overall, longitudinal mixed-design repeated-measures ANOVA demonstrated significant main effects of time, group, and time $\times$ group interaction for neurological, cognitive, motor, and ADLs outcomes (all $p < 0.001$), with moderate to large effect sizes indicating significantly greater recovery trajectories in the CMR group than in CG.

Discussion

AIS remains a major cause of neurological disability and functional dependence worldwide. MT has become a standard treatment for selected patients with large vessel occlusion because of its ability to rapidly restore cerebral perfusion and improve clinical outcomes¹⁷. However, despite successful recanalization, many patients continue to experience persistent neurological, motor, and cognitive impairments, highlighting the importance of structured post-procedural rehabilitation strategies¹⁸. In the absence of timely rehabilitation intervention, patients remain at risk for complications including muscle atrophy, joint stiffness, cognitive decline, and deep vein thrombosis, which may adversely affect long-term recovery and quality of life^{19, 20}. Therefore, identifying clinically feasible rehabilitation strategies following MT remains an important area of investigation.

The potential benefits of early rehabilitation are commonly explained through the concept of neuroplasticity, whereby repetitive functional stimulation may facilitate neural reorganization and functional adaptation after ischemic injury²¹. In addition, restoration of cerebral perfusion after MT may create a favorable physiological environment for neurological recovery²². Initiating multidisciplinary rehabilitation early after thrombectomy, followed by continuation throughout the subsequent rehabilitation pathway, may support neurological and functional recovery across both the acute and subacute phases after stroke²³. However, because the present intervention incorporated multiple rehabilitation components, including cognitive training, motor rehabilitation, ADLs training, electrical stimulation, acupuncture, massage, and adjunctive physical therapies, the study design does not permit identification of the relative contribution or mechanistic effect of individual therapeutic modalities.

Table 7

Comparison of exploratory patient satisfaction outcomes and complication incidence between the continuous multidisciplinary rehabilitation (CMR) and control groups

Variable	Group		χ^2 (df)	p-value	Odds ratio (95% CI)
	CMR	control			
Patient satisfaction	47 (94.0)	37 (74.0)	$\chi^2(1) = 6.353$	0.012	5.50 (1.42–21.28)
Complication incidence	4 (8.0)	12 (24.0)	$\chi^2(1) = 5.004$	0.025	0.28 (0.08–0.93)

df – degrees of freedom; CI – confidence interval.

Note: Data are presented as numbers (percentages). Comparisons between groups were performed using the Chi-square test. Odds ratios with 95% CIs are reported for between-group comparisons. Patient satisfaction was assessed using an institutionally developed questionnaire and should be interpreted as an exploratory secondary outcome.

Table 8

Comparison of favorable prognosis rates between groups at 3 months after intervention

Group	Favorable prognosis	Unfavorable prognosis	χ^2 (df)	p-value	Effect size (Cramér V)	Odds ratio (95% CI)
CMR	38 (76.0)	12 (24.0)	$\chi^2(1) = 7.111$	0.008	0.267	3.43 (1.46–8.08)
Control	24 (48.0)	26 (52.0)	–	–	–	–

CMR – continuous multidisciplinary rehabilitation; *df* – degrees of freedom; CI – confidence interval.

Note: Data are presented as numbers (percentages). Comparisons between groups were performed using the chi-square test. Effect size is reported as Cramér V. Odds ratio with 95% CI represents the likelihood of achieving a favorable prognosis in the continuous multidisciplinary rehabilitation group relative to the control group. Favorable prognosis was defined according to the predefined functional recovery criteria based on Montreal Cognitive Assessment (MoCA), Motor Assessment Scale (MAS), and Fugl-Meyer Assessment (FMA) outcomes.

In the present study, no statistically significant baseline differences were observed between groups for major functional indicators, suggesting acceptable comparability between groups prior to intervention. During follow-up, patients in the CMR group demonstrated significantly greater improvements in MoCA, MAS, FMA, BI, and NIHSS scores than those in CG, with significant time $\times$ group interaction effects observed across all longitudinal outcome measures. These findings suggest that CMR may be associated with improved short-term neurological and functional recovery following MT. Similar findings have been reported in previous rehabilitation studies involving patients with AIS^{24,25}. However, because the intervention comprised multiple rehabilitation components delivered as an integrated package, the results should be interpreted as exploratory and association-based rather than as evidence of the efficacy of any specific rehabilitation modality. The observed improvements may also have been influenced by greater treatment intensity, increased therapist–patient interaction, closer clinical supervision, and other nonspecific effects^{26,27}. Therefore, the findings support the potential clinical value of a CMR pathway but do not establish a causal relationship between any individual rehabilitation component and the observed outcomes.

An important consideration when interpreting these findings is that the intervention extended well beyond the acute post-stroke period. Although the multidisciplinary rehabilitation program was initiated within 24–72 hrs after MT, it continued as a structured rehabilitation pathway throughout the subsequent 3-month follow-up period. Therefore, the superior neurological and functional outcomes observed at 3 months should be interpreted as reflecting the cumulative effects of sustained multidisciplinary rehabilitation initiated early after stroke, rather than the isolated effect of early rehabilitation alone. This distinction matters clinically because recovery after AIS is a dynamic process spanning the acute and subacute phases and shaped by ongoing rehabilitation exposure over time.

An additional consideration is that rehabilitation practices following MT vary across institutions and healthcare systems. At our center, a structured multidisciplinary rehabilitation pathway was not routinely available as part of standard post-procedural care during the study period. Consequently, CG reflected the prevailing institutional standard of care rather than an intentional withholding of rehabilitation. In healthcare settings where structured multidisciplinary rehabilitation is routinely incorporated into standard care, the magnitude of the treatment effect observed in the present study may differ. Therefore, our findings should be interpreted within the context of the local rehabilitation practice in which the study was conducted.

The CMR group demonstrated significantly greater improvement in MoCA scores than CG during follow-up. These findings were associated with participation in the comprehensive multidisciplinary rehabilitation program. Because cognitive rehabilitation was delivered together with multiple other rehabilitation components, including motor

rehabilitation, ADLs training, electrical stimulation, acupuncture, massage, and other adjunctive therapies, the present study cannot determine the independent contribution of cognitive training to the observed cognitive improvements²⁸. Therefore, these findings should be interpreted as reflecting the overall multidisciplinary rehabilitation program rather than the effect of any individual rehabilitation component.

Motor dysfunction is a common sequela following AIS and frequently contributes to long-term disability and reduced independence in ADLs²⁹. In the present study, patients in the CMR group demonstrated significantly improved MAS and FMA scores compared with CG during follow-up, suggesting potential benefits of progressive multidisciplinary motor rehabilitation after MT. The staged rehabilitation protocol incorporated passive mobilization, active motor exercises, balance training, gait rehabilitation, and fine motor training according to patient recovery status. This structured progression may have supported motor recovery while minimizing excessive physical burden during the early rehabilitation period³⁰.

ADLs recovery is an important determinant of functional independence and social reintegration after AIS³¹. In the present study, patients receiving the CMR program showed significantly higher BI scores than controls at follow-up, suggesting improved daily living performance. The rehabilitation protocol incorporated individualized training related to feeding, dressing, personal hygiene, mobility, and bowel and bladder management³². Although patients receiving the CMR program demonstrated better ADLs outcomes than CG, these findings should be interpreted as reflecting the overall multidisciplinary rehabilitation program rather than the isolated effect of ADLs training³³.

Patients in the CMR group additionally demonstrated significantly lower NIHSS scores during follow-up, indicating less severe neurological impairment than CG. However, because multiple rehabilitation modalities were delivered simultaneously as part of a comprehensive multidisciplinary rehabilitation program, the present study cannot determine which intervention components, if any, were primarily responsible for the observed neurological improvements³⁴. Furthermore, patients in the CMR group received greater therapist contact, structured supervision, and rehabilitation intensity than those receiving routine care alone³⁵. Therefore, some of the observed benefits may have been influenced by nonspecific treatment effects, increased patient engagement, or attention-related factors rather than the effects of any individual rehabilitation modality³⁶.

Complication prevention represents an important component of post-stroke rehabilitation management because complications may delay recovery, prolong hospitalization, and adversely affect prognosis¹⁹. In the present study, the incidence of complications including pressure ulcers, pulmonary infection, deep vein thrombosis, and joint stiffness was significantly lower in the CMR group. This finding may be related to earlier mobilization, structured nursing support, rehabilitation monitoring, and continuous supervised physical activity during follow-up³⁷.

Patients in the CMR group reported higher satisfaction than those in CG. However, this finding should be interpreted cautiously because satisfaction was assessed using an institutional questionnaire that has not undergone external validation. Furthermore, greater therapist contact, increased rehabilitation intensity, closer clinical supervision, and enhanced patient engagement in the CMR group may have contributed to higher satisfaction ratings independent of the specific rehabilitation intervention. Therefore, patient satisfaction should be regarded as an exploratory secondary outcome rather than definitive evidence of treatment superiority. In addition, the favorable prognosis rate at 3 months was significantly higher in the CMR group than in CG.

Overall, the findings of the present study suggest that a CMR pathway initiated after MT may be associated with improved short-term neurological, motor, cognitive, and ADLs outcomes in patients with AIS. These findings are generally consistent with previous rehabilitation studies involving stroke populations¹⁹. Nevertheless, given the multicomponent intervention design and methodological limitations of the present study, further rigorously controlled multicenter studies are required to clarify causal relationships and determine the independent contribution of specific rehabilitation strategies.

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, the follow-up period was limited to 3 months, precluding evaluation of long-term neurological recovery, functional outcomes, and recurrence. Third, the rehabilitation protocol comprised a multicomponent multidisciplinary intervention, making it difficult to determine the independent contribution of each rehabilitation modality. Fourth, because blinding of participants and therapists was not feasible, nonspecific treatment effects, including attention bias, increased therapist contact, enhanced patient engagement, closer clinical monitoring, and differences in rehabilitation intensity, may have influenced the observed outcomes. Consequently, part of the observed treatment benefit may reflect enhanced therapeutic attention rather than the specific effects of the rehabilitation program. Future randomized controlled trials incorporating attention-matched CGs are warranted to better distinguish the effects of rehabilitation intensity from those of individual rehabilitation interventions. Furthermore, although rehabilitation was initiated during the acute phase, the intervention continued throughout the subacute recovery period. Therefore, the present study was not designed to distinguish the independent effect of early rehabilitation initiation from the cumulative effect of sustained multidisciplinary rehabilitation over the 3-month intervention period. Fifth, no formal *a priori* sample size calculation was performed. Instead, the sample size was determined by the number of eligible patients recruited during the study period and the feasibility of completing the trial. Consequently, the study may have been underpowered to detect smaller between-

group differences, and the findings should be interpreted as exploratory. Sixth, the study was not prospectively registered in a public clinical trial registry before participant enrollment. Although the study protocol, including the routine-care control condition and the structured multidisciplinary rehabilitation intervention, was prospectively reviewed and approved by the Institutional Ethics Committee before recruitment, and the intervention protocol, outcome measures, and statistical analysis plan were predefined, the absence of prospective trial registration limits independent verification of the prespecified protocol and should be considered an important methodological limitation. Seventh, because structured multidisciplinary rehabilitation was not routinely incorporated into standard post-thrombectomy care at our institution during the study period, the comparator reflected the prevailing local standard of care. Consequently, the findings may not be directly generalizable to healthcare systems in which comprehensive multidisciplinary rehabilitation is routinely provided following MT. Future multicenter randomized controlled trials comparing structured multidisciplinary rehabilitation with contemporary standard rehabilitation across different healthcare settings are warranted to confirm the external validity of our findings. In addition, several clinically relevant thrombectomy-related variables, including pre-stroke mRS score, baseline Alberta Stroke Program Early CT Score – ASPECTS, stroke etiology, reperfusion success measured by the modified Thrombolysis in Cerebral Infarction – mTICI score, and time to recanalization, were not systematically collected. Consequently, residual confounding related to baseline stroke severity and procedural characteristics cannot be completely excluded. Future prospective studies should incorporate these variables to enable more comprehensive adjustment for potential confounders. Furthermore, the favorable prognosis endpoint was based on an exploratory composite functional outcome rather than the standard 90-day mRS, which remains the most widely accepted functional outcome measure in the AIS research. Therefore, these findings should be interpreted cautiously and confirmed in future prospective multicenter studies. Similarly, patient satisfaction was assessed using an institutionally developed questionnaire that demonstrated acceptable internal consistency but lacked external validation. Accordingly, these findings should also be considered exploratory until validated using standardized, validated patient-reported outcome measures. Future multicenter randomized studies with larger sample sizes, longer follow-up durations, prospective clinical trial registration, standardized rehabilitation protocols, formal *a priori* sample size calculations, and attention-matched CGs are warranted to further evaluate the long-term safety and effectiveness of CMR pathways initiated early after MT in patients with AIS and to confirm the present findings.

Conclusion

A continuous multidisciplinary rehabilitation program initiated within 24–72 hrs after mechanical thrombectomy and maintained throughout a 3-month rehabilitation pathway was associated with improved short-term

neurological, cognitive, motor, and activities of daily living outcomes in patients with acute ischemic stroke. Patients receiving this structured rehabilitation program also demonstrated lower complication rates and higher favorable prognosis rates during short-term follow-up. Because the intervention extended beyond the acute phase into the subacute recovery period, the observed benefits should be interpreted as reflecting the cumulative effects of sustained multidisciplinary rehabilitation rather than the isolated effect of early initiation alone. Furthermore, given the comprehensive multicomponent nature of the rehabilitation program and the methodological limitations

of this exploratory single-center study, these findings should be regarded as hypothesis-generating and association-based rather than definitive evidence of the efficacy of a specific rehabilitation protocol. The present findings support the potential clinical value of a continuous multidisciplinary rehabilitation pathway following mechanical thrombectomy. Further rigorously designed, prospectively registered, multicenter randomized controlled trials with standardized rehabilitation protocols are warranted to confirm these findings and to determine the independent contribution of individual rehabilitation components.

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Toxoplasmosis in the Autonomous Province of Vojvodina, Serbia: a 29-year surveillance data analysis (1997–2025)

Toksoplazmoza u Autonomnoj pokrajini Vojvodini, Srbija: analiza podataka nadzora tokom 29 godina (1997–2025)

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Abstract

Background/Aim. Toxoplasmosis is a widespread zoonotic infection with significant public health implications, particularly for pregnant women and immunocompromised individuals. The aim of the study was to analyze the epidemiological characteristics and temporal trends of toxoplasmosis in the Autonomous Province (AP) of Vojvodina, Serbia, over 29 years (1997–2025). **Methods.** A descriptive, population-based study was conducted using routine surveillance data on reported toxoplasmosis cases for the 1997–2025 period. Incidence rates were calculated *per* 100,000 inhabitants. Demographic, topographic, and seasonal differences among patients were analyzed. The association between the reported incidence of toxoplasmosis and climatic variables was evaluated using Spearman's correlation coefficient. **Results.** A total of 389 cases of toxoplasmosis were reported during the study period, with no fatal outcomes registered. The annual reported incidence ranged from 0.06 to 1.49 *per* 100,000 inhabitants. Segmented log-linear regression analysis identified two statistically significant trend changes in the reported toxoplasmosis incidence: a significant increase during the 2001–2007 period [annual percent change (APC) = 32.1%, 95% confidence interval (CI): 14.0–53.0; $p = 0.005$] and a decrease during the 2015–2019 period (APC = -35.1%,

95% CI: -49.8–-16.2; $p = 0.013$). The highest proportion of cases was observed in the 15–29 years (34.19%) age group, followed by 0–14 years (23.14%) and 30–39 years (21.59%). Females accounted for 60.15% of cases and were significantly older than males (30.56 ± 14.74 vs. 22.88 ± 15.02 years; $p < 0.001$). The majority of cases (66.07%) were recorded in urban areas. No statistically significant difference was found in seasonal distribution, although the highest proportion of cases (38.30%) occurred in the period January–March. A statistically significant negative correlation was found between reported incidence of toxoplasmosis and average temperature ($\rho = -0.5111$, $p = 0.0046$), whereas correlations with precipitation, humidity, and the number of rainy days were not statistically significant ($p > 0.05$). **Conclusion.** The reported incidence of toxoplasmosis in the AP of Vojvodina showed a declining trend over the observed period. Despite the decrease in reported incidence, enhancing surveillance, improving the recognition of congenital toxoplasmosis, and considering standardized prenatal screening could contribute to more effective prevention and early detection of congenital infection.

Keywords: epidemiology; incidence; serbia; toxoplasmosis; zoonoses.

Apstrakt

Uvod/Cilj. Toksoplazmoza je široko rasprostranjena zoonoza od značaja za javno zdravlje, naročito kod trudnica i imunokompromitovanih osoba. Cilj rada bio je da se analiziraju epidemiološke karakteristike i vremenski trendovi toksoplazmoze u Autonomnoj Pokrajini (AP) Vojvodini, Srbija, u periodu od 29 godina (1997–2025). **Metode.** Sprovedena je deskriptivna populaciona studija korišćenjem podataka o prijavljenim slučajevima toksoplazmoze iz rutinskog sistema nadzora za period 1997–2025. Stope incidencije izračunate su na 100 000

stanovnika. Analizirane su demografske, topografske i sezonske razlike među obolelima. Povezanost između utvrđene učestalosti toksoplazmoze i klimatskih varijabli procenjena je primenom Spearman-ovog koeficijenta korelacije. **Rezultati.** Registrovano je ukupno 389 slučajeva toksoplazmoze tokom studije, bez registrovanih smrtnih ishoda. Godišnja utvrđena incidencija kretala se od 0,06 do 1,49 na 100 000 stanovnika. Segmentiranjem log-linearnom regresionom analizom utvrđene su dve statistički značajne promene trenda registrovane incidencije toksoplazmoze: značajan porast u periodu 2001–2007. godine [godišnja procentualna promena (*annual percent*

change – APC) = 32,1%; 95% interval poverenja (confidence interval – CI): 14,0–53,0; $p = 0,005$] i pad u periodu 2015–2019. godine (APC = -35,1%; 95% CI: -49,8–-16,2; $p = 0,013$). Najveći udeo registrovanih slučajeva zabeležen je u grupi životnog doba 15–29 godina (34,19%), zatim 0–14 godina (23,14%) i 30–39 godina (21,59%). Žene su činile 60,15% svih slučajeva i bile su statistički značajno starije od muškaraca ($30,56 \pm 14,74$ naspram $22,88 \pm 15,02$ godina; $p < 0,001$). Većina slučajeva (66,07%) registrovana je u urbanim sredinama. Nije nađena statistički značajna razlika u sezonskoj distribuciji, mada je najveći udeo (38,30%) slučajeva zabeležen u periodu januar–mart. Nađena je statistički značajna negativna povezanost između prijavljene incidencije toksoplazmoze i prosečne temperature ($\rho = -0,5111$; $p = 0,0046$),

dok povezanost sa padavinama, vlažnošću i brojem kišnih dana nije bila statistički značajna ($p > 0,05$). **Zaključak.** Prijavljena incidencija toksoplazmoze u AP Vojvodini pokazala je opadajući trend tokom posmatranog perioda. Uprkos sniženju prijavljene učestalosti, unapređenje nadzora, poboljšanje prepoznavanja kongenitalne toksoplazmoze i razmatranje standardizovanog prenatalnog skrininga mogu doprineti efikasnijoj prevenciji i ranom otkrivanju kongenitalne infekcije.

Cljučne reči:
epidemiologija; incidencija; srbija; toksoplazmoza; zoonoze.

Introduction

Toxoplasma (T.) gondii is a ubiquitous intracellular protozoan parasite with a global distribution, capable of infecting virtually all warm-blooded animals, including humans^{1, 2}. Humans typically acquire infection by ingesting tissue cysts in undercooked meat, consuming food or water contaminated with oocysts shed by felids, or through congenital transmission from mother to fetus^{2–4}. Although infection is often asymptomatic in immunocompetent individuals, toxoplasmosis represents a significant public health concern due to its potential to cause severe disease in immunocompromised persons and adverse pregnancy outcomes, including miscarriage, stillbirth, and congenital anomalies^{1, 5}.

Globally, it is estimated that approximately one-third of the human population has been exposed to *T. gondii*, although seroprevalence varies widely depending on geographic, climatic, socioeconomic, and cultural factors^{3, 6, 7}. Higher prevalence rates have been reported in regions with warm and humid climates, as these conditions favor the survival of oocysts in the environment^{8, 9}. In contrast, lower prevalence is typically observed in high-income countries with improved hygiene standards and food safety practices¹⁰. In recent decades, several studies have suggested a declining trend in seroprevalence in many developed countries, likely reflecting changes in dietary habits, meat production, and public health measures^{7, 11}.

In Europe, toxoplasmosis remains an important zoonotic infection, with considerable heterogeneity in both seroprevalence and incidence across countries^{8, 12}. Cases of congenital toxoplasmosis (CT) across 30 European Union and European Economic Area countries increased slightly in 2021 after the decrease from 2017 to 2020. This was mainly due to the increased number of cases reported by France, which accounted for the majority (78%) of all the reported cases during that period. Surveillance systems across Europe are not uniform, and toxoplasmosis is not mandatorily reported in all countries, which complicates direct comparisons¹². Nevertheless, CT continues to be a major concern, and some countries have implemented systematic prenatal screening programs to reduce disease burden^{5, 13}.

Environmental and climatic factors, including temperature and humidity, as well as food consumption patterns (e.g., raw or undercooked meat), are recognized as key determinants of infection risk in European populations^{8, 9}.

In Serbia, data on toxoplasmosis are relatively limited and primarily derived from seroepidemiological studies^{14–16}. However, comprehensive long-term analyses of incidence trends, age-specific patterns, and environmental determinants of toxoplasmosis in Serbia remain scarce¹⁷.

Similar to other European countries, changes in lifestyle, dietary habits, and improved hygienic conditions have likely contributed to a gradual decline in infection rates over time. However, this trend may increase the proportion of susceptible women of reproductive age, thereby raising the risk of primary infection during pregnancy and, consequently, CT¹⁶. Surveillance of toxoplasmosis has been conducted since 1991. Notably, no cases of CT were officially reported in the Autonomous Province (AP) of Vojvodina, Serbia, during the period 1991–2024¹⁸.

Understanding temporal trends and epidemiological characteristics of toxoplasmosis at the regional level is essential for guiding public health interventions, particularly in settings where systematic surveillance data are available over extended periods.

By providing a comprehensive assessment of toxoplasmosis epidemiology in this region, the study aims to enhance understanding of disease dynamics in Southeast Europe and inform future prevention and control strategies.

Therefore, the aim of this study was to analyze the epidemiological characteristics of toxoplasmosis in the AP of Vojvodina, Serbia, over a 29-year period, including temporal trends, age- and gender-specific distribution, seasonal patterns, topographic variation, gender differences, and associations with selected meteorological variables.

Methods

This descriptive study was conducted in the AP of Vojvodina, Serbia, a northern region of Serbia with approximately 1.7 million inhabitants¹⁹.

Data were retrieved from the regional communicable disease surveillance system, coordinated by the Institute of Public Health of Vojvodina (IPHV), Novi Sad, Serbia.

The study was based on anonymized surveillance data routinely collected for public health purposes. Therefore, approval from an Ethics Committee was not required under Serbian regulations.

Reporting of toxoplasmosis is mandatory in Serbia and is based on standardized notification forms completed by healthcare providers. All toxoplasmosis cases reported between 1 January 1997 and 31 December 2025 were included in the comprehensive analysis. Given that the majority of toxoplasmosis infections are asymptomatic^{1–7}, it is likely that most reported cases were detected through routine screening programs, testing requested by primary care physicians or gynecologists before or during pregnancy, or patient-initiated testing.

Laboratory testing was performed following referral by a primary care physician, gynecologist, or infectious disease specialist, as well as upon the patient's own request. Confirmation was based on serological evidence of acute or recent *T. gondii* infection, characterized by the presence of toxoplasma-specific immunoglobulin (Ig) M antibodies, low-avidity IgG antibodies, or both. All serological testing was performed at the Center for Virology of the IPHV. During the study period, different serological methods were used for the laboratory diagnosis of toxoplasmosis. From 1997 to 2002, diagnosis was based on the complement fixation test (CFT). Between 2002 and 2007, serological testing was performed using the SERION enzyme-linked immunosorbent assays (ELISA) classic *T. gondii* IgG and IgM assays (SERION Diagnostics GmbH, Würzburg, Germany). From 2007 to 2025, serum samples were analyzed for *T. gondii*-specific IgM and IgG antibodies using ELISA with EUROIMMUN reagents (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Assessment of IgG antibody avidity was not routinely available throughout the study period. IgG avidity testing was introduced in 2016 and was subsequently performed only in selected pregnant women upon referral by their primary care physician or gynecologist^{18, 20}.

Demographic data (age, gender, place of residence), topographic information (district of residence), and temporal data (year and month of diagnosis) were extracted for all reported cases.

Annual population data for the AP of Vojvodina were obtained from official census data and inter-census estimates provided by the Statistical Office of the Republic of Serbia¹⁹. Crude incidence rates (IRs) were calculated as the number of reported cases *per* 100,000 inhabitants *per* year. Age-specific IRs were calculated for the following groups: 0–14, 15–29, 30–39, 40–49, 50–59, and ≥ 60 years.

Data on meteorological variables, including average annual temperature (°C), total annual precipitation (mm), average relative humidity (%), and number of rainy days (≥ 0.1 mm precipitation) for the period 1997–2025, were obtained from the Republic Hydrometeorological Service of Serbia²¹. These variables were analyzed to assess their potential association with reported annual toxoplasmosis incidence.

Statistical analysis

Descriptive statistics were used to summarize the demographic and epidemiological characteristics of toxoplasmosis cases. Continuous variables were expressed as means $\pm$ standard deviations, while categorical variables were presented as frequencies and percentages. Differences between groups were assessed using the chi-square (χ^2) test for categorical variables and the independent samples *t*-test for continuous variables.

Correlation between reported annual IRs and climatic variables was assessed using Spearman's rank correlation coefficient (ρ), given the non-normal distribution of the data.

All statistical analyses were conducted using SPSS software, version 21 (IBM Corp., Armonk, NY, USA).

Temporal trends in toxoplasmosis IRs were assessed using segmented log-linear regression analysis²². Annual IRs *per* 100,000 inhabitants were logarithmically transformed and modeled separately for predefined time intervals corresponding to visually identified changes in the direction of the trend. For each segment, the annual percent change (APC) was estimated from the slope of the regression line according to the formula: $APC = [\exp(\beta) - 1] \times 100$, where β represents the regression coefficient for calendar year. Statistical significance of temporal trends was evaluated using *t*-tests of the regression coefficients. Corresponding 95% confidence interval (CI) and *p*-values were calculated for each APC estimate.

A two-sided value of $p < 0.05$ was considered statistically significant.

Results

Between 1997 and 2025, a total of 389 toxoplasmosis cases were reported in the AP of Vojvodina, Serbia, with no fatal outcomes registered. In addition, no cases of CT were reported through the surveillance system during the study period. The overall reported annual IR fluctuated between 0.06 and 1.49 *per* 100,000 inhabitants, without a consistent long-term increasing trend.

In the late 1990s and early 2000s, reported IRs were relatively variable, with an initial peak observed in 1998 (1.49 *per* 100,000), followed by a decline in the period 1999–2002 (range: 0.25–0.45 *per* 100,000). A second period of elevated reported IR was recorded between 2004 and 2008, with values exceeding 1.0 *per* 100,000 in most years and reaching 1.38 *per* 100,000 in 2007.

From 2009 onwards, a gradual decline in reported incidence was observed, with rates generally remaining below 1.0 *per* 100,000. This downward trend became more pronounced after 2016, with consistently low reported incidence levels recorded between 2017 and 2025 (range: 0.06–0.57 *per* 100,000), including the lowest value in 2025 (0.06 *per* 100,000) (Figure 1).

The overall long-term trend for the entire study period (1997–2025) indicated a decreasing pattern (average APC = -7.9% *per* year).

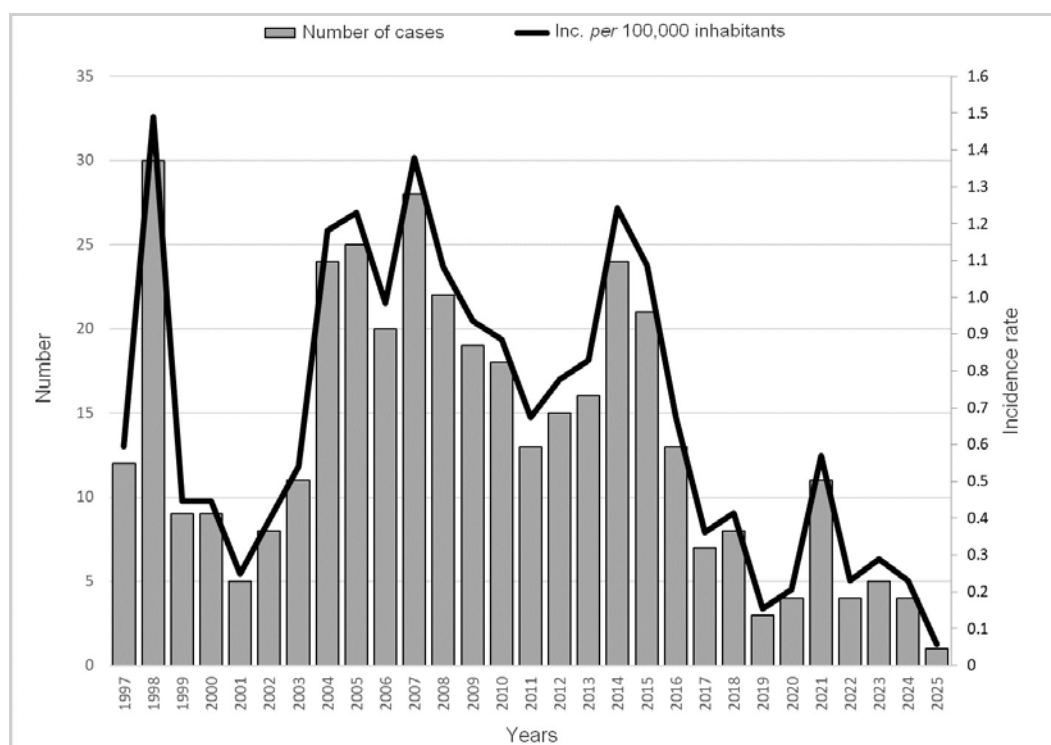


**Fig. 1 – Toxoplasmosis in the Autonomous Province of Vojvodina, Serbia (1997–2025).
Inc. – incidence.**

Table 1

**Segment-specific annual percent changes in toxoplasmosis incidence rates
in the Autonomous Province of Vojvodina, Serbia (1997–2025)**

Period	Trend phase	APC	95% CI	<i>p</i> -value	Interpretation
1997–2001	Early decline	-25.5	-56.2–26.5	0.175	Rapid initial decrease in incidence
2001–2007	Epidemic increase	32.1	14.0–53.0	0.005	Marked increase with epidemic-like fluctuations
2007–2015	Plateau/mild decline	-1.5	-8.5–6.1	0.643	Relative stabilization with slight downward trend
2015–2019	Sharp decline	-35.1	-49.8–-16.2	0.013	Pronounced and sustained decrease
2019–2021	Rebound increase	88.7	-86.9–2,612.2	0.203	Short-term resurgence, possibly outbreak-related
2021–2025	Final decline	-36.3	-60.1–-1.7	0.055	Strong decline to lowest observed incidence

APC – annual percent change; CI – confidence interval.

Values that differ significantly ($p < 0.05$) are marked in bold.

Segmented log-linear regression analysis revealed a significant increase in toxoplasmosis incidence during the 2001–2007 period (APC = 32.1%; 95% CI: 14.0–53.0; $p = 0.005$), followed by a period of relative stability during the 2007–2015 period (APC = -1.5%; 95% CI: -8.5–6.1; $p = 0.643$). A significant decline was observed during the 2015–2019 period (APC = -35.1%; 95% CI: -49.8–-16.2; $p = 0.013$). No statistically significant changes were identified during the remaining periods (Table 1).

Among children aged 0–14 years, reported IRs fluctuated markedly, with several pronounced peaks, including the

highest value observed in 2013 (2.88 *per* 100,000), followed by 2010 (2.48 *per* 100,000), and 2005 and 2008 (both 2.17 *per* 100,000). After 2016, reported incidence in this age group declined substantially, reaching zero in 2022 and 2023, and remaining low thereafter (Figure 2A).

In the 15–29 age group, reported IRs were generally more stable but showed intermittent increases, with notable peaks in 2011 (2.20 *per* 100,000), 2014 (2.48 *per* 100,000), and a maximum in 2015 (3.30 *per* 100,000). Following this peak, a declining trend was observed, with low reported incidence values recorded in the most recent years (Figure 2A).

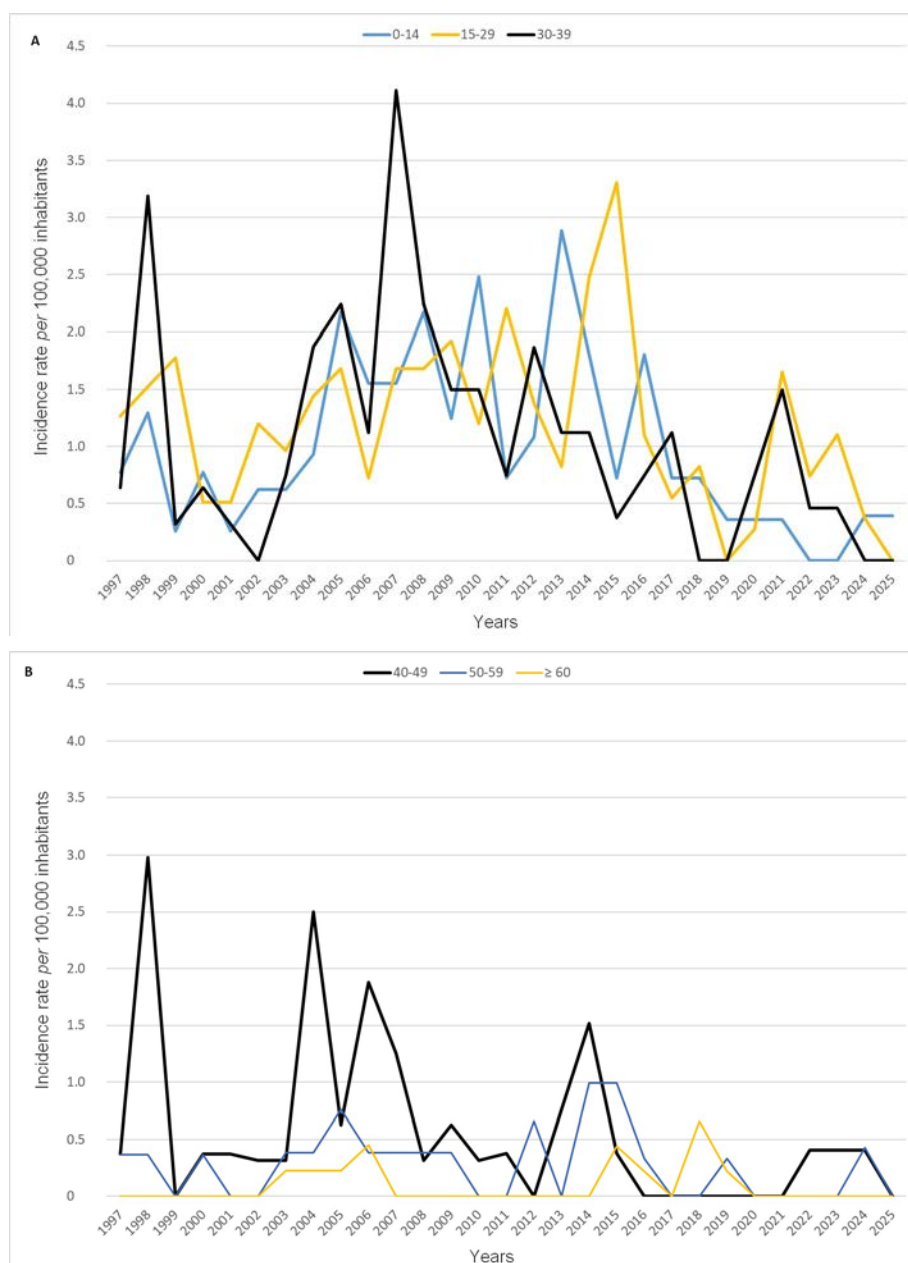


Fig. 2 – Age-specific incidence rates of toxoplasmosis among individuals aged: A) 0–14, 15–29, and 30–39 years and B) 40–49, 50–59, and ≥ 60 years, in the Autonomous Province of Vojvodina, Serbia (1997–2025).

The 30–39 age group exhibited the greatest variability, including the highest single-year reported incidence overall in 2007 (4.11 *per* 100,000). Additional elevated values were recorded in 1998 (3.19 *per* 100,000) and 2005 and 2008 (both 2.24 *per* 100,000). However, reported incidence declined markedly after 2012, with several years of zero reported incidence (2018, 2019, 2024, and 2025) (Figure 2A).

In the 40–49 age group, reported IRs showed intermittent peaks, most notably in 1998 (2.98 *per* 100,000) and 2004 (2.50 *per* 100,000), followed by moderately elevated values in 2006 (1.88 *per* 100,000) and 2014 (1.52 *per* 100,000). After 2016, reported incidence declined markedly, with predominantly zero values and only occasional low rates recorded between 2022 and 2024 (0.40 *per* 100,000) (Figure 2B).

In the 50–59 age group, reported incidence remained consistently low. The highest values were observed in 2014 and 2015 (0.99 *per* 100,000), while most other years were characterized by either very low or zero reported incidence (Figure 2B).

Among individuals aged ≥ 60 years, reported incidence was minimal throughout the entire period, with only sporadic cases resulting in low reported incidence values. The highest recorded rate was 0.66 *per* 100,000 in 2018, while most years showed zero reported incidence, particularly after 2020 (Figure 2B).

The highest proportion of toxoplasmosis cases was recorded in January (19%), followed by February (12%) and November (9%). In contrast, the lowest proportion was observed in December (5%). During the spring and summer

months, the distribution was relatively stable, with monthly proportions ranging from 6% to 7% between March and October, suggesting a more even occurrence of cases during this period (Figure 3).

Age-specific distribution of toxoplasmosis cases showed marked monthly variation (Figure 4), with the 15–29 age group predominating in most months. This group accounted for the highest proportions in February (21/46; 45.7%), May (9/29; 31.0%), July (9/25; 36.0%), August

(10/25; 40.0%), September (11/28; 39.3%), November (14/35; 40.0%), and December (9/20; 45.0%), indicating consistent dominance throughout the year, particularly in late summer and winter.

The 0–14 age group predominated only in October (11/28; 39.3%), while the 30–39 years was leading in June (9/23; 39.1%). In January, the 15–29 and 30–39 years shared the highest proportions (21/74; 28.4% each), with a substantial contribution from children (19/74; 25.7%) (Figure 4).

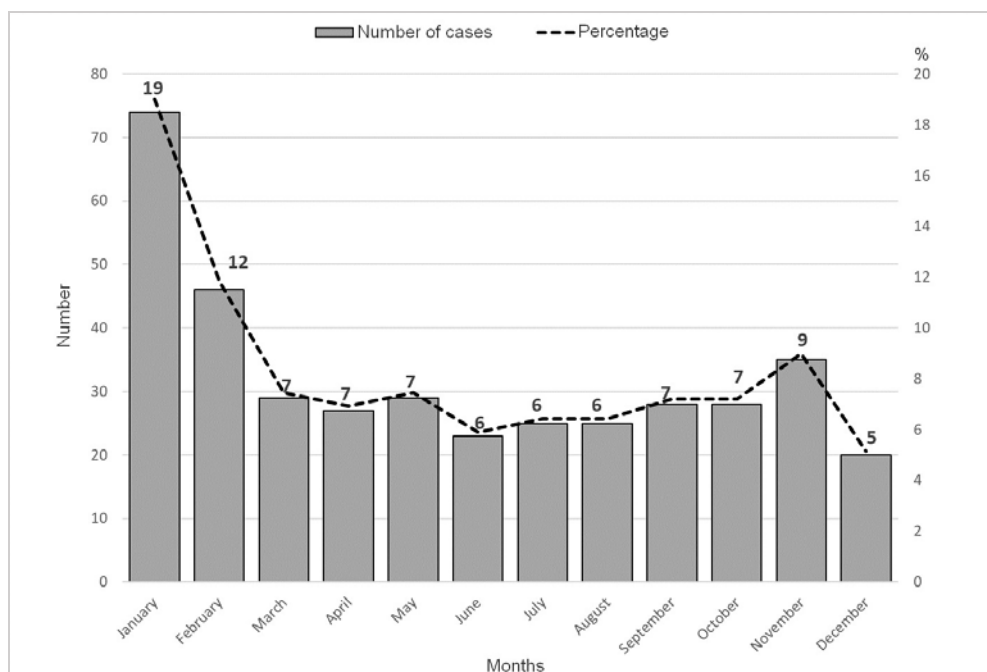


Fig. 3 – Seasonal distribution of toxoplasmosis in the Autonomous Province of Vojvodina, Serbia (1997–2025).

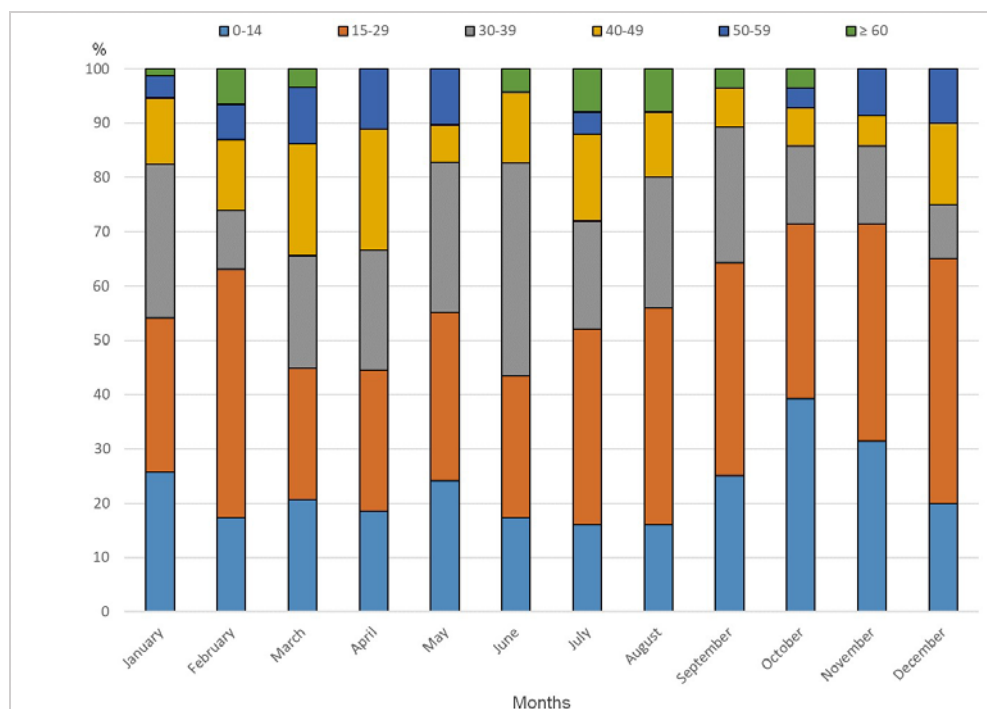


Fig. 4 – Monthly predominance of age groups in toxoplasmosis cases in the Autonomous Province of Vojvodina, Serbia (1997–2025).

Older age groups (40–49, 50–59, and ≥ 60 years) did not predominate in any month and consistently accounted for lower proportions of cases (Figure 4).

Gender-specific characteristics of toxoplasmosis cases during the period of 1997–2025 are shown in Table 2.

A total of 389 cases of toxoplasmosis were analyzed, including 155 (39.85%) males and 234 (60.15%) females. The mean age of all cases was 27.50 ± 15.32 years, with males being significantly younger than females (22.88 ± 15.02 vs. 30.56 ± 14.74 years, respectively; $p < 0.001$).

Regarding age distribution, the highest proportion of cases was observed in the 15–29 age group (34.19%), followed by the 0–14 (23.14%) and 30–39 (21.59%) age groups. Significant differences in age distribution between males and females were observed ($p = 0.0001$), with males more frequently represented in younger age groups.

A statistically significant difference in place of residence was observed between sexes ($p = 0.0048$), with a higher proportion of urban residence among females (71.79%) compared to males (57.42%).

Regarding topographic distribution within the AP of Vojvodina, the highest proportion of cases was recorded in South Bačka District (36.76%), followed by West Bačka (25.19%) and North Banat (11.31%). Distribution across districts differed significantly between males and females ($p = 0.0027$).

Seasonal distribution showed no statistically significant variation ($p = 0.7817$), with the highest proportion of cases observed in the January–March period (38.30%), followed by relatively similar proportions across the remaining quarters of the year.

A statistically significant moderate inverse correlation was observed between annual toxoplasmosis incidence and average temperature ($\rho = -0.5111$, $p = 0.004608$), indicating that higher temperatures were associated with lower reported IRs over the study period (Figure 5A).

No statistically significant association was observed between reported annual toxoplasmosis incidence and average precipitation ($\rho = 0.2216$, $p = 0.247987$), indicating a weak and non-significant positive correlation over the study period (Figure 5B).

Although this association did not reach statistical significance ($p = 0.051243$), a moderate positive correlation was observed between reported annual toxoplasmosis incidence and average relative humidity ($\rho = 0.3654$), suggesting a borderline relationship between humidity levels and reported incidence over the study period (Figure 5C).

A weak positive correlation was found between reported annual toxoplasmosis incidence and the number of rainy days ($\rho = 0.2985$), which did not reach statistical significance ($p = 0.115758$), indicating no clear association between precipitation frequency and disease-reported incidence over the study period (Figure 5D).

Table 2

**Gender-specific characteristics of toxoplasmosis cases
in the Autonomous Province of Vojvodina, Serbia (1997–2025)**

Variable	Total (n = 389)	Male (n = 155)	Female (n = 234)	p-value
Age, years	27.50 ± 15.32	22.88 ± 15.02	30.56 ± 14.74	< 0.001
Age, range				
0–14	90 (23.14)	51 (32.90)	39 (16.67)	0.0001
15–29	133 (34.19)	60 (38.71)	73 (31.20)	
30–39	84 (21.59)	20 (12.90)	64 (27.35)	
40–49	48 (12.34)	15 (9.68)	33 (14.10)	
50–59	22 (5.66)	6 (3.87)	16 (6.84)	
≥ 60	12 (3.08)	3 (1.94)	9 (3.85)	
Place of residence				
urban	257 (66.07)	89 (57.42)	168 (71.79)	0.0048
rural	132 (33.93)	66 (42.58)	66 (28.21)	
District of AP of Vojvodina				
North Bačka	33 (8.48)	18 (11.61)	15 (6.41)	0.0027
West Bačka	98 (25.19)	42 (27.10)	56 (23.93)	
South Bačka	143 (36.76)	42 (27.10)	101 (43.16)	
North Banat	44 (11.31)	23 (14.84)	21 (8.97)	
Central Banat	16 (4.11)	3 (1.94)	13 (5.56)	
South Banat	36 (9.25)	15 (9.68)	21 (8.97)	
Srem	19 (4.88)	12 (7.74)	7 (2.99)	
Quarter of the year				
January–March	149 (38.30)	60 (38.71)	89 (38.03)	0.7817
April–June	79 (20.31)	28 (18.06)	51 (21.79)	
July–September	78 (20.05)	31 (20.00)	47 (20.09)	
October–December	83 (21.34)	36 (23.23)	47 (20.09)	

n – number; AP – Autonomous Province.

Values are given as mean $\pm$ standard deviation and numbers (percentages).

Values that differ significantly ($p < 0.05$) are marked in bold.

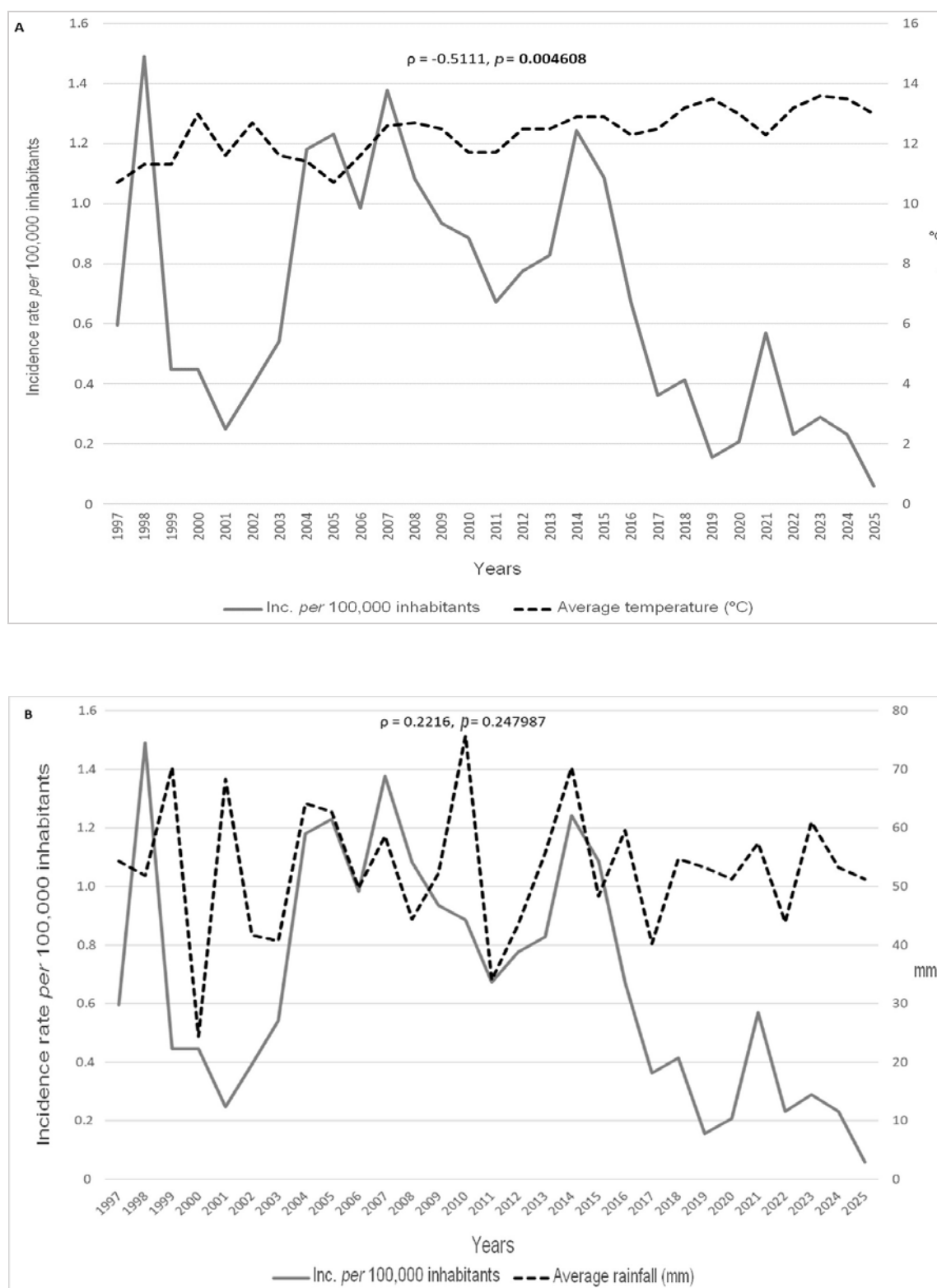


Fig. 5 – Temporal trends in toxoplasmosis incidence and association with: A) average annual temperature; B) average annual precipitation; C) average annual relative humidity; and D) average annual rainy days, in the Autonomous Province of Vojvodina, Serbia (1997–2025). Inc. – incidence.

Note: Fig. 5 continued on next page.

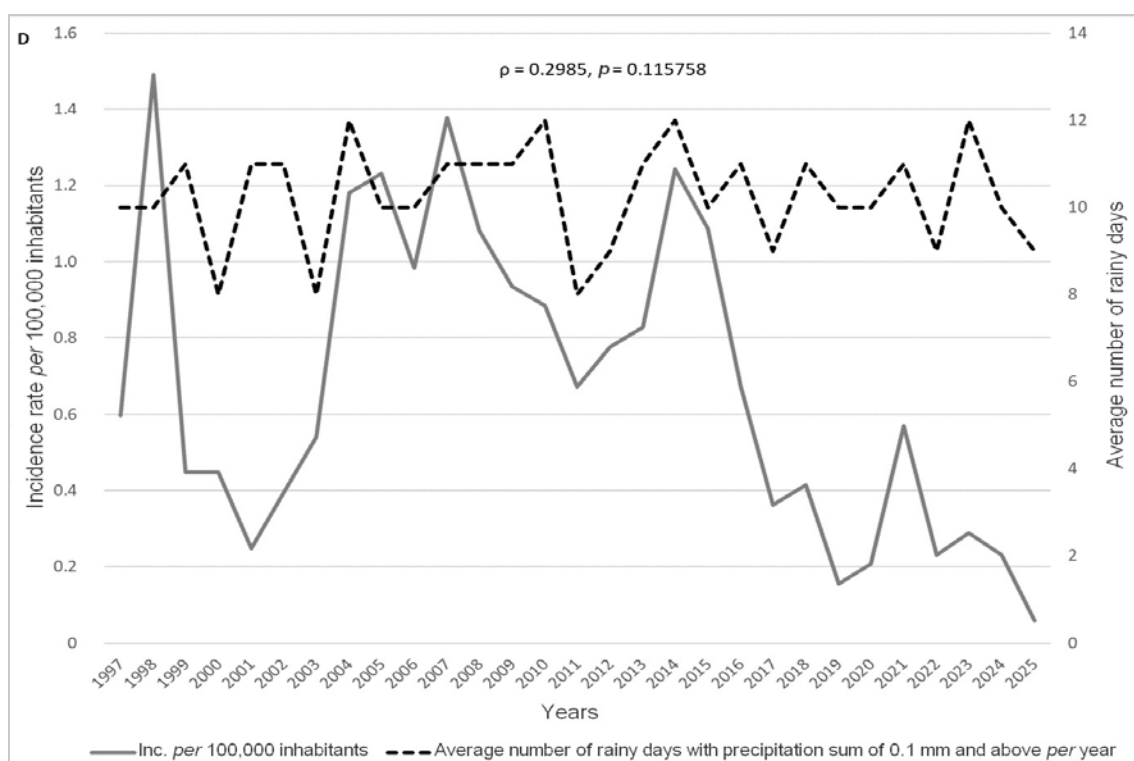
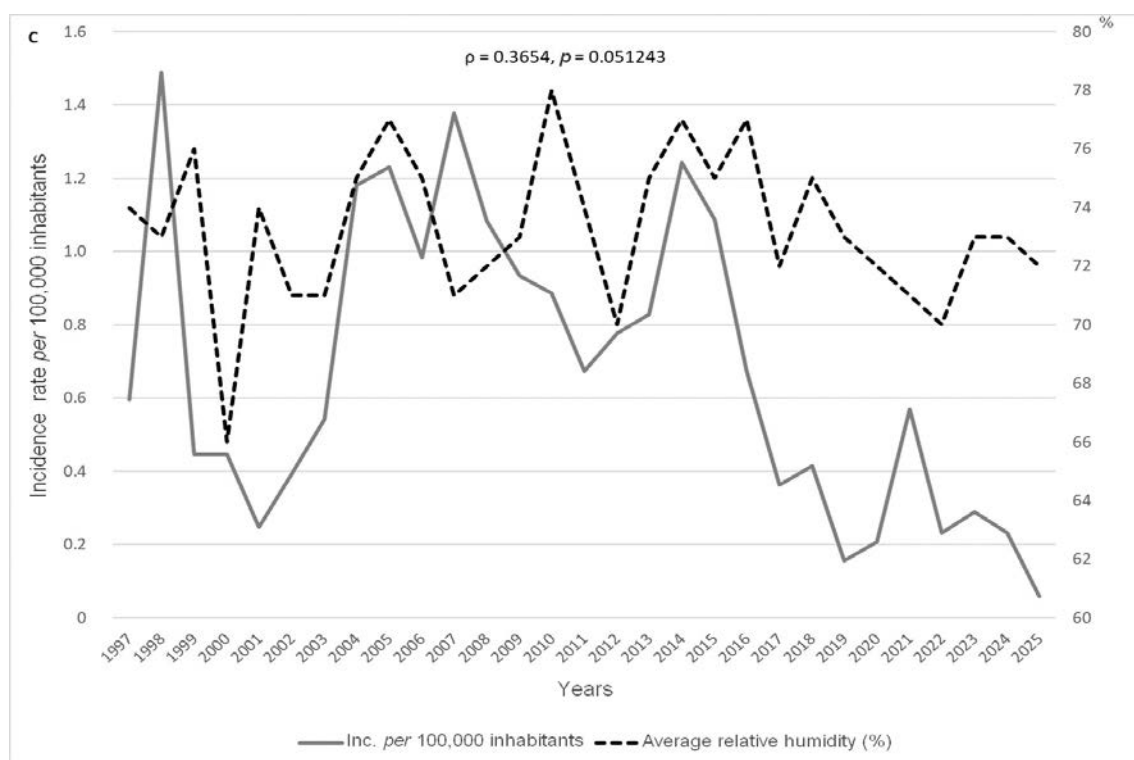


Fig. 5 (Continued) – Temporal trends in toxoplasmosis incidence and association with: A) average annual temperature; B) average annual precipitation; C) average annual relative humidity; and D) average annual rainy days, in the Autonomous Province of Vojvodina, Serbia (1997–2025). Inc. – incidence.

Discussion

Our findings indicate that, despite a sustained downward trend, toxoplasmosis epidemiology remains dynamic and influenced by changes in exposure pathways, surveillance practices, and environmental conditions, consistent with a previous study¹⁰.

Segmented log-linear regression analysis revealed substantial fluctuations in toxoplasmosis incidence over the study period. Incidence increased significantly during the 2001–2007 period, remained relatively stable between 2007 and 2015, and declined markedly during the 2015–2019 period. Although a temporary increase was observed between 2019 and 2021, this trend was not statistically significant and should be interpreted cautiously because of the small number of reported cases. A further decline after 2021 resulted in the lowest IRs recorded during the study period. The observed temporal variations likely reflect a combination of factors, including changes in diagnostic practices, testing intensity, healthcare-seeking behavior, surveillance sensitivity, and reporting completeness, rather than true changes in transmission alone. Given that toxoplasmosis is predominantly asymptomatic, reported incidence is strongly influenced by screening activities, particularly among women before and during pregnancy. Therefore, fluctuations in reported incidence may partly represent changes in case detection rather than actual changes in disease occurrence.

Similar to our findings, the observed decline in incidence is consistent with broader European trends and is likely attributable to improvements in food safety, meat processing, and hygiene standards^{2, 3, 12}. However, reported incidence represents only a fraction of total infections, as most cases remain asymptomatic and are not captured through routine surveillance systems. Consequently, reductions in notified cases do not necessarily reflect proportional declines in population-level exposure, particularly given the lifelong persistence of latent infection. This discrepancy between reported incidence and true prevalence has been well documented across European settings^{2, 5, 12, 23, 24}.

The findings of this study should also be considered in the context of the broader epidemiological role of latent toxoplasmosis, as highlighted by Flegr et al.²⁵, who demonstrated substantial global variation in seroprevalence across 88 countries and its potential association with population-level disease burden. The marked decline in reported incidence observed after 2015, together with consistently low rates in recent years, may reflect improvements in hygiene, food safety, and public awareness—the key determinants of transmission also emphasized in global analyses. However, as latent infection persists lifelong, reductions in reported incidence do not necessarily correspond to immediate declines in population-level prevalence^{1, 3–5}.

Recent European evidence supports the declining trend observed in our study. A systematic review and meta-analysis covering the period 2000–2020 reported an overall

seroprevalence of 32.1% in Europe, with marked heterogeneity between countries and a general decrease over time²⁶. More recent modeling data (2000–2021) confirm substantial regional variation with higher seroprevalence in Western (48%) and Eastern (50%) Europe and considerably lower levels in Northern Europe (18%), reflecting differences in environmental conditions, dietary practices, and exposure risk²⁷.

In addition, the decreasing reported incidence observed in this study is consistent with previously reported declines in seroprevalence in our country. Earlier data from the AP of Vojvodina (2006–2008), including the study by Brkić et al.²⁸, indicated substantially higher seroprevalence levels (38.1%) in the general population. In contrast, more recent studies conducted between 2017 and 2018¹⁴ and between 2008 and 2021²⁰ have reported lower levels (20.5–23.5%), supporting a long-term reduction in transmission. This concordance between decreasing seroprevalence and declining reported incidence suggests a sustained epidemiological shift towards lower exposure to toxoplasmosis in the population.

At the same time, the predominance of cases among younger age groups in our study (34.19% in the 15–29 age group and 21.59% in the 30–39 age group) is consistent with evidence of lower seroprevalence in younger cohorts and increasing seropositivity with age, indicating delayed primary infection. From a public health perspective, this shift may result in a growing proportion of susceptible individuals, particularly among women of reproductive age, potentially increasing the risk of primary infection during pregnancy and consequent congenital infection, as highlighted in global burden estimates^{5–7}. No cases of CT were reported through the surveillance system during the study period; however, this finding may reflect underdiagnosis or insufficient systematic screening rather than the true absence of congenital infection.

The predominance of females (60.15%) among reported cases is likely influenced by differences in healthcare-seeking behavior and targeted prenatal screening, as women of reproductive age are routinely tested due to the risk of CT in Europe^{29–31}. In our study, females were also significantly older than males, which likely reflects detection bias rather than true gender-specific differences in infection risk. Women are more frequently diagnosed through screening in adulthood, particularly during pregnancy, whereas men are more likely to be tested only when symptomatic, contributing to the lower average age observed among males. This pattern is consistent with findings from other European settings^{32, 33}. In countries with systematic screening programs, such as Austria and France, women account for the majority of diagnosed cases, reflecting the impact of antenatal testing on case detection^{31, 34}.

An interesting finding of this study was the significant difference in the distribution of cases by gender and place of residence. Women living in urban areas accounted for nearly three-quarters of all female cases, whereas the distribution among men was more balanced between urban and rural settings. This pattern is unlikely to reflect a true difference in

susceptibility to *T. gondii* infection. Rather, it may be explained by differences in healthcare utilization and diagnostic practices, as women of reproductive age in urban areas are more likely to undergo routine serological testing during prenatal care or infertility evaluations and generally have better access to specialized healthcare services. Nevertheless, differences in exposure to environmental or dietary risk factors between urban and rural populations cannot be excluded and warrant further investigation.

Seasonal variation in reported toxoplasmosis incidence is not consistently demonstrated across European studies and may differ depending on transmission route, surveillance sensitivity, and population behavior. Although environmental survival of *T. gondii* oocysts is generally favored by moderate temperatures and higher humidity, human infection is predominantly driven by foodborne exposure, particularly consumption of contaminated meat, which may not follow strict seasonal patterns^{2, 8, 12}. Although the observed difference was not statistically significant, the higher proportion of cases during winter months may reflect increased consumption of undercooked meat during holiday periods, as well as potential clustering of healthcare utilization and diagnostic testing following symptom onset in colder months. Similar weak or inconsistent seasonality has been reported in European surveillance systems, suggesting that climatic effects are limited in regions with stable food supply chains and widespread refrigeration practices¹². Consistent with this observation, we found an inverse correlation between reported incidence and temperature, indicating higher incidence during colder periods. Although the possibility of an “ecological fallacy” cannot be excluded, this finding may suggest that low winter temperatures in the AP of Vojvodina are associated with certain cultural practices, such as traditional pig slaughtering and the consumption of insufficiently cooked meat products during this period. While environmental survival of *T. gondii* oocysts is generally favored by moderate temperatures and humidity, the relationship between climate and human infection remains complex and context-dependent. This likely reflects the predominance of foodborne transmission pathways over direct environmental exposure in contemporary European settings^{20, 25–27}.

We observed topographical differences across the AP of Vojvodina, with the highest proportion (36.76%) of cases reported in the South Bačka District. This finding is likely influenced, at least in part, by referral patterns, as the South Bačka District is home to the University Clinical Center of Vojvodina, the only tertiary-level healthcare facility in the province, which serves as a regional referral center for more severe or complex cases³⁵. In addition, South Bačka is the most populous district in the AP of Vojvodina, accounting for 607,454 inhabitants in 2022 (18.7% of the provincial population)¹⁹, which may further contribute to the higher number of reported cases.

The main strength of this study lies in its long observation period (29 years) and the use of population-based surveillance data, enabling robust analysis of temporal trends and epidemiological patterns. The inclusion of age-

specific, seasonal, topographic, and climatic analyses provides a comprehensive overview of toxoplasmosis epidemiology in the region.

Limitations of the study

Several limitations should be acknowledged. First, the study relies on passive surveillance data, which may be subject to underreporting, particularly regarding asymptomatic or mild cases that seroepidemiological research can better detect. Second, changes in diagnostic methods over the study period, including the transition from the CFT to ELISA-based assays and the later introduction of IgG avidity testing, may have influenced case detection. In addition, because information on testing indications was not available in the surveillance database, the potential impact of changes in testing practices over time on the observed temporal trends cannot be excluded. Third, the lack of individual-level data on risk factors (e.g., dietary habits, contact with animals, socioeconomic status) limits the ability to identify specific determinants of infection. Fourth, the official infectious disease surveillance records lacked data on comorbidities (e.g., immunodeficiency) and pregnancy status, precluding the evaluation of these variables in the analysis. Fifth, our findings suggest the presence of potential detection bias and a demographic artifact. Specifically, women accounted for the majority of reported cases (60.15%), were significantly older than men, and most infections occurred among individuals aged 15–39 years. This distribution is unlikely to reflect a true biological susceptibility or greater exposure to infection among women; rather, it is probably a consequence of differences in testing practices. Women of reproductive age are routinely screened during prenatal care or infertility evaluations, whereas men are generally tested only when clinical symptoms, such as lymphadenopathy, develop. Therefore, the observed demographic patterns should be interpreted in light of these differences in diagnostic ascertainment. Finally, ecological analysis of climatic variables does not allow for causal inference and may be influenced by unmeasured confounders.

Conclusion

The epidemiology of toxoplasmosis in the Autonomous Province of Vojvodina reflects a broader European transition characterized by declining reported incidence alongside persistent underlying transmission and population susceptibility. The absence of reported congenital toxoplasmosis cases during the study period may reflect underrecognition rather than the true absence of disease. Strengthening surveillance, improving the detection and reporting of congenital toxoplasmosis, and considering the implementation of standardized prenatal screening may contribute to earlier diagnosis and more effective prevention of congenital infection. Further research integrating epidemiological, environmental, and behavioral data is needed to better understand disease transmission and inform evidence-based public health strategies.

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Value of three-dimensional transvaginal ultrasonography and contrast-enhanced ultrasonography in assessing prognosis of lauromacrogol sclerotherapy for cesarean scar pregnancy

Značaj transvaginalne trodimenzionalne ultrasonografije i ultrasonografije sa kontrastom u proceni prognoze skleroterapije lauromakrogolom kod trudnoće u ožiljku carskog reza

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Abstract

Background/Aim. When cesarean scar pregnancy (CSP) is diagnosed, it is proposed in clinical practice to remove the pregnancy tissue as soon as possible. Contrast-enhanced ultrasonography (CEUS) has higher diagnostic value for CSP compared to three-dimensional transvaginal ultrasonography (3D-TVUS). The aim of the study was to assess the efficiency of combining 3D-TVUS with CEUS in the prognosis of lauromacrogol sclerotherapy (LSC) for CSP. **Methods.** The study included patients with CSP admitted to our hospital and treated with LSC from January 2023 to January 2024 (CSP group, $n = 150$), and women with normal pregnancy after cesarean section (normal pregnancy group, $n = 150$). The CSP group was additionally divided into good and poor prognosis groups. Both groups received 3D-TVUS and CEUS, with quantitative parameters measured [3D-TVUS parameters: vascular index (VI), flow index (FI), and vascular FI (VFI)]. CEUS parameters included the area under the

time-intensity curve (AUC_{TIC}), arrival time (AT), time to peak (TTP), and peak intensity (PI). **Results.** After treatment, both CSP and normal pregnancy groups exhibited significant decreases in VI, FI, VFI, PI, and AUC_{TIC} , alongside a significant increase in TTP compared with baseline ($p < 0.05$). Conversely, compared with the good prognosis CSR group, the poor prognosis CSR group demonstrated higher VI, FI, VFI, PI, and AUC_{TIC} , as well as a shorter TTP, both before and after treatment ($p < 0.05$). The combined parameters of 3D-TVUS and CEUS showed the best predictive performance of LSC prognosis in CSP, significantly outperforming any single parameter. **Conclusion.** A considerable proportion of patients with CSP had poor short-term prognosis after LSC. Pretreatment 3D-TVUS and CEUS parameters may help in the early evaluation of short-term prognosis.

Keywords:

cesarean section; polidocanol; pregnancy, ectopic; prognosis; sclerotherapy; ultrasonography, prenatal.

Apstrakt

Uvod/Cilj. Kada se dijagnostikuje trudnoća u ožiljku od prethodnog carskog reza (*cesarean scar pregnancy* – CSP), u kliničkoj praksi se predlaže što ranije uklanjanje tkiva embriona. Ultrasonografija sa kontrastom (*contrast-enhanced ultrasonography* – CEUS) ima veću dijagnostičku vrednost za CSP u poređenju sa transvaginalnom trodimenzionalnom ultrasonografijom (*three-dimensional transvaginal ultrasonography* – 3D-TVUS). Cilj rada bio je da se proceni efikasnost kombinacije 3D-TVUS i CEUS u prognozi skleroterapije lauromakrogolom (*lauromacrogol sclerotherapy* – LSC) kod CSP. **Metode.** Istraživanje je uključilo pacijentkinje koje su imale CSP, primljene u našu bolnicu i lečene pomoću LSC od januara 2023. do januara 2024.

godine (grupa CSP, $n = 150$), kao i žene sa normalnom trudnoćom posle carskog reza ($n = 150$). Grupa CSR je dodatno podeljena na grupu sa dobrom i grupu sa lošom prognozom. Obe grupe su pregledane pomoću 3D-TVUS i CEUS, uz određivanje kvantitativnih parametara [parametri 3D-TVUS: vaskularni indeks (*vascular index* – VI), indeks protoka (*flow index* – FI) i indeks vaskularnog protoka (*vascular FI* – VFI)]. Parametri CEUS uključivali su *area under the time-intensity curve* (AUC_{TIC}), *arrival time* (AT), *time to peak* (TTP) i *peak intensity* (PI). **Rezultati.** Nakon tretmana, u obe grupe (CSP i sa normalnom trudnoćom) zabeleženo je značajno smanjenje vrednosti VI, FI, VFI, PI i AUC_{TIC} , uz značajno povećanje TTP u poređenju sa početnim vrednostima ($p < 0.05$). Nasuprot tome, u poređenju sa CSR grupom sa dobrom

prognozom, CSR grupa sa lošom prognozom pokazala je više vrednosti VI, FI, VFI, PI i AUC_{TIC}, kao i kraće TTP, kako pre tako i posle tretmana ($p < 0,05$). Kombinovani parametri 3D-TVUS i CEUS pokazali su najbolje prediktivne performanse u proceni prognoze LSC za CSP, značajno nadmašujući bilo koji pojedinačni parametar. **Zaključak.** Značajan broj pacijentkinja koje su

imale CSP imao je lošu kratkoročnu prognozu posle LSC. Parametri 3D-TVUS-a i CEUS pre tretmana mogu pomoći u ranoj proceni kratkoročne prognoze.

Ključne reči:

carski rez; polidokanol; trudnoća, ektopična; prognoza; skleroterapija; ultrasonografija, prenatalna.

Introduction

Cesarean scar pregnancy (CSP), a familiar complication after cesarean section (CS), displays no specific clinical manifestations in the early stage. Symptoms such as painless vaginal bleeding and lower abdominal pain may occur in only a few patients, which, if not diagnosed and intervened in time, increase the risk of events such as uterine rupture, placenta implantation, and uncontrollable intraperitoneal hemorrhage, serving as a serious threat to the safety of patients. In clinical practice, therefore, it is proposed to remove pregnancy tissues as soon as possible once CSP has been diagnosed^{1,2}. At present, curettage is a crucial method for treating CSP; however, vascular rupture easily occurs since tissues at the incision sites of CS in the lower uterine segment are weak, with poor flexibility and *vasa vasorum* going deep into the muscular layer, thus elevating the risk of massive hemorrhage³. Lauromacrogol, a foam hardener, exhibits high affinity for vascular endothelial cells and exerts a sclerosing effect by triggering vascular inflammation, thereby promoting cell fibrosis and necrosis after injection⁴. Considering its pharmacological characteristics, lauromacrogol is used clinically for the treatment of CSP. It is believed that satisfactory efficacy can be achieved by combining lauromacrogol sclerotherapy (LSC) to block local blood vessels at the scar site with curettage within 24 hrs⁵. At our center, LSC combined with curettage is a preferred treatment option for eligible type I/II CSP because it can directly reduce local trophoblastic blood supply before evacuation, thereby potentially decreasing intraoperative bleeding risk. Compared with more invasive surgical procedures or uterine artery embolization, this approach is minimally invasive, relatively easy to perform under ultrasound guidance, and may help preserve uterine integrity and reproductive potential in selected patients. Based on our institutional experience and available evidence, patients with type I/II CSP and suitable clinical conditions were managed using this treatment strategy. However, outcomes vary among patients because the implantation depth of the gestational sac within the scar differs⁶. Therefore, early assessment of LSC prognosis in patients with CSP has become a critical focus of current clinical research.

Currently, the evaluation of CSP relies predominantly on conventional two-dimensional (2D) transvaginal ultrasonography (TVUS) – 2D-TVUS. However, this method is limited in its ability to fully evaluate overall blood flow at the pregnancy site, so it fails to make an accurate assessment. In contrast, three-dimensional (3D) TVUS – 3D-TVUS can reflect the overall blood supply around scars, thus making a

more objective evaluation⁷. Contrast-enhanced ultrasonography (CEUS), a novel ultrasonic examination approach in which microbubble contrast agents enhance the backscatter of ultrasonic sound waves, has a stronger ability to assess microvascular perfusion at the site of CSP, offering higher diagnostic value for CSP⁸. The above studies have shown that 3D-TVUS and CEUS have high sensitivity and specificity for diagnosing CSP, but whether they can predict LSC prognosis in patients with CSP remains unclear.

Therefore, 3D-TVUS and CEUS were utilized in this study for the first time to predict LSC prognosis in patients with CSP, aiming to provide a reference for future research.

Methods

Subjects

A total of 150 patients with CSP, treated with LSC in our hospital from January 2023 to January 2024, were selected as the CSP group. Our hospital is a regional tertiary maternal and child health center receiving referred CSP cases from surrounding hospitals. They had undergone 1–3 previous CSs. As to disease types, there were 65 type I and 85 type II cases of CSP. The study was approved by the Ethics Committee of the Affiliated Maternal and Child Health Hospital of Nantong University, Nantong, Jiangsu, China (from January 18, 2023).

Inclusion and exclusion criteria

Patients were included if they met the following criteria: a documented history of CS with type I/II CSP confirmed by color Doppler ultrasonography or pelvic magnetic resonance imaging (MRI); an amenorrhea duration of ≥ 6 weeks with positive urine beta-human chorionic gonadotropin (β -HCG); normal cervical morphology and length; and a request for pregnancy termination *via* LSC, with written informed consent provided prior to study enrollment.

Exclusion criteria were as follows: type III CSP; severe hepatic, renal, or cardiopulmonary dysfunction or severe underlying systemic comorbidities; active local or systemic infection; history of prior CSP; other ectopic pregnancy locations (e.g., tubal or ovarian); those allergic to contrast agents; those intolerant to vaginal ultrasonography; or those with mental or communication disorders.

Recent imaging and vascular studies indicate that implantation into a previous CS scar is associated with

altered local vascular supply and scar niche formation⁹. In addition, the presence of a scar niche in the lower uterine segment may affect uterine perfusion and endometrial-myometrial integration¹⁰. Therefore, by selecting women with prior CS but with a current normal intrauterine pregnancy as the control group, we aimed to balance the surgical scar background while isolating the specific effect of scar-site implantation.

One hundred and fifty women with normal pregnancy after CS were included as a normal pregnancy group (NPG). They had undergone 1–3 previous CS and had a normal pregnancy confirmed by ultrasonography. All patients in the CSP group successfully completed LSC and the scheduled follow-up. No patient was lost to follow-up, and none were excluded after enrollment.

Three-dimensional transvaginal ultrasonography and contrast-enhanced ultrasonography

All subjects were examined using a color Doppler ultrasound system (Resona 7S, Mindray) equipped with a V11-3Hu intracavitary probe at a probe frequency of 5–9 MHz. Specifically, the gestational sac size, yolk sac, embryo, and fetal heart were observed first. Then, the 3D energy Doppler scanning was started in the uterus to obtain 3D blood flow imaging of the *vasa vasorum* at the implantation site of the gestational sac. The vascular index (VI), flow index (FI), and vascular FI (VFI) were calculated using VOCAL software, and the mean of three measurements was taken. Thereafter, the contrast mode was started: SonoVue contrast agent (Bracco, Italy) was mixed with 5 mL of 0.9% sodium chloride injection to prepare an ultrasonic microbubble contrast agent, and 1.2 mL of that agent was then injected through the elbow vein. Afterwards, the muscular layer at the scar underneath the serosa of the anterior isthmus was selected as the region of interest, in which the whole process of contrast agent perfusion was analyzed; a time-intensity curve was plotted, and CEUS parameters were obtained, including the area under the time-intensity curve (AUC_{TIC}), arrival time (AT), time to peak (TTP), and peak intensity (PI). The results were judged by two radiologists with 5 years of experience.

Lauromacrogol sclerotherapy

LSC was performed as follows: before LSC, patients underwent routine blood and urine examinations, coagulation function examination, hepatic and renal function examination, and gynecological examination, and were instructed to empty their bladder. Then, lauromacrogol was slowly injected into the muscular layer and peripheral contraction points around the gestational sac by transvaginal puncture in patients in a lithotomy position. The volume of raw lauromacrogol solution was 10–20 mL. The injection was stopped when the gestational sac showed sheet or annular enhancement with scarce peripheral blood flow. Curettage was performed within 24 hrs after LSC by the same team of physicians. After that, conventional postoperative anti-infection therapy, oxytocin-

induced uterine contraction, symptomatic fluid replacement, and other supportive treatments were carried out. In addition, patients were followed up by outpatient visits after surgery, during which postoperative vaginal bleeding, menstruation recovery, and β -HCG decline were monitored, and ultrasonography was conducted.

Evaluation of prognosis of lauromacrogol sclerotherapy

In this study, prognosis referred to short-term treatment response within one month after LSC. All patients with CSP followed the same follow-up protocol. Pre-treatment ultrasonography was performed within 24 hrs before lauromacrogol injection, and follow-up assessment was performed one month after the procedure. During follow-up, vaginal bleeding, menstrual recovery, serum β -HCG decline, and ultrasonographic findings were recorded.

Poor short-term prognosis was defined as the presence of residual gestational tissue with visible blood-flow signals on ultrasonography at one month after treatment, accompanied by an unsatisfactory decline in serum β -HCG and the need for additional treatment. The decision for retreatment was made by two experienced gynecologists based on the predefined clinical, serological, and ultrasonographic criteria. Patients without residual vascularized tissue and without the need for additional treatment were classified as having a good short-term prognosis.

Ultrasonographic images and parameters were evaluated by two radiologists with 5 years of experience. When disagreement occurred, a final decision was reached by discussion and consensus. According to this definition, 150 patients with CSP were divided into a good prognosis group (GPG; $n = 108$) and a poor prognosis group (PPG; $n = 42$).

Statistical analysis

SPSS 25.0 software was used for statistical analysis. Measurement data were expressed as mean $\pm$ standard deviation and compared using the independent-samples t -test between groups and the paired t -test within groups. Count data were expressed as frequency and percentage and compared using the χ^2 test. Receiver operating characteristic (ROC) curves were plotted with poor short-term prognosis after LSC as the state variable to evaluate the predictive performance of 3D-TVUS and CEUS parameters. Collinearity among candidate ultrasonographic parameters was assessed using the variance inflation factor (VIF), with $VIF < 5$ indicating no obvious multicollinearity. Multivariable logistic regression analysis was performed to identify independent predictors of poor short-term prognosis. The combined model was constructed using the predicted probability derived from the multivariable logistic regression equation. Internal validation was performed using 1,000 bootstrap resampling procedures, and calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test and the Brier score. A two-sided $p < 0.05$ was considered statistically significant.

Results

Patients with CSP were 25–36 years old, with a mean age of 30.12 ± 2.54 years, while women with normal pregnancy were 25–35 years old, with a mean age of 30.19 ± 2.42 years. The baseline data showed no significant differences between the two groups ($p > 0.05$).

The CSP group had higher VI, FI, and VFI, as well as PI and AUC_{TIC}, and shorter TTP than the NPG ($p < 0.05$), whereas no significant difference was shown in the AT value between the two groups ($p > 0.05$) (Table 1).

Baseline characteristics, including age, BMI, gravidity, number of previous CSs, type of CSP, time since the last CS, days of amenorrhea, gestational sac diameter, myometrial thickness between the gestational sac and bladder, and β -HCG levels, showed no statistically significant differences ($p > 0.05$) between the GPG and PPG, indicating good comparability between the two cohorts (Table 2).

The VI, FI, and VFI dropped in both the PPG and the GPG after treatment compared with those before treatment ($p < 0.05$), and they were higher in the PPG than in the GPG both before and after treatment ($p < 0.05$) (Table 3).

Table 1

Parameters of 3D-TVUS and CEUS in normal pregnancy and CSP groups

Group	Parameter of 3D-TVUS			Parameter of CEUS			
	VI	FI	VFI	AT, s	TTP, s	PI, dB	AUC _{TIC} , dB×s
Normal pregnancy (n = 150)	8.21 ± 0.37	29.90 ± 3.11	4.13 ± 0.29	15.63 ± 2.31	23.76 ± 2.31	18.92 ± 2.13	190.09 ± 54.63
CSP (n = 150)	21.98 ± 2.43	43.54 ± 4.65	12.19 ± 2.14	15.21 ± 2.14	16.49 ± 1.78	27.87 ± 3.42	657.76 ± 54.56
t-test	68.610	29.860	45.710	1.672	30.530	27.210	74.190
p-value	< 0.001	< 0.001	< 0.001	0.095	< 0.001	< 0.001	< 0.001

3D-TVUS – three-dimensional transvaginal ultrasonography; **CEUS** – contrast-enhanced ultrasonography; **CSP** – cesarean scar pregnancy; **VI** – vascular index; **FI** – flow index; **VFI** – vascular flow index; **AT** – arrival time; **TTP** – time to peak; **PI** – peak intensity; **AUC_{TIC}** – area under the time-intensity curve; **n** – number. Values are given as mean $\pm$ standard deviation.

Table 2

Baseline data of CSP patients with different outcomes of LSC

Baseline characteristics	Poor prognosis group (n = 42)	Good prognosis group (n = 108)	χ^2/t	p-value
Age, years	30.19 ± 2.67	30.23 ± 2.85	0.079	0.938
BMI, kg/m ²	23.87 ± 2.19	23.90 ± 2.31	0.072	0.942
Gravidity				
2	16 (38.10)	39 (36.11)		
3	14 (33.33)	39 (36.11)	0.106	0.949
4	12 (28.57)	30 (27.78)		
Previous CS frequency				
1	16 (38.10)	62 (57.41)		
2	26 (61.90)	45 (41.67)	5.174	0.075
3	0	1 (0.93)		
CSP type				
I	19 (45.24)	46 (42.59)		
II	23 (54.76)	62 (57.41)	0.086	0.769
Time from the last CS, years	3.42 ± 0.29	3.49 ± 0.30	1.295	0.197
Number of amenorrhea days	46.56 ± 3.98	46.92 ± 4.12	0.485	0.628
Average diameter of the gestational sac, mm	20.12 ± 1.67	19.97 ± 1.85	0.458	0.648
Thickness of the gestational sac and bladder myometrium, mm	3.00 ± 0.12	3.02 ± 0.16	0.733	0.465
Blood β -HCG value, mIU/mL	$4,235.54 \pm 387.96$	$4,238.98 \pm 390.90$	0.048	0.961

CSP – cesarean scar pregnancy; **LSC** – lauromacrogol sclerotherapy; **n** – number; **BMI** – body mass index; **CS** – cesarean section; **β -HCG** – beta-human chorionic gonadotropin; **IU** – international units. Values are given as mean $\pm$ standard deviation and numbers (percentages).

Table 3

Parameters of 3D-TVUS in CSP patients with different outcomes of LSC before and after treatment

Group	VI		FI		VFI	
	before	after	before	after	before	after
Good prognosis (n = 108)	18.65 ± 2.23	$9.19 \pm 0.76^*$	39.65 ± 4.53	$30.42 \pm 2.98^*$	9.12 ± 1.03	$5.00 \pm 0.43^*$
Poor prognosis (n = 42)	23.43 ± 2.31	$13.32 \pm 1.85^*$	47.67 ± 6.98	$37.87 \pm 3.41^*$	14.34 ± 2.51	$7.18 \pm 0.26^*$
t-test	11.670	19.430	8.286	13.190	18.110	30.710
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

3D-TVUS – three-dimensional transvaginal ultrasonography; **CSP** – cesarean scar pregnancy; **LSC** – lauromacrogol sclerotherapy; **VI** – vascular index; **FI** – flow index; **VFI** – vascular flow index; **n** – number. Values are given as mean $\pm$ standard deviation.

Note: * $p < 0.05$ vs. before treatment.

Table 4**Parameters of CEUS in CSP patients with different outcomes of LSC before and after treatment**

Group	AT, s		TTP, s		PI, dB		AUC _{TIC} , dB×s	
	before	after	before	after	before	after	before	after
Good prognosis (n = 108)	15.34 ± 1.22	15.24 ± 1.09	20.12 ± 2.07	23.41 ± 2.21*	22.12 ± 2.19	19.43 ± 1.75*	500.32 ± 54.64	231.23 ± 25.46*
Poor prognosis (n = 42)	15.28 ± 1.31	15.11 ± 1.17	12.32 ± 1.54	21.08 ± 1.32*	30.14 ± 3.45	20.28 ± 2.14*	700.98 ± 76.69	307.86 ± 54.59*
t-test	0.265	0.643	22.140	6.395	16.960	2.210	17.930	11.710
p-value	0.791	0.522	< 0.001	< 0.001	< 0.001	0.029	< 0.001	< 0.001

CEUS – contrast-enhanced ultrasonography; CSP – cesarean scar pregnancy; LSC – lauromacrogol sclerotherapy; AT – arrival time; TTP – time to peak; PI – peak intensity; AUC_{TIC} – area under the time-intensity curve; n – number.

Values are given as mean ± standard deviation.

Note: **p* < 0.05 vs. before treatment.

Table 5**Early predictive value of 3D-TVUS and CEUS parameters for LSC prognosis in CSP**

Index	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %	<i>p</i> -value
VI	0.850 (0.793–0.925)	20.76	84.29	80.42	< 0.001
FI	0.804 (0.756–0.893)	43.12	81.50	76.90	< 0.001
VFI	0.800 (0.734–0.904)	11.47	81.00	76.87	< 0.001
TTP	0.869 (0.793–0.930)	16.56 s	85.45	81.43	< 0.001
PI	0.823 (0.734–0.912)	25.45 dB	82.13	80.98	< 0.001
AUC _{TIC}	0.887 (0.768–0.934)	589.92 dB×s	87.76	83.32	< 0.001
Combination	0.923 (0.856–0.987)	-	90.41	87.98	< 0.001

3D-TVUS – three-dimensional transvaginal ultrasonography; CEUS – contrast-enhanced ultrasonography; LSC – lauromacrogol sclerotherapy; CSP – cesarean scar pregnancy; AUC – area under the curve; CI – confidence interval; VI – vascular index; FI – flow index; VFI – vascular flow index; TTP – time to peak; PI – peak intensity; AUC_{TIC} – area under the time-intensity curve.

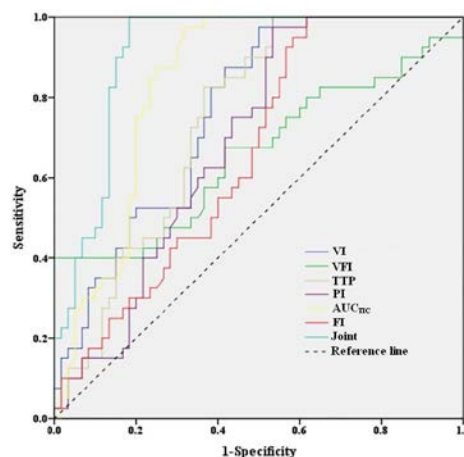


Fig. 1 – Receiver operating characteristic curves of parameters of three-dimensional transvaginal ultrasonography and contrast-enhanced ultrasonography in evaluating prognosis of lauromacrogol sclerotherapy for cesarean scar pregnancy.

VI – vascular index; VFI – vascular flow index; TTP – time to peak; PI – peak intensity; AUC_{TIC} – area under the time-intensity curve; FI – flow index.

Compared with pre-treatment, TTP was prolonged, while PI and AUC_{TIC} decreased in both groups after treatment (*p* < 0.05). In contrast to the GPG, the PPG showed shorter TTP (*p* < 0.05), higher PI and AUC_{TIC} (*p* < 0.05), and a comparable AT value (*p* > 0.05) before and after treatment (Table 4).

Collinearity analysis showed that the VIF values of the candidate ultrasonographic parameters were < 5, indicating no obvious multicollinearity. Therefore, VI, FI, VFI, TTP, PI, and AUC_{TIC} were included in the multivariate logistic regression model. The results showed that VI [odds ratio (OR) = 1.38, 95% confidence interval (CI): 1.12–1.69, *p* = 0.003], TTP (OR = 0.82, 95% CI: 0.71–0.94, *p* = 0.005), and AUC_{TIC} (OR = 1.015, 95% CI: 1.006–1.024, *p* = 0.001) were independent predictors of poor short-term prognosis after LSC.

The combined model was constructed using the predicted probability derived from the multivariable logistic regression equation. According to the ROC curves plotted with the prognosis of LSC in patients with CSP as the state variable and the ultrasonic parameters of significant differences between the PPG and the GPG (VI, FI, VFI, TTP, PI, and AUC_{TIC}) as the test variables, AUC_{TIC} performed the best in prediction among all the parameters, with AUC, sensitivity, and specificity of 0.887, 87.76%, and 83.32%, respectively. It was further found that the performance of the combination of all parameters in predicting the LSC prognosis in CSP was superior to that of any single parameter, and its AUC, sensitivity, and specificity were 0.923, 90.41%, and 87.98%, respectively (*p* < 0.05) (Table 5 and Figure 1). After 1,000

bootstrap resampling procedures, the optimism-corrected AUC was 0.911, suggesting good internal stability of the model. The calibration curve showed acceptable agreement between predicted and observed probabilities. Hosmer-Lemeshow test showed no significant lack of fit ($p = 0.428$), and the Brier score was 0.086, indicating acceptable calibration performance.

Discussion

According to the investigation, the incidence rate of CSP is about 6% among females with a history of CS, which is increasing annually. It is now accepted that the development of CSP is associated with endometrial injury and poor postoperative incision healing¹¹. In the case of CSP, the risks of uterine rupture and massive hemorrhage are relatively high due to the relatively thin myometrium where CSP develops and due to the relatively poor contractility of scar tissue. Even if a few pregnant patients successfully enter the third trimester, most of them suffer from dangerous complications such as placenta previa, placenta implantation, and other serious adverse events¹². Currently, the treatment of CSP mainly aims to terminate pregnancy as soon as possible to avoid the occurrence of serious adverse events. LSC, a common treatment for CSP, involves injecting lauromacrogol near veins to damage vascular endothelial cells and trigger an aseptic inflammatory response. This leads to venous occlusion and blocks local blood supply^{13–15}. The results of this study revealed that among the 150 patients with CSP, 42 had a poor prognosis after LSC, signifying that LSC is effective for CSP, but some patients still have a poor prognosis and recovery. This is attributed to the fact that scar tissue is weak, leading to deeper villous implantation.

As the preferred examination method for CSP, TVUS is characterized by easy operation, non-invasiveness, and high sensitivity, and does not require bladder filling compared with traditional transabdominal ultrasonography¹⁶. In 3D ultrasonography, 3D images are acquired based on 2D ultrasonography, in which the Y and Z planes are obtained from the X plane, thereby revealing the coronal section that cannot be displayed by 2D ultrasonography. As a result, relationships among the uterine cavity, gestational sac, mass, and incision are revealed from multiple sections^{17–19}. Huang et al.²⁰ compared the diagnostic efficacy of 3D-TVUS and MRI in CSP and discovered no significant difference in the detection rate of CSP between the two examination methods. Nevertheless, the detection rates of the yolk sac, embryo, and heartbeat with 3D-TVUS were significantly higher than those with MRI. In the present study, women with normal intrauterine pregnancies after CS were included as an external physiological reference. This design aimed to provide baseline values for 3D and CEUS parameters under normal conditions. By contrasting these physiological parameters with those observed in scar pregnancies, we were able to better characterize the abnormal vascularization and perfusion associated with scar implantation and further explore their

relationship with clinical prognosis in patients treated with LSC. Using this approach, 3D-TVUS was performed in both groups. The results revealed that the VI, FI, and VFI values were significantly higher in patients with CSP than in those with NPG. Possible reasons include the following: the gestational sac is implanted near the incision site of the previous CS, and the villi erode peripheral tissues to form a venous fistula. In addition, the muscle wall at the incision is thin, but blood vessels and fibrous tissues in this region are rich. As a result, the VI at this site is high, and there is more blood flow, increasing FI. Moreover, differences in 3D-TVUS parameters in patients with different LSC outcomes were further analyzed in this study. It was found that the VI, FI, and VFI rose in patients with poor prognosis, indicating more vessels and deeper implanted villi at the implantation location of the gestational sac in patients with poor prognosis. The ROC curves were plotted in this study to analyze the cut-off value of 3D-TVUS parameters in the early prediction of the LSC prognosis. The results showed that the optimal prediction performance was obtained when the values of VI, FI, and VFI were 20.76, 43.12, and 11.47, respectively, suggesting that in clinical practice, the prognosis of LSC can be predicted early by analyzing the quantitative parameters of 3D-TVUS before treatment.

CEUS can effectively distinguish the difference in blood perfusion between lesions and normal tissues, identify blood flow signals that conventional ultrasonography cannot distinguish, and inhibit peripheral tissue signals under a low mechanical index, thereby judging the relationship between the gestational sac and the perimetrium layer^{21, 22}. After CS, adhesion is observed in the fundus and abdominal wall, and the uterus and cervix are elongated, thereby limiting the application of TVUS²³. However, the above phenomena do not restrict the application of CEUS, i.e., the blood flow in the trophoblast can be displayed in CEUS. The results of the present study showed that the AT was not significantly different between the CSP group and the NPG, whereas shorter TTP and higher PI and AUC_{TIC} were found in the CSP group compared with the NPG. These results officially reflected the rich blood flow of the gestational sac at the incision in CSP. Wu et al.²⁴ found that CEUS could guide the selection of the location of lauromacrogol injection for patients with CSP, and the postoperative morphological indices could fully predict the curative effect after curettage and guide clinical treatment. In this study, CEUS was used to evaluate the prognosis of LSC in patients with CSP. The results revealed that TTP was prolonged, and PI and AUC_{TIC} declined after treatment compared with before treatment, demonstrating that LSC is markedly effective in the treatment of CSP. The PPG displayed shortened TTP and higher PI and AUC_{TIC} than the GPG both before and after treatment. It is possibly because patients with poor prognosis have more blood vessels in and around the gestational sac, as well as faster local blood flow, so part of the injection may flow away rapidly through the villous space when LSC is performed. As a result, complete

sclerosis fails, resulting in poor prognosis. Furthermore, to determine the cut-off values of CEUS parameters for predicting LSC prognosis, ROC curves were plotted, and it was found that when TTP, PI, and AUC_{TIC} were 16.56 s, 25.45 dB, and 589.92 dB×s, respectively, the optimal predictive performance was obtained. Among them, AUC_{TIC} displayed the best prediction performance, and its AUC, sensitivity, and specificity were 0.887, 87.76%, and 83.32%, respectively. These results suggest that early prediction of the prognosis of LSC can be achieved clinically by observing blood vessels and local blood flow.

Additionally, we found that for predicting the LSC prognosis in CSP, the combination of 3D-TVUS and CEUS was more effective than single parameters. This shows that 3D-TVUS with CEUS is more helpful for improving the early evaluation of the prognosis of LSC in CSP. This is possibly ascribed to the following facts: the blood supply to the pregnancy focus is reflected by various blood flow parameters in 3D-TVUS, whereas CEUS reveals blood flow information in smaller vessels. The two examination methods complement each other's advantages, thereby enhancing prediction performance. From a clinical perspective, these ultrasound parameters may help translate imaging findings into individualized perioperative management. Higher VI, FI, VFI, PI, and AUC_{TIC} values, together with shortened TTP, indicate richer local vascularization and faster perfusion around the gestational sac, suggesting a higher risk of incomplete devascularization, residual vascularized tissue, and unsatisfactory β -HCG decline after LSC. Therefore, patients with parameters exceeding the proposed cut-off values, especially those with elevated AUC_{TIC} or a high combined ultrasound assessment, may be considered at higher risk of poor short-term response. In practical management, such patients may benefit from more detailed preoperative counseling, closer preparation for possible bleeding, treatment by an experienced operator, and more intensive post-treatment monitoring of serum β -HCG and ultrasonographic residual blood-flow signals²⁵. In contrast, patients with lower-risk ultrasound features may undergo routine follow-up after treatment. Thus, the combined use of 3D-TVUS and CEUS may provide a practical imaging-based framework for early risk stratification, although treatment decisions should still be made comprehensively according to clinical symptoms, β -HCG trends, and follow-up ultrasound findings²⁶.

These findings should also be interpreted in the context of recent international guidelines and expert recommendations. The 2022 Society for Maternal-Fetal Medicine – SMFM Consult Series #63 recommends against expectant management of CSP and suggests operative resection or ultrasound-guided uterine aspiration as potential surgical approaches, while sharp curettage alone should be avoided. Intra-gestational methotrexate may be administered with or without other treatment modalities, whereas systemic methotrexate alone is not recommended²⁵. Since this guideline does not specifically evaluate LSC and acknowledges that the optimal treatment strategy remains uncertain, the present findings provide

complementary evidence for pretreatment risk stratification and post-treatment monitoring within a minimally invasive sclerotherapy protocol, rather than establishing a universally preferred treatment approach. Meanwhile, an international Delphi consensus on the sonographic assessment of first-trimester CSP recommends TVUS at 6–7 weeks of gestation and standardized documentation of gestational-sac size, vascularity, its relationship with the uterine vessels, residual myometrial thickness, and its location relative to the uterine cavity and serosa; routine MRI was not considered to provide additional diagnostic value²⁷. Our findings extend this standardized anatomical assessment by demonstrating the potential prognostic value of quantitative 3D vascular indices and CEUS perfusion parameters. More recently, the 2026 Royal College of Obstetricians and Gynaecologists Green-Top Guideline recognized CSP as a potential precursor of placenta previa and placenta accreta spectrum and emphasized expert counseling regarding these risks when a live pregnancy persists beyond the first trimester²⁸. Although the patients in the present study underwent pregnancy termination and the continuation of pregnancy was not evaluated, this guideline perspective further reinforces the importance of early diagnosis, individualized counseling, and preparedness for severe hemorrhage in patients with marked vascularity. Therefore, the proposed ultrasonographic cut-off values should complement, rather than replace, comprehensive assessment based on clinical symptoms, serum β -HCG trends, residual myometrial thickness, gestational characteristics, and local treatment expertise.

Limitations of the study

This study has several limitations. First, this was a single-center retrospective study with a relatively limited sample size, which may restrict the generalizability of the findings. Second, the NPG was used only as a physiological reference group for ultrasonographic perfusion characteristics, rather than as a true prognostic control group. Therefore, the main prognostic conclusions should be interpreted based on the comparison between good- and poor-prognosis patients within the CSP cohort. Third, the follow-up period was limited to one month, so the outcome evaluated in this study should be regarded as short-term treatment response rather than final long-term treatment success. Longer follow-up is needed to assess complete lesion resolution, recurrence, and reproductive outcomes. Fourth, although ultrasound assessment was performed by experienced radiologists and disagreements were resolved by consensus, formal interobserver variability analysis was not conducted. Finally, although multivariable logistic regression, internal validation, and calibration analysis were added, this was still a single-center retrospective study without external validation. Further prospective multicenter studies with larger samples are required to confirm the independent predictive value and generalizability of these parameters.

Conclusion

A considerable proportion of patients with cesarean scar pregnancy had poor short-term prognosis after lauromacrogol sclerotherapy. Pretreatment transvaginal three-dimensional ultrasonography and contrast-enhanced

ultrasonography parameters may help in the early evaluation of short-term prognosis after lauromacrogol sclerotherapy.

Conflict of interest

The author declares no conflict of interest.

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An *in vitro* comparative evaluation of the osteogenic potential of antibiotics or neem-infused titanium platelet-rich fibrin

In vitro uporedna procena osteogenog potencijala antibiotika ili neem-a u infuziji titanijumom-pripremljenog fibrina obogaćenog trombocitima

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Abstract

Background/Aim. Titanium-prepared platelet-rich fibrin (T-PRF) has superior properties with respect to resorption time, thickness, growth factor holding capacity, and gradual release at the surgical site. The aim of this study was to evaluate the cell viability and osteogenic differentiation of an osteoblastic cell line MG-63 following exposure to eluates obtained from T-PRF clots injected with antibiotic gels [amoxicillin/clavulanic acid – amoxiclav gel (AMX), metronidazole – metronidazole gel (MET)], or herbal extracts (neem gel). **Methods.** In this *in vitro* study, blood was collected, and T-PRF clots were prepared. Antibiotic/herbal gels along with T-PRF clots were added to Dulbecco's Modified Eagle Medium – DMEM to obtain eluate. This eluate was later added to the cultured osteoblastic cells to check for cell viability using the [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] (MTT) assay on days 1, 3, and 7. Gene expression [alkaline phosphatase (ALP), osteopontin (OSP), bone morphogenetic protein-2 (BMP-2)] was checked on day 7 using quantitative polymerase chain reaction (qPCR), and alizarin red S (ARS) staining was used for late-stage osteogenic differentiation and matrix mineralization on day 21. Statistical analysis was performed using GraphPad Prism

software. One-way analysis of variance (ANOVA) was used for the MTT assay and for ARS staining, with Tukey's *post-hoc* test applied only for significant ANOVA results (on day 7 MTT and day 21 ARS data). The Kruskal-Wallis test was used for qPCR analysis. **Results.** Cell viability was significantly higher in the T-PRF + neem group on day 3 ($p = 0.0229$) and day 7 ($p = 0.0034$), while the T-PRF + AMX group showed significantly higher cell viability on day 7 ($p = 0.0142$) compared with T-PRF alone. The qPCR analysis showed significantly increased expression of OSP ($p = 0.0422$), ALP ($p = 0.0203$), and BMP-2 ($p = 0.0290$) in all tested groups (T-PRF + AMX/MET/neem) compared with the control group (T-PRF alone). Intergroup comparisons showed no significant differences, except between the T-PRF + neem group and the control group. ARS analysis at 21 days revealed the highest mineralization in T-PRF + neem, followed by T-PRF + MET and T-PRF + AMX. **Conclusion.** Both antibiotic- and herbal-extract-incorporated T-PRF clots demonstrated superior osteogenic potential compared to plain T-PRF alone. Therefore, T-PRF can serve as a sustained drug delivery system.

Keywords:

amoxicillin; clavulanic acid; drug delivery systems; plants; platelet-rich plasma.

Apstrakt

Uvod/Cilj. Titanijumom-pripremljen fibrin obogaćen trombocitima (*titanium-prepared platelet-rich fibrin* – T-PRF) ima superiorna svojstva u pogledu vremena resorpcije, debljine, kapaciteta zadržavanja faktora rasta i postepenog oslobađanja na mestu hirurške intervencije. Cilj rada bio je da se procene vijabilnost ćelija i osteogeno diferentovanje osteoblastne ćelijske linije MG-63 posle izlaganja eluatima

dobijenim iz ugrušaka T-PRF u koje su ubrizgani gelovi sa antibioticima [amoksicilom/klavulanskom kiselinom – amoksiklav gel (AMX), metronidazolom – metronidazol gel (MET)] ili biljnim ekstraktima (*neem* gel). **Metode.** U ovoj *in vitro* studiji, sakupljena je krv i pripremljeni su T-PRF ugrušci. Antibiotički/biljni gelovi, zajedno sa T-PRF ugrušcima, dodati su u *Dulbecco* modifikovani *Eagle* medijum – DMEM kako bi se dobio eluat. Ovaj eluat je kasnije dodat kultivisanim osteoblastnim ćelijama kako bi se procenila

vijabilnost ćelija 1, 3. i 7. dana primenom [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] (MTT) testa. Ekspresija gena [alkalna fosfataza (ALP), osteopontin (OSP), koštani morfogenetski protein-2 (*bone morphogenetic protein-2* – BMP-2)] proverena je 7. dana korišćenjem kvantitativne lančane reakcije polimeraze (*quantitative polymerase chain reaction* – qPCR), dok je bojenje alizarin crvenim S (*alizarin red S* – ARS) korišćeno za kasnu fazu osteogene diferencijacije i mineralizaciju matriksa 21. dana. Statistička analiza izvršena je korišćenjem GraphPad Prism softvera. Jednofaktorska analiza varijanse (*analysis of variance* – ANOVA) korišćena je za MTT test i za bojenje ARS, pri čemu je Tukey-ev *post-hoc* test primenjen samo za značajne rezultate ANOVA (podaci MTT testa 7. dana i bojenje ARS 21. dana). Kruskal-Wallis-ov test korišćen je za analizu qPCR. **Rezultati.** Značajno veća vijabilnost ćelija zabeležena je u grupi T-PRF + *neem* 3. dana ($p = 0,0229$) i 7. dana ($p = 0,0034$), dok je u grupi T-PRF + AMX značajno

veća vijabilnost pokazana 7. dana ($p = 0,0142$) u poređenju sa samim T-PRF. Analiza qPCR pokazala je značajno povećanu ekspresiju OSP ($p = 0,0422$), ALP ($p = 0,0203$) i BMP-2 ($p = 0,0290$) u svim testiranim grupama (T-PRF + AMX/MET/*neem*) u poređenju sa kontrolnom grupom (samo T-PRF). Međugrupna poređenja nisu pokazala značajne razlike, osim između grupe T-PRF + *neem* i kontrolne grupe. Analizom ARS 21. dana otkrivena je najveća mineralizacija u T-PRF + *neem*, zatim u T-PRF + MET i T-PRF + AMX. **Zaključak.** Ugrušci T-PRF kako sa dodatkom antibiotika tako i sa dodatkom biljnog ekstrakta pokazali su superiorniji osteogeni potencijal u poređenju sa samim T-PRF. Zbog toga, T-PRF se može primenjivati kao sistem za produženo oslobađanje lekova.

Ključne reči:

amoksicilin; klavulanska kiselina; lekovi, sistemi za isporuku; biljke; plazma bogata trombocitima.

Introduction

Platelet concentrates are simple, autologous therapies used to treat periodontitis (soft and hard tissue defects) and are applied in membrane¹, clot², or liquid³ forms depending on clinical need. Due to the presence of adhered cells and gradual release of various growth factors—such as platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor (TGF)- β , epidermal growth factor, and insulin-like growth factor (IGF)—at the surgical site, better healing was observed with improved clinical and radiographic parameters⁴. Specifically, a decrease in probing pocket depth, a gain in clinical attachment level, and bone fill in intra-bony defects were noted^{5, 6}; additionally, a reduction in recession depth and width, alongside an increase in keratinized tissue width, occurred in gingival recession treatments^{7, 8}.

The utilization of medications as part of surgical and non-surgical periodontal therapy has been employed for many years. Medications such as amoxicillin, metronidazole, doxycycline, clindamycin, metformin, atorvastatin, and rosuvastatin have been administered systemically (as oral tablets) or locally (*via* local drug delivery systems) during non-surgical therapies, reporting positive clinical outcomes^{9, 10}. These medications affect osteogenesis differently and interact with osteoblastic cells in distinct ways. For instance, the antibiotic metronidazole activates the bone morphogenetic protein (BMP)-2 and runt-related transcription factor 2 (Runx2) pathways, aiding in bone cell differentiation and activity. Additionally, it reduces inflammation, which can change the environment and promote new bone formation with bone cells¹¹.

Casellas et al.¹² showed that amoxicillin/clavulanate had a positive effect on active ulcerative colitis, reducing inflammatory mediator production and, in turn, intraluminal interleukin (IL)-8 and other inflammatory mediators. Furthermore, Balcazar-Ochoa et al.¹³ claimed that clavulanate has both anti-inflammatory and pain-relieving qualities. It was never easy to maintain the prolonged release

of these medications at the surgical site. Because of this, scientists have employed several delivery methods, such as collagen membranes, microspheres, gelatin sponges, carbopolymers, etc.¹⁴. However, due to concerns regarding synthetic production, increased antigenicity, and potential allergic responses, there has been a continuous search for superior, autogenous materials to develop sustained drug delivery systems (SDDS). Leukocyte- and platelet-rich fibrin – PRF (L-PRF), a second-generation platelet concentrate, has been shown to enhance soft and hard tissue healing while reducing postoperative pain. Its three-dimensional fibrin structure serves as a reservoir for growth factor, enabling their sustained and gradual release. It can be prepared easily without adding anticoagulants and is purely autogenous¹⁵. Based on these promising findings, Polak et al.¹⁶ incorporated vancomycin, penicillin, and other antibiotics into L-PRF and evaluated its antimicrobial activity against *Staphylococcus* (*S.*) *aureus* and *Fusobacterium nucleatum*. They found that antibiotic-loaded L-PRF exhibited significantly greater antimicrobial activity than L-PRF alone. In their study on third molar surgery, Bennardo et al.¹⁷ evaluated antibiotics combined with L-PRF; their findings indicated lower postoperative pain and infection rates compared to L-PRF alone.

Despite the promising clinical outcomes associated with L-PRF, its relatively short resorption time, the potential for silica contamination, and the risk of glass test tube fracture prompted the development and investigation of improved biomaterials. As a result, scientists were drawn to titanium (Ti), which has seen widespread use in medicine and dentistry. Tunali et al.¹⁸ introduced Ti-prepared PRF (T-PRF) in 2013. This inert substance passivates into a layer of Ti dioxide, which triggers platelet activation and the creation of a three-dimensional fibrin clot. T-PRF is a second-generation platelet concentrate made by centrifuging blood in medical-grade Ti tubes. Compared to L-PRF, T-PRF eliminates silica contamination and exhibits a longer resorption time of up to 21 days. The tubes are sterile, reusable, and unbreakable.

Previous histological investigations^{19–22} comparing T-PRF and L-PRF found that T-PRF had more fibrin, a denser fibrin network, and superior cellular entrapment compared to L-PRF. Studies have shown that T-PRF persists at the surgical site for approximately 21 days, indicating a longer resorption time than earlier PRF preparations¹⁹. Due to the hard and soft tissue characteristics of T-PRF when used as a biomaterial, Ercan et al.²³ chose to employ T-PRF as their SDDS. They used doxycycline hyclate liquid injected into T-PRF clots and tested medication release and antimicrobial activity. They concluded that T-PRF retained the medication better, showed a sustained release profile based on drug kinetics, and exhibited superior antimicrobial activity as evidenced by its impact on *S. aureus* and *Pseudomonas (P.) aeruginosa* bacteria. The successful impregnation of drugs into T-PRF was verified by scanning electron microscopy (SEM).

In a related experiment, Indurkar and Purohit²⁴ administered a 3 mg/mL doxycycline solution into the T-PRF. The antimicrobial activity of the T-PRF membrane was also evaluated using *P. aeruginosa* and *S. aureus*. They concluded that T-PRF alone has antimicrobial properties and that the addition of antibiotics improved them. They also stated that T-PRF's activity may be used to treat peri-implantitis and periodontitis. Herbal items such as aloe vera, neem, curcumin, and mangosteen were also used as mouthwashes, gels, etc., and the results were positive. Clinical variables such as the plaque index, gingival index, enhanced clinical attachment level, and reduced probing pocket depth were assessed²⁵. Neem, curcumin, and aloe vera gel were used in a number of trials^{26–29} and produced positive outcomes. Therefore, in chronic periodontitis, topical applications of curcumin or aloe vera may be regarded as adjuncts to scaling and root planing.

It is always preferable to experiment on cell lines before starting a clinical trial of a new treatment protocol. As a result, test groups may interact with cultured cells in an environment that allows researchers to determine whether the experiment had any negative effects on the cells³⁰. Additionally, researchers can determine whether the cells are actively dividing when they are treated. Sumida et al.³¹ conducted an *in vitro* experiment in 2019 on rat cells that showed PRF boosted osteoprotegerin levels in MC3T3-E1 cell cultures, indicating that PRF played a significant role in the initial stages of osteogenesis by regulating osteoblastic differentiation. Esmaeilnejad et al.³² investigated the effects of L-PRF and advanced PRF (A-PRF) on the osteoblastic cell line MG-63. Their findings demonstrated that A-PRF was more effective at promoting osteoblastic proliferation and mineralization, whereas L-PRF predominantly stimulated cellular proliferation. According to the authors, no research has been done on the osteogenic potential of T-PRF clots containing neem gel or antibiotics (amoxicillin/clavulanate or metronidazole) in osteoblast cells.

The aim of this *in vitro* study was to compare the osteogenic differentiation of osteoblastic cell lines exposed to neem-embedded T-PRF and antibiotic-embedded T-PRF.

Methods

Study design, sample collection, and ethical clearance

This study was an *in vitro* experiment approved by the Department of Periodontics, Saveetha Institute of Technical and Medical Sciences (SIMATS), and Saveetha Dental College and Hospital, Chennai, India. The study was conducted in accordance with the protocol authorized by the institutional and/or licensing Committees of Saveetha Dental College and Hospital and was properly reviewed by Saveetha University, Chennai, India (No. SRB/SDC/PhD/PERIO-2251/24/245, from August 8, 2024). Blood sample collection adhered to proper protocols and regulations. The study followed appropriate ethical norms and was conducted in accordance with the Declaration of Helsinki. Confidentiality was maintained to safeguard the volunteers' privacy. Verbal and written informed consent was obtained from the subjects and/or legal guardians before conducting the study. The pros and cons of the study protocol were explained, and after acceptance, the blood-drawing procedure was performed.

Inclusion and exclusion criteria

A healthy, 31-year-old male with no blood-related problems, willing to donate blood and take part in the study, was recruited for the study. As this was an initial exploratory study, only one individual's blood was considered. Subjects who were not interested, unwilling to donate blood, or on any sort of medication were excluded.

Study groups

Groups consisted of T-PRF + AMX, T-PRF + MET, T-PRF + neem, and T-PRF clot without any additional substances, which was used as the control.

Procedure and total sample size of study

Triplication was done for each group, so a total of 12 experiments (i.e., 36 tests) were performed for the [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] (MTT) assay at three different time intervals (on day 1, 3, and 7) for viability of osteoblast cells (MG-63). For quantitative polymerase chain reaction (qPCR), 12 experiments were done on day 7 [gene expression for osteopontin (OSP), alkaline phosphatase (ALP), and BMP-2]. Triplication was also performed for alizarin red S (ARS) staining. For mineralization assessment, 12 experiments were done on day 21. The study adhered to the Checklist for Reporting *In-vitro* Studies – CRIS guidelines to the extent possible.

Antibiotic gels and neem gel preparations

Amoxiclav gel (AMX) (TBD003-1vl Biomall, India), metronidazole (Cat No. 210340050, Thermo Fisher Scientific), and neem gel were commercially prepared from Periobiologics Lab, Hyderabad, India, in insulin syringes using

weight/weight percentage. AMX was prepared by mixing amoxicillin, clavulanate, carboxymethyl cellulose (Cat No. 332640010, Thermo Fisher Scientific), magnesium stearate (Cat No. PA13 66000 Pharmaffiliates), polycarbophil powder, and glycerine (Cat No. 2143-03, Thermo Fisher Scientific) to form an 18% concentration. Metronidazole gel (MET) was prepared using Poloxamer 123 (Cat No. FP106449, Biosynth Inc.), propylene glycol, methylisothiazolinone (CAS No. 2682-20-4, Santa Cruz, India), and distilled water in the required quantity to prepare a 30% concentration. To prepare 100 g of neem extract, 20 g of neem leaves (CAS No. 84696-25-3, Bureau of Indian Standards) were boiled in 1 L of water; further, 20 g Carbopol 934P (Himedia), calcium chloride 0.02%, and sodium chloride 0.9% were added to form a 12% concentration neem gel.

Preparation of titanium-prepared platelet-rich fibrin clots and their incorporation with antibiotic gels or neem gel to form eluates

Twenty mL of blood was collected through the antecubital vein from a healthy volunteer and centrifuged at 3,500 rotations *per min* for 15 min (Remi 8C, New Delhi, India) (modified Tunali et al.¹⁸ protocol) to obtain T-PRF in sterile medical-grade Ti tubes (Grade 2, Supra Alloys Company, Camarillo, USA). This T-PRF preparation protocol was adapted from the previously published articles of Bhattacharya et al.^{21, 22}. Clots were carefully retrieved using sterile tweezers from Ti tubes and placed on Petri dishes, which were later transferred to Eppendorf tubes for lyophilization. Lyophilized T-PRF clots were mixed with antibiotic gel or neem gel in Dulbecco's Modified Eagle Medium (DMEM), incubated to obtain a proper eluate, and exposed

to the wells of osteoblast cells on days 1, 3, and 7 to perform the MTT assay and qPCR on day 7.

Osteoblast culture

DMEM supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin was used to culture MG-63 (osteosarcoma cell line) osteoblast cells obtained from the National Center for Cell Science, Pune, India. They were grown in a humidified atmosphere at 37 °C with 5% CO₂.

MTT assay

The MTT assay was used to assess cell viability and proliferation on days 1, 3, and 7. After seeding 10,000 osteoblast cells *per well* in 96-well plates, the corresponding T-PRF clot eluate containing AMX, MET, and neem was incubated separately, and the results were compared to the T-PRF clot eluate alone. Cells were cultured for 4 hrs after adding MTT solution (5 mg/mL) at all time points, then incubated again for 4 hrs at 37 °C to calculate percentage viability. Dimethyl sulfoxide was used to dissolve the formazan crystals, and absorbance at 570 nm was measured using a microplate reader [Synergy™ hybrid Multi-Mode Reader (BioTek, Winooski, VT, US)] (Figures 1 and 2).

The percent cell viability was calculated using the resulting equation³³:

$$\text{Cell viability (\%)} = \frac{\text{OD}(\text{test sample}) - \text{OD}(\text{blank})}{\text{OD}(\text{PC}) - \text{OD}(\text{blank})} \times 100$$

where OD stands for optical density, and PC is the positive control.

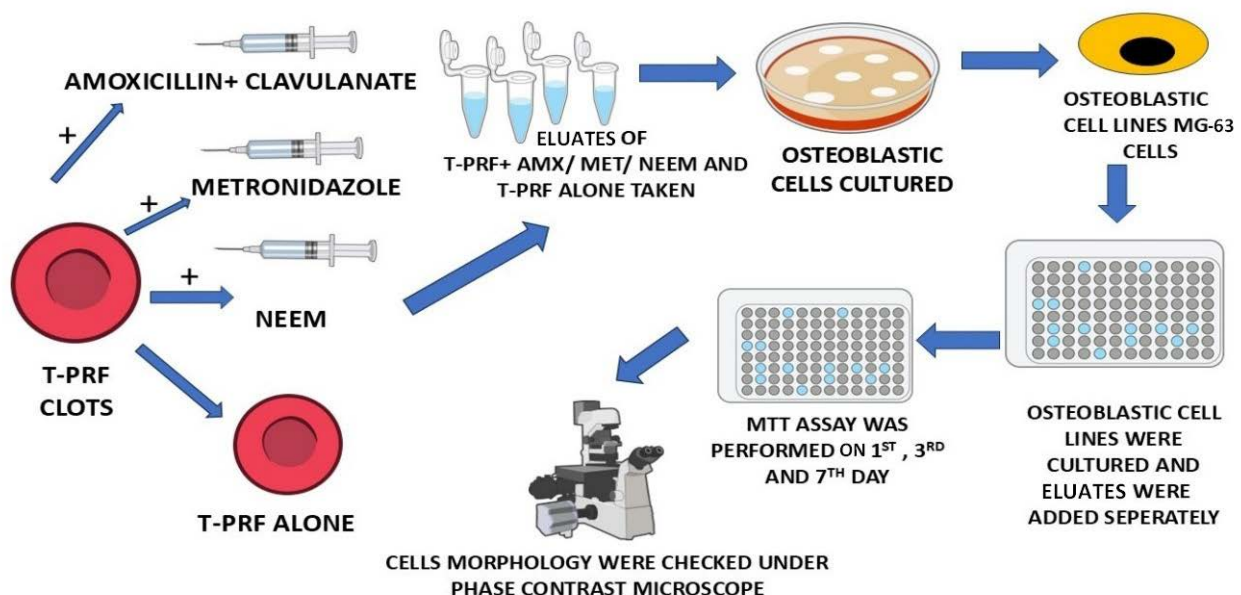


Fig. 1 – Schematic representation of the MTT assay procedure of the study and the method of incorporating T-PRF and AMX/MET/neem eluates.

MTT – [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide];

T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

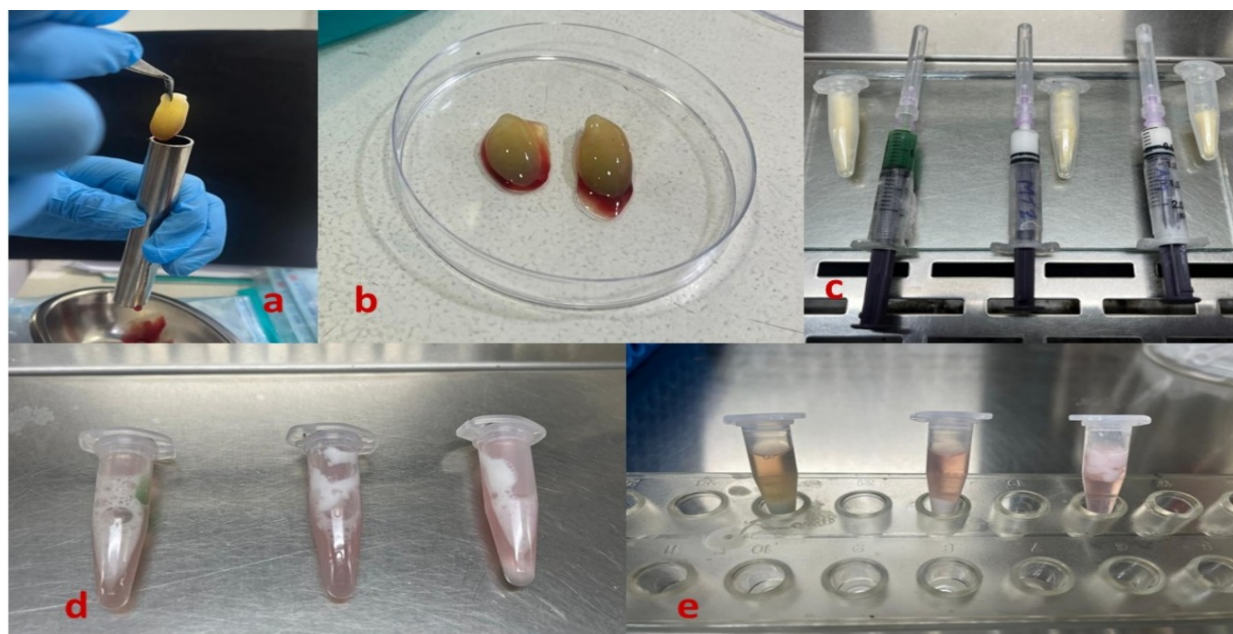


Fig. 2 – a) T-PRF retrieval from tubes; b) T-PRF placed on culture plates; c) T-PRF sample eluate in Eppendorf tubes; d) the immediate (AMX/MET) and neem eluates mixed with T-PRF eluate after vortexing; e) the final eluates of both test and control groups in an Eppendorf tube, which was ready to use on MG-63 osteoblastic cells.
T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

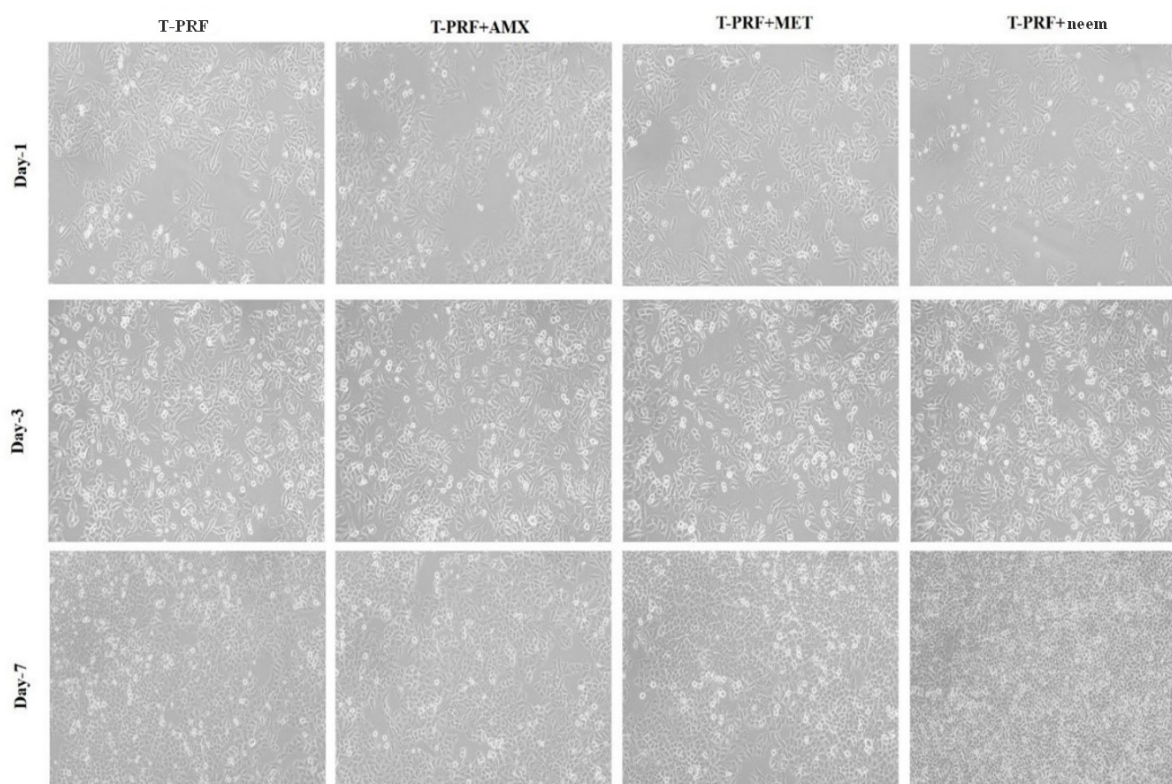


Fig. 3 – The biocompatibility of T-PRF gels incorporated with AMX, MET, and neem gels, along with the control group on osteoblast cells, was evaluated by morphological assessment using phase contrast (×20 magnification).
T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

Further biocompatibility of T-PRF eluate incorporated with AMX/MET/neem, along with T-PRF eluate alone, on os-

teoblasts was morphologically assessed and visualized under a phase contrast microscope at ×20 magnification (Figure 3).

Ribonucleic acid isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for osteogenic markers

Total ribonucleic acid (RNA) was extracted using TRIzol™ reagent (Thermo Fisher Scientific, Waltham, MA, USA). A NanoDrop™ ND 100 (Thermo Fisher Scientific, Waltham, MA, USA) was used for ultraviolet spectrometry at 260 and 280 nm to measure RNA quantity and purity. In addition, 500 ng of RNA was converted into complementary deoxyribonucleic acid – DNA (cDNA) using the PrimeScript™ RT Reagent Kit (Perfect Real Time, Takara, Shiga, Japan). Using Bio-Rad Connect real-time PCR equipment (Bio-Rad, Hercules, CA, USA) and KAPA SYBR™ Fast Master Mix (2×) Universal (Kapa Biosystems, Sigma, Darmstadt, Germany), qPCR analysis was conducted. Three indicators associated with osteo-

genesis were selected for analysis: BMP-2, OSP, and ALP, and primer design was employed using primer blast software (Table 1). β-actin was used as a housekeeping gene (Figure 4). Three independent qPCR experiments were carried out for three osteogenic markers: OSP, ALP, and BMP-2. To maximize PCR amplification properties, primer sets were employed. OSP and BMP-2 were subjected to a real-time PCR reaction at 95 °C for 3 min, followed by 40 amplification cycles at 95 °C for 3 sec and 58 °C for 30 sec. After the reaction was run at 95 °C for 3 min, 40 amplification cycles were carried out for ALP at 95 °C for 3 seconds and 60 °C for 30 sec. The information was assessed using Bio-Rad CFX Manager software version 3.0. Relative expression levels of OSP, ALP, and BMP-2 were quantified using the double delta cycle threshold ($2^{-\Delta\Delta Ct}$) method, which was the most convenient way of assessment ^{34, 35}.

Table 1
The forward and reverse primers used for checking the PCR in the present study

Gene	Forward 5'-3'	Reverse 5'-3'
β-actin (housekeeping)	TCGTGTTGGATTCTGGGGAC	ACGAAGGAATAGCCACGCTC
OSP	CCTGGCTGAATTCTGAGGGAC	TATAGGATCTGGGTGCAGGCT
ALP	TGGGCATTGTGACTACCACTCGG	CCTCTGGTGGCATCTCGTTATCC
BMP-2	TGCTAGTAACTTTGGCCATGATG	GCGTTCCGCTGTTTGTGTT

PCR – polymerase chain reaction; OSP – osteopontin; ALP – alkaline phosphatase; BMP-2 – bone morphogenetic protein-2.

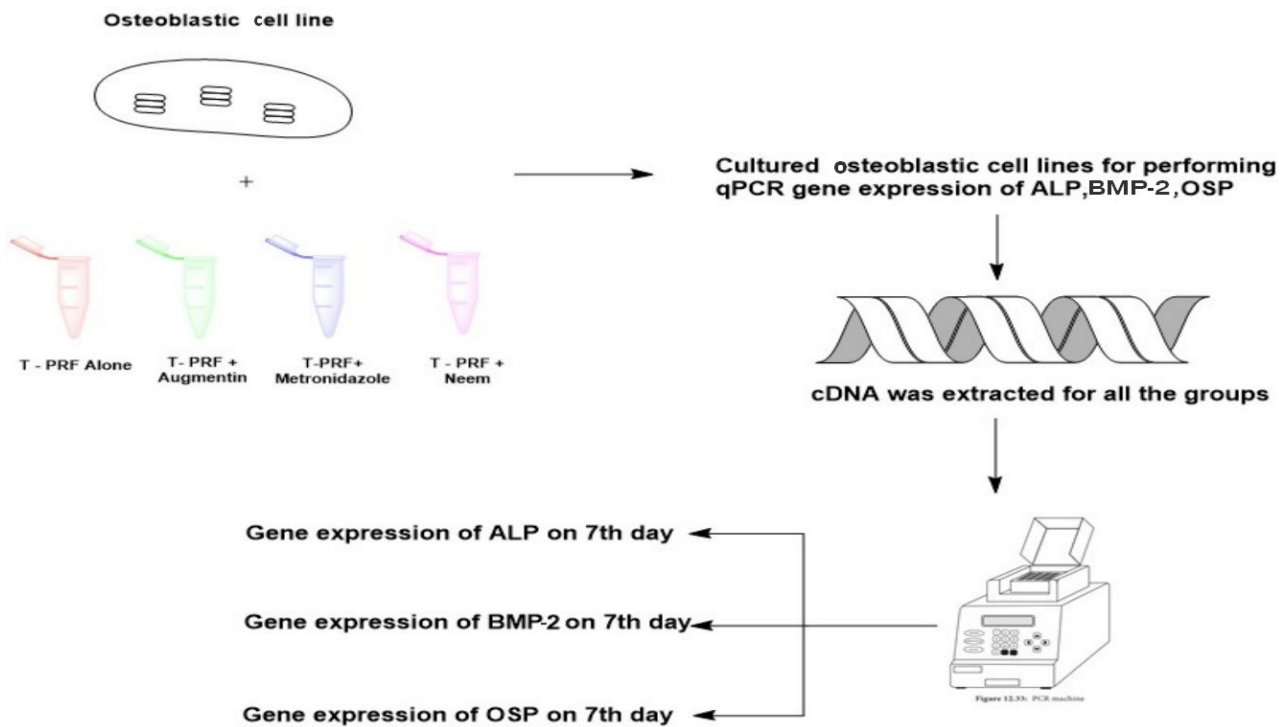


Fig. 4 – Schematic representation of the qPCR protocol of the study.
T-PRF – titanium-prepared platelet-rich fibrin; qPCR – quantitative polymerase chain reaction; ALP – alkaline phosphatase; BMP-2 – bone morphogenetic protein; OSP – osteopontin; cDNA – complementary deoxyribonucleic acid.

Alizarin red S staining assay

To evaluate late-stage osteogenic differentiation and matrix mineralization, ARS staining was performed on day 21. Human osteoblasts were cultured in 24-well plates and exposed to four experimental conditions: T-PRF alone, amoxiclav-incorporated (AMX) T-PRF, metronidazole-incorporated (MET) T-PRF, and neem gel-incorporated T-PRF clots. At the end of the incubation period, cells were washed twice with phosphate-buffered saline, fixed with 4% paraformaldehyde for 15 min at room temperature, and subsequently stained with 2% ARS (pH 4.2) for 20 min. Excess dye was removed by washing with distilled water. For quantification, bound dye was solubilized using 10% cetylpyridinium chloride, and the absorbance was measured at 405 nm using a microplate reader. Mineralization levels were compared across groups to assess calcium deposition and osteogenic potential.

Statistical analysis

GraphPad Prism software was used for statistical analysis, with all collected data imported directly into the program. One-way analysis of variance (ANOVA) test was performed to assess the time changes for MTT assay (on day 1, 3, and 7) with *post-hoc* Fisher's least significant difference – LSD test on day 7. Data from qPCR analysis (7th

day) and ARS (21st day) were initially checked for normalization using the Shapiro-Wilk test. Because the data were non-normally distributed, the Kruskal-Wallis test was employed for qPCR analysis. Dunn's *post-hoc* multiple comparison test with Bonferroni adjustment was also performed for qPCR. One-way ANOVA test with *post-hoc* honestly significant difference – HSD test was performed for ARS analysis. The value of $p < 0.05$ was considered statistically significant.

Results*MTT assay (days 1, 3, and 7)*

On days 1 and 3, overall ANOVA results were non-significant ($p > 0.05$); therefore, *post-hoc* comparisons were not performed. Compared with T-PRF alone, T-PRF + AMX/MET showed no statistically significant differences on days 1 and 3. In contrast, the T-PRF + neem group showed a statistically significant difference on day 3 ($p = 0.0229$) compared with T-PRF alone. On day 7, overall ANOVA results showed a significant difference among groups ($F = 9.42$, $p = 0.001$). *Post-hoc* and ANOVA analyses revealed significantly higher viability for T-PRF + neem ($p = 0.0034$) and T-PRF + AMX ($p = 0.0142$) when compared with T-PRF alone. Comparisons between test groups (neem vs. AMX or MET) were non-significant ($p > 0.05$) (Tables 2 and 3 and Figure 5).

Table 2

Mean percentages of MTT assay of T-PRF injected with antibiotics, neem gel, and control on days 1, 3, and 7

Day	T-PRF control, %	T-PRF + AMX, %	T-PRF + MET, %	T-PRF + neem, %	<i>F</i>	<i>p</i> -value
1	100 ± 0	91.64 ± 4.82	97.86 ± 3.40	97.10 ± 2.92	2.334	0.150
<i>p</i> -value	0	0.070	0.079	0.1517		
3	102.36 ± 0	103.96 ± 5.39	106.58 ± 1.40	111.51 ± 3.41	2.827	0.107
<i>p</i> -value	0	0.325	0.286	0.0229*		
7	108.66 ± 0	120.29 ± 4.94	122.12 ± 1.93	125.55 ± 2.50	9.42	0.001*
<i>p</i> -value	0	0.142*	0.286	0.0034*		

MTT – [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide]; **T-PRF** – titanium-prepared platelet-rich fibrin; **AMX** – amoxiclav gel; **MET** – metronidazole gel.

Values are given as mean ± standard error.

Note: *Statistically significant ($p < 0.05$).

Table 3

Least significant difference (LSD) *post-hoc* comparisons among the groups on day 7

Pairwise comparison	<i>p</i> -value
T-PRF + AMX vs. T-PRF alone	0.0142*
T-PRF + MET vs. T-PRF alone	0.286
T-PRF + neem vs. T-PRF alone	0.0034*
T-PRF + AMX vs. T-PRF + MET	0.603
T-PRF + AMX vs. T-PRF + neem	0.136
T-PRF + MET vs. T-PRF + neem	0.821

T-PRF – titanium-prepared platelet-rich fibrin;

AMX – amoxiclav gel; **MET** – metronidazole gel.

Note: *Statistically significant difference ($p < 0.05$) based on Fisher's LSD *post-hoc* test.

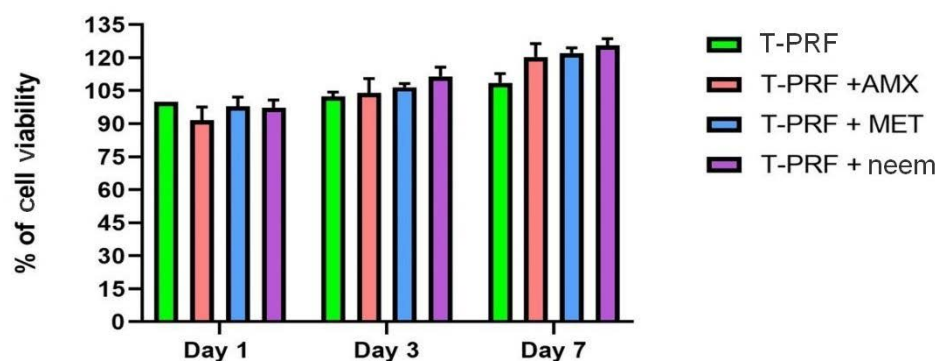


Fig. 5 – Biocompatibility of T-PRF clots incorporated with AMX, MET, and neem gels, along with the control group (T-PRF alone), on osteoblast cells was evaluated on days 1, 3, and 7 by MTT assay. T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel; MTT – [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide].

Table 4

Mean gene expression levels of OSP, ALP, and BMP-2 measured by qPCR in the T-PRF treatment groups on day 7

Groups	OSP	ALP	BMP-2
T-PRF alone	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
T-PRF + AMX	3.43 ± 0.80	3.03 ± 0.11	2.53 ± 0.75
T-PRF + MET	4.83 ± 1.09	3.49 ± 0.29	3.20 ± 0.85
T-PRF + neem	5.33 ± 0.39	13.31 ± 2.03	6.61 ± 1.59
<i>H</i> -test	8.191	9.804	9.024
<i>p</i> -value	0.0422*	0.0203*	0.0290*

OSP – osteopontin; ALP – alkaline phosphatase; BMP-2 – bone morphogenetic protein-2; qPCR – quantitative polymerase chain reaction; T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

Values are given as mean ± standard error.

Note: *Statistically significant difference ($p < 0.05$). *H* – primary test statistic for the Kruskal-Wallis test.

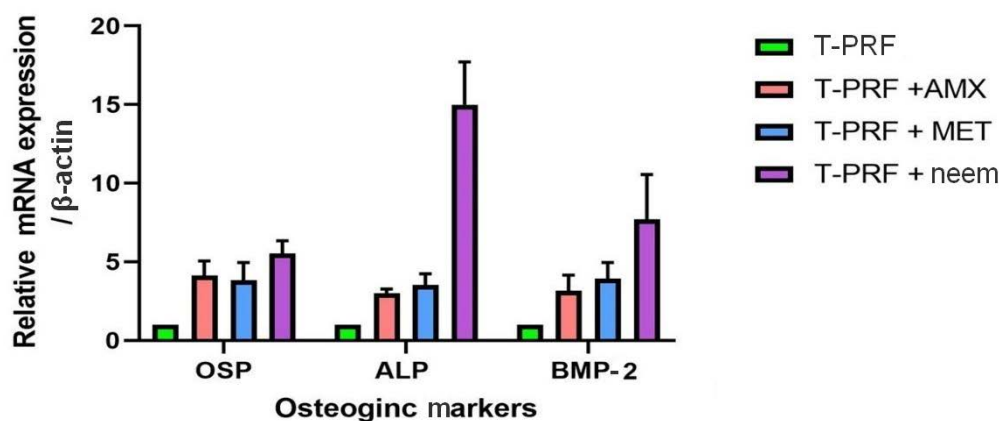


Fig. 6 – Expression of selected osteoblastic markers based on relative mRNA levels (qPCR) in osteoblast cells continuously treated with T-PRF clots incorporated with AMX, MET, and neem gels, along with the control group, for 7 days.

Note: Average values ± standard deviation are plotted, indicating significantly higher levels in the T-PRF + neem group when compared with other groups.

mRNA – messenger ribonucleic acid; qPCR – quantitative polymerase chain reaction; T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

Quantitative polymerase chain reaction (day 7)

All test groups (T-PRF + AMX/MET/neem) showed a significant increase in OSP expression ($p = 0.0422$)

compared to the control group (T-PRF alone). A similar trend of significantly increased ALP ($p = 0.0203$) and BMP-2 ($p = 0.0290$) levels across all test groups was noted compared with the control group (Table 4 and Figure 6).

However, when Dunn's *post-hoc* test was performed for group comparison, all differences were non-significant, except for the comparison between the T-PRF + neem group and T-PRF alone (Table 5).

Alizarin red S staining (day 21)

On day 21, ARS analysis revealed statistically significant differences among all groups ($p < 0.001$). T-PRF + neem demonstrated the highest mineralization and calcium deposition, followed by T-PRF + MET and T-PRF + AMX, while T-PRF alone showed the lowest values (Table 6).

Post-hoc tests confirmed significant differences for each test group compared to T-PRF alone ($p < 0.05$). When comparisons were performed among the test groups, a

significant difference ($p = 0.0133$) was reported for T-PRF + neem compared with T-PRF + AMX, while the remaining comparisons were non-significant ($p > 0.05$) (Table 7). The results showed increased calcium deposition in both T-PRF + AMX and T-PRF + MET compared to T-PRF alone. The neem-incorporated T-PRF group demonstrated relatively higher mineralization than the T-PRF + AMX/MET groups, suggesting enhanced late-stage osteogenic activity. This mineralization evidence provides functional confirmation of osteogenic differentiation when observed under a phase-contrast microscope (Figure 7). Thus, the T-PRF + neem group exhibited greater mineralization and a stronger osteoinductive effect than T-PRF alone. The second highest representation was recorded for T-PRF + MET, followed by T-PRF + AMX, with T-PRF alone showing the lowest values (Figure 8).

Table 5

Dunn's *post-hoc* multiple comparison test (Bonferroni-adjusted) for gene expression of qPCR analysis

Comparisons	OSP	ALP	BMP-2
T-PRF vs. T-PRF + AMX	1.0000	1.0000	1.0000
T-PRF vs. T-PRF + MET	0.2407	0.4085	0.4085
T-PRF vs. T-PRF + neem	0.0372*	0.0125*	0.0182*
T-PRF + AMX vs. T-PRF + MET	1.0000	1.0000	1.0000
T-PRF + AMX vs. T-PRF + neem	1.0000	0.4085	0.6624
T-PRF + MET vs. T-PRF + neem	1.0000	1.0000	1.0000

qPCR – quantitative polymerase chain reaction; OSP – osteopontin; ALP – alkaline phosphatase; BMP-2 – bone morphogenetic protein; T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

Note: *Statistically significant ($p < 0.05$).

Table 6

Mean values of ARS staining among the groups on day 21 (one-way ANOVA)

Values	T-PRF	T-PRF + AMX	T-PRF + MET	T-PRF + neem	<i>F</i>	<i>p</i> -value
Mean ± SE	0.33 ± 0	0.82 ± 0.02	1.01 ± 0.06	1.50 ± 0.04	266.154	< 0.0001*
<i>p</i> -value	0	0.0018*	0.0023*	0.0006*		

ARS – alizarin red S; ANOVA – analysis of variance; T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel; SE – standard error.

Note: *Statistically significant ($p < 0.05$).

Table 7

The *post-hoc* pairwise comparisons among all the groups for ARS staining on day 21

Group-wise comparison	<i>p</i> -value
T-PRF + AMX vs. T-PRF + MET	0.7484
T-PRF + AMX vs. T-PRF + neem	0.0133*
T-PRF + MET vs. T-PRF + neem	0.0645

ARS – alizarin red S; T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

Note: *Statistically significant ($p < 0.05$).

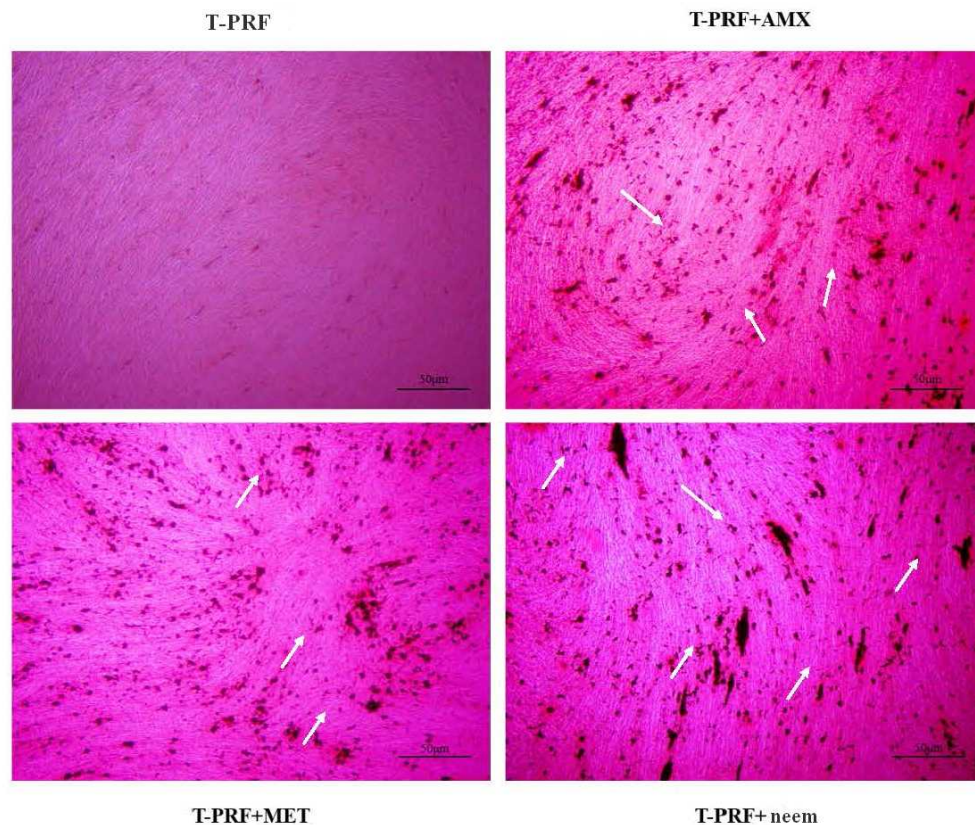


Fig. 7 – Alizarin red S staining of osteoblast cultures treated with T-PRF alone (control), antibiotic-incorporated (AMX/MET) T-PRF, and neem-incorporated T-PRF clots after 21 days of incubation at 50 µm scale bar at ×20 magnification. The staining and white arrows indicate calcium-rich mineral deposition, a hallmark of osteogenic differentiation and suggesting an enhanced osteogenic potential.

T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel.

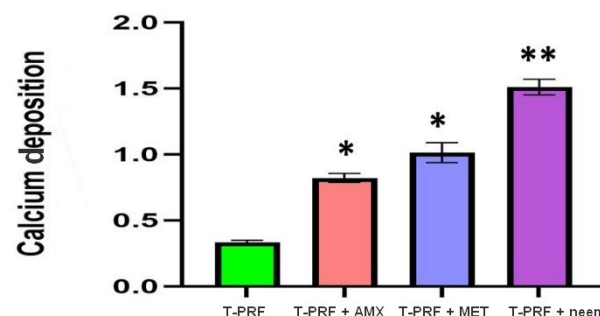


Fig. 8 – Quantitative analysis of mineral deposition by measuring ARS stain eluted from osteoblast cultures at day 21. Absorbance was recorded at 405 nm. Both the antibiotic + T-PRF and neem + T-PRF groups showed significantly higher calcium deposition compared to the control, with the neem group exhibiting the highest mineralization.

Data represent mean ± standard error.

ARS – alizarin red S; T-PRF – titanium-prepared platelet-rich fibrin; AMX – amoxiclav gel; MET – metronidazole gel; n – number.

Note: ** Indicates statistical significance for the T-PRF + neem group compared with all groups, and * also indicates statistical significance of T-PRF + AMX/MET when compared with T-PRF alone (control).

Discussion

To assess cell viability in osteoblastic cells, the current study looked at a novel therapy regimen that included herbal extract and antibiotics in T-PRF for gene expression and

differentiation potential. Cell viability and differentiation were determined using MTT; qPCR was used to assess gene expression, and verification of mineralization was done by ARS staining. The study utilized MG-63 cells—human osteosarcoma cells. These osteosarcoma cell lines, in

contrast to normal osteoblast cells, have a different route that includes fewer or faulty interactions with immune cells. This immunological response may also be influenced by the microenvironment of carcinoma cell lines and the absence of significant expression of major histocompatibility complex class I (MHC I). This may partially explain the absence of detectable abnormalities in the tests and the lack of observed immunological reactions³⁶. This research offers a method for identifying T-PRF as SDDS. The specific antibiotics used in the current study were selected because they represent established standard treatments for periodontitis³⁷.

Additionally, the rationale behind utilizing neem extract in the current study was its antibacterial, antioxidant, and analgesic effects. The current study used MG-63 osteosarcoma cells because of their replication as osteoblastic cells and their assistance with the study protocols. According to a histological analysis by Gummaluri et al.³⁸, a thin fibrin network formed when neem gel was injected into T-PRF clots. These characteristics were not statistically significant for T-PRF alone, and the cells had a 26–75% score with a scattered or layered arrangement and a wider distribution. This suggested that herbal extracts could be indirectly integrated into T-PRF without significantly changing the fibrin structure.

Gummaluri et al.³⁹ further conducted a histological study evaluating the incorporation of antibiotic gels, such as AMX and MET, and neem herbal extract gel into T-PRF. The research examined the results of adding AMX/MET or neem to T-PRF using light microscopy and SEM. It was determined that adding drugs or herbal extract into clots did not change the fibrin structure. The haziness captured in the SEM images suggested that these antibiotics or herbal extracts had successfully penetrated and adhered to the T-PRF fibrin surface, creating a system for continuous drug release. Since platelet concentrates are known to retain growth factors and release them over a period of time, Gummaluri et al.⁴⁰ conducted another study in which they incorporated neem and *Triphala (T.) indica* gels into T-PRF clots. The release of IGF-1, vitamin C, and azadirachtin was evaluated at 6 hrs, 72 hrs, 7 days, and 14 days using individual assay methods. The findings indicated that both herbal groups demonstrated consistent release of vitamin C and azadirachtin throughout the duration of the experiment, with no significant differences between them. Even IGF-1 production was not suppressed. This demonstrates that T-PRF may be employed as an SDDS. The majority of published studies were limited to light microscopy and SEM. Previous research findings were connected with the literature on the interactions or combination forms of antibiotics and T-PRF^{38–40}. Due to the scarcity of information on T-PRF clots on osteoblast cells treated with AMX, MET, or neem, the present study was performed.

The current study findings show a mixed pattern compared with Manzano-Moreno et al.⁴¹, in terms of the results of AMX-incorporated T-PRF. Manzano-Moreno et al.⁴¹ concluded in their study that plain amoxicillin had a beneficial impact on osteoblastic differentiation at concentrations up to 400 µg/mL, which resulted in higher

expression of TGF-β receptor type 1, type I collagen, and osteocalcin. They also found that 150 g/mL clindamycin is far more effective at sterilizing the intraoral autogenous grafts. Their research also showed that clindamycin was a superior antibiotic. These two studies differed mostly in the type of materials utilized and the distinct application of T-PRF, which potentially demonstrated increased viability and greater gene expression of BMP-2, ALP, and OSP. Given the favorable findings regarding cell viability and gene expression in the present study, it is possible that the anti-inflammatory effects of amoxicillin/clavulanate contributed to the observed effects in the T-PRF + AMX group, potentially explaining the favorable gene expression and osteogenic effects observed in this group^{12, 13}. The current results regarding the T-PRF + AMX group can be compared to Bilginaylar et al.⁴², who stated that antibiotic-loaded PRF had better outcomes—including accelerated tissue healing and reduced postoperative pain and trismus—compared to PRF alone. They also concluded that antibiotic-incorporated PRF reduced inflammation and bacterial load. The difference from the previously quoted study was the type of platelet concentrates used; the method of injecting antibiotics or herbal extract was different, but the addition of antibiotics and herbal extract had good cell viability and gene expressions.

The current study findings of MET integrated T-PRF for qPCR and MTT assay were similar to those of Duggal et al.⁴³, where they came to the conclusion that at 7 days, MET showed positivity for ALP and BMP activity, along with increased osteoblastic differentiation. Osteogenic differentiation and the development of mineralized nodules were additional outcomes seen three weeks after therapy. When MET was employed, the messenger ribonucleic acid – mRNA levels of osteogenic genes were elevated in bone mesenchymal stem cells. The nitroimidazole ring and the diminished anti-inflammatory impact may be responsible for the beneficial osteogenic action of the current study. The MET effect may also involve activation of the Wnt pathway, which is thought to be responsible for the expression of osteogenic indicators such as BMP-2 or Runx2, essential transcription factors for hard tissue formation, development, differentiation, and regeneration.

Current research indicates that both neem-incorporated (T-PRF + neem) and antibiotic-incorporated (T-PRF + AMX/MET) clots significantly enhance osteoblast viability and osteogenic differentiation compared to the control. Notably, the T-PRF + neem group demonstrated substantially superior outcomes numerically. The bioactive substances in neem, such as azadirachtin, nimbidin, nimbolide, and nimbin, may be responsible for the neem-incorporated T-PRF's better performance, which have been demonstrated to regulate cell responses, improve antibacterial and anti-inflammatory qualities, and aid bone healing. Neem components also reduce inflammatory mediators like tumour necrosis factor (TNF)-α, IL-1, and IL-6 that would decrease osteogenesis. It also shows down-regulation of receptor activator of nuclear factor kappa beta ligand – RANKL and helps in up-regulation of osteoprotegerin⁴⁴.

The results of the current study were further supported by qPCR analysis, which revealed higher gene expression of key osteogenic markers, such as OSP, ALP, and BMP-2, in all test groups compared with T-PRF alone, with the highest expression observed in the T-PRF + neem group. The T-PRF + neem group showed significantly higher expression of all three markers than the T-PRF-alone group. Neem-integrated T-PRF is indicative of a more positive osteogenic environment, as it supports bone formation and osteoblast differentiation. Neem gel produced better osteogenic potential than the antibiotic-incorporated T-PRF gels. Though the presence of antibiotics may have contributed to the positive osteogenic potential, the discrepancy among group results may be attributed to prolonged periods of cellular stress or restricted proliferation^{45, 46}.

In the present study, the antibiotics and the herbal extract were added post-centrifugation. Cell viability and gene expression were then evaluated in osteoblastic cell lines, demonstrating promising outcomes. These findings can be indirectly compared to a recent systematic review by Niemczyk et al.⁴⁶, which examined 13 *in vitro* experiments. In those studies, antibiotics were incorporated into the PRF using various techniques, including direct addition prior to blood centrifugation⁴⁷. It was determined that systemic administration of antibiotics was the most effective approach. The addition of antibiotics prior to centrifugation enhanced the antimicrobial activity of PRF and resulted in larger zones of inhibition. The antimicrobial properties of PRF were superior to those of many alternative materials, including collagen sponges. As a result, they also claimed that antibiotic-loaded PRF is a powerful tool for targeted antimicrobial delivery and may be a viable choice in clinical practice. Thus, the current study demonstrates that the T-PRF + neem group provides a treatment strategy for dentistry to utilize herbal extracts in future non-surgical and surgical periodontal treatments. Neem components have many positive properties, such as anti-inflammatory, antibacterial, antioxidant, and antiviral, which are very important for good wound healing (both soft and hard tissues)⁴⁸. Assessing the ALP, BMP-2, and OSP gene expressions with late-stage ARS mineralization assays was a key strength of the current research to determine the importance of this novel treatment protocol and also to use T-PRF as SDDS.

Current study results of herbal extract neem shared a similar pattern with that of a recent study performed by Gummaluri et al.⁴⁹. *Mangosteen rind* and *T. indica* gel extracts were injected into T-PRF clots and checked for MTT assay, qPCR gene expressions, and ARS staining, where they concluded both that the treatment groups showed similar cell viability and that T-PRF + *T. indica* showed better gene expression. They also concluded that

these herbal extracts added to T-PRF and subjected to future human trials might be better for hard tissue healing. Therefore, this suggests that T-PRF can be used as SDDS.

Limitation of the study

A limitation of the present study was the small sample size. The MTT assay, gene expressions, and ARS analyses performed in the current study had restricted gene and time points, which may affect the study outcomes. Checking gene expressions both pre- and post-treatment at multiple time points can help in the proper establishment of a comprehensive picture of the biological process (i.e., multi-time-point gene expression would offer deeper insight into the kinetics of osteogenic differentiation). Another limitation of the study was the use of MG-63 osteosarcoma cells instead of primary human osteoblasts. Further, a larger sample size with more replicates would improve statistical validity. Experiments with human osteoblast cells and *in vivo* trials might have enhanced the outcomes of the study. This study was an initial exploratory study, and *in vitro* model applicability is confined to testing the initial lab findings. Translating *in vitro* results to clinical settings through animal and clinical trials will provide better-established scientific outcomes to roll out this treatment strategy of T-PRF as SDDS into routine dental practice.

Conclusion

The present study concluded that both antibiotic (amoxicillin/clavulanate or metronidazole) and neem-incorporated titanium-platelet rich fibrin clots demonstrated osteogenic potential compared with titanium-platelet rich fibrin clots alone. Given the growing concern over antibiotic resistance, neem extract offers a promising alternative for bone regeneration without compromising antimicrobial properties. The enhanced osteoblast proliferation and elevated expression of osteogenic markers with neem extract suggest its potential as a biocompatible, osteogenic agent for regenerative therapies. Further *in vitro* studies using primary cells and additional blood donors, as well as subsequent validation in animal models and randomized clinical trials for *in vivo* use, are recommended to confirm these findings.

Conflicts of interest

The authors declare no conflict of interest.

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Development and preliminary psychometric evaluation of three context-specific workplace scales among Serbian community pharmacists

Razvoj i preliminarna psihometrijska procena tri kontekstualno specifične skale radnog okruženja među farmaceutima u javnim apotekama u Srbiji

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Abstract

Background/Aim. Pharmaceutical practice requires not only technical expertise but also interpersonal, motivational, and leadership-related competencies. However, there is a lack of context-specific instruments for assessing these constructs in pharmacy practice. The aim of this study was to develop and conduct an initial psychometric evaluation of the Workplace Personality Scale (WPS), the Achievement Motivation Scale (AMS), and the Agile Leadership Scale (ALS) among Serbian community pharmacists. **Methods.** Items were developed through a literature review and expert input, followed by content review and pilot testing. The final instruments were administered to 633 pharmacists. Internal consistency was evaluated using Cronbach α coefficient, while exploratory factor analysis (EFA) was conducted to examine the internal structure. Correlation analyses and group comparisons were performed using appropriate statistical tests. **Results.** All scales demonstrated satisfactory to high internal consistency ($\alpha = 0.855–0.947$). EFA revealed a

four-factor structure for the WPS and predominantly unidimensional structures for AMS and ALS. Participants reported relatively high workplace-related behavioral tendencies and achievement motivation, while perceptions of supervisory support and adaptive leadership behaviors were more moderate. Positive to strong associations were observed among the scales ($r = 0.495–0.711$). Differences were found according to gender, age, and professional experience, while other demographic variables showed no consistent associations. **Conclusion.** The study provides preliminary evidence on the internal structure and reliability of three context-specific instruments developed for Serbian community pharmacists. Further validation is required, including confirmatory factor analysis, test-retest reliability, and assessment of external validity, before broader application.

Keywords:

data interpretation, statistical; motivation; pharmacy; pharmacists; serbia; surveys and questionnaires; workplace.

Apstrakt

Uvod/Cilj. Farmaceutska praksa zahteva ne samo tehničku ekspertizu, već i interpersonalne, motivacione i liderske kompetencije. Međutim, postoji nedostatak kontekst-specifičnih instrumenata za procenu ovih osobina u farmaceutskoj praksi. Cilj rada bio je razvoj i inicijalna psihometrijska evaluacija Skale ličnosti na radnom mestu (*Workplace Personality Scale* – WPS), Skale motivacije za postignuća (*Achievement Motivation Scale* – AMS) i Skale agilnog liderstva (*Agile Leadership Scale* – ALS) kod farmaceuta zaposlenih u javnim apotekama u Srbiji. **Metode.** Stavke su razvijene na osnovu pregleda literature i ekspertске procene, nakon čega su sprovedeni pregled sadržaja i pilot testiranje. Konačni instrumenti primenjeni su na uzorku od 633 farmaceuta. Interna

konzistentnost procenjena je pomoću Cronbach-ovog α koeficijenta, dok je za ispitivanje interne strukture sprovedena eksploratorna faktorska analiza (EFA). Analize korelacije i poređenja između grupa sprovedene su primenom odgovarajućih statističkih testova. **Rezultati.** Sve skale pokazale su zadovoljavajuću do visoku internu konzistentnost ($\alpha = 0.855–0.947$). EFA je ukazala na četvorofaktorsku strukturu WPS i pretežno jednodimenzionalne strukture za AMS i ALS. Učesnici su prijavili relativno učestale biheioralne tendencije povezane sa radnim mestom i motivaciju za postignućem, dok su percepcije podrške supervizora i adaptivnog liderskog ponašanja bile ređe. Primećene su pozitivne do jake povezanosti između skala ($r = 0,495–0,711$). Utvrđene su razlike u odnosu na pol, starost i profesionalno iskustvo, dok druge demografske varijable

nisu pokazale konzistentne povezanosti. **Zaključak.** Studija pruža preliminarne dokaze o unutrašnjoj strukturi i pouzdanosti tri kontekst-specifična instrumenta razvijena za farmaceute zaposlene u javnim apotekama u Srbiji. Pre njihove šire primene potrebna je dalja validacija, uključujući konfirmatornu faktorsku analizu,

procenu pouzdanosti metodom test-retest i procenu spoljašnje validnosti.

Ključne reči:

statistička analiza podataka; motivacija; apoteka; farmaceuti; srbija; ankete i upitnici; radno mesto.

Introduction

Pharmaceutical practice is a demanding and dynamic profession that requires not only technical expertise but also strong interpersonal, motivational, and leadership skills to ensure high-quality patient care and organizational effectiveness. Previous research has examined various psychological and motivational factors in healthcare professionals, highlighting the influence of personality traits, achievement motivation, and leadership on work performance and professional development¹⁻⁵.

However, most studies have examined these dimensions in isolation, and few instruments specifically measure these phenomena in pharmacy practice, particularly in community and industrial pharmacy settings^{6,7}. Existing tools are either too general or adapted from other healthcare or organizational contexts, limiting their relevance for evaluating pharmacists' professional behavior, motivation, and perceptions of leadership⁸⁻¹⁰.

The relationship between workplace personality, achievement motivation, and perceived leadership can be theoretically understood through shared work-related behavioral and motivational processes. Employees with more positive work-related behavioral tendencies may be more likely to demonstrate higher levels of achievement motivation, as both constructs reflect goal-directed engagement, responsibility, and proactive work behavior. In addition, perceived leadership support may further reinforce motivational processes by shaping the work environment, enhancing autonomy, and facilitating adaptive responses to workplace demands. From this perspective, these constructs are expected to be interrelated as part of a broader pattern of work-related functioning within healthcare settings. However, the present study adopts an exploratory approach, without testing predefined directional hypotheses.

To address this gap, the present study has two main objectives. First, it aims to develop and conduct a preliminary psychometric evaluation of three novel scales specifically adapted to the Serbian pharmaceutical context: the Workplace Personality Scale (WPS), the Achievement Motivation Scale (AMS), and the Agile Leadership Scale (ALS). These instruments are conceptualized as context-specific measurement tools capturing work-related behavioral tendencies, motivational orientations, and perceived leadership support within pharmacy practice, rather than as direct operationalizations of established global trait models. The WPS was not developed as an alternative to established personality models such as the Five-Factor Model (FFM)¹¹ or the honesty-humility (H), emotionality (E), extraversion (X), agreeableness (A), conscientiousness (C), and openness to experience (O) –

HEXACO model¹². Rather, it was designed to assess context-specific behavioral and attitudinal tendencies expressed in pharmacy practice, including responsibility, cooperation, work engagement, and professional orientation. Consequently, the construct labeled workplace personality should be understood as a contextualized pattern of work-related behavioral dispositions rather than a measure of stable global personality traits. The AMS was informed by the broader achievement motivation tradition, drawing conceptually on theories that emphasize goal striving, persistence, personal responsibility, and self-directed effort in professional contexts¹³⁻¹⁷. This tradition encompasses several theoretical perspectives, including need-based, expectancy-value, and self-determination approaches to achievement behavior. Rather than attempting to distinguish between specific theoretical components such as intrinsic motivation, self-efficacy, or goal orientation, the present study adopts a pragmatic and context-specific approach, conceptualizing achievement motivation as a general tendency toward achievement-oriented behavior in pharmacy practice. The ALS was developed to assess employees' perceptions of supervisory behaviors characterized by adaptability, support, responsiveness, and facilitation of work processes in dynamic healthcare environments. The construct is conceptually related to established leadership frameworks, including perceived supervisor support¹⁸, transformational leadership¹⁹, and servant leadership²⁰. However, the term agile leadership is used in this study to emphasize leadership behaviors that facilitate adaptation to changing workplace demands and support collaborative problem-solving within pharmacy teams. It is acknowledged that conceptual boundaries between these constructs remain subject to ongoing theoretical discussion. In this study, agile leadership is conceptualized as perceived adaptive supervisory support (as measured by the ALS), including flexibility, communication, support, and facilitation of work processes in pharmacy teams. The term 'agile leadership' is used as a pragmatic descriptor rather than a distinct theoretical leadership model. Second, the study seeks to examine the relationships among personal characteristics, achievement motivation, and perceptions of supervisory support. Rather than testing causal relationships or predictive models, it explores preliminary associations among these constructs, offering a contextualized understanding of the factors that support professional development, effective management, and employee engagement. These constructs are not intended to represent distinct classical psychological taxonomies but context-specific workplace-relevant composites.

By integrating scale development with empirical investigation, this study provides initial evidence on the structure and relationships of workplace personality, achievement motivation, and perceived agile leadership within

the Serbian pharmacy context. The focus is on exploratory rather than confirmatory conclusions. The developed instruments represent early-stage, context-specific measures that require further validation, including confirmatory factor analysis (CFA), test-retest reliability, and external criterion validation in future research. All three instruments should be interpreted as context-specific behavioral and perceptual measures rather than operationalizations of established global trait or leadership taxonomies.

Methods

Ethical approval

The study protocol was reviewed and approved by the Ethics Committee of the Pharmaceutical Chamber of Serbia (No. 30/1–4, from February 7, 2025). All participants provided informed consent prior to participation. Data were collected anonymously and treated confidentially in accordance with ethical guidelines for human research.

Scale development and preliminary psychometric evaluation

The study involved developing and preliminarily psychometrically evaluating three novel scales designed specifically for the Serbian pharmaceutical context: the WPS, AMS, and ALS. The scale development process comprised five sequential phases. First, during item generation, items for each scale were generated based on an extensive review of the literature on workplace personality traits^{21–27}, achievement motivation^{28–31}, and agile leadership^{32–35}, combined with qualitative input from practicing pharmacists. These items were drafted to reflect behaviors and attitudes relevant to community pharmacy settings as perceived within this specific occupational context. Second, content validation was conducted by a panel of six experts, including one psychologist, one human resources professional with expertise in organizational behavior, one faculty professor from the School of Pharmacy, and three practicing pharmacists. The panel independently reviewed the item pool to assess relevance, clarity, and comprehensiveness, resulting in minor wording revisions without item additions or deletions. Content validity relied on qualitative expert evaluation, as quantitative indices such as the Content Validity Index (CVI) were not calculated in this study and remain recommended for future validation research. Third, during linguistic and contextual development, items were developed in Serbian within the target occupational context, ensuring linguistic and conceptual appropriateness for the Serbian pharmacy setting. All three scales were created and contextually and linguistically adapted according to established guidelines for scale development and adaptation^{36, 37}. Fourth, pilot testing was conducted, with the preliminary scales administered to a small sample of pharmacists ($n = 10$) to assess item clarity, comprehension, and feasibility. This phase was limited to

face validity assessment and was not used for psychometric analysis. Minor revisions were made based on participant feedback. Finally, reliability assessment involved evaluating the internal consistency of each scale using Cronbach α . Exploratory factor analysis (EFA) was performed to examine the underlying factor structure as an initial exploratory step in scale development rather than confirmatory validation. The present study represents an initial stage of scale development based on EFA, while CFA was not conducted and is recommended for future validation studies using independent samples.

Participants and sample size

The final survey was administered to a sample of 633 pharmacists working in community pharmacy settings in Serbia. Participants were recruited through professional associations and workplace contacts. Inclusion criteria required participants to be licensed pharmacists actively working in a professional pharmacy setting.

Sample size was determined to ensure adequate power for both factor analysis and correlational studies, following standard recommendations for scale validation (at least 5–10 participants *per item*) and population-based sampling considerations.

This study represents a cross-sectional convenience sample and is not intended for population-level generalization.

Instruments

Participants completed three measurement instruments. First, the WPS assessed work-related behavioral tendencies and attitudes in pharmacy practice. Items reflected behavioral and attitudinal patterns associated with work engagement, responsibility, cooperation, and task orientation in the workplace context. The scale does not operationalize established personality taxonomies, but rather reflects context-specific work-related behavioral patterns. Here, the term ‘workplace personality’ is used as a contextual label reflecting patterns of work-related behavioral tendencies rather than stable personality traits as conceptualized in major trait theories. Second, the AMS assessed work-related motivational orientation in pharmacy practice, including goal striving, persistence, self-directed effort, and responsibility for achieving professional outcomes. The scale was developed based on established theoretical frameworks of achievement motivation, encompassing goal-directed behavior and self-regulatory processes in professional settings. Finally, the ALS assessed perceptions of supervisory behaviors related to support, adaptability, communication, and facilitation of work processes in pharmacy teams. In this study, this construct is conceptualized as perceived adaptive supervisory support rather than a distinct theoretical leadership framework. The scale reflects perceived adaptive and supportive leadership in dynamic healthcare environments and overlaps conceptually with broader constructs, such as perceived supervisor support

and transformational leadership. All scales used a 5-point Likert scale (1 – Never, 5 – Always). Mean scores were computed for each subscale and the overall scale to allow for subsequent analyses. The full versions of these instruments are provided in Supplementary Table 1.

Table 1**Demographic characteristics of the sample**

Variable	Values
Gender	
female	579 (91.5)
male	54 (8.5)
Marital status	
single	409 (64.6)
married	139 (22.0)
divorced	79 (12.5)
widowed	6 (0.9)
Educational level	
pharmacy graduate	525 (82.9)
specialist studies	103 (16.3)
PhD	5 (0.8)
Age, years	46.58 ± 10.45
Experience, years	19.02 ± 9.86

PhD – Doctor of Philosophy.

All values are given as numbers (percentages) or mean ± standard deviation.

Statistical analysis

Data were analyzed using SPSS (IBM SPSS Statistics 31.0.2.0). Descriptive statistics were performed [mean ± standard deviation (SD), range] for all items and scales. Reliability analysis was conducted using Cronbach α for internal consistency. EFA was conducted using principal axis factoring with varimax rotation to examine the underlying factor structure. Correlation analysis was conducted to examine associations among workplace-related constructs. For the comparative analysis, differences in the WPS, AMS, and ALS scores across demographic variables (gender, marital status, age, work experience, and educational level) were assessed using statistical tests appropriate to the data distribution. Normality of each variable was examined, and depending on the distribution, independent *t*-tests or one-way analysis of variance – ANOVA were used for normally distributed data, while the Mann-Whitney *U* or Kruskal-Wallis *H* tests were applied for non-normally distributed data. Given observed non-normality, non-parametric tests were ultimately applied for all comparative analyses.

Results

Participant characteristics

The final sample consisted of 633 pharmacists working in community pharmacies. The majority of participants were female (91.5%, *n* = 579) and single (64.6%, *n* = 409). The participants' educational levels varied, with most holding a pharmacy degree (82.9%, *n* = 525), followed by specialist studies (16.3%, *n* = 103) and Doctor of Philosophy (PhD) degrees (0.8%, *n* = 5). Participants' ages ranged from 25 to 77

years (mean = 46.58, SD = 10.45), with work experience ranging from 1 to 44 years (mean = 19.02, SD = 9.86) (Table 1).

For comparative analyses, age was categorized into five groups: group 1 (25–29 years), group 2 (30–40 years), group 3 (41–50 years), group 4 (51–60 years), and group 5 (61–77 years). Years of professional experience were similarly categorized: group 1 (1–5 years), group 2 (6–10 years), group 3 (11–20 years), group 4 (21–30 years), and group 5 (31–44 years).

Descriptive statistics of scale items

Descriptive statistics for all items from the three newly developed scales – the WPS, AMS, and ALS (reflecting perceived adaptive supervisory support) – were calculated. Means and SDs were obtained for each item, with responses scored on a 5-point Likert scale (1 – Never, 5 – Always).

For the WPS (15 items), participants reported generally high self-ratings of work-related behavioral tendencies related to collaboration, responsibility, and motivation, with mean scores ranging from 3.50 to 4.80 (SD = 0.46–0.90). The highest-rated items were those reflecting responsibility for work and willingness to learn new techniques.

For the AMS (7 items), participants indicated strong achievement motivation in professional practice, with mean scores ranging from 4.00 to 4.61 (SD = 0.55–0.95). Participants particularly endorsed items reflecting effort to achieve the best results and belief in their ability to succeed.

For the ALS (7 items), ratings of supervisors' perceived adaptive supervisory support behaviors were moderate, with mean scores ranging from 3.21 to 3.50 (SD = 1.11–1.33). The highest-rated behaviors included support for professional development and flexibility in adapting to new challenges.

Scale-level descriptive statistics

Means and SDs were also calculated at the scale level by averaging responses across all items within each scale. Participants demonstrated high scores on the WPS and AMS, while the ALS ratings were more moderate. This pattern suggests that, in general, pharmacists perceive themselves as motivated and responsible professionals, whereas their evaluation of leadership behaviors is more variable.

The range indicates the minimum and maximum possible scores for each item (1 – Lowest, 5 – Highest). Means and SDs were calculated across all items within each scale.

Detailed item-level descriptive statistics are provided in Supplementary Table 2.

Scale development and linguistic/contextual adaptation

All three scales (WPS, AMS, and ALS) were developed based on a thorough review of relevant literature and theoretical frameworks. Items were generated to capture all relevant dimensions of each construct, and expert review ensured clarity, conceptual relevance, and coverage of the constructs. The scales were subsequently developed directly within the Serbian-language healthcare context, with

linguistic refinement to ensure clarity and comprehensibility for the target population. Although the scales were originally developed in Serbian, back-translation procedures were used to support potential future cross-cultural application rather than to establish cross-cultural equivalence in the current study. Pilot testing with a small sample of Serbian community pharmacists ($n = 10$) confirmed comprehension and relevance of all items. The final versions retained the original item structure and response format, ensuring both conceptual fidelity and cultural appropriateness.

Reliability and item analysis

Internal consistency of the three scales was examined using Cronbach α , and descriptive statistics were computed for each scale (Table 2, Supplementary Table 2).

All three scales demonstrated high internal consistency. The item-total correlations were satisfactory across all scales, indicating that each item contributed appropriately to the total scale score. Inter-item correlations were moderate to high, suggesting coherent scale structures without redundancy. Notably, no item deletion substantially increased Cronbach α , confirming that all items were retained in the final scales.

Descriptive statistics showed that respondents generally rated high on the WPS, particularly on items related to task efficiency and responsibility. The AMS revealed a strong orientation toward personal goal setting and professional improvement, while the ALS reflected moderate perceptions

of supervisor support and leadership flexibility in the workplace.

Overall, the adapted scales demonstrated strong reliability and satisfactory preliminary psychometric properties, supporting their potential usefulness for research on Serbian community pharmacists.

Inter-scale correlations were examined as preliminary evidence regarding associations among the measured constructs. Pearson correlations revealed that all three scales are positively related ($p < 0.001$), indicating that workplace personality, achievement motivation, and agile leadership are associated constructs in the context of Serbian community pharmacists. The strongest correlation was observed between the WPS and AMS ($r = 0.711$), suggesting that conscientiousness, responsibility, and efficiency are closely linked to professional motivation. The ALS correlated moderately with both the WPS ($r = 0.495$) and AMS ($r = 0.538$), demonstrating that leadership behaviors are related to, but distinct from, personal traits and motivation. These results provide preliminary evidence of relationships among the constructs, but do not support conclusions regarding full construct validity.

Exploratory factor analysis results

EFA was conducted for all three scales. The results indicated adequate factorability and interpretable factor solutions for each instrument (Tables 3–5). The WPS

Table 2

Descriptive statistics and reliability of the adapted scales

Scale	Number of items	Mean $\pm$ SD	Cronbach α	Cronbach α (standardized)
Workplace Personality Scale	15	66.94 $\pm$ 5.89	0.855	0.870
Achievement Motivation Scale	7	30.48 $\pm$ 4.02	0.878	0.883
Agile Leadership Scale	7	23.82 $\pm$ 7.47	0.947	0.949

SD – standard deviation.

Table 3

Rotated factor loadings and communalities – Workplace Personality Scale

Item	F1	F2	F3	F4	h^2
I am satisfied with my work when I can help users with their problems	0.566	0.277	0.175	-	0.428
I enjoy collaborating with colleagues to solve problems	0.563	0.160	0.101	-	0.353
I help new colleagues adapt to the work environment	0.548	-	0.346	0.324	0.525
I like to take responsibility for my work	0.494	0.167	0.204	0.281	0.392
I try to maintain a positive attitude with users, even in stressful situations	0.472	0.292	0.365	-	0.443
I feel calm even when I have many tasks to complete	0.465	0.302	-	0.114	0.326
I usually find it easy to adapt to new situations	0.447	0.439	-	0.117	0.415
I strive to develop my organizational skills	0.152	0.888	0.205	-	0.854
I feel motivated to always provide the best service to users	0.337	0.634	0.108	-	0.530
I understand how my behavior affects the workplace atmosphere	0.210	0.402	0.213	0.230	0.303
I am willing to learn new techniques and improve my skills	0.337	0.369	0.333	-	0.366
I enjoy completing tasks efficiently and accurately	-	0.306	0.733	-	0.638
I strive to always make accurate and fair decisions	0.173	-	0.596	0.135	0.405
I believe that teamwork is essential for achieving good results	0.426	0.186	0.493	0.221	0.508
I take responsibility for all mistakes that occur at work	-	-	-	0.783	0.636

Note: Extraction method – principal axis factoring. Rotation method – Varimax with Kaiser normalization. Factor loadings ≥ 0.30 are shown. h^2 – extracted communalities. Kaiser–Meyer–Olkin = 0.846; Bartlett’s test of sphericity: $\chi^2(105) = 3,238.80$, $p < 0.001$. Total variance explained by the four-factor solution = 47.47%.

Table 4

Factor loadings and communalities – Achievement Motivation Scale

Item	F1	h^2
I continue working toward goals even when facing difficulties or uncertainty	0.800	0.641
I am always motivated to improve my knowledge and skills in pharmaceutical practice	0.768	0.590
I am willing to take responsibility for achieving important results in my work	0.743	0.553
I am motivated to contribute to improving pharmaceutical services and work quality	0.730	0.533
I regularly set challenging goals that I aim to accomplish in pharmaceutical practice	0.710	0.504
I believe in my ability to succeed in the tasks assigned to me	0.666	0.444
I invest maximum effort to achieve the best possible results in my work	0.621	0.386

Note: Extraction method – principal axis factoring. Rotation not applicable. Factor loadings ≥ 0.30 are shown. h^2 – extracted communalities. Kaiser–Meyer–Olkin = 0.848; Bartlett’s test of sphericity: $\chi^2(21) = 2,181.93$, $p < 0.001$. Total variance explained = 52.14%.

Table 5

Factor loadings and communalities – Agile Leadership Scale

Item	F1	h^2
My supervisor effectively resolves conflicts within the team	0.922	0.850
My supervisor demonstrates flexibility in adapting to new challenges in pharmaceutical practice	0.887	0.787
My supervisor has a clear vision for the development of pharmaceutical practice and communicates it regularly	0.879	0.773
My supervisor makes an effort to understand the personal and professional challenges I face	0.876	0.767
My supervisor encourages me to take initiative and strive for better results	0.865	0.748
My supervisor clearly defines work tasks and priorities	0.843	0.710
My supervisor supports my development through additional training and education	0.691	0.478

Note: Extraction method – principal axis factoring. Rotation not applicable. Factor loadings ≥ 0.30 are shown. h^2 – extracted communalities. Kaiser–Meyer–Olkin = 0.926; Bartlett’s test of sphericity: $\chi^2(21) = 4,270.88$, $p < 0.001$. Total variance explained = 73.04%.

demonstrated adequate factorability, as indicated by a Kaiser–Meyer–Olkin (KMO) measure of 0.846 and a significant Bartlett’s test of sphericity [$\chi^2(105) = 3,238.80$, $p < 0.001$]. The solution revealed a four-factor structure explaining 47.47% of the total variance. This empirical structure reflects item clustering in the sample and is not intended to represent a predefined theoretical structure. Factor 1 reflected general positive work attitudes and adaptive functioning; Factor 2 reflected organizational skills and motivation; Factor 3 reflected task efficiency, accuracy, and decision-making; and Factor 4 reflected responsibility for mistakes, with some items showing cross-loadings and partial overlap between dimensions.

The AMS demonstrated adequate factorability, as indicated by a KMO measure of 0.848 and a significant Bartlett’s test of sphericity [$\chi^2(21) = 2,181.93$, $p < 0.001$]. The analysis revealed a unidimensional structure, with all seven items loading on a single factor. This factor accounted for 52.14% of the total variance. Factor loadings ranged from 0.621 to 0.800, indicating that all items contributed substantially to the overall construct of achievement motivation. This structure reflects empirical item aggregation rather than confirmation of a predefined theoretical model. Communalities ranged from 0.386 to 0.641, supporting the adequacy of the factor solution. As only one factor was extracted, rotation was not applicable.

The ALS showed a high level of factorability, as evidenced by a KMO measure of 0.926 and a significant Bartlett’s test of sphericity [$\chi^2(21) = 4,270.88$, $p < 0.001$]. The analysis revealed a unidimensional structure, with all seven items loading on a single factor. This factor explained

73.04% of the total variance. Factor loadings ranged from 0.691 to 0.922, indicating strong contributions of each item to the underlying construct of agile leadership. Communalities ranged from 0.478 to 0.850, supporting the adequacy of the factor solution. Since only one factor was extracted, rotation was not applicable. This structure reflects empirical item aggregation rather than a distinct theoretical leadership framework.

Comparative analyses across demographic groups

The distribution of scores for all three scales was examined across demographic variables, including gender, age group, marital status, years of experience, and educational level. Normality was assessed using both the Kolmogorov–Smirnov and Shapiro–Wilk tests. Results indicated that, for nearly all groups and scales, the Shapiro–Wilk test was significant ($p < 0.05$), suggesting that the assumption of normality was violated. This pattern was consistent across gender, age, marital status, experience, and educational level. It should be noted that very small subgroups, such as participants with a PhD ($n = 5$) or widowed participants ($n = 6$), further limit the reliability of normality testing in these specific groups. Due to the non-normal distribution of the scale scores across demographic groups, non-parametric methods are required for subsequent comparative analyses. Specifically, the Mann–Whitney U test was applied to compare two groups (e.g., gender), whereas the Kruskal–Wallis H test was applied to compare more than two groups (e.g., age, marital status, experience, educational level). These analyses allowed examination of potential

differences in workplace personality, achievement motivation, and agile leadership while accounting for the distributional characteristics of the data.

Nonparametric analyses were conducted to examine potential differences in the three scales based on gender, using the Mann-Whitney U test due to the non-normal distribution of all dependent variables.

For the WPS, no significant difference was observed between female ($n = 579$) and male ($n = 54$) participants ($U = 17,573.50$, $Z = 1.516$, $p = 0.129$), indicating similar scores on the WPS across genders. For the AMS, a significant difference was found between genders ($U = 18,793.50$, $Z = 2.474$, $p = 0.013$), with males reporting higher AMS scores (median = 4.59) than females (median = 4.38). For ALS, gender differences were also significant ($U = 19,455.50$, $Z = 2.978$, $p = 0.003$), with males reporting higher ALS scores (median = 3.80) compared to females (median = 3.39).

These results should be interpreted cautiously given the gender imbalance in the sample and the exploratory nature of the analyses. Nonparametric analyses using the Kruskal-Wallis H test were conducted to examine potential differences in the three scales across marital status categories (single, married, divorced, widowed) due to the non-normal distribution of the dependent variables.

For the WPS, a statistically significant difference was observed across marital status groups ($H = 10.392$, $df = 3$, $p = 0.016$). However, pairwise comparisons with Bonferroni correction did not reveal statistically significant differences between individual groups (all adjusted p -values > 0.05), including the comparison between single and divorced participants (adjusted $p = 0.123$). For the AMS, no significant difference was found among marital status groups ($H = 4.502$, $df = 3$, $p = 0.212$), indicating no statistically significant differences in the AMS scores across marital status categories. For the ALS, significant differences were observed across marital status groups ($H = 22.408$, $df = 3$, $p < 0.001$). Pairwise comparisons showed that single participants scored higher than married (adjusted $p = 0.009$), divorced (adjusted $p = 0.043$), or widowed participants (adjusted $p = 0.012$) in the ALS scores. These findings indicate statistical differences across marital status groups; however, results should be interpreted as exploratory due to small subgroup sizes. Nonparametric analyses using the Kruskal-Wallis H test were conducted to examine potential differences in the three scales across educational level categories (pharmacy graduate, specialist studies, PhD), due to the non-normal distribution of the dependent variables.

For the WPS, no significant difference was observed among educational level groups ($H = 0.689$, $df = 2$, $p = 0.709$). Pairwise comparisons with Bonferroni correction indicated no statistically significant differences between any of the groups.

For the AMS, the Kruskal-Wallis test also revealed no significant differences across educational levels ($H = 2.221$, $df = 2$, $p = 0.329$), with pairwise comparisons confirming the absence of significant contrasts.

For the ALS, results again indicated no significant differences among educational level groups ($H = 0.447$, $df = 2$, $p = 0.800$), and pairwise comparisons showed no statistically significant differences.

These findings suggest that educational level is not associated with differences in the WPS, AMS, or ALS scores within the sample. Nonparametric analyses using the Kruskal-Wallis H test were conducted to examine potential differences in the three scales across age groups, due to the non-normal distribution of the dependent variables.

For the WPS, the Kruskal-Wallis test indicated a statistically significant difference among age groups ($H = 12.595$, $df = 4$, $p = 0.013$). Pairwise comparisons with Bonferroni correction suggested that the differences were most pronounced between certain groups, although after adjustment, some contrasts did not reach the conventional significance threshold (adjusted $p > 0.05$).

For the AMS, the Kruskal-Wallis test also revealed significant differences among age groups ($H = 15.780$, $df = 4$, $p = 0.003$). Pairwise comparisons indicated that the oldest age groups tended to report higher AMS_sum scores compared with younger groups, with some adjusted p -values approaching significance.

For the ALS, significant differences were observed across age groups ($H = 16.900$, $df = 4$, $p = 0.002$). Pairwise comparisons showed that differences were mainly between the youngest and oldest groups, with adjusted p -values confirming the contrasts between certain groups (e.g., groups 1 vs. 4: adjusted $p = 0.049$; groups 4 vs. 5: adjusted $p = 0.032$).

Age was associated with differences in the WPS, AMS, and ALS scores, with older age groups generally reporting higher AMS and ALS scores. Nonparametric analyses using the Kruskal-Wallis H test were conducted to examine potential differences in scales across experience groups, due to the non-normal distribution of the dependent variables.

For the WPS, the Kruskal-Wallis test revealed a statistically significant difference among experience groups ($H = 30.367$, $df = 4$, $p < 0.001$). Pairwise comparisons with Bonferroni correction indicated that participants in group 3 differed significantly from groups 2, 4, and 5, suggesting that mid-career participants reported higher or lower scores compared with other experience levels.

For the AMS, significant differences were observed among experience groups ($H = 34.248$, $df = 4$, $p < 0.001$). Pairwise contrasts showed significant differences between group 3 and most other groups (groups 1, 2, 4, and 5), with Bonferroni-adjusted p -values confirming the contrasts between group 3 and the other experience levels.

For the ALS, the Kruskal-Wallis test indicated significant differences across experience groups ($H = 17.317$, $df = 4$, $p = 0.002$). Pairwise comparisons showed that group 3 differed significantly from groups 1, 4, and 5 in the ALS scores. Professional experience was associated with differences in the WPS, AMS, and ALS scores, with mid-career participants differing from other experience groups.

Discussion

The present study provides an initial psychometric evaluation of three context-specific scales designed to assess workplace-related behavioral tendencies, achievement motivation, and perceived leadership support among Serbian community pharmacists. The results provide preliminary evidence of internal consistency and acceptable factor structures, supporting the potential usefulness of these instruments for future research in pharmacy practice.

The observed associations among workplace personality, achievement motivation, and perceived agile leadership can be interpreted within a shared framework of work-related behavioral and motivational functioning. The findings suggest that more positive work-related behavioral tendencies are associated with higher achievement motivation, while perceived leadership support may further contribute to motivational processes by shaping the work environment. These relationships are consistent with the proposed conceptual overlap between the constructs, although the cross-sectional design does not allow causal interpretations.

The WPS demonstrated good internal consistency, consistent with findings from previous cross-cultural studies. High mean scores across items related to efficiency, responsibility, and collaboration suggest that Serbian pharmacists perceive themselves as conscientious and service-oriented. Some items showed slightly lower item-total correlations (e.g., “I take responsibility for all mistakes that occur at work”), indicating potential variation in how respondents experience certain aspects of workplace behavior. These discrepancies are common in multidimensional behavioral scales and may inform future refinement of item wording or conceptual clarity³⁷. Overall, the findings suggest that the scale captures several related workplace-oriented behavioral and attitudinal dimensions within the studied sample. EFA of the WPS revealed a multidimensional empirical structure that reflects several workplace-related behavioral domains identified within the studied sample^{35, 37}. The naming of factors reflects *post hoc* interpretative labeling based on item content rather than a predefined theoretical structure. The identified factors, reflecting user- and teamwork-oriented behaviors, organizational skills and motivation, task efficiency and accuracy, and responsibility for mistakes, represent empirically derived workplace behavior dimensions consistent with prior organizational behavior literature. These findings suggest that the adapted scale captures distinct but related aspects of professional behavior, indicating that the identified factors represent related but distinguishable dimensions of workplace functioning. The prominence of teamwork and service-oriented items resonates with the professional norms in pharmacy practice, where collaboration and attention to user needs are emphasized³⁵. Similarly, factors related to organizational skills and task performance highlight the importance of self-regulation and accuracy in professional settings. The factorial structure of the WPS reflects empirical clustering of

work-related behavioral and attitudinal items rather than a predefined theoretical structure based on the FFM. Therefore, the identified factors should be interpreted as context-specific dimensions of workplace functioning rather than direct representations of global personality traits. This empirical structure provides preliminary support for the internal organization of the scale and serves as a basis for further refinement and validation in future studies. Workplace personality in this study refers to context-specific work-related behavioral tendencies rather than stable personality traits as defined in major trait models.

The AMS exhibited high internal consistency, demonstrating that all items reliably measure motivation toward professional achievement. Participants reported strong engagement in goal setting, skill development, and responsibility for outcomes, reflecting a high level of professional proactivity. These findings support the potential relevance of achievement motivation as a measurable workplace construct within the pharmacy profession^{38, 39}.

EFA suggests that the AMS may be interpreted as a predominantly unidimensional measure within this sample. The single-factor solution, explaining over 50% of the variance, suggests that the items collectively capture a coherent construct encompassing perseverance, goal-setting, responsibility, and continuous professional development.

These findings align with theoretical perspectives on achievement motivation, which emphasize the importance of goal-directed behavior, self-efficacy, and the willingness to exert effort to attain high standards^{40, 41}. In the context of pharmaceutical practice, motivation to achieve may be particularly relevant for ensuring high-quality patient care, adherence to professional standards, and engagement in continuous learning.

The high communalities and strong factor loadings of all items indicate that the scale is reliable and that each item contributes meaningfully to the measurement of the construct. These findings provide preliminary justification for examining total scale scores in future research.

Moreover, the unidimensional structure simplifies interpretation and suggests that interventions aimed at enhancing achievement motivation, such as training programs, goal-setting workshops, and feedback systems, could be evaluated using the total scale score. Future research may examine the predictive validity of the AMS in relation to work performance, professional development outcomes, and engagement in quality improvement initiatives.

Overall, the AMS demonstrated promising psychometric characteristics that warrant further validation.

Although labeled as agile leadership, the ALS includes items that reflect broader supervisory support behaviors (e.g., communication, employee support, task coordination). This reflects the applied operationalization of agile leadership in the healthcare context, where agility is expressed through supportive, adaptive, and responsive leadership behaviors rather than purely strategic or technological agility frameworks. Future studies should further refine the construct and compare it with established leadership scales to

enhance discriminant validity. The ALS demonstrated high internal consistency, reflecting strong inter-item correlations and coherent measurement of supervisory support, flexibility, and guidance. Mean scores indicated moderate perceptions of leadership behaviors, which may reflect organizational realities, such as variations in leadership style or supervisory support. The high internal consistency suggests that the items consistently assess perceptions of supervisory support and leadership-related behaviors^{42, 43}.

The findings indicate that the ALS suggests a unidimensional structure of perceived supervisory support among community pharmacists within the present sample. The single-factor solution, accounting for over 70% of the variance, demonstrates that items collectively capture the overarching construct of supportive supervisory behaviors, including guidance, encouragement, flexibility, conflict management, and facilitation of professional growth.

These results are consistent with theoretical frameworks emphasizing the role of supervisors in promoting employee development, motivation, and engagement^{44, 45}. In the context of pharmaceutical practice, supervisor support is particularly relevant for ensuring effective team collaboration, adherence to professional standards, and continued competence development.

The high communalities and strong factor loadings suggest that each item contributes meaningfully to the measurement of supervisor support, supporting the use of a total scale score for research and practical applications. Furthermore, the unidimensional structure simplifies interpretation and may facilitate the assessment of interventions aimed at enhancing supervisory practices, such as leadership training programs, structured feedback, and mentorship initiatives.

Overall, the ALS demonstrated promising internal consistency and preliminary psychometric properties and may provide a useful basis for future research examining perceptions of supervisory support in pharmacy settings. Accordingly, the scale reflects supervisory support behaviors rather than a comprehensive theoretical model of leadership. The unidimensional structure of both the AMS and ALS simplifies their practical application, allowing researchers and practitioners to use total scale scores as reliable indicators of professional motivation and perceived supervisory support. This structure suggests that the items consistently capture a single, coherent construct, enhancing interpretability and comparability across studies. In contrast, the multi-dimensional nature of the WPS reflects the complex interplay of individual traits in professional behavior, highlighting specific areas, such as teamwork, organizational skills, and task accuracy, that may benefit from targeted interventions. Together, these factor structures provide nuanced insights into individual and leadership-related characteristics in community pharmacy practice, supporting both research and applied development initiatives.

All three scales exhibit satisfactory to excellent internal consistency ($\alpha > 0.855$). While the WPS and AMS showed good reliability, the ALS achieved the highest α , suggesting

particularly strong coherence among items. Descriptive data indicate that participants report high levels of workplace personality traits and achievement motivation, but more moderate perceptions of agile leadership behaviors. These patterns are consistent with the internal structure observed within the scales, although additional validation studies are required. The relatively high correlation between the WPS and the AMS may reflect conceptual proximity between work-related behavioral tendencies and motivational orientations in professional settings. Several items across the two scales share emphasis on effort, responsibility, and goal-oriented work behavior, which may contribute to their empirical overlap. This pattern suggests the possibility of partial construct redundancy or a broader underlying work-related engagement dimension. However, given the cross-sectional and single-method design, further validation using more distinct measurement approaches and higher-order models is needed to clarify the discriminant validity of these constructs. A single higher-order factor was not tested in the present study; therefore, the possibility that a general underlying work-related disposition explains the observed correlations cannot be ruled out.

The observed associations among the three constructs are broadly consistent with findings reported in previous organizational and healthcare research^{35, 37, 43}. High conscientiousness and goal-oriented behavior may facilitate engagement and proactive problem-solving, although the degree of conceptual overlap among the constructs requires further investigation in future validation studies. These insights underscore the importance of integrated development approaches that target both individual capacities and leadership quality to optimize team functioning in pharmacy settings. High conscientiousness and goal-oriented behavior may facilitate engagement and proactive work-related functioning, which could partly explain the observed associations among the three constructs. However, the degree of conceptual overlap among the scales requires further investigation in future validation studies. The observed correlations suggest the possibility of a broader underlying workplace-related disposition, which should be examined in future research using higher-order or bifactor models. This overlap suggests that future iterations of the instruments should further differentiate behavioral (WPS) and motivational (AMS) content to reduce semantic redundancy. The findings should be interpreted as context-specific and preliminary, referring to Serbian community pharmacists. While the developed scales may have potential applications in research and professional development, further validation in different healthcare settings and cultural contexts is required before broader generalization. The constructs measured in this study are context-specific and should not be interpreted as direct representations of established personality or leadership taxonomies.

The interpretation of the observed relationships among workplace personality, achievement motivation, and perceived supervisory support can be further situated within established theoretical frameworks of organizational behavior and leadership research. The positive associations

between these constructs are consistent with prior evidence linking work-related behavioral tendencies and motivational orientations with perceived organizational and supervisory support, as well as leadership behaviors that facilitate employee development and engagement^{1, 6, 13, 18, 19, 46}. From a broader theoretical perspective, these relationships can be understood through transformational and supportive leadership frameworks, which emphasize the role of leaders in shaping motivation, engagement, and adaptive work behaviors^{19, 20, 43, 45}. In addition, perceived organizational support theory suggests that employees' motivation and work-related attitudes are influenced by the extent to which they perceive their organization and supervisors as valuing their contribution and well-being^{18, 42, 46}.

Furthermore, the conceptual proximity between workplace personality and achievement motivation observed in this study may reflect a shared underlying dimension of positive work engagement or proactive work orientation, as suggested in contemporary organizational psychology and motivation literature^{5, 16, 26, 31}. However, the present findings remain exploratory and do not allow for definitive conclusions regarding the underlying structure of these constructs. Future research should further examine these relationships using more complex structural models, including higher-order and bifactor approaches, as well as longitudinal and multi-source designs, in order to clarify the directionality and distinctiveness of these associations^{47, 48}.

Taken together, these findings provide preliminary support for the relevance of integrating individual dispositional factors and leadership-related perceptions in understanding professional functioning in pharmacy practice, particularly in relation to employee motivation, work engagement, and adaptive supervisory environments^{3, 8, 41}.

Limitations of the study

Several limitations should be acknowledged. First, the study employed a cross-sectional self-report design, which may have increased the risk of common method variance. In addition, all three instruments were assessed using a single-method, single-occasion design, which limits the ability to control for common method bias and does not allow for multi-trait multi-method (MTMM) validation. As a result, observed relationships among constructs may be partially inflated by shared method variance. Therefore, the present study does not support strong conclusions regarding convergent or discriminant validity, and validity evidence is restricted to preliminary internal structure and association-level findings.

From a psychometric perspective, EFA was conducted within a single sample, and CFA was not performed. Therefore, the identified factor structures should be considered preliminary and sample-specific. Furthermore, test-retest reliability was not assessed, and temporal stability of the instruments remains unverified. Similarly, criterion-related validity (e.g., associations with job performance, job satisfaction, burnout, turnover intention, or supervisor-rated performance) was not examined, and convergent and

discriminant validity against established external instruments were not assessed. Accordingly, validity evidence in this study is limited to EFA and inter-scale correlations, which provide only initial support and do not establish full construct validity. Future research should incorporate CFA on independent samples, MTMM designs, and external criterion measures to strengthen validation.

The present study also has theoretical and construct-related limitations. The observed mismatch between the theoretical framework used for item generation and the empirically derived factor structure highlights the exploratory nature of this scale development, particularly for the WPS, which should be interpreted as a context-specific behavioral construct rather than a direct operationalization of established personality models. In addition, the ALS demonstrates conceptual overlap with broader constructs such as general supervisory support and traditional leadership frameworks, indicating the need for further refinement and discriminant validation in future studies. The observed intercorrelations among the three scales may also reflect partial construct overlap or a broader underlying work-related disposition, which should be examined in future higher-order or bifactor models.

An additional limitation relates to potential response biases. The high mean scores observed for the WPS and AMS may indicate ceiling effects and socially desirable responding, particularly given the normative desirability of items related to responsibility, teamwork, accuracy, and professional development. Since no social desirability measure was included, this potential bias could not be controlled statistically and may have influenced score distributions and inter-construct relationships.

Regarding the sample, the study was conducted on Serbian community pharmacists, and the findings may not be generalizable to other healthcare professions or cultural contexts without further validation. The sample was predominantly female (91.5%), reflecting the structure of the profession; however, this imbalance should be considered when interpreting results. The subgroup of participants with PhD degrees was very small ($n = 5$), limiting statistical power for educational comparisons. In addition, the pilot testing phase included a small sample ($n = 10$), which was used only for preliminary assessment of item clarity and feasibility and does not provide psychometric evidence.

Finally, future validation studies should incorporate additional psychometric indices, including McDonald's omega, composite reliability, average variance extracted, parallel analysis, and test-retest reliability, as well as more rigorous multi-stage validation procedures. Quantitative content validity indices (e.g., CVI) should also be considered in future research to further strengthen measurement quality.

Conclusion

The present study provides an initial psychometric evaluation of three context-specific instruments designed to assess workplace-related behavioral tendencies, achievement motivation, and perceived leadership support among Serbian

community pharmacists. The findings indicate satisfactory internal consistency and preliminary evidence regarding the internal structure of the proposed scales, supporting their potential usefulness for future research in pharmacy practice.

Together, these instruments offer a context-specific approach to examining selected psychological, motivational, and leadership-related aspects of professional work in pharmacy settings. The observed associations among the scales contribute to understanding how these workplace-related constructs may interact within the professional environment.

Several limitations should be considered when interpreting the findings. The study relied exclusively on self-report measures collected at a single time point, which may have increased the influence of common method variance and social desirability bias. In addition, test-retest reliability, criterion validity, convergent validity with established instruments, and confirmatory factor analysis were not examined. Therefore, the findings should be regarded as preliminary and require further validation.

Future research should evaluate the scales in independent samples, examine temporal stability and

external validity, and investigate relationships with relevant professional outcomes such as job performance, employee engagement, and professional development. Such studies will help determine the broader applicability and usefulness of these instruments within pharmacy and other healthcare contexts.

The developed scales provide preliminary, context-specific tools for assessing workplace personality, achievement motivation, and perceived agile leadership among Serbian community pharmacists. Their application is currently limited to this professional context, and further validation in other healthcare settings and cultural environments is necessary before broader use can be recommended.

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Supplementary Table 1**Final versions of the scales****Scale 1: Workplace Personality Scale (WPS) in pharmaceutical practice**

Instructions: For each statement, please indicate how often it describes your professional behavior in your workplace. Use the following scale: 1 – Never, 2 – Rarely, 3 – Sometimes, 4 – Often, 5 – Always.

Item	Statement
1	I am satisfied with my work when I can help users with their problems.
2	I feel calm even when I have many tasks to complete.
3	I enjoy collaborating with colleagues to solve problems.
4	I try to maintain a positive attitude with users, even in stressful situations.
5	I help new colleagues adapt to the work environment.
6	I take responsibility for all mistakes that occur at work.
7	I am willing to learn new techniques and improve my skills.
8	I enjoy completing tasks efficiently and accurately.
9	I feel motivated to always provide the best service to users.
10	I strive to develop my organizational skills.
11	I usually find it easy to adapt to new situations.
12	I like to take responsibility for my work.
13	I understand how my behavior affects the workplace atmosphere.
14	I believe that teamwork is essential for achieving good results.
15	I strive to always make accurate and fair decisions.

Scale 2: Achievement Motivation Scale (AMS) in pharmaceutical practice

Instructions: For each statement, please indicate how often it describes your professional behavior in your workplace. Use the following scale: 1 – Never, 2 – Rarely, 3 – Sometimes, 4 – Often, 5 – Always.

Item	Statement
1	I invest maximum effort to achieve the best possible results in my work.
2	I regularly set challenging goals that I aim to accomplish in pharmaceutical practice.
3	I am always motivated to improve my knowledge and skills in pharmaceutical practice.
4	I continue working toward goals even when facing difficulties or uncertainty.
5	I believe in my ability to succeed in the tasks assigned to me.
6	I am motivated to contribute to improving pharmaceutical services and work quality.
7	I am willing to take responsibility for achieving important results in my work.

Scale 3: Agile Leadership Scale (ALS) in pharmaceutical practice

Instructions: For each statement, please indicate how often it describes your supervisor's behavior in your workplace. Use the following scale: 1 – Never, 2 – Rarely, 3 – Sometimes, 4 – Often, 5 – Always.

Item	Statement
1	My supervisor makes an effort to understand the personal and professional challenges I face.
2	My supervisor clearly defines work tasks and priorities.
3	My supervisor encourages me to take initiative and strive for better results.
4	My supervisor effectively resolves conflicts within the team.
5	My supervisor demonstrates flexibility in adapting to new challenges in pharmaceutical practice.
6	My supervisor has a clear vision for the development of pharmaceutical practice and communicates it regularly.
7	My supervisor supports my development through additional training and education.

Note: All items are rated on a 5-point Likert scale (1 – Never, 5 – Always). Scales are culturally adapted for the context of community pharmacy practice. These instruments were developed specifically for this study and have been validated for internal consistency and reliability.

Supplementary Table 2

Descriptive statistics for the three scales

Scale	Number of items	Mean $\pm$ SD	Range
Workplace Personality Scale	15	4.52 $\pm$ 0.49	1–5
Achievement Motivation Scale	7	4.39 $\pm$ 0.52	1–5
Agile Leadership Scale	7	3.40 $\pm$ 0.62	1–5

Scale/Item	Range	Mean $\pm$ SD
Workplace Personality Scale (n = 633)		
I am satisfied with my work when I can help users with their problems.	1–5	4.61 $\pm$ 0.74
I feel calm even when I have many tasks to complete.	1–5	3.50 $\pm$ 0.88
I enjoy collaborating with colleagues to solve problems.	2–5	4.54 $\pm$ 0.70
I try to maintain a positive attitude with users, even in stressful situations.	1–5	4.43 $\pm$ 0.61
I help new colleagues adapt to the work environment.	1–5	4.75 $\pm$ 0.48
I take responsibility for all mistakes that occur at work.	1–5	4.01 $\pm$ 0.99
I am willing to learn new techniques and improve my skills.	1–5	4.70 $\pm$ 0.61
I enjoy completing tasks efficiently and accurately.	1–5	4.80 $\pm$ 0.46
I feel motivated to always provide the best service to users.	1–5	4.31 $\pm$ 0.90
I strive to develop my organizational skills.	2–5	4.52 $\pm$ 0.68
I usually find it easy to adapt to new situations.	2–5	3.93 $\pm$ 0.78
I like to take responsibility for my work.	2–5	4.78 $\pm$ 0.46
I understand how my behavior affects the workplace atmosphere.	3–5	4.56 $\pm$ 0.61
I believe that teamwork is essential for achieving good results.	2–5	4.68 $\pm$ 0.62
I strive to always make accurate and fair decisions.	3–5	4.81 $\pm$ 0.42
Achievement Motivation Scale (n = 633)		
I invest maximum effort to achieve the best possible results in my work.	2–5	4.51 $\pm$ 0.60
I regularly set challenging goals that I aim to accomplish in pharmaceutical practice.	1–5	4.00 $\pm$ 0.85
I am always motivated to improve my knowledge and skills in pharmaceutical practice.	1–5	4.36 $\pm$ 0.80
I continue working toward goals even when facing difficulties or uncertainty.	2–5	4.25 $\pm$ 0.81
I believe in my ability to succeed in the tasks assigned to me.	2–5	4.61 $\pm$ 0.55
I am motivated to contribute to improving pharmaceutical services and work quality.	1–5	4.23 $\pm$ 0.94
I am willing to take responsibility for achieving important results in my work.	1–5	4.52 $\pm$ 0.67
Agile Leadership Scale (n = 633)		
My supervisor makes an effort to understand the personal and professional challenges I face.	1–5	3.43 $\pm$ 1.17
My supervisor clearly defines work tasks and priorities.	1–5	3.50 $\pm$ 1.11
My supervisor encourages me to take initiative and strive for better results.	1–5	3.40 $\pm$ 1.33
My supervisor effectively resolves conflicts within the team.	1–5	3.21 $\pm$ 1.26
My supervisor demonstrates flexibility in adapting to new challenges in pharmaceutical practice.	1–5	3.46 $\pm$ 1.13
My supervisor has a clear vision for the development of pharmaceutical practice and communicates it regularly.	1–5	3.31 $\pm$ 1.24
My supervisor supports my development through additional training and education.	1–5	3.50 $\pm$ 1.30

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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

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

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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.

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

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

ORIGINAL ARTICLE

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

PRELIMINARY REPORT

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

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

REVIEW ARTICLES

GENERAL (NARRATIVE) LITERATURE REVIEW

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

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

MINI-REVIEW ARTICLE

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

SYSTEMATIC LITERATURE REVIEW

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

META-ANALYSIS

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

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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

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

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

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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.

SCIENTIFIC MEETING REPORT

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

MANUSCRIPT LENGTH

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

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

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

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MANUSCRIPT PREPARATION

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The first page of the manuscript should include the following:

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4. At the bottom of the page, provide the name and surname, postal address, email address, and phone number (mobile/Viber or WhatsApp) of the author responsible for correspondence.

ABSTRACT

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

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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.

KEYWORDS

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

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

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

A Case Report must not have more than five authors.

QUESTIONNAIRES

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

TABLES AND FIGURES

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

Tables

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

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

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Figures include all forms of graphical material (photographs, drawings, diagrams, and graphs). Figures should be embedded in the manuscript at the end of the text, after the References and after the Tables (if any). Figures should be numbered with Arabic numerals in the order in which they appear in the text. Capital letters A, B, C, etc., should be used to designate parts of multipart figures. Letters, numbers, and symbols must be clear, consistent, and of sufficient size to remain legible after reduction. All elements shown in figures must be saved as images (not as editable graphic objects) so that their position cannot be altered, ensuring the accuracy of the data presented. Only digital images with a minimum resolution of 300 dpi and in JPEG, PNG, or PDF format are accepted. Figures that do not meet these requirements will not be accepted for publication. The dimensions of submitted figures should be approximately the same as the dimensions at which they will be published. If authors are unable to provide digital photographs, original images should be scanned at a resolution of 300 dpi and at their original size and submitted in that form. All text in diagrams and graphs should be written in a sans-serif font for

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

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

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ACKNOWLEDGEMENTS

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

STATISTICAL ANALYSIS

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

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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**

Metcalfe 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 AD. 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 *Asestant*. 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://asestant.ceon.rs/index.php/vsp/user>. Техничко упутство за коришћење система електронске пријаве доступно је на: <https://asestant.ceon.rs/index.php/vsp/about/submissions>.

Уколико имате проблем са подношењем рукописа путем платформе *Asestant* 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 саопшteni 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 cm а преостале три 2 cm. Текст куцати без дељења речи (хифениације), 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. Избежавати 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 обилм заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или субскрипту.

Избежавати фонтове **bold** и курзив (*italic*) јер су резервисани за поднаслове. Изузети 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 избежавати 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. Избежавати 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 индекси.

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 писану 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 *SCIndex 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 професионалних 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 формирању, 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.

Недовосмислено 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 са рецензијом (M20) из области прегледног рада.

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МЕТА-АНАЛИЗА

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

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

АКТУЕЛНА ТЕМА

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

У ФОКУСУ

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

КАЗУИСТИКА

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

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

УВОДНИК

Уводници су нерцензирани 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

Нерцензирани 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/рубике 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 (значи *, †, ‡, §, ||, ¶, **, †† ... итд. показују редом установе у којима аутори раде);
4. На дну странице навести име и презиме, адресу за контакт, е-маил адресу и број телефона (мобилног/Viber или WhatsApp) аутора задуженог за кореспонденцију.

АПСТРАКТ

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

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

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

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

КЉУЧНЕ РЕЧИ

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

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

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

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

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

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

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

ПРИЛОЗИ

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

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 фонтот *Times New Roman*, величином слова 12, с једноструким поредом. 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 графикани). 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 задовољавају 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 фонтот 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 резом (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 графикана 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, ултразвук, 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 избежавати 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 резом (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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