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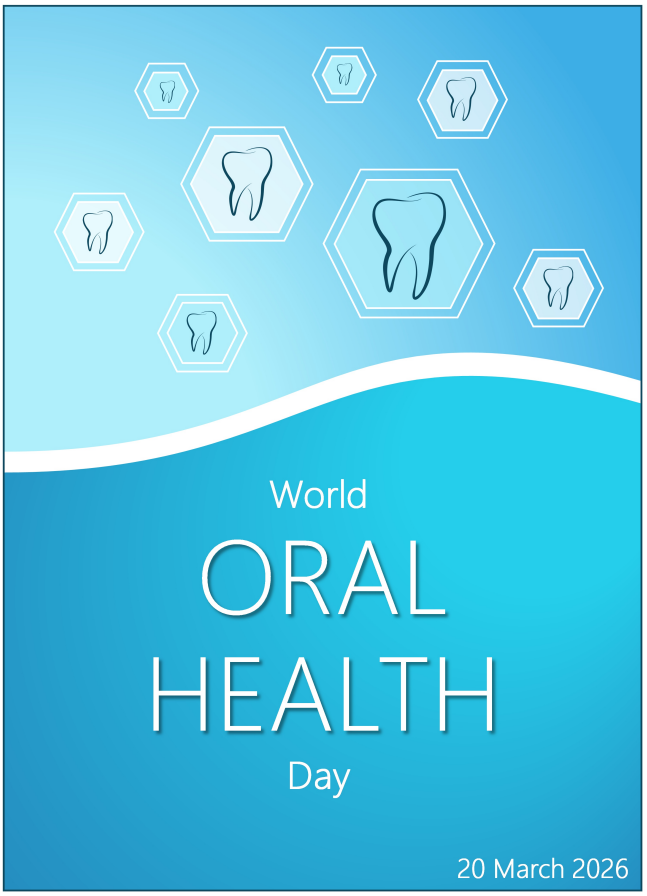

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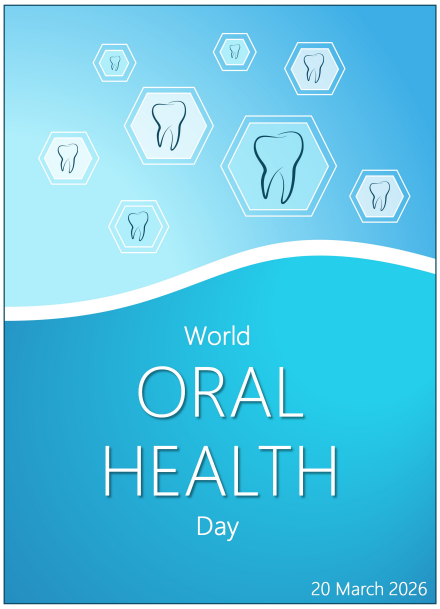
CONTENTS / SADRŽAJ



Oral diseases, affecting over 3.5 billion people, represent one of the most common health problems worldwide. Good oral health contributes to overall health and plays a significant role in everyday activities and social interactions. World Oral Health Day is observed annually on 20 March, initiated by the World Dental Federation, with the aim of raising awareness on the importance of oral health in maintaining quality of life. The theme of this year's campaign is "A happy mouth is a happy life".

Bolesti usta i zuba, koje pogađaju preko 3,5 milijardi ljudi, predstavljaju jedan od najučestalijih zdravstvenih problema u svetu. Dobro oralno zdravlje doprinosi opštem zdravlju organizma i ima značajnu ulogu u svakodnevnim aktivnostima i društvenim odnosima pojedinca. Svetski dan oralnog zdravlja obeležava se 20. marta svake godine na inicijativu Svetske federacije stomatologa kako bi se podigla svest o značaju zdravlja usta i zuba u očuvanju kvaliteta života. Tema ovogodišnje kampanje je „Srećna usta su srećan život".

GENERAL REVIEW

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Genetic polymorphism and pharmacokinetics/toxicokinetics of carbamazepine: a general review

Genetički polimorfizam i farmakokinetika/toksikokinetika karbamazepina: opšti pregled

Aleksandra Kovačević*†, Vladan Lukić‡, Bojana Cikota Aleksić*, Slobodan M. Janković§, Viktorija Dragojević-Simić*†

Military Medical Academy, *Center for Clinical Pharmacology, ‡National Poison Control Center, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; §University Clinical Center Kragujevac, Department of Clinical Pharmacology, Kragujevac, Serbia

Abstract

Carbamazepine (CBZ) is a widely used medication in treating epilepsy, bipolar disorder, and neuropathic pain. Its pharmacokinetic profile is highly variable due to slow absorption, extensive metabolism, and auto-induction. Genetic polymorphisms affect transporters and metabolic enzymes, additionally modifying therapeutic response, and lead to nonlinear and unpredictable toxicokinetics in overdose. CBZ is primarily metabolized by cytochrome P450 (CYP) enzymes CYP3A4, CYP3A5, and CYP2C8 to the active metabolite CBZ-10, 11-epoxide (CBZ-E), with subsequent hydrolysis by epoxide hydrolase 1 (EPHX1) and glucuronidation by uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7), with other CYPs additionally contributing to the formation of reactive intermediates and inactive CBZ metabolites. Polymorphisms in genes encoding enzymes (EPHX1, CYP3A4/5, and UGT2B7) and transporters (ABCB1, ABCC2, RALBP1) can affect CBZ and CBZ-E exposure, maintenance dose, and the risk of adverse drug reactions. In overdose cases, CBZ exhibits saturable epoxidation, which leads to the accumulation of the drug and its active metabolite, prolonged elimination, and neurotoxicity, while serum concentrations correlate poorly with clinical findings. Therapeutic monitoring of the drug and its active metabolite in patients' blood, together with pharmacogenetic testing, could improve both the individualization of therapy and the management of overdose.

Keywords:
carbamazepine; cytochrome p-450 enzyme system; drug monitoring; metabolism; pharmacokinetics; poisoning; polymorphism, genetic; toxicokinetics.

Apstrakt

Karbamazepin (KBZ) je široko korišćen lek u terapiji epilepsije, bipolarnog poremećaja i neuropatskog bola. Njegov farmakokinetički profil je veoma varijabilan zbog spore resorpcije, ekstenzivnog metabolizma i autoindukcije. Genetički polimorfizmi utiču na transportere i metaboličke enzime, dodatno modifikujući terapijski odgovor i dovode do nelinearne i nepredvidive toksikokinetike u slučaju predoziranja. KBZ se prvenstveno metaboliše putem citohrom P450 (CYP) enzima CYP3A4, CYP3A5 i CYP2C8 do aktivnog metabolita KBZ-10, 11-epoksida (KBZ-E), nakon čega sledi hidroliza pomoću epoksid hidrolaze 1 (EPHX1) i glukuronidacija pomoću uridin difosfat glukuronil transferaze 2B7 (UGT2B7). Drugi CYP enzimi dodatno doprinose stvaranju reaktivnih intermedijera i neaktivnih metabolita KBZ. Polimorfizmi u genima koji kodiraju enzime (EPHX1, CYP3A4/5 i UGT2B7) i transportere (ABCB1, ABCC2, RALBP1) mogu uticati na izloženost organizma KBZ i KBZ-E, dozu održavanja i rizik od neželjenih reakcija na lek. U slučajevima predoziranja lekom dolazi do zasićenja procesa epoksidacije, što dovodi do akumulacije leka i njegovog aktivnog metabolita, produžene eliminacije i neurotoksičnosti, dok serumske koncentracije slabo koreliraju sa kliničkim nalazima. Praćenje koncentracije leka i njegovog aktivnog metabolita u krvi bolesnika, zajedno sa farmakogenetičkim testiranjem, moglo bi poboljšati i individualizaciju terapije i lečenje predoziranja.

Ključne reči:
karbamazepin; citohrom p-450; lekovi, monitoring; metabolizam; farmakokinetika; trovanje; polimorfizam, genetički; toksikokinetika.

Correspondence to: Aleksandra Kovačević, Military Medical Academy, Center for Clinical Pharmacology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: alexandra.kova@gmail.com

Introduction

Carbamazepine (CBZ) is a medication chemically related to tricyclic antidepressants that has been used for decades as an anticonvulsant in monotherapy for partial and generalized tonic-clonic seizures. Its use in combination regimens is generally reserved for patients who cannot be adequately controlled with previous monotherapy, and it is usually ineffective in the myoclonic and absence seizures [1–3]. Additionally, the established indications include the treatment of bipolar disorders and trigeminal neuralgia. Regarding its mechanism of action, it stabilizes hyperexcitable neuronal membranes by blocking voltage-gated sodium channels, inhibiting repetitive neuronal firing, and reducing the propagation of synaptic excitatory impulses. Its action also involves calcium channel inhibition, inhibition of excitatory amines, and gamma-aminobutyric acid agonism. By modulating inhibitory and excitatory neurotransmission, CBZ exerts a mood-stabilizing effect and exhibits an antineuralgic effect. It also has anticholinergic, antidiuretic, muscle-relaxant, and antiarrhythmic properties [3-6].

CBZ has a complex and variable pharmacokinetic profile, characterized by slow absorption, extensive protein binding, liver enzyme-mediated metabolism, and autoinduction, which necessitates careful monitoring to minimize the risk of adverse reactions [2, 5, 7]. In overdose cases, the elimination of CBZ follows zero-order kinetics, leading to symptoms of prolonged toxicity coinciding with the peak serum levels of CBZ and its active metabolites [8–10].

Scope and methodology approach

The aim of this manuscript was to synthesize current knowledge on how genetic variations influence the pharmacokinetics of CBZ at therapeutic doses and the risk of adverse drug reactions (ADRs), with a focus on clinical interpretation rather than quantitative analysis. Additionally, the toxicokinetics of CBZ in overdose patients differ from its behavior within the therapeutic range, focusing on how the body influences CBZ and its metabolites at concentrations exceeding the therapeutic level.

For this general review, a literature search was conducted from September to December 2025 to obtain newly available clinical data and regulatory information. The literature search was performed in PubMed and EBSCO databases, with Google Scholar used as a complementary tool for cross-checking and identification of supplementary publications. The following criteria were applied to identify English-language publications published up to 2025: (“carbamazepine“ OR “anticonvulsants“ OR „antiepileptic drugs” OR “carbamazepine metabolites” OR “carbamazepine-10,11-epoxide”) AND (“pharmacokinetics” OR “population pharmacokinetics” OR “toxicokinetics” OR “CYP450” OR “poisoning” OR “overdosage” OR “genetic polymorphism” OR “metabolism” OR “elimination kinetics”). We also included reference lists from recent systematic reviews, meta-analyses, and clinical guidelines as additional sources. Only relevant full texts were evaluated. Furthermore, we reviewed regulatory databases from the United States Food and Drug Administration and the European Medicines Agency, as well as authoritative drug information sources (drugs.com and the Electronic Medicines Compendium). Commentaries, opinion articles, editorials, and conference abstracts were excluded.

Pharmacokinetics of carbamazepine

Absorption, distribution, and transport protein genetic polymorphisms

CBZ is a lipophilic molecule that shows slow and variable but almost complete absorption [2, 5, 7]. After oral administration, peak plasma concentrations occur 2–8 hrs after ingestion of immediate-release formulations and 12–24 hrs after ingestion of CBZ sustained-release formulations (single dose) and 4–8 hrs after multiple doses [9]. The absorption of CBZ could additionally be delayed due to its weak anticholinergic properties and decreased gastrointestinal motility [5, 11]. Its bioavailability ranges from 75% to 85% for sustained-release formulations and up to 90% for immediate-release formulations [7, 12, 13].

Due to the moderate to high lipid solubility of CBZ, the apparent volume of distribution in adults and older children generally ranges between 0.59 and 2 L/kg [4, 7, 14]. The drug binds to plasma proteins at 70–80%, primarily to albumin and α1-acid glycoprotein. In neonates, the free fraction is higher, ranging from 30 to 35%, due to lower plasma protein concentrations [4, 15].

The transport of CBZ across biological membranes occurs *via* adenosine triphosphate (ATP)-binding cassette sub-family B member 1 (ABCB1), ATP-binding cassette sub-family C member 2 (ABCC2), and Ral-binding protein 1 (RALBP1) transport proteins, which significantly contribute to the pharmacokinetic variability of many drugs and play a crucial role in the efflux of CBZ [16, 17]. These proteins participate in the transport of CBZ across the intestinal barrier and the blood-brain barrier. They are also expressed in the liver, where they are involved in ATP-dependent efflux of CBZ and its metabolites from hepatocytes. Transporters substantially influence intracellular drug availability and contribute to interindividual variability in therapeutic response [16]. ABCB1 is a major efflux transporter at the intestinal and blood-brain barrier surfaces. The ABCC2 transporter contributes to the efflux of xenobiotics and metabolites [18]. RALBP1 is also an ATP-dependent transporter implicated in the removal of CBZ conjugates. Boughrara and Chentouf [16] concluded that genetic variability in *ABCB1*, *ABCC2*, and *RALBP1* contributes to interindividual differences in CBZ response, but the overall evidence is still inconsistent across populations. Djordjevic et al. [19] also discussed *ABCB1* polymorphisms, the most extensively studied efflux transporter, which showed that haplotypes like c.3435C>T (cytosine replaced by thymine), c.2677G>T/A (guanine replaced by thymine or adenine), and c.1236C>T (cytosine replaced by thymine) are associated with altered CBZ transport and treatment outcomes.

Additionally, the 1236T–2677T–3435T haplotype is associated with increased clearance in pediatric patients, therefore showing more efficient efflux and lower systemic exposure [19]. Wang et al. [20] have concluded that the *ABCB1* rs2032582, rs10234411, and rs2032582-rs10234411 AT, CA haplotype is significantly associated with the ratio of CBZ-10,11-epoxide (CBZ-E) to CBZ when CBZ is used in therapy in combination with other anticonvulsants, such as phenobarbitone or phenytoin. In addition, several authors suggested that the *ABCB1* gene polymorphism increases the risk of poor treatment response [21], particularly the rs1128503 polymorphism, which was significantly associated with CBZ pharmacoresistance [22]. *ABCC2* polymorphisms included c.-24C>T, c.1249G>A, and c.3972C>A, and several *RALBP1* variants were examined for their potential association with drug resistance [16]. RALBP1 is a multifunctional protein, a non-ATP-binding cassette important transporter for CBZ at the human blood-brain barrier, where its expression is increased in patients with drug-resistant epilepsy [23]. On the contrary, other authors have not found an association between *RALBP1* expression and resistance to antiepileptic drugs [24].

Metabolism and genetic polymorphism of metabolic enzymes

CBZ is extensively metabolized in the liver by microsomal cytochrome P450 (CYP) enzymes – CYP450, and excreted in feces (28%), with only 1–3% of the dose excreted as unchanged drug in urine, while the rest, about 70%, is excreted as metabolites [2, 7, 17, 25]. The major pathway of CBZ metabolism is oxidation mediated by CYP3A4, with involvement of CYP2C8 and CYP3A5, forming the pharmacologically active metabolite CBZ-E, which contributes to both its efficacy and toxicity. CBZ-E undergoes further metabolism *via* microsomal epoxide hydrolase 1 (EPHX1) to trans-10,11-dihydro-10,11-dihydroxycarbamazepine, CBZ-diol, a pharmacologically inactive metabolite. After oxidative metabolism and further hydrolysis by EPHX1, phase II metabolism involves the glucuronidation of CBZ and CBZ-E by uridine diphosphate glucuronosyltransferases (UGTs), particularly UGT2B7, which facilitates renal excretion of both CBZ and CBZ-E [26].

Several reactive intermediates are formed during the minor oxidative pathways of CBZ. One branch involves the generation of an epoxide intermediate, CBZ-2,3-epoxide, which is further oxidized by multiple CYP isoenzymes to 2-hydroxycarbamazepine (2-OH-CBZ) and by CYP3A4 and CYP2B6 to 3-hydroxycarbamazepine (3-OH-CBZ). The 2-OH-CBZ undergoes secondary oxidation *via* CYP3A4 to form 2-hydroxyiminostilbene, which is further converted to iminoquinone, a highly reactive electrophilic species capable of forming covalent adducts with protein residues, including CYP450 enzymes. The formation of such adducts may inactivate enzymes and contribute to immune-mediated hypersensitivity reactions, such as Stevens-Johnson syndrome [27]. The 3-OH-CBZ metabolite is further oxidized by CYP2C19, CYP3A4, and CYP3A5 to CBZ catechol and subsequently to CBZ o-quinone. In parallel, through myeloperoxidase-mediated oxidation, 3-OH-CBZ can generate free radicals and reactive oxygen species, which enhance oxidative stress and promote tissue injury [28] (Figure 1). Lukic et al. [10] supposed that CYP3A activity decreased, while CYP2B6 activity increased, accompanied by increased free radicals and reactive oxygen species, leading to increased markers of inflammation in patients with acute self-poisoning with CBZ.

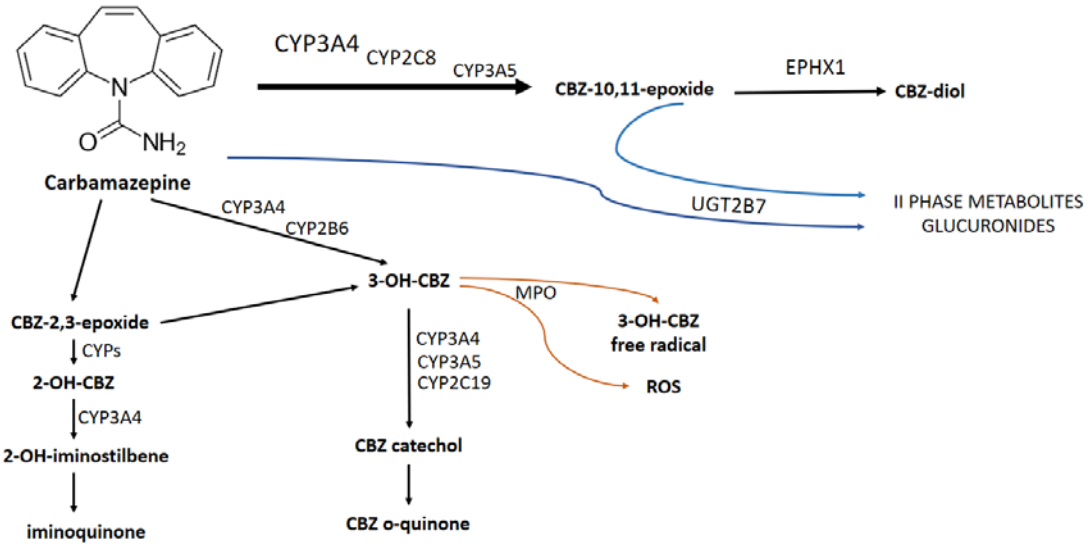

Fig. 1 – **Hepatic biotransformation pathways of CBZ and associated enzymes. Adapted from the ClinPGx CBZ pathway, pharmacokinetics [28].**
CBZ – carbamazepine; CYP – cytochrome P450 enzymes; EPHX1 – epoxide hydrolase 1; 2-OH-CBZ – 2-hydroxycarbamazepine; 3-OH-CBZ – 3-hydroxycarbamazepine; 2-OH-iminostilbene – 2-hydroxyiminostilbene, UGT2B7 – uridine diphosphate glucuronosyltransferase-2B7; MPO – myeloperoxidase; ROS – reactive oxygen species.
***Note*: CYP3A4, CYP3A5, CYP2C8, CYP2C19, CYP2B6 – specific CYP450 isoenzymes involved in the CBZ metabolism.**

CBZ exhibits autoinduction within the first few weeks of therapy, accelerating its own metabolism and decreasing its elimination half-life over time [7, 29]. This process corresponds to CBZ binding to nuclear receptors NR1|2 and NR1|3, which upregulate CYP1A2 during autoinduction. The −163C>A polymorphism alters CYP1A2 inducibility and contributes to interindividual differences in CBZ metabolism [30]. Although CYP1A2 is not directly involved in the biotransformation of CBZ, its induction amplifies the broader hepatic autoinduction response, contributing to increased expression and activity of both CYP3A4 and CYP2B6, two primary enzymes responsible for CBZ metabolism and several co-administered drugs [15, 25, 31].

Investigating the genetic polymorphisms of CBZ-metabolizing enzymes and transport pathways can significantly impact individual drug responses, leading to safer and more effective personalized dosing [32]. In the previous section, it was shown that three main groups of enzymes are involved in CBZ elimination: oxidative metabolism and hydrolysis enzymes of phase I *via* CYP3A4, CYP3A5, CYP2C8, and EPHX1, and phase II glucuronidation *via* UGT2B7 (Figure 1). Therefore, the influence of genetic polymorphisms as predictors of responsiveness to CBZ therapy is exerted by genes that affect CYP3A4, CYP3A5, CYP2C19, and CYP2C8 activity, as well as the *EPHX1* gene [33].

The most significant association exists between the *EPHX1* gene polymorphism and the maintenance dose of CBZ [33, 34]. Two common polymorphic sites in the gene affect EPHX1 activity and, subsequently, CBZ and CBZ-E plasma levels. The presence of the 337C allele instead of 337T (when the amino acid tyrosine is replaced by histidine) is associated with decreased microsomal epoxide hydrolytic activity. Lower EPHX1 activity leads to increased CBZ-E (less conversion to CBZ-diol), while variability in the CBZ-diol/CBZ-E ratio could be a good indicator of hydrolase catalytic activity. Furthermore, increased concentrations of CBZ-E in patients with epilepsy may lead to more frequent skin reactions and severe ADRs, such as Stevens–Johnson syndrome and toxic epidermal necrolysis [35].

An increased enzymatic activity is observed in the presence of the 416G allele, rather than the 416A, when histidine is replaced by arginine, which accelerates CBZ-E hydrolysis, resulting in reduced CBZ-E levels and a lower CBZ-diol/CBZ-E ratio as CBZ-diol declines much faster due to glucuronidation and renal excretion [33, 34, 36]. On the contrary, population pharmacokinetic modeling by Yip et al. [37] showed that the *EPHX1* 416G/G genotype was associated with a 50% decrease in the overall clearance of CBZ-E, reflecting the influence of conversion rate, downstream glucuronidation, and systemic elimination processes. CBZ-E retains anticonvulsant activity, but elevated plasma concentrations have been associated with reduced tolerability to CBZ treatment and increased adverse events, such as blurred vision, dizziness, and fatigue.

CYP3A4 is essential for the oxidation of CBZ to active metabolites such as CBZ-E, which is then deactivated by other enzymes [2, 7, 15, 25, 38]. Several *CYP3A4* polymorphisms, such as *CYP3A4*1G* (rs2242480) and rs4646440, modify enzyme activity and may accelerate CBZ clearance, although studies have shown an inconsistent causal relationship with steady-state plasma concentrations. Some studies report that carriers of *CYP3A4*1G* variant alleles exhibit higher metabolic activity and lower adjusted CBZ levels. However, overall, the effect of *CYP3A4* genetic variation on CBZ efficacy appears to be modest [39]. Other authors have noted that *CYP3A4* has limited polymorphism, and its inducibility outweighs genetic effects on enzyme activity. Therefore, genetic variations in *CYP3A4* have less influence than those in other CYP enzymes, such as *CYP3A5*, which shows higher genetic variability [40]. A meta-analysis by Zhao et al. [41] regarding the *CYP3A4* rs2242480 polymorphism revealed that carriers of the AG genotype had significantly lower plasma CBZ concentrations than those with the GG genotype, indicating a genotype-dependent effect on drug exposure. Overall, the data suggest that the G allele of this single-nucleotide polymorphism (SNP) could reduce plasma CBZ levels. At the same time, individuals with the AA genotype display a lower CBZ-E/CBZ ratio than GG or AG+GG carriers, without any influence on CBZ-E. Wang et al. [20] investigated *CYP3A4* gene polymorphisms in the Chinese population, focusing on rs2242480 and rs4646440, which are common SNPs and may affect CYP3A4 catalytic activity. Authors have concluded that the examined *CYP3A4* genotypes were not associated with CBZ plasma concentrations, neither in the monotherapy group nor in the polytherapy group. On the other hand, *CYP3A5* rs776746 and rs15524 might affect CBZ metabolism and were significantly associated with CBZ plasma concentrations [20]. In the research by Jamil et al. [42], which assessed *CYP3A5* rs15524 rather than specific *CYP3A4* SNPs, it was concluded that *CYP3A5* polymorphisms are an important genetic factor influencing CBZ serum concentrations and the need for dose adjustments. In White populations, the failure of *CYP3A* polymorphisms to predict the maintenance dose due to low activity of the *CYP3A4*-392 G allele may be compensated by functional *CYP3A5* in individuals carrying at least one copy of the 6986A allele. Such influence is not expected in Japanese individuals, who generally lack the *CYP3A4*-392 G allele, and in whom *CYP3A5* c. 6986A>G polymorphism is a major contributor to interindividual variability in CBZ pharmacokinetics [34]. While investigating *CYP3A5* genetic polymorphism, Ganesapandian et al. [43] concluded that the impact of this polymorphism on CBZ metabolism showed discrepant results in different populations. According to their results, the *CYP3A5*3* polymorphism significantly influenced CBZ metabolism in Indian epileptic patients, consistent with studies performed in Serbian, Chinese, and Korean populations. Additionally, Seo et al. [44] concluded, based on a study with Japanese patients with epilepsy, that the *CYP3A5*3* genotype increased CBZ oral clearance by only about 8% compared with *CYP3A5*1* carriers, indicating a minimal effect on overall CBZ pharmacokinetics. Some other authors have concluded that genetic variability in *CYP3A5* and *EPHX1* moderately influences plasma

concentrations, which are usually insufficient to cause significant clinical effects [26]. Ragia et al. [45] suggested that, despite the lack of definitive conclusions on whether the CYP3A5 enzyme has similar affinity and metabolic capacity to CYP3A4 for psychiatric drugs, the role of CYP3A5 should be more thoroughly investigated in the future due to its potential to catalyze alternative metabolic pathways and form intermediate metabolites with unknown pharmacological properties that influence CBZ bioavailability. Additionally, studies of CBZ epoxidation and the influence of *CYP3A5*3* on pharmacokinetic parameters were examined. Wild-type *CYP3A5*1/*1* liver microsomes have the highest maximum velocity (V_{max}) and maximum clearance (CL_{max}), meaning strong catalytic activity. Heterozygous *CYP3A5*1/*3* microsomes show markedly reduced activity, less than half of the wild-type, and homozygous *CYP3A5*3/*3* microsomes show activities similar to wild-type. These results suggest that the *CYP3A5*3* polymorphism has a negligible effect on CBZ epoxidation in an *in vitro* system using human liver microsomes, primarily because *in vivo* CYP3A4 is the dominant enzyme for epoxidation processes [46].

Glucuronidation plays an important role in the elimination of CBZ [3, 26]. The enzyme UGT2B7 is responsible for the glucuronidation of both CBZ and CBZ-E. The *UGT2B7*2* (802C>T) variant has been shown to influence CBZ clearance, as demonstrated in a study of 62 patients on CBZ monotherapy, in which carriers of the *1/*2 and *2/2 genotypes exhibited lower steady-state CBZ concentrations and required higher maintenance doses than wild-type individuals [33, 47]. In contrast, the *UGT2B7*3* (211G>T) polymorphism did not affect steady-state levels nor dosing adjustments. On the other hand, other studies have not reported a significant association between *UGT2B7* variants and CBZ pharmacokinetics [33, 47, 48]. Population pharmacokinetic modeling allows clinicians to predict drug clearance in patients using only a few clinical characteristics [49]. It is a helpful tool for optimizing dosing regimens of drugs with high variability, autoinduction, and a narrow therapeutic window, like CBZ [25]. Jankovic et al. [25] demonstrated in their study that physiological covariates, such as age and weight, as well as drug–drug interactions (DDI) in polytherapy with valproate, are the primary drivers of CBZ clearance in Serbian patients. The low residual variability suggests that many unexplained differences could originate from genetic polymorphisms, highlighting the need for future pharmacogenomic work in this population. A systematic review of the population pharmacokinetics of CBZ by Methaneethorn et al. [11] highlights that the most frequently identified covariates, such as age, weight, CBZ dose, and concomitant therapy with other anticonvulsants (phenytoin, phenobarbitone, valproate), influence the clearance of CBZ. Only two studies evaluated genetic predictors, and the *CYP1A2*-163A/A variant had a small but statistically significant influence on clearance, while the *CYP2C8*3* variant showed no clinically significant effect. The authors highlight that although the pharmacokinetic determinants of CBZ disposition are well defined, the relationship between pharmacokinetic variability and pharmacodynamic outcomes remains largely inconclusive and highly dependent on the target population [11].

According to Kanojia et al. [50], CBZ can induce *CYP1A1 via* aryl hydrocarbon receptor-dependent transcriptional regulation, which contributes to the clinically relevant interindividual variability in response to CBZ therapy.

In a systematic review, Zhang et al. [51] found that the *ABCB1* c.3435C>T and *EPHX1* c.416A>G gene polymorphisms significantly affected CBZ concentrations, indicating their critical role in CBZ pharmacokinetics and pharmacodynamics. Various confounding factors, such as ethnicity, age, and differences in dosing and treatment duration, could explain the observed inconsistencies across studies, suggesting that considerable caution is needed when transferring to other populations.

Therapeutic drug monitoring

CBZ reaches steady state very slowly, typically within 4 to 30 days. Its half-life at steady state ranges from 20 to 36 hrs in adults and 8 to 14 hrs in children, again reinforcing the need for individualised dose titration [4, 7]. The initial half-life of the epoxide metabolite is 25–43 hrs [4]. Due to its narrow therapeutic window and non-linear pharmacokinetics, therapeutic drug monitoring (TDM) is essential for achieving safe and effective dosing of CBZ [11, 52]. The most commonly applied methods for routine CBZ determination in biological materials are high-performance liquid chromatography with ultraviolet or photodiode detection [high-performance liquid chromatography-ultraviolet (HPLC-UV) and high-performance liquid chromatography–photodiode array (HPLC-PDA)] and immunoassay [fluorescence polarization immunoassay (FPIA)] [53]. The therapeutic CBZ reference range is 4–12 mg/L, although individual variation exists, with the minimum toxic level of 10 mg/L [4, 54, 55]. The usual plasma concentration range of CBZ-E, 0.2–2 mg/L, should be considered in case of intoxication [55]. When CBZ is prescribed with other anticonvulsants, the therapeutic range is from 4 to 8 mg/L. Therefore, TDM is also critical for assessing the effect of co-administration of other drugs, since CBZ can induce CYP3A4 or other oxidative enzymes and enhance glucuronyltransferase activity, and, as a result, accelerate metabolism of these drugs and decrease their concentrations in the body (like warfarin, oral contraceptives, tricyclic antidepressants, antipsychotics) [3, 56, 57]. In contrast, felbamate, oxcarbazepine, phenobarbital, phenytoin, primidone, and rufinamide induce its clearance, resulting in lower CBZ concentrations [56, 57].

Moreover, there are suggestions related to genotyping patients in order to evaluate its effects on CBZ TDM. Belhekar et al. [58] conducted a study demonstrating that adding *CYP3A5* genotyping to TDM did not improve the prediction of CBZ plasma levels or reduce ADRs compared with TDM alone. They observed substantial interindividual variability in CBZ plasma concentrations and CYP3A5 status, but no significant associations were noticed with trough plasma levels at 1, 3, 6, or 12 months of therapy, nor

with the occurrence of CBZ-related adverse effects. Their findings indicate that *CYP3A5* polymorphism, although biologically relevant to CBZ metabolism, does not substantially influence TDM outcomes in epileptic patients. They suggest routine genotyping for patients who develop unexpected toxicity of CBZ.

The complex pharmacokinetic CBZ behavior increases the potential for ADRs. Hypersensitivity reactions may occur in approximately 10% of patients [37]. The primary cause of ADRs may be metabolism *via* CYP3A4, as it is highly prone to auto- and hetero-induction or inhibition, making CBZ susceptible to a wide range of DDI that may increase or decrease its serum levels. Anticonvulsants such as clobazam and stiripentol inhibit CBZ metabolism, thereby increasing its plasma concentration and potentially leading to ADRs [3, 56, 57].

Some authors have highlighted clear benefits of combining TDM with genotyping to reduce ADRs. CBZ TDM is routinely done to optimize dosing, but genetic polymorphisms significantly influence patients' risk of CBZ toxicity or CBZ-induced hypersensitivity [58]. In a systematic review, Jaramillo et al. [59] noted that *HLA-B**15:02 and *HLA-A**31:01 are strongly linked to severe skin-related ADRs. They recommend *HLA-B**15:02 genotyping for Asian patients to prevent severe ADRs. Relying only on TDM is insufficient because it cannot predict the CBZ immune-mediated reactions.

CBZ has an established role in epilepsy treatment, but up to 40% of patients may still demonstrate pharmacoresistance [60]. A study by Puranik et al. [61] showed that genetic variation in *CYP3A4*, *CYP3A5*, *EPHX1*, *UGT2B7*, *ABCB1*, and *ABCC2* significantly contributes to interindividual differences in CBZ pharmacokinetics, emphasizing a clear genotype-dependent covariate in population pharmacokinetics. These factors also contributed to altered CBZ-E/CBZ and CBZ-diol/CBZ-E ratios. Transporter polymorphisms were associated with decreased central nervous system penetration and inadequate seizure control.

A study by Kang et al. [27] did not directly determine the genetic polymorphisms of *CYP3A4*; however, it demonstrated that functional variability in the activity of this enzyme could have a substantial influence on patients' susceptibility to form reactive covalent adducts and develop severe idiosyncratic ADRs.

Fuhr et al. [62] developed a physiologically based pharmacokinetic model of CBZ and CBZ-E that included autoinduction of CYP3A4, CYP2C8, CYP2B6, and UGT2B7, which could predict plasma and saliva concentration-time profiles and DDI predictions. As CBZ is a known inducer of various enzymes and transporters, modelling methods for sensitive substrates could have future clinical implications.

Toxicokinetics of carbamazepine

In acute CBZ poisoning, the risk of severe outcomes depends on the ingested dose and the plasma concentrations of CBZ and its metabolite CBZ-E [63].

When doses of CBZ exceed 24 g in adults, it is correlated with fatal outcomes. Signs and symptoms of toxicity appear up to 3 hrs after ingestion, starting with neuromuscular disturbances, followed by impaired consciousness, which leads to coma, tremor, restlessness, psychomotor disturbances, dizziness, and drowsiness. Initial hyperreflexia is progressing to hyporeflexia, and ingested doses higher than 60 g led to severe cardiac dysfunction. Additionally, the presence of respiratory depression, abnormalities in the electrocardiogram, shock, and urinary retention demands intensive patient monitoring. The measures for overdose treatment are focused on the elimination of CBZ and include vomiting, gastric lavage, therapy with activated charcoal, forced diuresis, and extracorporeal therapy, such as hemodialysis or plasmapheresis. If seizures occurred, then treatment with benzodiazepines is recommended [4, 5, 64–66].

However, there are insufficient data on the relationship between CBZ dose, its pharmacokinetic behavior, and the severity of clinical manifestations [8, 9, 67]. CBZ pharmacokinetics is complex and variable even at therapeutic doses. In cases of large CBZ ingestions, it is additionally unpredictable, characterized by slow absorption, delayed peak concentrations, and prolonged elimination, resulting in zero-order kinetics [68, 69]. Serum levels of CBZ may increase up to 72–96 hrs, depending on the formulation used, due to impaired gastrointestinal motility, as a result of its anticholinergic effect, and poorly soluble mass of drug, whose dissolution rate is a limiting factor in the absorption process, since CBZ is a poorly water-soluble drug [70–72]. Compared with solid-dose CBZ formulations, which exhibit slow and variable absorption, the CBZ suspension for children and adults demonstrates predictable, rapid absorption, with a short time to reach peak drug levels [73]. Liquid formulations can cause severe clinical manifestations in cases of acute overdose due to rapid absorption and distribution. Based on the elimination rate of these patients, they recover much faster than others with CBZ solid formulation overdoses. However, although the toxic CBZ concentrations in patients are not entirely consistent with clinical manifestations, measuring serum CBZ concentrations is a standard method used to confirm drug exposure, and the minimum toxic level is 10 mg/L, while severe intoxication occurs at serum levels > 20 mg/L [4, 54, 55]. In children, the toxicity range is much lower. Monitoring of serum CBZ concentration in acute poisoning is a routine clinical procedure. Djordjević et al. [67] have demonstrated that salivary CBZ levels correlate well with serum concentrations in cases of overdosing, suggesting the possibility of extrapolating concentrations to their corresponding serum values. Clinical data from a study that analyzed acute mood stabilizer poisonings indicate that CBZ is associated with variable toxicity severity and clinical outcomes, emphasizing significant interindividual differences in the toxicokinetic behavior of CBZ that are not entirely predictable from dose alone [74].

Additionally, in overdose situations, CBZ reached markedly high serum concentrations due to saturation

kinetics in the epoxidation [75]. Namely, it is well known that processes which depend on specific proteins are capacity-limited, and due to that, metabolism in drug overdose is saturable in a dose-dependent manner [8]. According to Vree et al. [75], serum concentrations of CBZ in acute poisoning are consistently plateau-like, with delayed decreases and extended half-lives of CBZ and its metabolite, CBZ-E. Moreover, prolonged serum CBZ monitoring in severe overdose is necessary due to the possible clinical deterioration as a result of delayed toxicity caused by the rebound phenomenon due to redistribution of the drug from the tissue to the serum after some therapeutic measures, such as hemoperfusion [65, 75]. Nevertheless, it is necessary to monitor CBZ and CBZ-E serum concentrations serially, as prolonged absorption and enterohepatic recirculation may lead to late rises [76].

It was noted that there was an apparent lack of correlation between CBZ serum concentrations and clinical manifestations of toxicity, which could be partially attributed to the formation of active metabolites [65, 77]. Nonetheless, Winnicka et al. [9] have not found a statistically significant influence of CBZ-E levels on the duration of coma in poisoned patients. Perhaps the CBZ metabolites cannot be excluded as contributing factors to toxicity, since their protein binding is lower, and at high concentrations, they could have a significant impact. The study of Hundt et al. [8] suggests that CBZ metabolites are not more potent than the parent drug, but their additive effects can prolong or intensify clinical manifestations during overdose. In accordance with such findings, a case report was published describing the additional influence of CBZ-E in a case of fatal CBZ ingestion, and a dramatically higher ratio of CBZ-E/CBZ was observed, pointing to a significant contribution of CBZ-E serum concentrations quantification in overdose cases [63]. Therefore, the active metabolite CBZ-E accumulates alongside the parent drug and additionally contributes to neurotoxicity. In order to determine patients at risk for moderate to severe toxicity according to available clinical data at the time of initial admittance to the poison control center, Montgomery et al. [78] conducted a six-year study on patients with CBZ overdose. The most important finding regarding CBZ toxicokinetics was a confirmation of a weak but significant correlation between outcome and peak CBZ level for each observed age group, with severe toxicity exhibited in children at slightly lower CBZ levels than in adults. Clinical pharmacokinetic study of CBZ and its metabolites after an acute drug overdose indicated that CBZ and CBZ-E clearance values were low, and their dependency on urine flow supported the assumption that both were excreted by glomerular filtration minus tubular reabsorption [75]. Further population toxicokinetics research focused on estimating the clearance and volume of distribution of CBZ, CBZ-E concentrations, and the sum of these values. Lukic et al. [10] discussed the factors influencing the elimination of CBZ and CBZ-E in adult patients after acute poisoning with this drug. They concluded that elimination kinetics is strongly associated with high C-reactive protein and aspartate aminotransferase levels, as well as with treatment with sedating agents. Additionally, the elevated aspartate aminotransferase was not only an indicator of hepatic impairment, but also a consequence of possible rhabdomyolysis in patients with seizures or prolonged coma. Creatine kinase values in poisoned patients were significantly higher than in the healthy population.

When an overdose occurs after long-term exposure to CBZ, the toxicokinetic profile is very different from that of an acute overdose. Chronic use induces the liver CYP system continuously, so concentrations of CBZ may remain within the therapeutic range even when toxicity develops [76].

Conclusion

Carbamazepine exhibits significant pharmacokinetic and toxicokinetic variability due to its slow and variable absorption, extensive and complex hepatic metabolism, and pronounced autoinduction of the enzymes involved in its metabolism. Additionally, there is wide interindividual variability influenced by genetic polymorphisms in genes encoding the most important carbamazepine metabolic proteins, such as CYP3A4, CYP3A5, CYP2C8, EPHX1, UGT2B7, and transporters like ABCB1, ABCC2, and RALBP1, which influence carbamazepine absorption, distribution, metabolism, and excretion. The combination of routine therapeutic drug monitoring and pharmacogenetic testing could improve the safety and personalization of therapy. However, therapeutic drug monitoring remains the central tool in routine clinical practice, while the routine implementation of pharmacogenetic testing is currently limited to specific clinical situations and selected patient populations. After an overdose, the toxicokinetics of carbamazepine exhibit prominent nonlinearity due to prolonged absorption, saturable epoxidation in the liver, delayed clearance, and extended toxicity, as a result of accumulation of both parent compound and active metabolites. All of these lead to unpredictable elimination kinetics. Further well-designed prospective studies are needed to clarify genotype-phenotype relationships and their contribution to the precision of dosing strategies, both for therapeutic use and overdose treatment.

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Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Marson AG, Al-Kharusi AM, Alwaidh M, Appleton R, Baker GA, Chadwick DW,* et al. The SANAD study of effectiveness of carbamazepine, gabapentin, lamotrigine, oxcarbazepine, or topiramate for treatment of partial epilepsy: an unblinded randomised controlled trial. Lancet 2007; 369(9566): 1000–15. DOI: 10.1016/S0140-6736(07)60460-7.
2. *American Society of Health-System Pharmacists.* AHFS drug information 2016. 58th ed. Bethesda (US): American Society of Health-System Pharmacists; 2016. p. 3840.
3. *Metcalf CS, Smith MD, Wilcox KS.* Pharmacotherapy of the Epilepsies. In: *Brunton LL, Knollmann BC,* editors. Goodman & Gilman's The pharmacological basis of therapeutics. 14th ed. NY: McGrawHill; 2023. p. 385–411.
4. *Leikin JB, Paloucek FP.* Poisoning and Toxicology Handbook. 4th ed. New York: Informa Healthcare; 2008. p. 167–9.
5. *Doyon S.* Anticonvulsants. In: *Nelson LS, Lewin NA, Howland MA, Hoffman RS, Goldfrank LR, Flomenbaum NE,* editors. Goldfrank's Toxicologic Emergencies. 9th ed. New York: McGraw-Hill; 2011. p. 698–710.
6. *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.
7. *Ragueneau-Majlessi I, Yu J, Isoherranen N.* Design and optimization of dosage regimens: pharmacokinetic data. In: *Brunton LL,* editor. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 14th ed. New York: McGrawHill; 2023. p. 1533–89.
8. *Hundt HK, Aucamp AK, Müller FO.* Pharmacokinetic aspects of carbamazepine and its two major metabolites in plasma during overdosage. Hum Toxicol 1983; 2(4): 607–14. DOI: 10.1177/096032718300200405.
9. *Winnicka RI, Łopaciński B, Szymczak WM, Szymańska B.* Carbamazepine poisoning: elimination kinetics and quantitative relationship with carbamazepine 10,11-epoxide. J Toxicol Clin Toxicol 2002; 40(6): 759–65. DOI: 10.1081/clt-120015836.
10. *Lukic V, Jankovic SM, Petrovic NZ, Vucinic S, Jovic Stosic J, Djordjevic S,* et al. Population toxicokinetics of carbamazepine and its metabolite carbamazepine-10,11-epoxide in adults. Expert Opin Drug Metab Toxicol 2024; 20(8): 817–25. DOI: 10.1080/17425255.2024.2381555.
11. *Methaneethorn J, Lohitnavy M, Leelakanok N.* A Systematic Review of Population Pharmacokinetics of Carbamazepine. SRP 2020; 11(10): 653–73. DOI: 10.31838/srp.2020.10.98.
12. *Tolou-Ghamari Z, Zare M, Habibabadi JM, Najafi MR.* A quick review of carbamazepine pharmacokinetics in epilepsy from 1953 to 2012. J Res Med Sci 2013; 18(Suppl 1): S81–5.
13. *Schwarz A, Strakos C, Weihrich R.* A brief review on carbamazepine – history, pharmacological properties and environmental impact. Biochem Insights 2021; 1(4). DOI:10.33552/ICBC.2021.01.000519.
14. *Marvanova M.* Pharmacokinetic characteristics of antiepileptic drugs (AEDs). Ment Health Clin 2016; 6(1): 8–20. DOI: 10.9740/mhc.2015.01.008.
15. *Milovanovic JR, Jankovic SM.* Factors influencing carbamazepine pharmacokinetics in children and adults: population pharmacokinetic analysis. Int J Clin Pharmacol Ther 2011; 49(7): 428–36. DOI: 10.5414/cp201517.
16. *Boughrara W, Chentouf A.* The ABCB1, ABCC2 and RALBP1 polymorphisms are associated with carbamazepine response in epileptic patient: a systematic review. Acta Neurol Belg 2022; 122(4): 871–80. DOI: 10.1007/s13760-022-01920-5.
17. *Kiełbowski K, Król M, Bakinowska E, Pawlik A.* The Role of ABCB1, ABCG2, and SLC Transporters in Pharmacokinetic Parameters of Selected Drugs and Their Involvement in Drug–Drug Interactions. Membranes (Basel) 2024; 14(11): 223. DOI: 10.3390/membranes14110223.
18. *Jemnitz K, Heredi-Szabo K, Janossy J, Ioja E, Vereczkey L, Krajcsi P.* ABCC2/Abcc2: a multispecific transporter with dominant excretory functions. Drug Metab Rev 2010; 42(3): 402–36. DOI: 10.3109/03602530903491741.
19. *Djordjevic N, Cukic J, Dragas Milovanovic D, Radovanovic M, Radosavljevic I, Vuckovic Filipovic J,* et al. ABCB1 Polymorphism Is Associated with Higher Carbamazepine Clearance in Children. Pediatr Rep 2025; 17(1): 10. DOI: 10.3390/pediatric17010010.
20. *Wang P, Yin T, Ma HY, Liu DQ, Sheng YH, Wang C,* et al. Effects of CYP3A4/5 and ABCB1 genetic polymorphisms on carbamazepine metabolism and transport in Chinese patients with epilepsy treated with carbamazepine in monotherapy and bitherapy. Epilepsy Res 2015; 117: 52–7. DOI: 10.1016/j.eplepsyres.2015.09.001.
21. *Maqbool H, Saleem T, Sheikh N, Asmatullah, Mukhtar M, Javed I,* et al. Polymorphism in drug transporter gene ABCB1 is associated with drug resistance in Pakistani epilepsy patients. Epilepsy Res 2021; 178: 106814. DOI: 10.1016/j.eplepsyres.2021.106814.
22. *Fan YX, Zhang Z, Meng JR, Yin SJ, Wang P, Zhou T,* et al. Association of ABCB1 polymorphisms with carbamazepine metabolism and resistance in epilepsy: A meta-analysis. Epilepsy Res 2021; 177: 106785. DOI: 10.1016/j.eplepsyres.2021.106785.
23. *Awasthi S, Hallene KL, Fazio V, Singhal SS, Cucullo L, Awasthi YC,* et al. RLIP76, a non-ABC transporter, and drug resistance in epilepsy. BMC Neurosci 2005; 6: 61. DOI: 10.1186/1471-2202-6-61.
24. *Soranzo N, Kelly L, Martinian L, Burley MW, Thom M, Sali A,* et al. Lack of support for a role for RLIP76 (RALBP1) in response to treatment or predisposition to epilepsy. Epilepsia 2007; 48(4): 674–83. DOI: 10.1111/j.1528-1167.2007.00926.x.
25. *Jankovic SM, Jovanovic D, Milovanovic JR.* Pharmacokinetic modeling of carbamazepine based on clinical data from Serbian epileptic patients. Methods Find Exp Clin Pharmacol 2008; 30(9): 707–13. DOI: 10.1358/mf.2008.30.9.1323496.
26. *Thorn CF, Leckband SG, Kelsoe J, Leeder JS, Müller DJ, Klein TE,* et al. PharmGKB summary: carbamazepine pathway. Pharmacogenet Genomics 2011; 21(12): 906–10. DOI: 10.1097/FPC.0b013e328348c6f2.
27. *Kang P, Liao M, Wester MR, Leeder JS, Pearce RE, Correia MA.* CYP3A4-Mediated carbamazepine (CBZ) metabolism: formation of a covalent CBZ-CYP3A4 adduct and alteration of the enzyme kinetic profile. Drug Metab Dispos 2008; 36(3): 490–9. DOI: 10.1124/dmd.107.016501.
28. *ClinPGx.* Carbamazepine pathway, pharmacokinetics – pathway PA165817070 [Internet]. Leuven (BE): ClinPharGenomics; 2011 [cited 2025 Nov 11; accessed 2026 Jan 21]. Available from: https://www.clinpgx.org/pathway/PA165817070/overview
29. *Brodie MJ, Mintzer S, Pack AM, Gidal BE, Vecht CJ, Schmidt D.* Enzyme induction with antiepileptic drugs: cause for concern? Epilepsia 2013; 54(1): 11–27. DOI: 10.1111/j.1528-1167.2012.03671.x.
30. *Djordjevic N, Milovanovic DD, Radovanovic M, Radosavljevic I, Obradovic S, Jakovljevic M,* et al. CYP1A2 genotype affects carbamazepine pharmacokinetics in children with epilepsy. Eur J Clin Pharmacol 2016; 72(4): 439–45. DOI: 10.1007/s00228-015-2006-9.
31. *Alvarado AT, Muñoz AM, Varela N, Sullón-Dextre L, Pineda M, Bolarte-Arteaga M,* et al. Pharmacogenetic variants of CYP2C9 and CYP2C19 associated with adverse reactions induced by antiepileptic drugs used in Peru. Pharmacia 2023; 70(3): 603–18. DOI: 10.3897/pharmacia.70.e109011.

32. *Whirl-Carrillo M, Huddart R, Gong L, Sangkuhl K, Thorn CF, Whaley R,* et al. An evidence-based framework for evaluating pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther 2021; 110(3): 563–72. DOI: 10.1002/cpt.2350.
33. *Iannaccone T, Sellitto C, Manzo V, Colucci F, Giudice V, Stefanelli B,* et al. Pharmacogenetics of Carbamazepine and Valproate: Focus on Polymorphisms of Drug Metabolizing Enzymes and Transporters. Pharmaceuticals (Basel) 2021; 14(3): 204. DOI: 10.3390/ph14030204.
34. *Makmor-Bakry M, Sills GJ, Hitiris N, Butler E, Wilson EA, Brodie MJ.* Genetic variants in microsomal epoxide hydrolase influence carbamazepine dosing. Clin Neuropharmacol 2009; 32(4): 205–12. DOI: 10.1097/WNF.0b013e318187972a.
35. *He XJ, Jian LY, He XL, Tang M, Wu Y, Xu YY,* et al. Association of ABCB1, CYP3A4, EPHX1, FAS, SCN1A, MICA, and BAG6 polymorphisms with the risk of carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in Chinese Han patients with epilepsy. Epilepsia 2014; 55(8): 1301–6. DOI: 10.1111/epi.12655.
36. *Hung CC, Chang WL, Ho JL, Tai JJ, Hsieh TJ, Huang HC,* et al. Association of polymorphisms in EPHX1, UGT2B7, ABCB1, ABCC2, SCN1A and SCN2A genes with carbamazepine therapy optimization. Pharmacogenomics 2012; 13(2): 159–69. DOI: 10.2217/pgs.11.141.
37. *Yip VLM, Pertinez H, Meng X, Maggs JL, Carr DF, Park BK,* et al. Evaluation of clinical and genetic factors in the population pharmacokinetics of carbamazepine. Br J Clin Pharmacol 2021; 87(6): 2572–88. DOI: 10.1111/bcp.14667.
38. *Saiz-Rodríguez M, Almenara S, Navares-Gómez M, Ochoa D, Román M, Zubiaur P,* et al. Effect of the Most Relevant CYP3A4 and CYP3A5 Polymorphisms on the Pharmacokinetic Parameters of 10 CYP3A Substrates. Biomedicines 2020; 8(4): 94. DOI: 10.3390/biomedicines8040094.
39. *Zhao W, Meng H.* Effects of genetic polymorphism of drug-metabolizing enzymes on the plasma concentrations of antiepileptic drugs in Chinese population. Bioengineered 2022; 13(3): 7709–45. DOI: 10.1080/21655979.2022.2036916.
40. *Djordjevic N, Jankovic SM, Milovanovic JR.* Pharmacokinetics and Pharmacogenetics of Carbamazepine in Children. Eur J Drug Metab Pharmacokinet 2017; 42(5): 729–44. DOI: 10.1007/s13318-016-0397-3.
41. *Zhao GX, Zhang Z, Cai WK, Shen ML, Wang P, He GH.* Associations between CYP3A4, CYP3A5 and SCN1A polymorphisms and carbamazepine metabolism in epilepsy: A meta-analysis. Epilepsy Res 2021; 173: 106615. DOI: 10.1016/j.eplepsyres.2021.106615.
42. *Jamil A, Faisal MS, Zaman G, Khan M.* Decoding the genome: CYP3A5 rs15524 polymorphism and how it affects plasma carbamazepine levels in patients with epilepsy in Khyber Pakhtunkhwa. Afr J Biomed Res 2024; 31(1): 1187–94.
43. *Ganesapandian M, Ramasamy K, Adithan S, Narayan SK.* Influence of cytochrome P450 3A5 (CYP3A5) genetic polymorphism on dose-adjusted plasma levels of carbamazepine in epileptic patients in South Indian population. Indian J Pharmacol 2019; 51(6): 384–8. DOI: 10.4103/ijp.IJP_122_19.
44. *Seo T, Nakada N, Ueda N, Hagiwara T, Hashimoto N, Nakagawa K,* et al. Effect of CYP3A5*3 on carbamazepine pharmacokinetics in Japanese patients with epilepsy. Clin Pharmacol Ther 2006; 79(5): 509–10. DOI: 10.1016/j.clpt.2006.02.009.
45. *Ragia G, Dahl ML, Manolopoulos VG.* Influence of CYP3A5 polymorphism on the pharmacokinetics of psychiatric drugs. Curr Drug Metab 2016; 17(3): 227–36. DOI: 10.2174/1389200217666151210125831.
46. *Miyata-Nozaka Y, Zain SM, Taguchi M, Shigeyama M, Isobe T, Hanioka N.* Carbamazepine 10,11-epoxidation in human liver microsomes: influence of the CYP3A5*3 polymorphism. Pharmazie 2017; 72(12): 747–50. DOI: 10.1691/ph.2017.7754.
47. *Lu Q, Huang YT, Shu Y, Xu P, Xiang DX, Qu Q,* et al. Effects of CYP3A5 and UGT2B7 variants on steady-state carbamazepine concentrations in Chinese epileptic patients. Medicine (Baltimore) 2018; 97(30): e11662. DOI: 10.1097/MD.0000000000011662.
48. *Saleh Faisal M, Jamil A, Ali N, Alshahrani AM, Almarshad F.* Distribution pattern of UGT1A6 and UGT2B7 gene polymorphism and its impact on the pharmacokinetics of valproic acid and carbamazepine: Prospective genetic association study conducted in Pakistani patients with epilepsy. Gene 2024; 892: 147886. DOI: 10.1016/j.gene.2023.147886.
49. *Milovanovic J, Jankovic S.* Population pharmacokinetics. Vojnosanit Pregl 2005; 62(11): 847–50. (Serbian) DOI:10.2298/VSP0511847M.
50. *Kanojia N, Kukal S, Machahary N, Bora S, Srivastava A, Paul PR,* et al. Antiepileptic drugs carbamazepine and valproic acid mediate transcriptional activation of CYP1A1 via aryl hydrocarbon receptor and regulation of estrogen metabolism. J Steroid Biochem Mol Biol 2025; 248: 106699. DOI: 10.1016/j.jsbmb.2025.106699.
51. *Zhang ML, Chen XL, Bai ZF, Zhao X, Li WX, Wang XY,* et al. ABCB1 c.3435C > T and EPHX1 c.416A > G polymorphisms influence plasma carbamazepine concentration, metabolism, and pharmacoresistance in epileptic patients. Gene 2021; 805: 145907. DOI: 10.1016/j.gene.2021.145907.
52. *Patsalos PN, Spencer EP, Berry DJ.* Therapeutic drug monitoring of antiepileptic drugs in epilepsy: a 2018 update. Ther Drug Monit 2018; 40(5): 526–48. DOI: 10.1097/FTD.0000000000000546.
53. *Đorđević S, Kilibarda V, Stojanović T.* Determination of carbamazepine in serum and saliva samples by high performance liquid chromatography with ultraviolet detection. Vojnosanit Pregl 2009; 66(5): 347–52. DOI: 10.2298/vsp0905347d.
54. *Hojer J, Malmlund HO, Berg A.* Clinical features in 28 consecutive cases of laboratory confirmed massive poisoning with carbamazepine alone. J Toxicol Clin Toxicol 1993; 31(3): 449–58. DOI: 10.3109/15563659309000412.
55. *Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A.* Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Crit Care 2012; 16(4): R136. DOI: 10.1186/cc11441.
56. *Spina E, Pisani F, Perucca E.* Clinically significant pharmacokinetic drug interactions with carbamazepine. An update. Clin Pharmacokinet 1996; 31(3): 198–214. DOI: 10.2165/00003088-199631030-00004.
57. *Patsalos PN, Perucca E.* Clinically important drug interactions in epilepsy: general features and interactions between antiepileptic drugs. The Lancet Neurol 2003; 2(6): 347–56. DOI: 10.1016/s1474-4422(03)00483-6.
58. *Belhekar MN, Vinayak A, More S, Ambre S, Khimsuriya H.* A randomised controlled trial to evaluate genotyping and therapeutic drug monitoring vs only therapeutic drug monitoring as a strategy for risk minimisation in epileptic patients on carbamazepine therapy. Recent Trends Pharmacol 2025; 3(1): 27–35. DOI:10.62425/rtpharma.1614127.
59. *Jaramillo NM, Galindo IF, Vázquez AO, Cook HJ, LLerena A, López ML.* Pharmacogenetic potential biomarkers for carbamazepine adverse drug reactions and clinical response. Drug Metabol Drug Interact 2014; 29(2): 67–79. DOI: 10.1515/dmdi-2013-0046.
60. *Löscher W, Potschka H, Sisodiya SM, Vezzani A.* Drug resistance in epilepsy: clinical impact, potential mechanisms, and new innovative treatment options. Pharmacol Rev 2020; 72(3): 606–38. DOI: 10.1124/pr.120.019539.
61. *Puranik YG, Birnbaum AK, Marino SE, Ahmed G, Cloyd JC, Remmel RP,* et al. Association of carbamazepine major metabolism and transport pathway gene polymorphisms and pharma-

cokinetics in patients with epilepsy. Pharmacogenomics 2013; 14(1): 35–45. DOI: 10.2217/pgs.12.180.
62. *Fuhr LM, Marok FZ, Hanke N, Selzer D, Lehr T.* Pharmacokinetics of the CYP3A4 and CYP2B6 Inducer Carbamazepine and Its Drug-Drug Interaction Potential: A Physiologically Based Pharmacokinetic Modeling Approach. Pharmaceutics 2021; 13(2): 270. DOI: 10.3390/pharmaceutics13020270.
63. *Russell JL, Spiller HA, Baker DD.* Markedly Elevated Carbamazepine-10,11-epoxide/Carbamazepine Ratio in a Fatal Carbamazepine Ingestion. Case Rep Med 2015; 2015: 369707. DOI: 10.1155/2015/369707.
64. *Maan JS, Duong TvH, Saadabadi A.* Carbamazepine [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2025 Feb 14; accessed 2026 Jan 21]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482455/
65. *Wang L, Wang Y, Zhang RY, Wang Y, Liang W, Li TG.* Management of acute carbamazepine poisoning: A narrative review. World J Psychiatry 2023; 13(11): 816–30. DOI: 10.5498/wjp.v13.i11.816.
66. *Al Khalili Y, Sekhon S, Jain S.* Carbamazepine toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Feb 14; accessed 2026 Jan 21]. Available from: https://pubmed.ncbi.nlm.nih.gov/29939629/
67. *Djordjević S, Kilibarda V, Vucinić S, Stojanović T, Antonijević B.* Toxicokinetics and correlation of carbamazepine salivary and serum concentrations in acute poisonings. Vojnosanit Pregl 2012; 69(5): 389–93. DOI: 10.2298/VSP1205389D.
68. *Durelli L, Massazza U, Cavallo R.* Carbamazepine toxicity and poisoning. Incidence, clinical features and management. Med Toxicol Adverse Drug Exp 1989; 4(2): 95–107. DOI: 10.1007/BF03259906.
69. *Patel VH, Schindlbeck MA, Bryant SM.* Delayed elevation in carbamazepine concentrations after overdose: a retrospective poison center study. Am J Ther 2013; 20(6): 602–6. DOI: 10.1097/MJT.0b013e3182258e51.
70. *Rosenberg J, Benowitz NL, Pond S.* Pharmacokinetics of drug overdose. Clin Pharmacokinet 1981; 6(3): 161–92. DOI: 10.2165/00003088-198106030-00001.
71. *Graudins A, Peden G, Dowsett RP.* Massive overdose with controlled-release carbamazepine resulting in delayed peak serum concentrations and life-threatening toxicity. Emerg Med (Fremantle) 2002; 14(1): 89–94. DOI: 10.1046/j.1442-2026.2002.00290.x.
72. *Roberts DM, Buckley NA.* Pharmacokinetic considerations in clinical toxicology: clinical applications. Clin Pharmacokinet 2007; 46(11): 897–939. DOI: 10.2165/00003088-200746110-00001.
73. *Perez A, Wiley JF.* Pediatric carbamazepine suspension overdose-clinical manifestations and toxicokinetics. Pediatr Emerg Care 2005; 21(4): 252–4. DOI: 10.1097/01.pec.0000161471.57959.28.
74. *Abdel-Ghaffar Abdel-Dayem RS, Elshama SS, Maklad AI, Elmansy AM.* Assessment of the pattern, severity, and outcomes of acute mood stabilizer drug poisoning. Toxicol Res (Camb) 2024; 13(6): tfae193. DOI: 10.1093/toxres/tfae193.
75. *Vree TB, Janssen TJ, Hekster YA, Termond EF, van de Dries AC, Wijnands WJ.* Clinical pharmacokinetics of carbamazepine and its epoxy and hydroxy metabolites in humans after an overdose. Ther Drug Monit 1986; 8(3): 297–304. DOI: 10.1097/00007691-198609000-00011.
76. *Waseem M.* Carbamazepine toxicity: practice essentials [Internet]. Medscape; 2025 [cited 2025 Nov 14; accessed 2026 Jan 21]. Available from: https://emedicine.medscape.com/article/813654-overview
77. *Staniszewska A, Burda P, Waldman W, Anand JS.* Correlation between carbamazepine serum level and clinical condition of acute poisoned patient. Przegl Lek 2009; 66(6): 279–81. (Polish)
78. *Montgomery VL, Richman BJ, Goldsmith LJ, Rodgers GC Jr.* Severity and carbamazepine level at time of initial poison center contact correlate with outcome in carbamazepine poisoning. J Toxicol Clin Toxicol 1995; 33(4): 311–23. DOI: 10.3109/15563659509028916.

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A retrospective cohort study of survival in patients with healthcare-associated infection caused by *Klebsiella pneumoniae* – clinical, phenotypic, and genotypic predictors in a tertiary healthcare institution in Serbia (2022–2023)

Retrospektivna kohortna studija preživljavanja bolesnika sa bolničkom infekcijom izazvanom bakterijom *Klebsiella pneumoniae* – klinički, fenotipski i genotipski prediktori u tercijarnoj zdravstvenoj ustanovi u Srbiji (2022–2023)

Marko Simonović*, Bojan Rakonjac†, Aleksandra Nikolić‡, Jelena Dabić†, Duško Maksimović§, Momčilo Djurić†, Djordje Taušan‖, Vesna Šuljagić¶**

Military Medical Academy, ‖Treatment Sector, *Institute of Epidemiology, †Institute of Microbiology, §Clinic for Anesthesiology and Critical Care, ¶Department of Healthcare-Related Infection Control, Belgrade, Serbia; ‡University of Belgrade, Faculty of Medicine, Belgrade, Serbia; **University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. *Klebsiella* (*K.*) *pneumoniae* is a frequent cause of healthcare-associated infections (HAI), particularly in intensive care units. Carbapenem-resistant strains represent a serious threat due to high mortality and limited therapeutic options. The aim of this study was to identify clinical predictors of 30-day mortality and to determine the presence of carbapenemase genes among *K. pneumoniae* isolates. **Methods.** A retrospective cohort study was conducted at the Military Medical Academy in Belgrade, Serbia. It included 121 patients with HAI caused by *K. pneumoniae* between January 2022 and December 2023. Clinical data were collected through active HAI surveillance. Isolation and antimicrobial susceptibility testing were performed according to standard microbiological procedures, and detection of carbapenemase genes was carried out using multiplex polymerase chain reaction. Survival was analyzed using the Kaplan-Meier method, and predictors of mortality were assessed using Cox regression analysis. **Results**. Thirty-day mortality was 59.5%. High resistance rates were observed to aminoglycosides (84.3%), fluoroquinolones (94.2%), and carbapenems (95.9%), while 67.8% of isolates were multidrug-resistant. The most common gene was $bla_{OXA\text{-}48\text{-}like}$ (45.5% in 2022 and 65.9% in 2023), followed by bla_{NDM} (22.7% in 2022 and 4.5% in 2023), while bla_{KPC} was detected only in isolates from 2023 (23.9%). The most frequently detected combination of carbapenemase genes was bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ (31.8% in 2022 and 5.7% in 2023). Detected genes had no significant effect on survival. Age ≥ 70 years, bloodstream infection, and intensive care units stay were identified as independent predictors of 30-day mortality. **Conclusion**. The high mortality among patients with HAI caused by carbapenem-resistant *K. pneumoniae* strains was primarily associated with patient characteristics and disease severity rather than the presence of specific carbapenemase genes.

Keywords:
beta-lactamases; carbapenems; cross infection; drug resistance, bacterial; klebsiella pneumoniae; mortality; tertiary care centers.

Apstrakt

Uvod/Cilj. *Klebsiella* (*K.*) *pneumoniae* je čest uzročnik bolničkih infekcija (*healthcare-associated infections* – HAI), naročito u jedinicama intenzivne nege. Karbapenem-rezistentni sojevi predstavljaju ozbiljnu pretnju zbog visoke smrtnosti i ograničenih terapijskih mogućnosti. Cilj rada bio je da se identifikuju klinički prediktori 30-dnevne smrtnosti i da se utvrdi prisustvo gena za karbapenemaze kod izolata *K. pneumoniae*. **Metode**. Retrospektivna kohortna studija sprovedena je na Vojnomedicinskoj akademiji u Beogradu, Srbija. Studija je obuhvatila 121 bolesnika sa HAI izazvanim *K. Pneumoniae* od januara 2022. do decembra 2023. godine. Klinički podaci prikupljeni su aktivnim nadzorom nad HAI. Izolacija i ispitivanje osetljivosti na antimikrobne lekove urađeni su standardnim

Correspondence to: Marko Simonović, Military Medical Academy, Institute of Epidemiology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: markosimonovic84@gmail.com

mikrobiološkim procedurama, a detekcija gena za karbapenemaze urađena je metodom multipleks lančane reakcije polimeraze. Preživljavanje je analizirano Kaplan-Majerovom metodom, a prediktori smrtnosti Koksovom regresionom analizom. **Rezultati**. Tridesetodnevna smrtnost iznosila je 59,5%. Zabeležene su visoke stope rezistencije na aminoglikozide (84,3%), fluorohinolone (94,2%) i karbapeneme (95,9%), dok je 67,8% izolata bilo multirezistentno. Najčešći gen bio je *bla*$_{OXA\text{-}48\text{-}like}$ (45,5% u 2022. i 65,9% u 2023. godini), zatim *bla*$_{NDM}$ (22,7% u 2022. i 4,5% u 2023. godini), dok je *bla*$_{KPC}$ (23,9%) detektovan samo u 2023. godini. Najčešće otkrivena kombinacija gena za karbapenemaze bila je *bla*$_{NDM}$ + *bla*$_{OXA\text{-}48\text{-}like}$ (31,8% u 2022. i 5,7% u 2023. godini). Detektovani geni nisu imali značajan uticaj na preživljavanje. Starost ≥ 70 godina, infekcija krvotoka i boravak u jedinicama intenzivne nege identifikovani su kao nezavisni prediktori 30-dnevne smrtnosti. **Zaključak**. Visoka smrtnost bolesnika sa HAI izazvanom karbapenem-rezistentnim sojevima *K. pneumoniae* pre svega je bila povezana sa karakteristikama bolesnika i težinom osnovnog oboljenja, a ne sa prisustvom gena za karbapenemaze.

Ključne reči:
beta-laktamaze; karbapenemi; infekcija, intrahospitalna; lekovi, rezistencija mikroorganizama; klebsiella pneumoniae; mortalitet; zdravstvene ustanove, tercijarne.

Introduction

Klebsiella (*K.*) *pneumoniae* is a major cause of healthcare-associated infections (HAIs), including bloodstream infections (BSIs), pneumonia, and urinary tract infections. It represents one of the leading pathogens in intensive care units (ICUs) worldwide [1–3]. The emergence and spread of carbapenem-resistant *K. pneumoniae* (CRKP) have become a significant global public health challenge due to limited therapeutic options and high associated mortality rates [4, 5]. The World Health Organization (WHO) has identified carbapenem-resistant Enterobacterales, particularly CRKP, as critical priority pathogens requiring urgent research and development of new treatment options [5].

The prevalence of CRKP infections varies geographically, with the highest rates observed in Southern and Eastern Europe [6–8]. Data from the European Center for Disease Prevention and Control (ECDC) and the Central Asian and European Surveillance of Antimicrobial Resistance highlight persistently high resistance levels in the Balkan region, including Serbia, where carbapenem resistance rates among invasive *K. pneumoniae* isolates exceed 62.7% [7, 8]. Local studies confirm that CRKP strains predominate in Serbian hospitals, reflecting the regional epidemiological situation [9, 10].

The strong tendency of *K. pneumoniae* to acquire genetic material *via* horizontal gene transfer has facilitated the emergence of multidrug-resistant (MDR) strains, which are now predominant in hospital settings [1, 3]. Carbapenem resistance is primarily driven by the production of carbapenemases, such as New Delhi metallo-β-lactamase (NDM), oxacillinase-48 (OXA-48), *K. pneumoniae* carbapenemase (KPC), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase (IMP), which can hydrolyze carbapenems and other β-lactams [1, 3]. Studies assessing whether carbapenemase type influences survival in CRKP infections have shown inconsistent results. Some suggest higher mortality with metallo-β-lactamase producers, while others find that host factors and illness severity are more predictive than enzyme type [11–15].

In addition to host-related vulnerabilities, environmental and organizational factors, such as adherence to infection prevention and control (IPC) measures, staffing levels, and variability in nursing training, play a critical role in the acquisition and outcomes of HAIs caused by *K. pneumoniae*. These factors are particularly relevant in high-MDR healthcare settings, where systemic constraints may facilitate the transmission of CRKP. Several studies, including large systematic reviews, have shown that lower nurse-staffing levels and higher workloads are associated with increased risk of HAI, underscoring the importance of adequate staffing, adherence to IPC protocols, and organizational support in preventing CRKP spread [16, 17].

The aim of this study was to identify risk factors and mortality outcomes associated with *K. pneumoniae* HAIs and to provide molecular characterization of carbapenemase-producing isolates collected in a tertiary healthcare institution in Serbia.

Methods

This retrospective cohort study included 121 patients with registered HAIs caused by *K. pneumonia* between January 2022 and December 2023. The study was conducted at the Military Medical Academy (MMA), Belgrade, Serbia, a teaching hospital affiliated with the University of Defence, Belgrade. The MMA is a 1,000-bed tertiary university healthcare center, divided into 27 departments according to medical specialty. The study was approved by the Ethics Committee of the Faculty of Medicine MMA (No. 5/7/2024, from April 4, 2024).

Surveillance of healthcare-associated infection

The Department of Healthcare-Related Infection Prevention and Control performs continuous HAI surveillance among ICU and surgical patients hospitalized for more than 48 hrs. Patients were visited daily by an infection control nurse and a physician for data collection. The following variables were collected: age, sex, type of infection (pneumonia, BSI, urinary tract infection, surgical site infection), surgery within 30 days, ICU admission, primary diagnosis (cardiovascular, gastrointestinal, neurological, respiratory, cancer, injuries/intoxications, other), McCabe classification, pres-

ence of invasive devices [drain, central venous catheter (CVC), mechanical ventilation (MV), urinary catheter (UC)], and outcome. The ECDC definitions for HAIs translated into Serbian were applied [18].

Isolation and identification of Klebsiella pneumoniae

Clinical samples were collected from hospitalized patients with HAIs and processed according to standard operating procedures, including inoculation on appropriate culture media and incubation for 18–24 hrs under aerobic conditions at 37 °C. Isolate identification to the species level was performed using MALDI-TOF MS (Vitek® MS, bioMérieux, France). Isolates grown under aerobic conditions for 18–24 hrs on blood agar plates with 5% sheep blood were applied onto the analysis plate using a sterile loop, air-dried, and overlaid with matrix solution (Vitek® MS-CHCA, bioMérieux). The calibration strain *Escherichia coli* ATCC® 8739™ was analyzed in parallel. Non-repetitive *K. pneumoniae* isolates were included in further analyses.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and by minimum inhibitory concentration using an automated system (Vitek® 2, bioMérieux, France). Results were interpreted following EUCAST standards.

For the Kirby-Bauer disk diffusion method, Mueller-Hinton agar (pH 7.2–7.4) and disks of meropenem (10 μg), imipenem (10 μg), and ertapenem (10 μg) (Bio-Rad, France) were used. Bacterial suspensions were prepared to a density of 0.5 McFarland standard [$\approx 1 \times 10^8$ colony-forming units (CFU)/mL] and incubated at 37 °C for 18–24 hrs. *K. pneumoniae* isolates with inhibition zones for ertapenem and meropenem < 25 mm were selected for further testing.

Antibiotic susceptibility for selected strains was assessed using the automated Vitek® 2 system with AST-GN76 cards containing 12 antibiotics (piperacillin–tazobactam, ceftriaxone, ceftazidime, cefepime, ertapenem, imipenem, ciprofloxacin, levofloxacin, gentamicin, amikacin, trimethoprim–sulfamethoxazole, tigecycline) and phenotypic detection of extended-spectrum β-lactamases (ESBLs). Suspensions adjusted to 0.5 McFarland were prepared in sterile NaCl, and cards were incubated for 8–12 hrs with automatic readings every 15 min.

Colistin susceptibility was tested using the broth microdilution method (Liofilchem, Italy) according to EUCAST recommendations. Serial two-fold dilutions of colistin were prepared in cation-adjusted Mueller-Hinton broth in 96-well microtiter plates. Inocula of 0.5 McFarland suspensions were diluted to a final density of $\approx 5 \times 10^5$ CFU/mL. Plates were incubated at 37 °C for 18–20 hrs, and minimum inhibitory concentrations were recorded as the lowest concentrations showing no visible growth. Quality control was performed using *Escherichia coli* ATCC® 25922™.

Deoxyribonucleic acid isolation

Deoxyribonucleic acid (DNA) was extracted using the boiling method. Colonies from agar plates were transferred into Luria-Bertani broth and incubated overnight. A 1.5 mL aliquot was centrifuged at 12,000 revolutions *per* minute (rpm) for 2 min. The pellet was resuspended in 300 μL sterile distilled water, boiled for 10 min, cooled at -20 °C for 10 min, and centrifuged again at 12,000 rpm for 2 min. The supernatant containing DNA was transferred into a new tube and used for polymerase chain reaction (PCR) or stored at -20 °C.

Detection of carbapenemase genes by polymerase chain reaction

Carbapenemase genes (bla_{NDM} [19], bla_{KPC} [20], $bla_{OXA\text{-}48\text{-}like}$ [21], bla_{VIM} [22], and bla_{IMP} [22]) were detected using multiplex PCR. Primer sequences and amplicon sizes are listed in Table 1. PCR conditions included initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 59 °C for 60 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

Table 1

Primers used for carbapenemase gene detection

Primer	Sequence 5′-3′	Amplicon size (bp)	Reference
bla_{NDM} Fw	GGGCAGTCGCTTCCAACGGT	475	17
bla_{NDM} Rw	GTAGTGCTCAGTGTCGGCAT		
bla_{KPC} Fw	ATGTCACTGTATCGCCGTCT	893	18
bla_{KPC} Rw	TTTTCAGAGCCTTACTGCCC		
$bla_{OXA\text{-}48\text{-}like}$ Fw	TTGGTGGCATCGATTATCGG	744	19
$bla_{OXA\text{-}48\text{-}like}$ Rw	GAGCACTTCTTTTGTGATGGC		
bla_{VIM} Fw	GATGGTGTTTGGTCGCATA	390	20
bla_{VIM} Rw	CGAATGCGCAGCACCAG		
bla_{IMP} Fw	GGAATAGAGTGGCTTAATTCTC	188	20
bla_{IMP} Rw	CCAAACCACTACGTTATCT		

NDM – New Delhi metallo-β-lactamase; KPC – *Klebsiella pneumoniae* carbapenemase; OXA-48 – oxacillinase-48; VIM – Verona integron-encoded metallo-β-lactamase; IMP – imipenemase; Fw – forward primer; Rw – reverse primer; bp – base pairs.

Statistical analysis

Baseline differences in demographic and clinical characteristics were assessed across carbapenemase genes ($bla_{OXA-48-like}$, bla_{NDM}, bla_{KPC}, and bla_{NDM} + $bla_{OXA-48-like}$). Categorical variables were summarized as counts and percentages, and continuous variables as mean ± standard deviation (SD) or median (interquartile range), as appropriate. Differences in categorical variables were tested using Fisher's exact test with Monte Carlo simulation, while age differences were assessed using one-way analysis of variance. Two-sided *p*-values < 0.05 were considered statistically significant. The primary outcome was all-cause 30-day mortality following *K. pneumoniae* isolation. Survival was analyzed using Kaplan-Meier estimates stratified by carbapenemase genes, with group differences assessed using the log-rank test. Univariable Cox proportional hazards models were constructed for candidate predictors: age, sex, study year, infection type, surgery within 30 days, ICU admission, primary diagnosis, McCabe classification, invasive devices (drain, CVC, MV, UC), and carbapenemase genes. Since age did not satisfy the linearity assumption in the log-hazard, it was categorized as a binary variable (< 70 vs. ≥ 70 years), with the cut-off chosen based on the cohort median and clinical plausibility. Predictors with $p < 0.05$ in univariable analysis or considered clinically relevant were included in the multivariable model. Variables with fewer than 10 outcome events were excluded to avoid unstable estimates. McCabe classification was omitted due to the complete separation of outcomes. The proportional hazards assumption was evaluated graphically using log-minus-log plots. Model fit was assessed using likelihood ratio tests and -2 log-likelihood statistics. Analyses were conducted using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA).

Results

Characteristics of the studied cohort

A total of 121 patients with *K. pneumoniae* HAI were included in the study, with the majority (80.2%) detected in 2023 and 19.8% in 2022 (Table 2). The patients included 75 (62.0%) males and 46 (38.0%) females with a mean ± SD of 65.3 ± 17.3 years. Clinical characteristics of the patients are presented in Table 2. A mortality within 30 days occurred in 59.5% of patients.

Table 2

Distribution of carbapenemase gene types according to patient clinical characteristics

Parameter	All isolates (n = 121)	Most frequent carbapenemase genotypes (n = 110)	$bla_{OXA-48-like}$ (n = 68)	bla_{NDM} (n = 9)	bla_{KPC} (n = 21)	bla_{NDM} + $bla_{OXA-48-like}$ (n = 12)	*p*-value*
Study year							
2022	24 (19.8)	22 (20.0)	10 (45.5)	5 (22.7)	0 (0)	7 (31.8)	**< 0.001**
2023	97 (80.2)	88 (80.0)	58 (65.9)	4 (4.5)	21 (23.9)	5 (5.7)	
Age, years	65.3 (17.3)	65.0 (17.2)	64.5 (19.2)	74.7 (7.5)	67.1 (15.0)	57.2 (17.3)	0.148§
Gender							
male	75 (62.0)	69 (62.7)	42 (60.9)	6 (8.7)	11 (15.9)	10 (14.5)	0.374
female	46 (38.0)	41 (37.2)	26 (63.4)	3 (7.3)	10 (24.4)	2 (4.9)	
Previous stay in another hospital	28 (23.1)	26 (23.6)	17 (65.4)	1 (3.8)	5 (19.2)	3 (11.5)	0.909
Type of infection**							
pneumonia	35 (28.9)	33 (31.8)	19 (57.6)	3 (9.1)	9 (27.3)	2 (6.1)	0.421
BSI	49 (40.5)	45 (40.9)	29 (64.4)	2 (4.4)	10 (22.2)	4 (8.9)	0.579
UTIs	10 (8.3)	10 (9.1)	5 (50.0)	2 (20.0)	2 (20.0)	1 (10.0)	0.421
SSIs	13 (10.7)	11 (10.0)	5 (45.5)	2 (18.2)	2 (18.2)	2 (18.2)	0.275
Comorbidities							
diabetes mellitus	25 (20.7)	22 (20.0)	8 (36.4)	5 (22.7)	7 (31.8)	2 (9.1)	**0.007**
neoplasm	22 (18.2)	22 (20.0)	15 (68.2)	1 (4.5)	6 (27.3)	0 (0)	0.197
McCabe classification							
non-fatal disease	21 (17.4)	19 (17.3)	8 (42.1)	0 (0)	4 (21.1)	7 (36.8)	
ultimately fatal disease	26 (21.5)	23 (20.9)	14 (60.9)	4 (17.4)	5 (21.7)	0 (0)	**0.007**
rapidly fatal disease	74 (61.2)	68 (61.8)	46 (67.6)	5 (7.4)	12 (17.6)	5 (5.4)	
Presence of invasive devices***							
drain	55 (45.5)	50 (45.5)	33 (66.0)	1 (2.0)	11 (22.0)	5 (10.0)	0.170
central venous catheter	101 (83.5)	94 (85.4)	60 (63.8)	5 (5.3)	19 (20.2)	10 (10.6)	0.092
mechanical ventilation	101 (83.5)	93 (84.5)	61 (65.6)	4 (4.3)	20 (21.5)	8 (8.6)	**0.002**
urinary catheter	119 (98.3)	108 (98.2)	68 (63.0)	8 (7.4)	21 (19.4)	11 (10.2)	**0.035**
Pre-infection length of stay, day							
< 14	60 (49.6)	52 (47.3)	31 (59.6)	4 (7.7)	12 (23.1)	5 (9.6)	0.802
≥ 14	61 (50.4)	58 (52.7)	37 (63.8)	5 (8.6)	9 (15.5)	7 (12.1)	
Fatal outcome within 30 days	72 (59.5)	66 (60.0)	46 (69.7)	5 (7.6)	12 (18.2)	3 (4.5)	**0.048**
Surgery within 30 days	67 (55.4)	62 (56.4)	40 (64.5)	3 (4.8)	12 (19.4)	7 (11.3)	0.557

Table 2 (continued)

Parameter	All isolates (n = 121)	Most frequent carbapenemase genotypes (n = 110)	*bla*$_{OXA-48-like}$ (n = 68)	*bla*$_{NDM}$ (n = 9)	*bla*$_{KPC}$ (n = 21)	*bla*$_{NDM}$ + *bla*$_{OXA-48-like}$ (n = 12)	*p*-value*
Type of care							
non-ICU	12 (9.9)	11 (10.0)	7 (63.6)	2 (18.2)	1 (9.1)	1 (9.1)	0.471
ICU admission	109 (90.1)	99 (90.0)	61 (61.6)	7 (7.1)	20 (20.2)	11 (11.1)	
Primary diagnosis							
cardiovascular diseases	24 (19.8)	21 (19.1)	8 (38.1)	5 (23.8)	4 (19.0)	4 (19.0)	**0.008**
gastrointestinal diseases	15 (12.4)	15 (13.6)	11 (73.3)	0 (0)	2 (13.3)	2 (13.3)	0.686
neurological diseases	11 (9.1)	10 (9.1)	8 (80.0)	0 (0)	1 (10.0)	1 (10.0)	0.845
respiratory diseases	18 (14.9)	15 (13.6)	9 (60.0)	1 (6.7)	3 (20.0)	2 (13.3)	0.936
cancer	22 (18.2)	22 (20.0)	15 (68.2)	1 (4.5)	6 (27.3)	0 (0)	0.194
injuries and intoxications	22 (18.2)	20 (18.2)	12 (60.0)	1 (5.0)	5 (25.0)	2 (10.0)	0.906
other	9 (7.4)	7 (6.4)	5 (71.4)	1 (14.3)	0 (0)	1 (14.3)	0.433

BSI – bloodstream infection; UTI – urinary tract infection; SSI – surgical site infection; ICU – intensive care unit; n – number. For other abbreviations, see Table 1.
All values are given as numbers (percentages), except for age parameter, which is expressed as mean (standard deviation).
§*p*-value for one-way analysis of variance; other *p*-values derived from Fisher's exact test (Monte Carlo). Values that differ significantly ($p < 0.05$) are bolded.
***Note:* Percentages in the first column are based on all isolates (n = 121). *Comparative analyses and corresponding percentages refer to the four most prevalent carbapenemase groups only (n = 110). ** Some patients had multiple sites of infection. *** Some patients could have several invasive devices.**

Table 3

Antimicrobial susceptibility testing results of *Klebsiella pneumoniae*

Antimicrobial	Number of isolates tested	Susceptible	Susceptible, increased exposure	Resistant
Ceftriaxone/cefotaxime	121	0 (0)	0 (0)	121 (100)
Cefepime	121	1 (0.8)	0 (0)	120 (99.2)
Gentamicin	121	16 (13.2)	0 (0)	105 (86.8)
Amikacin	121	7 (5.8)	0 (0)	114 (94.2)
Ciprofloxacin	121	1 (0.8)	0 (0)	120 (99.2)
Levofloxacin	117	2 (1.7)	0 (0)	115 (95.0)
Imipenem	121	15 (12.4)	7 (5.8)	99 (81.8)
Meropenem	121	5 (4.1)	0 (0)	116 (95.9)
Trimethoprim-sulfamethoxazole	121	1 (0.8)	0 (0)	120 (99.2)
Colistin	119	47 (38.8)	0 (0)	72 (59.5)

All values are given as numbers (percentages).
***Note*: No isolates exhibit single resistance.**

Isolates were most often obtained from blood cultures (44.6%) and bronchial aspirates (38.8%), with smaller percentages from bronchoalveolar lavage (6.6%), urine (3.3%), wound swabs (2.5%), drains (1.7%), cerebrospinal fluid (1.7%), and brain abscess (0.8%).

Antimicrobial susceptibility testing

Antimicrobial susceptibility results are summarized in Table 3. A pronounced level of multidrug resistance was detected. Dual resistance to aminoglycosides (gentamicin and amikacin) was identified in 84.3% of isolates, when 15.7% remained susceptible to both agents. High resistance rates were also observed for fluoroquinolones, with 94.2% of isolates resistant to ciprofloxacin and levofloxacin, noting that levofloxacin testing was not performed on all isolates. Resistance to carbapenems was confirmed in 95.9% of isolates, with 18.2% interpreted as susceptible, including those classified as susceptible, and increased exposure according to EUCAST criteria. According to our study definition, *K. pneumoniae* isolates were considered carbapenem-resistant if they exhibited resistance to at least one of the two tested carbapenems (imipenem or meropenem). When resistance patterns were analyzed across four major antibiotic groups, including third-generation cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems, 67.8% of isolates exhibited resistance to all, highlighting the predominance of multidrug-resistant strains, while 32.2% retained at least partial susceptibility.

Distribution of carbapenemase genes during the study period

Of the 121 carbapenemase-producing isolates, 110 belonged to the four most frequent carbapenemase groups (*bla*$_{OXA-48-like}$, *bla*$_{NDM}$, *bla*$_{KPC}$, and *bla*$_{NDM}$ + *bla*$_{OXA-48-like}$), and were included in the comparative analysis. The distribution

of carbapenemase genes differed significantly between the two study years ($p < 0.001$) (Table 2).

The $bla_{OXA-48-like}$ gene was the most prevalent as the single carbapenemase gene detected in both years, with 45.5% of isolates in 2022 and 65.9% in 2023, followed by bla_{NDM}, with 22.7% in 2022 and 4.5% in 2023, while bla_{KPC} as a single carbapenemase gene was detected only in isolates from 2023 (23.9%). The most frequently detected combination of carbapenemase genes was bla_{NDM} + $bla_{OXA-48-like}$, with 31.8% in 2022 and 5.7% in 2023.

The remaining 11 isolates were excluded from the comparative analysis due to their small number and heterogeneity. Among these, the bla_{VIM} gene was detected only in combination with other carbapenemase genes, while the bla_{IMP} gene was not detected. Combinations $bla_{OXA-48-like}$ + bla_{KPC} and $bla_{OXA-48-like}$ + bla_{VIM} were each identified in two isolates in 2023. Single isolates carried bla_{NDM} + bla_{KPC} in 2022, and bla_{NDM} + bla_{VIM} and bla_{KPC} + bla_{VIM} in 2023. One isolate from 2022 harbored three carbapenemase genes (bla_{NDM} + $bla_{OXA-48-like}$ + bla_{KPC}). In addition, three isolates from 2023 did not carry any carbapenemase gene (Figure 1).

Association between carbapenemase genes and clinical characteristics of the patients

The association between carbapenemase genes and clinical characteristics of the patients is presented in Table 2. A statistically significant association was observed between carbapenemase gene distribution and diabetes mellitus status of the patients ($p = 0.007$). Non-diabetic patients were predominantly infected with *K. pneumoniae* carrying $bla_{OXA-48-like}$ (68.2%), whereas this carbapenemase was detected in 36.4% of isolates from diabetic patients. In contrast, diabetic patients were more frequently infected with *K. pneumoniae* carrying bla_{KPC} (31.8%) and bla_{NDM} (22.7%) carbapenemases.

In addition, a statistically significant association was observed between carbapenemase gene distribution and McCabe categories ($p = 0.007$) (Figure 2). In all categories, $bla_{OXA-48-like}$ was dominant (rapidly fatal disease 67.6%, ultimately fatal disease 60.9%, and non-fatal disease 42.1%); however, the relative contribution of other carbapenemase genes varied, and bla_{NDM} genes were not detected in the non-fatal disease category.

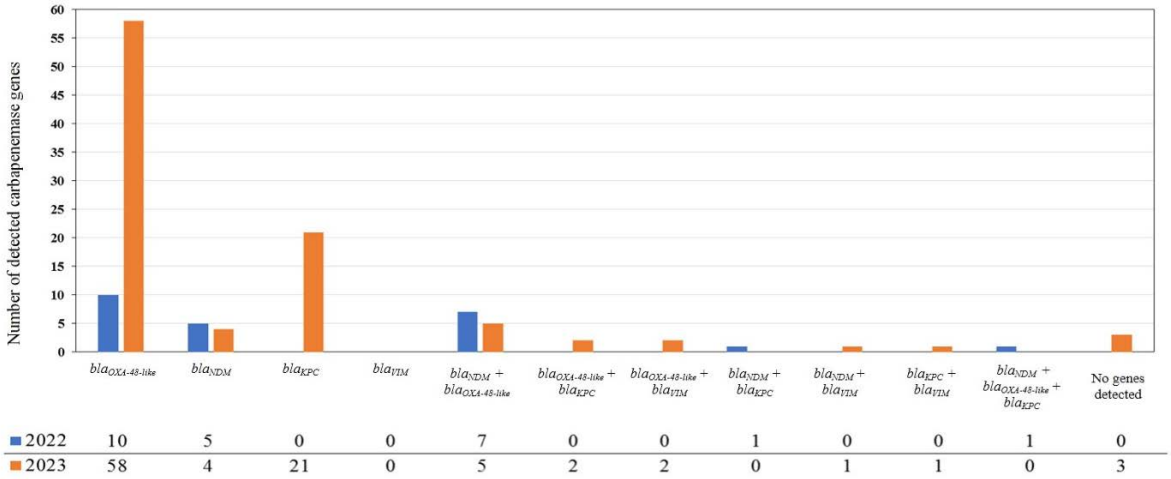

Fig. 1 – Distribution of carbapenemase genes during the study period. For abbreviations, see Table 1.

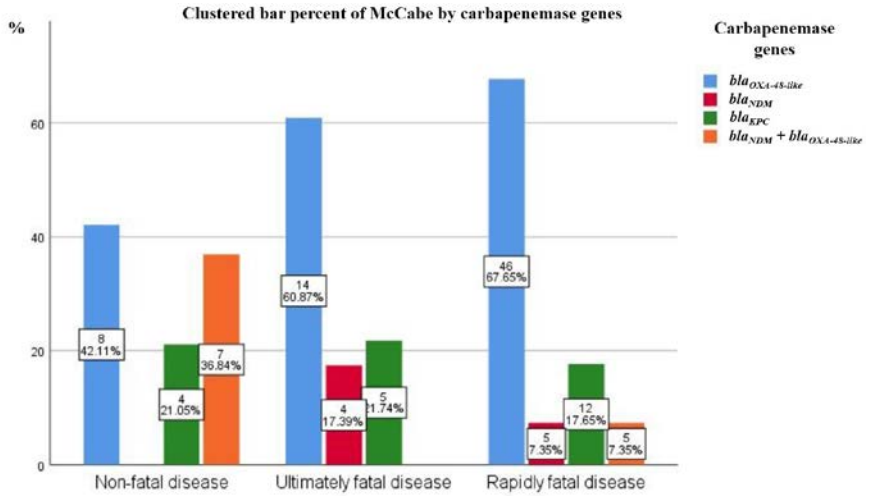

Fig. 2 – Distribution of carbapenemase genes according to McCabe classification at admission. For abbreviations, see Table 1.

Among patients on MV, the most common isolates were $bla_{OXA\text{-}48\text{-}like}$ (65.6%) and bla_{KPC} (21.5%), while bla_{NDM} was the least frequent (4.3%) compared with patients who were not on MV (p = 0.002). Urinary catheterization was nearly universal (98.3%), but the two patients without a UC carried bla_{NDM} and bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ (p = 0.035).

Overall, 59.5% of patients died within 30 days. Among non-survivors, $bla_{OXA\text{-}48\text{-}like}$ was most frequent (69.7%), whereas among survivors, $bla_{OXA\text{-}48\text{-}like}$ was isolated in 50.0% of patients. Among non-survivors, $bla_{OXA\text{-}48\text{-}like}$ was most frequent (69.7%), following bla_{KPC} (18.2%), bla_{NDM} (7.6%), and bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ (4.5%) (p = 0.048).

Kaplan-Meier analysis of 30-day survival

Kaplan-Meier survival analysis was performed to evaluate 30-day survival after *K. pneumoniae* isolation according to carbapenemase gene type (Figure 3). The overall median survival time was 13 days [95% confidence interval (CI): 8.7–17.3], while the mean survival was 16.1 days (95% CI: 13.8–18.4). In the subgroup analysis by carbapenemase gene type, the median survival was 12 days (95% CI: 7.5–16.5) for $bla_{OXA\text{-}48\text{-}like}$, 14 days (95% CI: 0–40.3) for bla_{NDM}, and 11 days (95% CI: 0–33.4) for bla_{KPC} producers. Median survival could not be estimated for the bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ group due to censoring, although the mean survival was longest in this group (22.8 days, 95% CI: 15.8–29.9) (Table 4).

The log-rank test showed no statistically significant difference in 30-day survival distributions across carbapenemase groups (χ^2 = 4.83, df = 3, p = 0.185), although isolates carrying the $bla_{OXA\text{-}48\text{-}like}$ gene demonstrated a clinically notable trend toward lower survival probabilities.

Cox regression analysis of predictors of 30-day survival

Results of univariable Cox regression analysis are presented in Table 5. In univariable Cox regression, age ≥ 70 years was associated with an increased risk of 30-day mortality [hazard ratio (HR): 1.96; 95% CI: 1.21–3.15; p = 0.006]. Among infection types, BSI was linked to a higher hazard of death (HR: 1.75; 95% CI: 1.01–2.78; p = 0.018), whereas pneumonia was associated with a lower risk (HR: 0.52; 95% CI: 0.29–0.91; p = 0.024). ICU admission (HR: 3.60; 95% CI: 1.13–11.46; p = 0.030), recent surgery within 30 days (HR: 1.82; 95% CI: 1.14–2.90; p = 0.013), and respiratory diseases as the primary diagnosis (HR: 1.80; 95% CI: 1.01–3.23; p = 0.049) were also significant predictors. In contrast, injuries and intoxications as the primary diagnosis appeared protective (HR: 0.45; 95% CI: 0.21–0.95; p = 0.036). Notably, predictors such as BSI and respiratory diseases had lower confidence-interval bounds close to 1.00 (1.01 and 1.01, respectively), indicating statistically fragile yet clinically plausible associations.

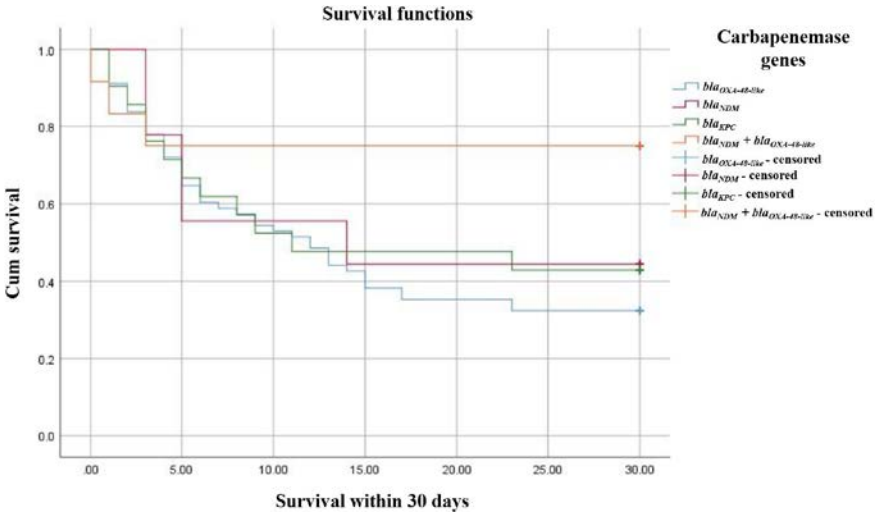

Fig. 3 – Kaplan-Meier survival estimates within 30 days after *Klebsiella pneumoniae* isolation according to carbapenemase type.
Cum – cumulative. For other abbreviations, see Table 1.
***Note*: The log-rank test showed no statistically significant difference in 30-day survival distributions across carbapenemase groups (χ^2 = 4.83, df = 3, p = 0.185).**

Table 4

Kaplan-Meier survival estimates within 30 days after *Klebsiella pneumoniae* isolation

Carbapenemase genes	Median survival (days)	95% CI for median	Mean survival (days)	95% CI for mean
$bla_{OXA\text{-}48\text{-}like}$	12	7.5–16.5	14.8	12.0–17.5
bla_{NDM}	14	0–40.3	16.7	8.6–24.7
bla_{KPC}	11	0–33.4	16.5	11.1–21.8
bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$*	–	–	22.8	15.8–29.9
Overall	13	8.7–17.3	16.1	13.8–18.4

CI – confidence interval. For other abbreviations, see Table 1.
***Note*: *Median not estimable as > 50% of patients were censored at 30 days.**

Table 5

Univariable Cox regression analysis of factors associated with 30-day mortality

Variable	Survivors (n = 49)	Non-survivors (n = 72)	HR (95% CI)	*p*-value
Study year				
2022	14 (28.6)	10 (13.9)	Ref –	–
2023	35 (71.4)	62 (86.1)	1.79 (0.91–3.49)	0.088
Age, years	57.8 ± 18.2	70.4 ± 14.7		
< 70	33 (67.3)	28 (38.9)	Ref –	–
≥ 70	16 (32.7)	44 (61.1)	1.96 (1.21–3.15)	0.006
Gender				
female	16 (32.7)	30 (41.7)	Ref –	–
male	33 (67.3)	42 (58.3)	0.74 (0.46–1.18)	0.211
Previous hospital stay	10 (20.4)	18 (25)	1.05 (0.61–1.79)	0.855
Type of infection				
pneumonia	20 (40.8)	15 (20.8)	0.52 (0.29–0.91)	0.024
BSI	15 (30.6)	34 (47.2)	1.75 (1.01–2.78)	0.018
UTI	5 (10.2)	5 (6.9)	0.82 (0.32–2.03)	0.664
SSI	8 (16.3)	5 (6.9)	0.54 (0.22–1.35)	0.191
Comorbidities				
diabetes mellitus	9 (18.4)	16 (22.2)	1.11 (0.64–1.94)	0.700
neoplasm	8 (16.3)	14 (19.4)	1.08 (0.60–1.94)	0.785
McCabe classification				
non-fatal	21 (42.9)	0 (0)	– –	–
ultimately fatal	15 (30.6)	11 (15.3)	– –	–
rapidly fatal	13 (26.5)	61 (84.7)	– –	–
Invasive devices				
drain	26 (53.1)	29 (40.3)	0.77 (0.48–1.23)	0.273
central venous catheter	43 (87.8)	58 (80.6)	0.88 (0.49–1.59)	0.683
mechanical ventilation	40 (81.6)	61 (84.7)	1.25 (0.66–2.38)	0.489
urinary catheter	47 (95.9)	72 (100)	1.82 (0.25–13.1)	0.553
Other predictors				
surgery within 30 days	35 (71.4)	32 (44.4)	1.82 (1.14–2.90)	0.013
pre-infection LOS ≥ 14 days	29 (59.2)	32 (44.4)	0.75 (0.47–1.19)	0.221
ICU admission	40 (81.6)	69 (95.8)	3.60 (1.13–11.46)	0.030
Primary diagnosis				
cardiovascular	9 (18.4)	15 (20.8)	0.97 (0.55–1.72)	0.927
gastrointestinal	5 (10.2)	10 (13.9)	1.52 (0.77–2.96)	0.223
neurological	6 (12.2)	5 (6.9)	0.60 (0.24–1.50)	0.276
respiratory	4 (8.2)	14 (19.4)	1.80 (1.01–3.23)	0.049
cancer	8 (16.3)	14 (19.4)	1.08 (0.60–1.94)	0.785
injuries/intoxications	14 (28.6)	6 (8.3)	0.45 (0.21–0.95)	0.036
other	3 (6.1)	6 (8.3)	1.30 (0.56–3.00)	0.537
Carbapenemase genes				
$bla_{OXA\text{-}48\text{-}like}$	22 (44.9)	46 (63.9)	1.50 (0.92–2.43)	0.097
bla_{NDM}	4 (8.2)	5 (6.9)	0.91 (0.36–2.24)	0.829
bla_{KPC}	9 (18.4)	12 (16.7)	0.96 (0.51–1.79)	0.903
bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$	9 (18.4)	3 (4.2)	0.33 (0.10–1.06)	0.063

LOS – length of stay; HR – hazard ratio; Ref – reference category.
For other abbreviations, see Tables 1, 2, and 4.
All values are given as numbers (percentages) or mean ± standard deviation.

Several variables did not reach statistical significance but showed HRs and CIs suggestive of potential clinical relevance. The presence of the $bla_{OXA\text{-}48\text{-}like}$ gene was associated with an elevated mortality risk (HR: 1.50; 95% CI: 0.92–2.43; $p = 0.097$), with the CI narrowly crossing 1.00.

McCabe classification showed complete HRs prediction of outcome: no deaths occurred among patients with non-fatal disease, whereas nearly all patients with rapidly fatal disease died within 30 days. Because of this complete separation, HR could not be reliably estimated for McCabe categories in univariable Cox regression, although descriptive analysis clearly demonstrated its strong prognostic value. For this reason, McCabe's classification was excluded from the multivariable analysis.

In the multivariable Cox regression model, age ≥ 70 years, BSI, and ICU admission remained independent predictors of 30-day mortality (Table 6). Patients aged ≥ 70 had nearly a two-fold increased hazard of death compared with those younger than 70 (HR: 1.98; 95% CI: 1.23–3.20; $p = 0.005$). BSI was associated with a 63% higher hazard (HR: 1.63; 95% CI: 1.02–2.61; $p = 0.040$), while ICU admission was associated with more than a three-fold increased hazard (HR: 3.35; 95% CI: 1.05–10.72; $p = 0.041$). Sex was not significantly associated with survival in this model.

Table 6

Univariable and multivariable Cox regression analysis of predictors of 30-day mortality

Variable	Univariable HR (95% CI)	*p*-value	Multivariable HR (95% CI)	*p*-value
Age ≥ 70 years	1.96 (1.21–3.15)	0.006	1.98 (1.23–3.20)	0.005
BSI	1.75 (1.01–2.78)	0.018	1.63 (1.02–2.61)	0.040
ICU admission	3.60 (1.13–11.46)	0.030	3.35 (1.05–10.72)	0.041
Pneumonia	0.52 (0.29–0.91)	0.024	–	n.s.
Surgery < 30 days	1.82 (1.14–2.90)	0.013	–	n.s.
Respiratory diagnosis	1.80 (1.01–3.23)	0.049	–	n.s.
Injuries/intoxications	0.45 (0.21–0.95)	0.036	–	n.s.

n.s. – not significant. For other abbreviations, see Tables 2, 4, and 5.

***Note*: Variables considered in the stepwise Cox regression were age ≥ 70 years, sex, BSI, ICU admission, pneumonia, recent surgery, and respiratory diagnosis. Only significant predictors are presented.**

Discussion

In this single-center cohort of hospitalized patients with HAI *K. pneumoniae* infections, 30-day all-cause mortality was high (59.5%). Independent predictors of poor outcome included age ≥ 70 years, BSI, and ICU admission, while carbapenemase genes did not independently influence survival. These findings emphasize that host and clinical severity remain the dominant determinants of short-term outcomes.

In our study, the mortality rate exceeds that reported in most multicenter European cohorts. It is important to differentiate healthcare between different countries. In the Serbian context, available data and literature indicate that death most often occurs in hospitals rather than at home. During the coronavirus disease 2019 pandemic, as many as 94.3% of deaths occurred in hospitals and only 5.7% at home [23]. Furthermore, the dying process has, in most cases, shifted from homes to healthcare facilities, highlighting that palliative care is still not fully integrated into everyday practice in Serbia [24].

However, Isler et al. [25] recently reported 30-day mortality of 44% in carbapenemase-harboring carbapenem-resistant *Klebsiella* spp. BSI infections, while a systematic review and meta-analysis indicated that more than 50% of ICU, HAI, CRKP, and ESBL-producing *K. pneumoniae* were associated with significantly higher 30-day mortality rates (estimated at ~ 29%) [26].

Similarly, Maraolo et al. [27] demonstrated an adjusted two-fold increase in death with CRKP vs. carbapenem-sensitive *K. pneumoniae* isolates. By contrast, northern European series, such as Fostervold et al. [28], documented substantially lower case-fatality (~12%), likely reflecting lower prevalence of resistance and more favorable baseline patient status. Our findings, therefore, align with the higher end of published mortality estimates, reflecting the severe case mix and therapeutic limitations in our setting.

Our results can also be compared with those of Soares de Moraes et al. [11], who investigated 107 patients with *K. pneumoniae* BSI in Brazil, of whom 50.5% carried carbapenem-resistant isolates. In their cohort, bla_{KPC} was the dominant carbapenemase, whereas in our study, $bla_{OXA\text{-}48\text{-}like}$ predominated, followed by bla_{KPC} and bla_{NDM}. Mortality was elevated in both studies, but different prognostic factors emerged. Soares de Moraes et al. [11] identified renal failure, liver failure, and extensively/pandrug-resistant status as predictors of death, while in our analysis, age ≥ 70 years, BSI, and ICU admission were most strongly associated with 30-day mortality. These results highlight that although resistance mechanisms differ across regions, mortality remains high and is largely determined by host condition and severity of illness rather than the presence of the carbapenemase genes alone.

Comparable mortality rates have been reported in several other high-risk cohorts. In Brazil, Andrey et al. [12] observed a 30-day mortality rate of 60% in patients with KPC-producing *K. pneumoniae* BSIs, with the sequence type 16 clone reaching nearly universal fatality. In Italy, Falcone et al. [29] reported a 30-day mortality rate of 40% in ICU patients with septic shock due to KPC-producing *K. pneumonia*. They demonstrated that a colistin-containing regimen, the use of two or more *in vitro* active antibiotics as definite therapy, and control of a removable source of infection were independently associated with a favorable outcome, while infection due to a colistin-resistant strain and an intra-abdominal source of infection were independently associated with death. Giacobbe et al. [30] further demonstrated that in the subgroup with colistin-resistant isolates, mortality reached ~ 50%, underscoring the impact of last-line resistance. Taken together, these findings confirm that our observed mortality lies at the upper range of global experience and is consistent with cohorts where advanced age, critical illness, and therapeutic limitations converge to drive poor outcomes.

Although in our cohort, the presence of the carbapenemase genes was not significantly associated with short-term survival, genetic information remains clinically relevant for antimicrobial selection. In particular, data from the Hellenic Ceftazidime/Avibactam Registry demonstrated markedly better outcomes among patients infected with KPC-producing *K. pneumoniae* compared with those harboring NDM or OXA-48 enzymes, underscoring the therapeutic importance of precise carbapenemase identification [31].

We observed high resistance rates to most of the tested antibiotics. Resistance to ceftriaxone/cefotaxime was universal (100%), far exceeding the European Union/European Economic Area (EU/EEA) population-weighted average of 34.8% reported by ECDC for 2023 [8]. Similarly, resistance to cefepime reached 99.2%, underscoring the diminished therapeutic value of extended-spectrum cephalosporins in our setting.

Very high resistance rates were also recorded for aminoglycosides, with 86.8% of isolates resistant to gentamicin and 94.2% to amikacin, compared to the European average of 23.6% [8]. Comparable patterns were observed for fluoroquinolones, with resistance rates of 99.2% for ciprofloxacin and 95.0% for levofloxacin; however, susceptibility testing was not performed for four isolates. By contrast, the European average was 33.7% [8]. These findings suggest that aminoglycosides and fluoroquinolones have lost nearly all clinical utility in our cohort, unlike in the broader European context.

Another major concern is the extremely high resistance to carbapenems, which have traditionally been considered last-line agents. Resistance to imipenem was observed in 81.8% of isolates, while resistance to meropenem reached 95.9%, compared with the EU/EEA average of 13.3% [8]. Although ECDC data indicated a gradual increase in carbapenem resistance over the 2019–2023 period, the levels observed in our study are considerably higher and align with reports of heavier resistance burdens in southern and eastern Europe [8].

High resistance was also observed for trimethoprim-sulfamethoxazole (99.2%).

Colistin showed variable results, with 59.5% of isolates being resistant and 38.8% remaining susceptible. Results are based on 119 isolates, as testing was unavailable for two isolates. This finding is clinically relevant given its role as a last-resort therapeutic option for carbapenem-resistant infections.

Dual resistance to both aminoglycosides was present in 84.3% of isolates, while 94.2% were resistant to both fluoroquinolones. Simultaneous resistance to both carbapenems was detected in 81.8% of isolates. In comparison, ECDC reported that 21.0% of *K. pneumoniae* isolates across the EU/EEA showed combined resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides, while 11.1% showed combined resistance to four groups of antibiotics, including carbapenems [8]. In our study, 67.8% of isolates were simultaneously resistant to all four groups, indicating a predominance of MDR *K. pneumoniae* strains. Similar trends were also reported in the Niš region (southern Serbia) during the 2014–2018 period, where *Klebsiella* spp. MDR isolates showed a decreasing trend in susceptibility to cephalosporins and fluoroquinolones, with resistance rates to ciprofloxacin and levofloxacin consistently above 60% [32].

The exceptionally high resistance rates observed in our study should be interpreted within the clinical context of a tertiary-care referral institution. These patients typically presented with severe or advanced clinical conditions, with nearly half diagnosed with BSI.

The majority required ICU management (90.1%) with exposure to invasive devices such as UC (98.3%), MV (83.5%), and CVC (83.5%), all of which are recognized risk factors for colonization and infection with MDR organisms. Extensive prior antimicrobial exposure combined with critical illness and invasive support creates strong selective pressure favoring the emergence and persistence of resistant strains. Therefore, the predominance of MDR *K. pneumoniae* isolates in our cohort is not only expected but also reflects the therapeutic challenges faced in high-risk clinical environments.

In the past period, there has been a rapid dissemination of MDR strains, including carbapenem-resistant strains [6–8]. A rapid and extensive dissemination of CRKP strains is caused by horizontal genetic transfer, since carbapenemase genes are mainly plasmid encoded [2].

During the study period, we detected $bla_{OXA\text{-}48\text{-}like}$ as the most dominant carbapenemase gene, followed by bla_{KPC}, bla_{NDM}, and bla_{VIM}. Moreover, the most common co-producers were bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$.

The distribution of carbapenemase genes in our study is consistent with previous reports from our country [33–35]. In the study by Zornic et al. [33], the majority of isolates carried $bla_{OXA\text{-}48\text{-}like}$, followed by bla_{KPC} and bla_{NDM} genes, while the majority of co-producers harbored $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM} genes. Additionally, bla_{NDM} + bla_{VIM} was detected in one isolate. In the other two studies, the most prevalent carbapenemase gene was $bla_{OXA\text{-}48\text{-}like}$, which was associated with the high-risk sequence type 101 clone. The plasmid that carried the $bla_{OXA\text{-}48\text{-}like}$ gene also carried the ESBL encoding gene $bla_{CTX\text{-}M\text{-}15}$ and several other resistance genes [34, 35]. In contrast to our results, a 2017 study of MDR *K. pneumoniae* strains reported bla_{NDM} was the most prevalent gene [36].

Our results are also related to the regional carbapenemase gene distribution, showing similar resistance profiles of *K. pneumoniae* strains with the predominant $bla_{OXA\text{-}48\text{-}like}$ carbapenemase circulating in neighboring countries. The predominance of $bla_{OXA\text{-}48\text{-}like}$ observed in our study is consistent with findings from several countries in the Balkan region [37–40].

In our study, the bla_{VIM} gene was detected in only four isolates from 2023, indicating its limited circulation compared with other carbapenemase genes. This finding is consistent with data from neighboring countries, where VIM-producing *K. pneumoniae* strains have been reported sporadically or replaced over time by other carbapenemase types [41]. Together, these findings suggest that in the Balkan region, bla_{VIM} genes occur at low frequencies and are being progressively displaced by the expansion of $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM}, mirroring the trend observed in our cohort.

With respect to comorbidities, diabetes mellitus was the only condition showing a statistically significant association with carbapenemase gene carriage. Among these patients, $bla_{OXA\text{-}48\text{-}like}$ and bla_{KPC} genes predominated, followed by bla_{NDM} and the bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ combination. A similar distribution of carbapenemase genes was observed among patients with neoplasms; however, this was not statistically significant. Given that these patients are typically immunocompromised, the presence of carbapenem-resistant isolates may further complicate treatment and increase the risk of adverse clinical outcomes. In contrast to our results, the study from Romania detected co-producers bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ as the dominant carbapenemase genes, but without a statistically significant association with diabetes mellitus and neoplasms [13].

A statistically significant association was also observed for the McCabe classification. The majority of cases were

classified as having a rapidly fatal disease, followed by an ultimately fatal disease. Within these categories, *bla*$_{OXA-48-like}$ and *bla*$_{KPC}$ were the most frequently detected carbapenemase genes, suggesting that these enzymes may be linked to more severe infections or reflect the higher vulnerability of critically ill patients. The majority of patients were treated in the ICU, frequently requiring MV and UC, both of which were significantly associated with carbapenemase gene distribution. Although our results confirm ICU stay, but not MV, CVC, or UC as risk factors for fatal outcome, other studies emphasize the importance of invasive procedures as potential risk factors for colonization or infection by CPKP [6, 10, 11].

Kaplan-Meier analysis showed differences in survival across carbapenemase genes, with the *bla*$_{NDM}$ + *bla*$_{OXA-48-like}$ group exhibiting the longest median survival, yet no statistically significant association with 30-day survival was detected. This aligns with a recent multicenter cohort where mortality did not differ between KPC- and NDM-producing isolates after adjustment for therapy and clinical severity [15]. In contrast, other cohorts reported worse outcomes with KPC compared with NDM producers [14]. A Spanish study focusing on OXA-48 producers further suggests that appropriate therapy, rather than the enzyme class *per se*, is the main determinant of short-term survival [42].

The marked shift in carbapenemase distribution between 2022 and 2023, characterized by a decline in NDM producers and a substantial increase in OXA-48 and KPC, may reflect changes in local epidemiology, plasmid transmission dynamics, selective antimicrobial pressure, and patient case mix. Similar year-to-year variability has been reported in tertiary hospitals across the Balkan region, where OXA-48 enzymes have rapidly become dominant over time [13]. Although our study design does not allow causal inference, these trends underscore the necessity of continuous molecular surveillance.

In addition to patient-related and microbiological factors, contextual characteristics of our healthcare system likely contribute to the high burden of CRKP and the poor outcomes observed. IPC programs are formally implemented in our hospital, but adherence to hand hygiene, contact precautions, and device-related bundles is often inconsistent in daily practice. These challenges are compounded by a chronic shortage of adequately trained nursing staff, with a considerable proportion of nurses newly qualified or retrained and with limited experience in caring for critically ill patients. Such constraints may reduce compliance with complex IPC procedures and delay early recognition of clinical deterioration, thereby further increasing the risk of adverse outcomes in this vulnerable population. Future studies should explicitly evaluate the impact of these environmental and organizational factors on CRKP transmission and mortality.

This study has several limitations. First, it was conducted in a single tertiary-care center, which may limit the generalizability of the findings. Second, the retrospective design is prone to selection and information bias, and some relevant clinical variables were not consistently available. Third, the relatively small sample size reduced statistical power, resulting in wide or imprecise CIs for several predictors. In some cases, the CIs were close to 1.00, including both statistically non-significant and statistically significant associations, indicating limited precision and the possibility that certain effects may be underestimated or overestimated. These findings should therefore be interpreted with caution and validated in larger cohorts. The limited number of events also affected age modeling, where different categorizations produced similar trends but varied in stability due to sparse events within strata. Additionally, although molecular characterization of carbapenemase genes was performed, whole-genome sequencing was not available and would have enabled a more detailed examination of resistance mechanisms and transmission dynamics. Finally, treatment-related data and infection prevention factors were not analyzed in depth, limiting conclusions regarding therapeutic effectiveness and environmental contributors to patient outcomes.

The strengths of our study include the integration of clinical outcomes with both phenotypic and genotypic characterization of CRKP isolates, the setting in a large tertiary-care center that reflects real-world practice, and the contemporaneous data capturing the ongoing regional shift toward NDM and OXA-48 producers. Moreover, our study represents a foundational effort to understand *K. pneumoniae* resistance in our hospital.

Conclusion

In this retrospective cohort of patients with healthcare-associated infections caused by carbapenem-resistant *Klebsiella pneumoniae*, 30-day mortality was high. Carbapenemase genes were not independently associated with short-term survival, whereas age ≥ 70 years, bloodstream infection, and intensive care unit stay emerged as the main predictors of mortality. These findings suggest that host factors and clinical severity, rather than specific carbapenemase genes, are the key determinants of outcome in this high-risk population. Our findings contribute valuable epidemiological insights from a Balkan tertiary-care setting, highlighting the regional predominance of New Delhi metallo-β-lactamase and oxacillinase-48 producers. Genes that encode *Klebsiella pneumoniae* carbapenemase and Verona integron-encoded metallo-β-lactamase carbapenemases were less prevalent and detected only in isolates from 2023, while the *bla imipenemase* gene was not detected in our study. Taken together, these observations reinforce the importance of coordinated local, regional, and international efforts to mitigate the impact of carbapenem-resistant *Klebsiella pneumoniae* and reduce its associated mortality.

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R E F E R E N C E S

1. *Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL.* Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 2012; 25(4): 682–707. DOI: 10.1128/CMR.05035-11.
2. *Pitout JD, Nordmann P, Poirel L.* Carbapenemase-Producing Klebsiella pneumoniae, a Key Pathogen Set for Global Nosocomial Dominance. Antimicrob Agents Chemother 2015; 59(10): 5873–84. DOI: 10.1128/AAC.01019-15.
3. *Caliskan-Aydogan O, Alocilja EC.* A Review of Carbapenem Resistance in Enterobacterales and Its Detection Techniques. Microorganisms 2023; 11(6): 1491. DOI: 10.3390/microorganisms11061491.
4. *Zhou R, Fang X, Zhang J, Zheng X, Shangguan S, Chen S,* et al. Impact of carbapenem resistance on mortality in patients infected with Enterobacteriaceae: a systematic review and meta-analysis. BMJ Open 2021; 11(12): e054971. DOI: 10.1136/bmjopen-2021-054971.
5. *Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E,* et al. The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. Lancet Infect Dis 2025; 25(9): 1033–43. DOI: 10.1016/S1473-3099(25)00118-5.
6. *Lin XC, Li CL, Zhang SY, Yang XF, Jiang M.* The Global and Regional Prevalence of Hospital-Acquired Carbapenem-Resistant Klebsiella pneumoniae Infection: A Systematic Review and Meta-analysis. Open Forum Infect Dis 2024; 11(2): ofad649. DOI: 10.1093/ofid/ofad649.
7. *European Centre for Disease Prevention and Control.* Rapid risk assessment -Carbapenem-resistant Enterobacterales: third update [Internet]. Stockholm: ECDC; 2025 [cited 2025 Dec 24]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/risk-assessment-carbapenem-resistant-enterobacterales-third-update-february-2025_0.pdf
8. *European Centre for Disease Prevention and Control, World Health Organization.* Antimicrobial resistance surveillance in Europe 2023: 2021 data. [Internet]. LU: Publications Office of the EU; 2023 [cited 2025 Sep 24; accessed 2025 Dec 24]. Available from: https://data.europa.eu/doi/10.2900/63495
9. *Brkic S, Cirkovic I.* Carbapenem-Resistant Enterobacterales in the Western Balkans: Addressing Gaps in European AMR Surveillance Map. Antibiotics (Basel) 2024; 13(9): 895. DOI: 10.3390/antibiotics13090895.
10. *Ćirković I, Marković-Denić L, Bajčetić M, Dragovac G, Đorđević Z, Mioljević V,* et al. Microbiology of Healthcare-Associated Infections: Results of a Fourth National Point Prevalence Survey in Serbia. Antibiotics (Basel) 2022; 11(9): 1161. DOI: 10.3390/antibiotics11091161.
11. *Soares de Moraes L, Gomes Magalhaes GL, Material Soncini JG, Pelisson M, Eches Perugini MR, Vespero EC.* High mortality from carbapenem-resistant Klebsiella pneumoniae bloodstream infection. Microb Pathog 2022; 167: 105519. DOI: 10.1016/j.micpath.2022.105519.
12. *Andrey DO, Pereira Dantas P, Martins WBS, Marques De Carvalho F, Almeida LGP, Sands K,* et al. An Emerging Clone, Klebsiellapneumoniae Carbapenemase 2-Producing K. pneumoniae Sequence Type 16, Associated With High Mortality Rates in a CC258-Endemic Setting. Clin Infect Dis 2020; 71(7): e141–50. DOI: 10.1093/cid/ciz1095.
13. *Lazar DS, Nica M, Dascalu A, Oprisan C, Albu O, Codreanu DR,* et al. Carbapenem-Resistant NDM and OXA-48-like Producing K. pneumoniae: From Menacing Superbug to a Mundane Bacteria; A Retrospective Study in a Romanian Tertiary Hospital. Antibiotics (Basel) 2024; 13(5): 435. DOI: 10.3390/antibiotics13050435.
14. *Seo H, Kim HJ, Kim MJ, Chong YP, Kim SH, Lee SO,* et al. Comparison of clinical outcomes of patients infected with KPC- and NDM-producing Enterobacterales: a retrospective cohort study. Clin Microbiol Infect 2021; 27(8): 1167.e1–8. DOI: 10.1016/j.cmi.2020.09.043.
15. *Dickstein Y, Yahav D, Tiseo G, Mussini C, Franceschini E, Santoro A,* et al. Carbapenemase type and mortality in blood-stream infections caused by carbapenemase-producing enterobacterales: a multicenter retrospective cohort study. Infection 2025; 53(6): 2491–501. DOI: 10.1007/s15010-025-02584-y.
16. *Mitchell BG, Gardner A, Stone PW, Hall L, Pogorzelska-Maziarz M.* Hospital Staffing and Health Care-Associated Infections: A Systematic Review of the Literature. Jt Comm J Qual Patient Saf 2018; 44(10): 613–22. DOI: 10.1016/j.jcjq.2018.02.002.
17. *Santos-Marques C, Ferreira H, Gonçalves Pereira S.* Infection prevention and control strategies against carbapenem resistant Enterobacteriaceae - a systematic review. J Infect Prev 2022; 23(4): 167–85. DOI: 10.1177/17571774211066762.
18. *Institute of Public Health of Serbia "Dr Milan Jovanović Batut".* Healthcare-Associated Infections Definitions. Belgrade: Ministarstvo zdravlja Republike Srbije; 2022. p. 116. (Serbian)
19. *Manchanda V, Rai S, Gupta S, Rautela RS, Chopra R, Rawat DS,* et al. Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii. Indian J Med Microbiol 2011; 29(3): 249–53. DOI: 10.4103/0255-0857.83907.
20. *Bratu S, Tolaney P, Karumudi U, Quale J, Mooty M, Nichani S,* et al. Carbapenemase-producing Klebsiella pneumoniae in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. J Antimicrob Chemother 2005; 56(1): 128–32. DOI: 10.1093/jac/dki175.
21. *Poirel L, Potron A, Nordmann P.* OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 2012; 67(7): 1597–606. DOI: 10.1093/jac/dks121.
22. *Ellington MJ, Kistler J, Livermore DM, Woodford N.* Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 2007; 59(2): 321–2. DOI: 10.1093/jac/dkl481.
23. *Stevanović A, Šantrić-Milićević M, Todorović J, Rajović N, Rosić N, Bjelobrk G.* COVID-19 premature mortality in Serbia: Does the place of death matter? Eur J Public Health 2024; 34(Suppl 3): ckae144.1921. DOI: 10.1093/eurpub/ckae144.1921.
24. *Maric DM.* Attitudes towards death and end-of-life care. Med Istraž 2023; 56(4): 81–5. DOI: 10.5937/medi56-46957.
25. *Isler B, Özer B, Çınar G, Aslan AT, Vatansever C, Falconer C,* et al. Characteristics and outcomes of carbapenemase harbouring carbapenem-resistant Klebsiella spp. bloodstream infections: a multicentre prospective cohort study in an OXA-48 endemic setting. Eur J Clin Microbiol Infect Dis 2022; 41(5): 841–7. DOI: 10.1007/s10096-022-04425-4.
26. *Li D, Huang X, Rao H, Yu H, Long S, Li Y,* et al. Klebsiella pneumoniae bacteremia mortality: a systematic review and meta-analysis. Front Cell Infect Microbiol 2023; 13: 1157010. DOI: 10.3389/fcimb.2023.1157010.
27. *Maraolo AE, Corcione S, Grossi A, Signori A, Alicino C, Hussein K,* et al. The Impact of Carbapenem Resistance on Mortality in Patients With Klebsiella Pneumoniae Bloodstream Infection: An Individual Patient Data Meta-Analysis of 1952 Patients. Infect Dis Ther 2021; 10(1): 541–58. DOI: 10.1007/s40121-021-00408-8.
28. *Fostervold A, Raffelsberger N, Hetland MAK, Bakksjø R, Bernhoff E, Samuelsen Ø,* et al. Risk of death in Klebsiella pneumoniae bloodstream infections is associated with specific phylogenetic

lineages. J Infect 2024; 88(5): 106155. DOI: 10.1016/j.jinf.2024.106155.
29. *Falcone M, Russo A, Iacovelli A, Restuccia G, Ceccarelli G, Giordano A,* et al. Predictors of outcome in ICU patients with septic shock caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Clin Microbiol Infect 2016; 22(5): 444–50. DOI: 10.1016/j.cmi.2016.01.016.
30. *Giacobbe DR, Del Bono V, Trecarichi EM, De Rosa FG, Giannella M, Bassetti M,* et al. Risk factors for bloodstream infections due to colistin-resistant KPC-producing Klebsiella pneumoniae: results from a multicenter case-control-control study. Clin Microbiol Infect 2015; 21(12): 1106.e1–8. DOI: 10.1016/j.cmi.2015.08.001.
31. *Karaiskos I, Daikos GL, Gkoufa A, Adamis G, Stefos A, Symbardi S,* et al. Ceftazidime/avibactam in the era of carbapenemase-producing Klebsiella pneumoniae: experience from a national registry study. J Antimicrob Chemother 2021; 76(3): 775–83. DOI: 10.1093/jac/dkaa503.
32. *Veličković-Radovanović R, Stefanović N, Damnjanović I, Kocić B, Mladenović-Antić S, Dinić M,* et al. Antibiotic utility and susceptibility changes of multidrug-resistant Escherichia coli and Klebsiella spp: 5-year experience in a tertiary healthcare centre. Eur J Hosp Pharm 2022; 29(2): 84–9. DOI: 10.1136/ejhpharm-2021-002758.
33. *Zornic S, Petrovic I, Lukovic B.* In vitro activity of imipenem/relebactam and ceftazidime/avibactam against carbapenem-resistant Klebsiella pneumoniae from blood cultures in a University hospital in Serbia. Acta Microbiol Immunol Hung 2023; 70(3): 187–92. DOI: 10.1556/030.2023.02108.
34. *Palmieri M, D'Andrea MM, Pelegrin AC, Mirande C, Brkic S, Cirkovic I,* et al. Genomic Epidemiology of Carbapenem- and Colistin-Resistant Klebsiella pneumoniae Isolates From Serbia: Predominance of ST101 Strains Carrying a Novel OXA-48 Plasmid. Front Microbiol 2020; 11: 294. DOI: 10.3389/fmicb.2020.00294.
35. *Novović K, Trudić A, Brkić S, Vasiljević Z, Kojić M, Medić D,* et al. Molecular Epidemiology of Colistin-Resistant, Carbapenemase-Producing Klebsiella pneumoniae in Serbia from 2013 to 2016. Antimicrob Agents Chemother 2017; 61(5): e02550–16. DOI: 10.1128/AAC.02550-16.
36. *Trudić A, Jelesić Z, Mihajlović-Ukropina M, Medić D, Zivlak B, Gusman V,* et al. Carbapenemase production in hospital isolates of multidrug-resistant Klebsiella pneumoniae and Escherichia coli in Serbia. Vojnosanit Pregl 2017; 74(8): 715–21. DOI**:** 10.2298/VSP150917260T.
37. *Šuto S, Bedenić B, Likić S, Kibel S, Anušić M, Tičić V,* et al. Diffusion of OXA-48 carbapenemase among urinary isolates of Klebsiella pneumoniae in non-hospitalized elderly patients. BMC Microbiol 2022; 22(1): 30. DOI: 10.1186/s12866-022-02443-y.
38. *Gajdács M, Ábrók M, Lázár A, Jánvári L, Tóth Á, Terhes G,* et al. Detection of VIM, NDM and OXA-48 producing carbapenem resistant Enterobacterales among clinical isolates in Southern Hungary. Acta Microbiol Immunol Hung 2020; 67(4): 209–15. DOI: 10.1556/030.2020.01181.
39. *Melinte V, Radu MA, Văcăroiu MC, Mîrzan L, Holban TS, Ileanu BV,* et al. Epidemiology of Carbapenem-Resistant Klebsiella Pneumoniae Co-Producing MBL and OXA-48-Like in a Romanian Tertiary Hospital: A Call to Action. Antibiotics (Basel) 2025; 14(8): 783. DOI: 10.3390/antibiotics14080783.
40. *Ludden C, Lötsch F, Alm E, Kumar N, Johansson K, Albiger B,* et al. Cross-border spread of blaNDM-1- and blaOXA-48-positive Klebsiella pneumoniae: a European collaborative analysis of whole genome sequencing and epidemiological data, 2014 to 2019. Euro Surveill 2020; 25(20): 2000627. DOI: 10.2807/1560-7917.ES.2020.25.20.2000627.
41. *Chatzidimitriou M, Kavvada A, Kavvadas D, Kyriazidi MA, Eleftheriadis K, Varlamis S,* et al. Carbapenem-resistant Klebsiella pneumoniae in the Balkans: Clonal distribution and associated resistance determinants. Acta Microbiol Immunol Hung 2024; 71(1): 10–24. DOI: 10.1556/030.2024.02230.
42. *Corbella L, Fernández-Ruiz M, Ruiz-Ruigómez M, Rodríguez-Goncer I, Silva JT, Hernández-Jiménez P,* et al. Prognostic factors of OXA-48 carbapenemase-producing Klebsiella pneumoniae infection in a tertiary-care Spanish hospital: A retrospective single-center cohort study. Int J Infect Dis 2022; 119: 59–68. DOI: 10.1016/j.ijid.2022.03.025.

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Clinical treatment of wounds using nano platelet-rich plasma

Kliničko lečenje rana primenom nano plazme obogaćene trombocitima

[1]Yongjun Xu*, [1]Haifeng Zhu[†], Jian Feng[‡], Bin Shen[§]

Hangzhou Normal University, Affiliated Xiaoshan Hospital, *Department of Hand and Foot Surgery, [‡]Department of Orthopedics, [§]Department of Burn and Plastic Surgery, Hangzhou, Zhejiang, China; [†]Zhejiang University School of Medicine, Affiliated Shaw Hospital, Department of Orthopedics, Hangzhou, Zhejiang, China

[1]The two authors contributed equally to this study.

Abstract

Background/Aim. Chronic refractory wounds are hard-to-heal lesions that are susceptible to bacterial and other pathogenic infections, leading to additional tissue damage and a significant reduction in patients' quality of life. The aim of this study was to examine the efficacy of nano platelet-rich plasma (PRP) – nano-PRP compared with conventional PRP in the clinical treatment of wounds. **Methods.** The study included a total of 96 patients with chronic refractory wounds, including wounds caused by trauma and pressure ulcers, admitted between June 2021 and June 2023. According to the treatment applied, the patients were divided into two groups: the observation group (OG) (n = 47) with nano-PRP treatment and the control group (CG) (n = 49) with conventional PRP treatment. Group differences were analyzed using independent-samples *t*-tests for continuous variables and Chi-square tests for categorical variables. **Results.** The overall treatment efficacy in the OG was significantly higher than that in the CG ($p < 0.05$). After treatment, the visual analog scale score decreased in both groups compared with baseline, with the OG showing significantly lower scores than the CG ($p < 0.05$). The wound healing rate was also significantly higher in the OG ($p < 0.05$). Moreover, nano-PRP-treated patients exhibited improved outcomes for scar hyperplasia and a shorter wound healing time relative to the conventional PRP-treated group ($p < 0.05$). **Conclusion.** Compared with conventional PRP, nano-PRP demonstrates superior wound healing efficacy in patients with chronic refractory wounds, is associated with greater pain relief, and results in improved aesthetic outcomes. These findings suggest that nano-PRP may represent a more effective therapeutic option than conventional PRP for the clinical management of difficult-to-heal wounds.

Keywords:
platelet-rich plasma; quality of life; treatment outcome; wound healing; wounds and injuries.

Apstrakt

Uvod/Cilj. Hronične refraktorne rane su teško zarastajuće lezije koje su podložne infekcijama uzrokovanim bakterijama i drugim patogenima, što dovodi do dodatnog oštećenja tkiva i značajnog smanjenja kvaliteta života bolesnika. Cilj rada bio je da se ispita efikasnost nano plazme obogaćene trombocitima (*platelet-rich plasma* – PRP) – nano-PRP u poređenju sa konvencionalnom PRP u kliničkom lečenju rana. **Metode.** U studiju je bilo uključeno ukupno 96 bolesnika sa hroničnim refraktornim ranama, uključujući rane izazvane traumom i dekubitalne ulkuse, primljenih u periodu od juna 2021. do juna 2023. godine. U skladu sa primenjenim tretmanom, bolesnici su bili podeljeni u dve grupe: opservacionu grupu (OG) (n = 47) tretiranu nano-PRP i kontrolnu grupu (KG) (n = 49) tretiranu konvencionalnom PRP. Razlike između grupa analizirane su korišćenjem *t*-testa za nezavisne uzorke za kontinuirane varijable i Hi-kvadrat testa za kategorijske varijable. **Rezultati.** Ukupna efikasnost lečenja u OG bila je značajno veća nego u KG ($p < 0,05$). Nakon tretmana, vrednost skora na vizuelnoj analognoj skali se smanjila u obe grupe u poređenju sa početnim vrednostima, pri čemu je OG pokazala značajno niže rezultate od KG ($p < 0,05$). Brzina zarastanja rana je takođe bila značajno veća u OG ($p < 0,05$). Takođe, bolesnici tretirani nano-PRP pokazali su bolje ishode hiperplazije ožiljaka i kraće vreme zarastanja rane u odnosu na grupu tretiranu konvencionalnom PRP ($p < 0,05$). **Zaključak.** U poređenju sa konvencionalnom PRP, nano-PRP pokazuje bolju efikasnost u zarastanju rana kod bolesnika sa hroničnim refraktornim ranama, povezan je sa većim ublažavanjem bola i dovodi do boljih estetskih ishoda. Ovi nalazi ukazuju na to da nano-PRP može predstavljati efikasniju terapijsku opciju od konvencionalne PRP za kliničko lečenje teško zarastajućih rana.

Ključne reči:
plazma bogata trombocitima; kvalitet života; lečenje, ishod; rana, zarastanje; rane i povrede.

Correspondence to: Bin Shen, Hangzhou Normal University, Affiliated Xiaoshan Hospital, Department of Burn and Plastic Surgery, Hangzhou 310 200, Zhejiang Province, China. E-mail: shenbinaxhhnu@gpnu-edu.cn

Introduction

Chronic refractory wounds (CRWs) are persistent, hard-to-heal lesions that fail to progress through the expected sequential phases of healing [1]. Owing to their prolonged duration and impaired healing capacity, these wounds are highly susceptible to bacterial and other pathogenic infections, which can further exacerbate tissue damage and significantly compromise patients' quality of life. In severe cases, persistent local infection may progress to systemic involvement, posing serious health risks [1, 2]. As the wound area enlarges, adjacent tissues may undergo structural damage or functional impairment, leading to limitations in daily activities, reduced mobility, and diminished self-care abilities [3]. Additionally, prolonged treatment courses often impose psychological burdens on patients, contributing to anxiety, depression, and reduced self-esteem [4].

Platelet-rich plasma (PRP) has been widely applied in recent years to promote the healing of CRWs. However, conventional PRP therapy has several limitations, including difficulty in fully filling irregular wound defects and the inability to inject PRP gel into deeper or uneven tissue structures. Nano-clay, particularly synthetic laponite, is a nanoscale silicate material that has been extensively investigated for biomedical applications due to its favorable biocompatibility, low cytotoxicity, and ability to support cell adhesion and proliferation [5, 6]. Previous studies have demonstrated that laponite-based hydrogels exhibit good biocompatibility and have been safely used as carriers for bioactive molecules and growth factors in tissue regeneration. In addition, nano-clay offers adjustable wettability and permeability, making it a promising carrier material [7, 8].

The aim of this study was to examine the clinical therapeutic effects of nano-PRP in the management of CRWs, to provide a more effective treatment strategy, and to improve patient outcomes and quality of life.

Methods

General data

A total of 96 patients with CRWs secondary to trauma or pressure ulcers, admitted to our hospital between June 2021 and June 2023, were enrolled in this prospective controlled study. All enrolled patients were followed for 90 days after treatment. The study was approved by the Ethics Committee of the Affiliated Xiaoshan Hospital, Hangzhou Normal University, Zhejiang, China (No. HZNUXS-2021-089, from June 03, 2021). All participants were fully informed about the study procedures and voluntarily signed written informed consent forms prior to enrollment.

Inclusion and exclusion criteria

Inclusion criteria were as follows: patients diagnosed with CRWs, defined as wounds persisting for more than 4 weeks despite appropriate standard wound care, including regular debridement, infection control, and pressure offloading when indicated [9]; age ≥ 18 years; absence of known immune system diseases. Exclusion criteria included: presence of local or systemic infection; severe anemia; poor treatment compliance or refusal to cooperate; coexisting malignant tumors or other contraindications to the study intervention.

Group allocation

Patients were allocated to two groups based on the treatment they received. Because treatment selection depended on patient preference and clinical suitability, randomization was not applied in this study. Therefore, assignment to the conventional PRP, i.e., the control group (CG) (n = 49), or nano-PRP, i.e., the observation group (OG) (n = 47), was non-randomized. To reduce subjective bias, outcome assessors were blinded to the type of treatment administered. Patients and treating physicians were not blinded due to the nature of the interventions.

Examinations and treatments

All patients in both groups underwent routine pretreatment assessments, including complete blood count, electrocardiogram, and chest X-ray or chest computed tomography. Baseline wound images (frontal and lateral views) were collected before intervention.

The patients in the CG were treated with conventional PRP. A total of 10–40 mL of venous blood was collected into vacuum blood collection tubes, with the amount based on the patient's clinical condition. The collected blood was centrifuged at 1,500 revolutions *per* minute (rpm) for 10 min to remove erythrocytes, followed by a second centrifugation at 2,200 rpm for 15 min to obtain PRP. The PRP was transferred into a 4 × 4 cm square groove at the bottom of a disposable dressing change box (Yangzhou Songtian Medical Instrument Co., Ltd., China). One box was used *per* tube of collected blood. Thrombin (Kunming Baima Pharmaceutical Co., Ltd., China) dissolved in 3 mL of normal saline was added to PRP at a 1 : 1 ratio to produce PRP gel. After thorough debridement of the wound bed, the PRP gel was applied to cover and fill the wound fully. Vaseline gauzes and medical semipermeable membranes were used for coverage, followed by appropriate pressure bandaging. The wound was left undisturbed for 5–7 days and then managed with routine dressing changes. If adequate healing was not achieved, PRP treatment was repeated once.

The patients in the OG were treated with nano-PRP, defined in this study as a deferoxamine mesylate (DFO)-containing platelet-laponite gel. Venous blood collection and PRP preparation were performed as in the CG. For nano-clay preparation, montmorillonite nano-clay (Laponite XLG, BYK Additives, Germany; particle size ~30 nm) was used. Nano-clay powder was dispersed in distilled water to prepare a 2% w/v suspension and stirred for 30 min to obtain a homogeneous dispersion. PRP was mixed with the nano-clay suspension at a ratio of 4 : 1 v/v using a mechanical stirrer (800 rpm, 2 min), followed by ultrasonic homogenization for 30 s to ensure uniform mixing. A calcium chloride solution

at a final concentration of 20 mM was added dropwise to induce rapid gelation and form a bioactive nano-PRP hydrogel within approximately 2–3 min. Following repeat debridement, the nano-PRP hydrogel was injected into the wound using a double syringe through a three-way tube. The remaining portion was applied to cover and fill the wound surface. As in the CG, wounds were dressed with vaseline gauze and sealed with medical semipermeable membranes, followed by appropriate compression. The wound remained undisturbed for 5–7 days, after which routine dressing changes were performed. Nano-PRP treatment could be repeated once if the wound did not reach the expected healing stage.

Collection of clinical data

The following clinical data of patients were collected: baseline characteristics (age, sex, wound size, smoking history, alcohol consumption, presence of diabetes, wound type and condition body mass index) and, clinical and pathological parameters [overall treatment efficacy, Visual Analog Scale (VAS) score, wound aesthetics, incidence of complications, wound healing rate, degree of scar hyperplasia, and wound healing time]. All outcome data were collected during the 90-day follow-up period.

Evaluation of outcomes

Clinical efficacy was categorized as follows: basically healed (the wound was nearly completely closed with no infection or other complications); markedly effective (the wound showed substantial improvement but was not fully healed, and additional treatment or extended healing time might be required); effective (the wound exhibited partial improvement, but residual symptoms or defects remained, requiring further time or supplementary measures for complete healing); ineffective (minimal or no improvement was observed after treatment). The total effective rate was calculated as (total cases – ineffective cases)/total cases × 100%.

Pain intensity was assessed using the VAS score before treatment and 90 days after treatment, with lower scores indicating less pain.

Wound aesthetics were jointly evaluated by clinicians and patients using a 10-point scale: > 9 indicated very high satisfaction, 8–9 indicated satisfaction, and < 8 indicated dissatisfaction. The satisfaction rate was calculated as (total cases – dissatisfied cases)/total cases × 100%.

The complication rate was determined by calculating the incidence of postoperative complications, including wound infection, numbness, sepsis, and other adverse events.

The wound healing rate was calculated based on wound area measurements obtained on days 10, 30, and 90 after treatment.

Scar hyperplasia was assessed using the Vancouver Scar Scale, a validated clinician-rated tool that evaluates four key scar characteristics: vascularity (0–3), pigmentation (0–2), pliability (0–5), and height (0–3). Total scores range from 0 to 13, with higher scores indicating more severe scar hyperplasia and poorer scar quality [10].

Wound healing time was defined as the duration from initial debridement to complete wound closure within the 90-day follow-up period.

Statistical analysis

Statistical analyses were performed using SPSS version 26.0. Measurement data were subjected to the normality test. The normally distributed measurement data were described using mean ± standard deviations, and compared between groups using an independent-samples *t*-test. Categorical variables were presented as numbers (percentages) and analyzed using the χ^2 test or Fisher's exact test when appropriate. A p-value < 0.05 was considered statistically significant.

Results

Baseline clinical data

The baseline demographic and clinical characteristics of the two groups were collected, including age, sex, body mass index, wound etiology, wound duration, wound size, and comorbidities. Both groups had similar baseline variables ($p > 0.05$), indicating good group comparability and homogeneity prior to treatment (Table 1).

Table 1

Baseline clinical data

Characteristics	Group		t/χ^2	p
	observation (n = 47)	control (n = 49)		
Age, year	58.42 ± 12.15	59.87 ± 11.69	0.623	0.535
Sex				
male	28 (59.57)	30 (61.22)	0.028	0.867
female	19 (40.43)	19 (38.78)		
BMI, kg/m^2	23.74 ± 3.18	24.06 ± 3.25	0.497	0.620
Wound etiology				
trauma	31 (65.96)	32 (65.31)	0.006	0.936
pressure ulcer	16 (34.04)	17 (34.69)		
Wound duration, months	3.82 ± 1.47	3.91 ± 1.52	0.301	0.764
Wound size, cm^2	18.46 ± 6.72	19.03 ± 7.15	0.423	0.673
Diabetes mellitus	12 (25.53)	13 (26.53)	0.011	0.916
Hypertension	15 (31.91)	14 (28.57)	0.138	0.711

BMI – body mass index; n – number.
All values are given as numbers (percentages) or mean ± standard deviations.

Clinical efficacy

At 90 days after treatment, the total effective rate of the OG was significantly higher than that in the CG ($p < 0.05$), indicating superior therapeutic outcomes with nano-PRP (Table 2).

Visual analog scale scores

Before treatment, there was no significant difference in the VAS scores between the two groups ($p > 0.05$). At 90 days after treatment, pain levels in both groups decreased compared with baseline, with the OG showing a significantly lower VAS score than the CG ($p < 0.001$) (Table 3).

Wound aesthetics

Following treatment, the OG demonstrated significantly better wound aesthetic outcomes compared with the CG ($p < 0.05$) (Table 4).

Complication rates

At 90 days after treatment, the OG exhibited a significantly lower incidence of complications compared with the CG ($p = 0.05$) (Table 5).

Wound healing rates

After treatment, the wound healing rate in the OG was significantly higher than that in the CG ($p < 0.001$) (Table 6).

Scar hyperplasia status

Following treatment, the OG showed significantly better outcomes for scar hyperplasia than the CG ($p < 0.001$) (Table 7).

Wound healing time

After treatment, the OG demonstrated a significantly shorter wound healing time than the CG (20.96 ± 5.37 vs.

Table 2

Clinical efficacy

Group	Basically healed	Markedly effective	Effective	Ineffective	Total effective rate
Observation (n = 47)	29 (61.70)	10 (21.28)	5 (10.64)	3 (6.38)	93.62%
Control (n = 49)	15 (30.61)	14 (28.57)	13 (26.53)	7 (14.29)	85.71%
χ^2					10.240
p					0.017

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

Table 3

Visual analog scale scores

Group	Treatment	
	before	90 days after
Observation (n = 47)	5.87 ± 1.78	1.56 ± 0.94
Control (n = 49)	5.96 ± 2.03	2.78 ± 1.03
t	0.818	6.054
p	0.231	< 0.001

n – number.
All values are given as mean ± standard deviations.

Table 4

Wound aesthetics

Group	Very satisfied	Satisfied	Dissatisfied	Satisfaction rate	χ^2	*p*
Observation (n = 47)	26 (55.32)	19 (40.43)	2 (4.26)	45 (95.74)	6.76	0.034
Control (n = 49)	16 (32.65)	25 (51.02)	8 (16.33)	41 (83.67)		

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

Table 5

Complication rates

Group	Wound infection	Numbness	Sepsis	Complication rate	**p*
Observation (n = 47)	0 (0)	2 (4.26)	0 (0)	2 (4.26)	<0.05
Control (n = 49)	4 (8.16)	5 (10.2)	0 (0)	9 (18.37)	

n – number.
All values are given as numbers (percentages).
***Note*: *Fisher's exact test.**

Table 6

Wound healing rates

Group	After treatment		
	10 days	30 days	90 days
Observation (n = 47)	58.26 ± 7.13	85.94 ± 5.46	95.58 ± 3.27
Control (n = 49)	51.45 ± 6.89	76.55 ± 7.16	87.84 ± 5.49
t	4.759	7.203	8.348
p	< 0.001	< 0.001	< 0.001

n – number.
All values are given as mean ± standard deviations.

Table 7

Scar hyperplasia status

Group	After treatment		
	10 days	30 days	90 days
Observation (n = 47)	8.78 ± 1.96	3.69 ± 1.34	2.13 ± 1.27
Control (n = 49)	10.21 ± 2.13	5.39 ± 1.86	3.24 ± 1.12
t	3.419	5.119	4.547
p	< 0.001	< 0.001	< 0.001

n – number.
All values are given as mean ± standard deviations.

29.89 ± 8.73 days, respectively), with a statistically significant difference ($t = 6.006$, $p < 0.05$).

Discussion

Common causes of wounds include trauma such as cuts, abrasions, and burns resulting from external forces. Some of the external forces include: surgery procedures, where incisions in the skin and underlying tissues create operative wounds; ulcers, which develop from tissue necrosis due to prolonged pressure, friction, or ischemia, as seen in pressure sores and leg ulcers; diabetic foot, where neuropathy and impaired circulation associated with diabetes predispose to foot ulceration; chronic inflammatory conditions, including eczema and other persistent inflammatory skin disorders [11–13].

CRWs often have more complex underlying mechanisms. These may include microcirculation disturbances, where inadequate local blood flow compromises oxygen and nutrient delivery, thereby impairing healing; chronic infection by bacteria, fungi, or other pathogens, leading to persistent inflammation that delays tissue repair; immunocompromise, which increases susceptibility to infection and slows regeneration; hyperglycemia, as seen in diabetes, which disrupts normal wound healing process; malnutrition, where insufficient intake of proteins, vitamins, and minerals hinders effective tissue repair [14–16].

Extracted from patients' own blood, PRP is widely used in the management of CRWs. Because it carries no risk of immunologic rejection or transmissible disease and allows for individualized therapy, PRP has gained considerable attention in clinical practice. Rich in platelets, growth factors, and other bioactive molecules, PRP provides an abundant source of mediators that promote angiogenesis, enhance collagen synthesis, and stimulate cellular proliferation, thereby accelerating wound repair and regeneration. However, PRP also has limitations and may not be suitable for all CRWs [17, 18]. Irregular wound cavities are often difficult to fill with PRP fully, and the gel form cannot be injected into deeper or structurally complex defects. Moreover, PRP releases growth factors rapidly, producing a strong but short-lived stimulatory effect on surrounding tissues [19, 20]. Although beneficial for promoting early healing, this rapid release may also lead to undesirable effects, such as excessive fibroblast proliferation and increased risk of hypertrophic scarring [21].

To address these shortcomings, this study employed, a bioactive hydrogel dressing composed of PRP and nano-clay, referred to as nano-PRP. Laponite nanosheets have been widely studied in regenerative medicine due to their ability to bind growth factors, form shear-thinning injectable hydrogels, and prolong growth-factor release [7, 22]. Previous studies have shown that laponite-based hydrogels create a pro-regenerative microenvironment, promote M2 macrophage polarization, enhance angiogenesis, and support sustained tissue repair [20, 23]. Furthermore, recent biomaterials research has demonstrated that nano-composite PRP hydrogels exhibit superior protein retention, improved mechanical stability, and enhanced biological activity compared with conventional PRP formulations [24, 25]. Despite these promising findings, only a limited number of clinical studies on nano-PRP have been published. Therefore, our results contribute additional clinical evidence to this emerging field.

The findings of this study demonstrated that the total effective rate in the OG was significantly higher than that in the CG. This improvement may be attributable to the hydrogel's capacity for controlled and sustained growth-factor release, enhanced wound-bed coverage, and superior bioavailability of incorporated agents such as DFO, which further promotes angiogenesis [20, 26]. After treatment, the VAS score in the OG was markedly reduced compared with baseline and significantly lower than that in the CG. In addition, patients in the OG exhibited better wound aesthetic outcomes, a

lower incidence of complications, a higher wound healing rate, more favorable scar hyperplasia status, and a shorter healing time. These results collectively indicate that nano-PRP not only alleviates pain but also inhibits excessive scarring and substantially enhances the overall healing process of chronic wounds. These findings align with a prior study reporting that nano-engineered hydrogels modulate inflammation and reduce fibroblast over-proliferation, thereby supporting more organized tissue repair [27].

Despite these promising findings, the use of nano-PRP is not without challenges. Although laponite-based hydrogels are generally regarded as biocompatible, potential risks include local immune reactions, variable degradation behavior, or altered inflammatory responses, particularly in infected or heavily exudative wounds [28]. Additionally, PRP composition varies across individuals, potentially affecting treatment consistency. The preparation of nano-PRP requires specific materials and controlled gelation conditions [29], which may limit its practicality in settings without adequate laboratory support. Further studies are required to standardize nano-PRP preparation, evaluate long-term safety, and determine optimal clinical indications.

Study limitations

The absence of a non-PRP CG limits our ability to assess the absolute treatment effect relative to standard care. The relatively small sample size and the single-center design may reduce statistical power and limit the generalizability of the findings. Subgroup analyses by wound type (e.g., traumatic wounds vs. pressure sores) could not be performed due to the small number of cases in each category, making it unclear whether the benefits of nano-PRP are consistent across different etiologies. The 90-day follow-up period may not fully capture long-term healing outcomes or scar progression. Despite these limitations, the study provides meaningful preliminary evidence supporting the comparative advantages of nano-PRP.

Conclusion

This non-randomized comparative study suggests that nano platelet-rich plasma is associated with improved clinical outcomes compared with conventional platelet-rich plasma in the treatment of chronic refractory wounds, including accelerated wound healing, greater pain reduction, and improved aesthetic outcomes. These findings should be interpreted in light of the non-randomized study design and indicate that nano platelet-rich plasma may represent a promising regenerative approach. Further large-scale, randomized controlled studies are warranted to confirm these preliminary observations.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

1. *Frykberg RG, Banks J.* Challenges in the Treatment of Chronic Wounds. Adv Wound Care (New Rochelle) 2015; 4(9): 560–82. DOI: 10.1089/wound.2015.0635.
2. *Izadi M, Bozorgi M, Hosseine MS, Khalili N, Jonaidi-Jafari N.* Health-related quality of life in patients with chronic wounds before and after treatment with medical ozone. Medicine (Baltimore) 2018; 97(48): e12505. DOI: 10.1097/MD.0000000000001250.
3. *Wu J, Chen LH, Sun SY, Li Y, Ran XW.* Mesenchymal stem cell-derived exosomes: The dawn of diabetic wound healing. World J Diabetes 2022; 13(12): 1066–95. DOI: 10.4239/wjd.v13.i12.1066.
4. *Su YJ, Liang SH.* Long-term mental health outcomes of severe burn injury: A 5-year follow-up of the 2015 Formosa Fun Coast Water Park fire disaster. Gen Hosp Psychiatry 2022; 75: 10–6. DOI: 10.1016/j.genhosppsych.2022.01.007.
5. *Das SS, Neelam, Hussain K, Singh S, Hussain A, Faruk A,* et al. Laponite-based Nanomaterials for Biomedical Applications: A Review. Curr Pharm Des 2019; 25(4): 424–43. DOI: 10.2174/1381612825666190402165845.
6. *Katti KS, Jasuja H, Jaswandkar SV, Mohanty S, Katti DR.* Nanoclays in medicine: a new frontier of an ancient medical practice. Mater Adv 2022; 3(20): 7484–500. DOI: 10.1039/d2ma00528j.
7. *Stealey ST, Gaharwar AK, Zustiak SP.* Laponite-Based Nanocomposite Hydrogels for Drug Delivery Applications. Pharmaceuticals (Basel) 2023; 16(6): 821. DOI: 10.3390/ph16060821.
8. *Liu B, Li J, Lei X, Miao S, Zhang S, Cheng P,* et al. Cell-loaded injectable gelatin/alginate/LAPONITE® nanocomposite hydrogel promotes bone healing in a critical-size rat calvarial defect model. RSC Adv 2020; 10(43): 25652–61. DOI: 10.1039/d0ra03040f.
9. *Eriksson E, Liu PY, Schultz GS, Martins-Green MM, Tanaka R, Weir D,* et al. Chronic wounds: Treatment consensus. Wound Repair Regen 2022; 30(2): 156–71. DOI: 10.1111/wrr.12994. Erratum in: Wound Repair Regen 2022; 30(4): 536. DOI: 10.1111/wrr.13035.
10. *Hiremath SCS, Ahmed Z.* Comparison of Two Entry Methods and Their Cosmetic Outcomes in Creating Pneumoperitoneum: A Prospective Observational Study. Surg J (N Y) 2022; 8(3): e239–44. DOI: 10.1055/s-0042-1756182.
11. *Vu NB, Nguyen HT, Palumbo R, Pellicano R, Fagoonee S, Pham PV.* Stem cell-derived exosomes for wound healing: current status and promising directions. Minerva Med 2021; 112(3): 384–400. DOI: 10.23736/S0026-4806.20.07205-5.
12. *Bonkemeyer Millan S, Gan R, Townsend PE.* Venous Ulcers: Diagnosis and Treatment. Am Fam Physician 2019; 100(5): 298–305.
13. *Roshangar L, Soleimani Rad J, Kheirjou R, Reza Ranjkesh M, Ferdowsi Khosroshahi A.* Skin Burns: Review of Molecular Mechanisms and Therapeutic Approaches. Wounds 2019; 31(12): 308–15.
14. *Williams M.* Wound infections: an overview. Br J Community Nurs 2021; 26(Sup6): S22–5. DOI: 10.12968/bjcn.2021.26.Sup6.S22.
15. *Swaney MH, Kalan LR.* Living in Your Skin: Microbes, Molecules, and Mechanisms. Infect Immun 2021; 89(4): e00695–20. DOI: 10.1128/IAI.00695-20.
16. *Gupta S, Paliczak A, Delgado D.* Evidence-based indications of platelet-rich plasma therapy. Expert Rev Hematol 2021; 14(1): 97–108. DOI: 10.1080/17474086.2021.1860002.
17. *Fang J, Wang X, Jiang W, Zhu Y, Hu Y, Zhao Y,* et al. Platelet-Rich Plasma Therapy in the Treatment of Diseases Associated

with Orthopedic Injuries. Tissue Eng Part B Rev 2020; 26(6): 571–85. DOI: 10.1089/ten.TEB.2019.0292.

18. *Sharara FI, Lelea LL, Rahman S, Klebanoff JS, Moawad GN.* A narrative review of platelet-rich plasma (PRP) in reproductive medicine. J Assist Reprod Genet 2021; 38(5): 1003–12. DOI: 10.1007/s10815-021-02146-9.
19. *Carr BJ.* Platelet-Rich Plasma as an Orthobiologic: Clinically Relevant Considerations. Vet Clin North Am Small Anim Pract 2022; 52(4): 977–95. DOI: 10.1016/j.cvsm.2022.02.005.
20. *Zhang J, Luo Q, Hu Q, Zhang T, Shi J, Kong L,* et al. An injectable bioactive dressing based on platelet-rich plasma and nanoclay: Sustained release of deferoxamine to accelerate chronic wound healing. Acta Pharm Sin B 2023; 13(10): 4318–36. DOI: 10.1016/j.apsb.2022.11.006.
21. *Yu Z, Li Y, Fu R, Xue Y, Zhao D, Han D.* Platycodin D inhibits the proliferation and migration of hypertrophic scar-derived fibroblasts and promotes apoptosis through a caspase-dependent pathway. Arch Dermatol Res 2023; 315(5): 1257–67. DOI: 10.1007/s00403-022-02513-1.
22. *Kim YH, Yang X, Shi L, Lanham SA, Hilborn J, Oreffo ROC,* et al. Bisphosphonate nanoclay edge-site interactions facilitate hydrogel self-assembly and sustained growth factor localization. Nat Commun 2020; 11(1): 1365. DOI: 10.1038/s41467-020-15152-9.
23. *Zhou L, Zhang C, Shi T, Wu D, Chen H, Han J,* et al. Functionalized 3D-printed GelMA/Laponite hydrogel scaffold promotes BMSCs recruitment through osteoimmunomodulatory enhance osteogenic via AMPK/mTOR signaling pathway. Mater Today Bio 2024; 29: 101261. DOI: 10.1016/j.mtbio.2024.101261.
24. *Zhang Y, Wang ZL, Deng ZP, Wang ZL, Song F, Zhu LL.* An extracellular matrix-inspired self-healing composite hydrogel for enhanced platelet-rich plasma-mediated chronic diabetic wound treatment. Carbohydr Polym 2023; 315: 120973. DOI: 10.1016/j.carbpol.2023.120973.
25. *Huang S, Hong X, Zhao M, Liu N, Liu H, Zhao J,* et al. Nanocomposite hydrogels for biomedical applications. Bioeng Transl Med 2022; 7(3): e10315. DOI: 10.1002/btm2.10315.
26. *Fan F, Saha S, Hanjaya-Putra D.* Biomimetic Hydrogels to Promote Wound Healing. Front Bioeng Biotechnol 2021; 9: 718377. DOI: 10.3389/fbioe.2021.718377.
27. *Zhuang ZM, Wang Y, Xu FW, Guo K, Cao LL, Feng ZX,* et al. Programmed nanozyme hydrogel enabling spatiotemporal modulation of wound healing achieves skin regeneration after biofilm infection. J Nanobiotechnology 2025; 23(1): 694. DOI: 10.1186/s12951-025-03773-5.
28. *Karimi Hajishoreh N, Dadashpour M, Akbarzadeh A.* Preparation and in vitro evaluation of biological agents based on Zinc-laponite- curcumin incorporated in alginate hydrogel. J Biol Eng 2023; 17(1): 73. DOI: https://doi.org/10.1186/s13036-023-00391-3.
29. *Gonzalez-Pujana A, Igartua M, Hernandez RM, Santos-Vizcaino E.* Laponite nanoclays for the sustained delivery of therapeutic proteins. Eur J Pharm Sci 2024; 201: 106858. DOI: 10.1016/j.ejps.2024.106858.

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Quantitative analysis of capsular microvasculature in relation to the thickness of chronic subdural hematoma

Kvantitativna analiza kapsularne mikrovaskularizacije u odnosu na debljinu hroničnog subduralnog hematoma

Boban Jelenković*†, Vesna Nikolov*†, Jovan Ilić†, Marija Ilić*, Aleksandar Marković†, Hristina Jocić†, Nikola Stojanović†, Pavle Pešić*

***University of Niš, Faculty of Medicine, Niš, Serbia; †University Clinical Center Niš, Department of Neurosurgery, Niš, Serbia**

Abstract

Background/Aim. Chronic subdural hematoma (CSDH) is an extra-axial, encapsulated, slow-growing hemorrhagic collection of blood, accompanied by local coagulopathy. This contributes to continuous re-bleeding from the newly formed capsule of hematoma, which leads to slow but progressive enlargement of the hematoma, with the potential to develop into a compressive intracranial lesion. The aim of this study was to investigate the relationship between the proliferation of sinusoid blood vessels and the growth and diameter of the CSDH. **Methods.** In this study, 33 cases of CSDH treated operatively were analyzed. A biopsy specimen (average size 3 × 3 mm) was obtained from the parietal capsule of the hematoma. The biopsied tissue samples were fixed in aqueous 4% buffered formaldehyde, routinely processed into paraffin-embedded slides, and immunohistochemically stained for the presence of the CD34 antigen. The profiles of microvascular bed blood vessels marked for CD34 were quantified, and the number of capillaries and sinusoids was expressed *per* one mm². **Results.** The variables age and number of blood vessels showed a statistically significant association with the increase in hematoma volume ($\beta = 0.422$, $p = 0.007$; $\beta = 0.486$, $p = 0.022$, respectively). Older patients had a higher risk of enlarged hematoma volume, as did patients with a larger number of sinusoidal blood vessels. **Conclusion.** The number of sinusoids in the parietal capsule of CSDH *per* surface unit of 1 mm² positively correlates with hematoma thickness, which emphasizes the importance of vascular theory in the development of a hematoma. Although CSDH is one of the most common neurosurgical diseases, its pathogenesis is still not fully understood. Further research in this field is necessary to develop potential new therapeutic options that would provide more comprehensive treatment modalities.

Keywords:
biopsy; blood vessels; capillaries; hematoma, subdural, chronic; immunohistochemistry.

Apstrakt

Uvod/Cilj. Hronični subduralni hematom (*chronic subdural hematoma* – CSDH) je ekstraaksijalna, inkapsulirana, sporo rastuća kolekcija krvi, praćena lokalnom koagulopatijom. To doprinosi kontinuiranom ponovnom krvarenju iz novoformirane kapsule hematoma, što dovodi do sporog, ali progresivnog uvećanja hematoma sa potencijalom da se razvije u kompresivnu intrakranijalnu leziju. Cilj rada bio je da se ispita odnos između proliferacije sinusoidnih krvnih sudova i rasta i prečnika CSDH. **Metode.** U ovoj studiji, analizirana su 33 slučaja CSDH koja su operativno lečena. Uzet je uzorak biopsije iz parijetalne kapsule hematoma (prosečne veličine 3 × 3 mm). Uzorci tkiva dobijeni biopsijom fiksirani su u 4% vodenom rastvoru formaldehida, rutinski su pripremljeni parafinski preseci na pločicama i obojeni imunohistohemijskim metodama u cilju detekcije prisutva CD34 antigena. Profili krvnih sudova mikrovaskularne mreže koji su ispoljavali CD34 su kvantifikovani, a broj CD34 pozitivnih kapilara i sinusoida izražen je po jednom mm². **Rezultati.** Varijable starost i broj krvnih sudova pokazale su statistički značajnu povezanost sa povećanjem zapremine hematoma ($\beta = 0{,}422$; $p = 0{,}007$; $\beta = 0{,}486$; $p = 0{,}022$, redom). Stariji bolesnici imali su veći rizik od uvećanja zapremine hematoma, kao i bolesnici sa većim brojem sinusoidnih krvnih sudova. **Zaključak.** Broj sinusoida u parijetalnoj kapsuli CSDH po jedinici površine od 1 mm² pozitivno korelira sa debljinom hematoma, što naglašava značaj vaskularne teorije u razvoju hematoma. Iako je CSDH jedno od najčešćih neurohirurških oboljenja, njegova patogeneza još uvek nije potpuno razjašnjena. Dalja

Correspondence to: Jovan Ilić, University Clinical Center Niš, Department of Neurosurgery, Bulevar dr Zorana Đinđića 48, 18 000 Niš, Serbia. E-mail: jovanilicneuro@gmail.com

istraživanja u ovoj oblasti neophodna su kako bi se razvile potencijalno nove terapijske opcije koje bi pružile sveobuhvatnije modalitete lečenja.

Ključne reči:
biopsija; krvni sudovi; kapilari; hematom, subduralni, hronični; imunohistohemija.

Introduction

Chronic subdural hematoma (CSDH) is defined as a chronically progressive accumulation of blood in the space between the *dura mater* and arachnoid, usually caused by minor, often unnoticed head injury, with the highest incidence in the elderly. The CSDH is accompanied by hypocoagulability, which results in slow but progressive enlargement of the hematoma, with the potential to develop into a compressive intracranial lesion [1–3]. In everyday clinical practice, CSDH is often considered one of the largest "imitators" due to the wide range of non-specific symptoms [3, 4]. Symptoms of CSDH in most cases include the following: a headache, cognitive alterations, dizziness, nausea, vomiting, lethargy, weakness, apathy, as well as epileptic seizures [2–4].

Meningeal (bridging) veins, measuring 1–3 mm in diameter, drain venous blood from the leptomeningeal region, traversing the subdural space in a relative length of 1–2 cm. Their wall thickness varies considerably in the subdural segment (10–600 μm) compared with their subarachnoid portion (50–200 μm). Variable wall thickness, together with a substantial presence of fibrous connective tissue in their walls, makes these veins susceptible to trauma [1, 2, 4].

At the *dura mater*–arachnoid interface (subdural space), there are also other constitutional weak spots potentially targeted by a traumatic event. *Dura mater* is composed of two indistinctly divided layers. The thick outer layer (periosteal) contains venous sinuses of the skull, larger blood and lymphatic vessels, and nerve fibers. The inner (meningeal) layer of the *dura mater* is thinner and composed of dense fibrous connective tissue with microvasculature. Facing the subdural space, the *dura mater* is lined by squamous-shaped fibroblasts-the border cell layer, which lacks the extracellular collagen, thus representing its structural *locus resistentiae minoris*, with potential for regional vulnerability [1–4].

In the course of subdural hematoma chronification, the formation of neomembrane (capsule) around its blood collection is a crucial event [4–8]. The capsule of developing CSDH has an outer (parietal) layer that contains numerous capillaries and sinusoids, with a vascular lumen wider than 40 μm in diameter. On the inner side of the hematoma, the visceral pseudomembrane is formed, which separates the clot from the arachnoid membrane [7, 8]. Based on ultrastructural observations, the visceral membrane is almost avascular, whereas the parietal layer of the capsule exhibits marked and likely more clinically significant pathological vascularization [4–8].

The aim of this study was to investigate the relationship between the proliferation of sinusoid blood vessels (SBV) and the growth and diameter of the CSDH.

Methods

The study included 33 patients surgically treated due to CSDH at the Clinic of Neurosurgery, University Clinical Center Niš, Niš, Serbia. The study was approved by the Ethics Committee of the University Clinical Center Niš (No. 9328, from January 19, 2024).

The examined population comprised 25 (75.8%) men and 8 (24.2%) women, and the results were analyzed in relation to three age groups. The first group included patients under 65 years of age, the second group patients between 65 and 80, and the third group patients over 85 years of age. An analysis of sinusoid vasculature *per* mm^2, hematoma osmolality, cerebrospinal fluid osmolality, and hematoma volume was performed. In terms of the number of SBV (NSBV)/mm^2, all patients were classified into three categories. The first category included patients with fewer than 30 sinusoids/mm^2 the second group comprised those with 30–80 sinusoids/mm^2, and the third group included patients with a vascular density greater than 80 sinusoids/mm^2. The largest number of patients, 20 (61%), had a value of this indicator between 30 and 80 mm^2, 12 (36%) patients had over 80 blood vessels/mm^2, while only one patient had a vascular density below 30/mm^2. Depending on the size of the hematoma, the patients were divided into three groups: the first with a volume of less than 50 mL, the second with a volume of 50 to 70 mL, and the third with a hematoma volume greater than 70 mL. In the majority of patients, 15 (46%), the size of the hematoma was between 50 and 70 mL, in 12 (36%) patients the volume was lower than 50 mL, while in 6 (18%) patients, it was greater than 70 mL.

All patients were operated on under general anesthesia. The decompression procedure consisted of a conventional burr hole craniotomy. One burr hole was placed at the tuber parietale of the parietal bone, after which a cross-shaped opening was made on the *dura mater*. The hematoma was irrigated. Drainage was placed subdurally, and the soft tissues were sewn *en bloc*. From each case of CSDH, a biopsy of the parietal hematoma capsule layer was taken, with average dimensions of 3 × 3 mm. Postoperatively, drainage was active for 1–3 days (typically 2). The biopsied tissue samples of the parietal layer were fixed in aqueous 4% buffered formaldehyde and routinely processed to paraffinized tissue slices, which were stained with hematoxylin and eosin, as well as immunohistochemically marked for the presence of CD34 antigen.

Immunohistochemistry

The adhered tissue sections were exposed to trypsin antigen retrieval for 60 min, and tissue peroxidases were

blocked with 3% hydrogen peroxide solution for 10 min. The monoclonal antibody to CD34 (anti-CD34, Dako, M716501, dilution 1 : 50) was applied to the rehydrated tissue sections overnight, at a temperature of 4 ºC, and then to the secondary antibody conjugated with horseradish peroxidase (EnVision-Flex, Dako). Between the mentioned steps, the preparations were washed with phosphate buffer (pH = 7.2). After exposure to a chromogen (diaminobenzidine), the tissue samples were counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted using Canada balsam and coverslips.

The microscopic slides were analyzed using a light microscope BX50 (Olympus, Japan), equipped with a digital camera Leica DFC295 (Leica Systems, Germany). Digital images of the whole hematoma capsule samples, immunohistochemically marked for CD34, were captured at ×200 magnification. Morphometric analysis of digital images of the CSDH capsule was performed using ImageJ version 1.53 (Wayne Rasband, National Institutes of Health, USA). The profiles of microvascular bed blood vessels marked on CD34 were quantified, and the number of macro-capillaries and sinusoids was expressed *per* one mm^2. From the standpoint of the NSBV/mm^2, all patients were classified into three categories. The first consists of patients whose value is less than 30. The second consists of patients whose value is between 30 and 80. The third consists of patients whose value of this indicator is higher than 80.

Statistical analysis

Data entry and tabulation were performed using Microsoft Excel 2016. The results of the statistical analysis were presented in tables. Statistical calculations were performed using SPSS software v23.0 (IBM Inc, USA). Of the basic descriptive statistical parameters, standard statistical methods were used for qualitative and quantitative assessment of the obtained results: absolute and relative numbers (%). Normality testing was performed using the Kolmogorov-Smirnov test. To test differences between variants that exhibited a normal distribution, the Student's test and analysis of variance were used when analyzing three or more groups. In cases where the distribution was not normal, the Mann-Whitney *U* test was used, as well as the Kruskal-Wallis test for three or more groups. The χ^2 test was used to test the statistical significance of the differences in absolute frequencies between samples. The interdependence between continuous variations was assessed using the Pearson correlation coefficient, and statistically significant correlations were subsequently presented. In order to determine predictive factors, univariate regression analysis was used, followed by multivariate multiple regression for variables that showed statistical significance. The statistical hypothesis was tested at the significance level for the risk of $\alpha = 0.05$, i.e., the difference between samples was considered significant if $p < 0.05$.

Results

The parietal membrane of the analyzed CSDH capsule consisted of immature dense connective tissue, with numerous fibroblast-like cells, as well as variable presence and density of mononuclear inflammatory cell infiltrates. In capsular connective tissue, structural components of the microvasculature, narrower capillaries and capsule sinusoids, were confirmed by the presence of CD34, immunohistochemically visualized with diaminobenzidine chromogen (Figure 1). The examined population consisted of 33 patients, composed of 25 (75.8%) men and 8 (24.2%) women. The average age was 71.30 ± 11.93 years, and the results were analyzed in relation to three age groups. Statistical analysis revealed no significant differences ($\chi^2 = 2.404$, $p = 0.301$) between men and women. Comparative analysis of the hematoma osmolality, cerebrospinal fluid osmolality, NSBV, and hematoma volume in relation to male and female genders is shown in Table 1. None of the examined parameters showed statistically significant differences based on gender.

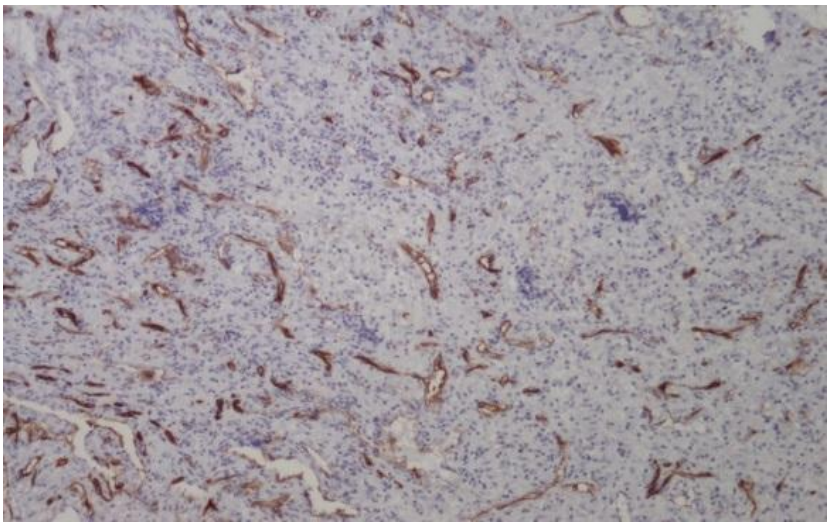

Fig. 1 – Numerous sinusoids in the hematoma capsule. Immunohistochemical staining for CD34 (×100 magnification).

Table 1

Hematoma osmolality, cerebrospinal fluid osmolality, number of sinusoid blood vessels, and hematoma volume in relation to gender

Parameters	Gender		χ^2/t^*	*p*-value
	male	female		
Hematoma osmolality				
< 300 mmoL	16 (64.0)	5 (62.5)		
300–400 mmoL	9 (36.0)	3 (37.5)	0.006	0.939
Cerebrospinal fluid osmolality	280.88 ± 3.03	282.13 ± 1.81	1.094*	0.283
Number of sinusoid blood vessels				
< 30/mm^2	1 (4.0)	0 (0)		
30–80/mm^2	16 (64.0)	4 (50.0)		
> 80/mm^2	8 (32.0)	4 (50.0)	1.056	0.590
Hematoma volume, mL				
< 50	7 (28.0)	5 (62.5)		
50–70	13 (52.0)	2 (25.0)		
≥ 70	5 (20.0)	1 (12.5)	3.143	0.208

All values are given as numbers (percentages) or mean ± standard deviation.
***Note:* *Student's *t*-test was used.**

Table 2

Hematoma osmolality, cerebrospinal fluid osmolality, number of sinusoid blood vessels, and hematoma volume in relation to patient age

Parameters	Age, years			χ^2/F	*p*-value
	< 65	65–80	≥ 85		
Hematoma osmolality					
300–400 mmoL	4 (66.7)	13 (61.9)	4 (66.7)		
> 400 mmoL	2 (33.3)	8 (38.1)	2 (33.3)	0.075	0.963
Cerebrospinal fluid osmolality	283.17 ± 2.86	280.90 ± 2.98	283.17 ± 0.75	2.129	0.137
Number of sinusoid blood vessels					
< 30/mm^2	0 (0)	0 (0)	1 (16.7)		
30–80/mm^2	3 (50.0)	14 (66.7)	3 (50.0)		
> 80/mm^2	3 (50.0)	7 (33.3)	2 (33.3)	5.225	0.265
Hematoma volume, mL					
< 50	4 (66.7)	6 (28.6)	2 (33.3)		
50–70	1 (16.7)	12 (57.1)	2 (33.3)		
≥ 70	1 (16.7)	3 (14.3)	2 (33.3)	4.740	0.315

***F* – Fisher's analysis of variance based on the F test.**
All values are given as numbers (percentages) or mean ± standard deviation.

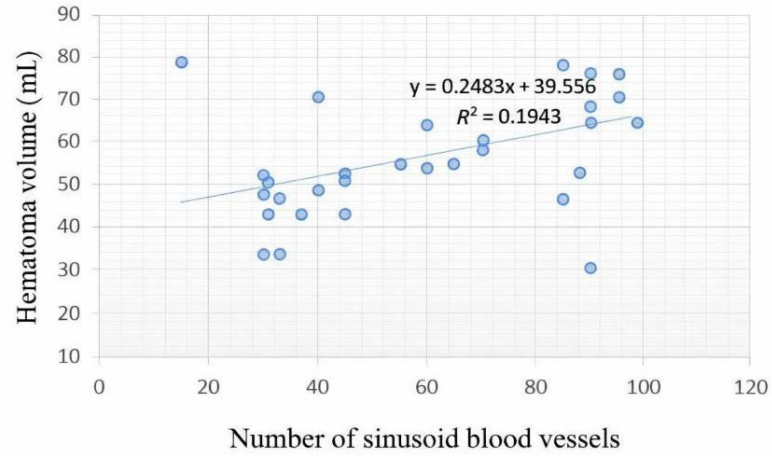

Fig. 2 – Correlation of the number of sinusoid blood vessels and hematoma volume.

Comparative results of hematoma osmolality, cerebrospinal fluid osmolality, NSBV, and hematoma volume according to the age distribution of the subjects, without a statistically significant association, are shown in Table 2.

Furthermore, a statistically significant correlation was proven between the NSBV and the hematoma volume (r = -0.441, p = 0.010) (Figure 2). As the number of sinusoids increases, so does the volume of the hematoma.

Table 3

Univariate multiple regression analysis of gender, age, biochemical parameters, hematoma osmolality, cerebrospinal fluid osmolality, and number of sinusoid blood vessels in relation to hematoma volume

Parameters	Unstandardized coefficient		Standardized coefficient ß	95% confidence interval bounds for ß		*p*-value
	B	SE		lower	upper	
Gender	-4.425	5.914	-0.133	-16.487	7.637	0.460
Age	-0.387	0.206	0.319	-0.034	0.807	0.070
Sodium	-0.036	0.512	-0.013	-1.079	1.008	0.945
Potassium	-0.069	0.094	-0.131	-0.260	0.122	0.469
Urea	-0.216	0.141	-0.265	-0.504	0.072	0.136
Glucose	0.200	0.112	0.305	-0.029	0.429	0.084
Hematoma osmolality	0.014	0.085	0.029	-0.159	0.186	0.871
Cerebrospinal fluid osmolality	-1.710	0.871	-0.332	-3.487	0.067	0.059
Number of sinusoid blood vessels	0.248	0.091	0.441	0.063	0.434	0.010*

SE – standard error.
***Note:* *Indicates statistical significance at $p < 0.05$.**

Table 4

Multivariate multiple regression analysis of age, glucose, cerebrospinal fluid osmolality, and number of blood vessels in relation to hematoma volume

Parameters	Unstandardized coefficient		Standardized coefficient ß	95% confidence interval bounds for ß		*p*-value
	B	SE		lower	upper	
Age	0.512	0.177	0.422	0.148	0.875	0.007*
Cerebrospinal fluid osmolality	0.130	0.095	0.198	-0.065	0.324	0.182
Number of sinusoid blood vessels	-1.265	0.753	-0.246	-2.809	0.278	0.104
Glucose	0.274	0.081	0.486	0.108	0.440	0.002*

SE – standard error.
***Note:* *Indicates statistical significance at $p < 0.05$.**

Gender, age, biochemical parameters (sodium, potassium, urea, and glucose), hematoma osmolality, cerebrospinal fluid osmolality, and the NSBV were analyzed using univariate multiple regression analysis as independent predictive factors in relation to hematoma volume. The only statistically significant independent factor for hematoma volume was the NSBV ($\beta = 0.441$, $p = 0.010$) (Table 3). Within the multivariate multiple regression model used to predict hematoma volume, NSBV and age were statistically significant as individual independent factors to the criterion of significance for $p < 0.01$ (Table 4). The entire model was statistically significant ($F = 6.758$, $p = 0.001$). The model as a whole explains 49.1% of the variance of hematoma volume (corrected $r^2 = 0.419$). The variables age ($\beta = 0.422$, $p = 0.007$) and NSBV ($\beta = 0.486$, $p = 0.022$) showed a statistically significant contribution to the model. Elderly patients were at greater risk of increased hematoma volume, as were the patients with a greater NSBV.

Discussion

In 1857, Virchow was the first to provide a precise histological description of the pathological entity of CSDH. The term *pachymeningitis hemorrhagica interna*, introduced by Virchow as "hemorrhagic inflammation of *dura mater* producing blood collection on its inner side", remains valid even today, in light of recent research highlighting the role of inflammatory cells in its structure [2]. The key features of CSDH, such as re-bleeding, progressive increase in size, rich vascularization, and inflammation of the parietal membrane, have been known since Virchow. In 1932, Gardner proposed that the osmotic gradient present between the hematoma and the adjacent capsular vessels may also be a driver of hematoma enlargement [9]. The CSDH growth based on continuous bleeding, caused by local hypocoagulation and hyperfibrinolysis, was previously precisely analyzed [8, 10]. The contents of most CSDHs are liquid. Increased levels of fibrin and fibrinogen degradation products in the hematoma contents, as well as the presence of tissue activator in the outer membrane of the hematoma, have been shown histologically [11]. Ito and others have found high concentrations of tissue plasminogen activator in the vessel walls of the outer membrane (parietal layer), whereas it was significantly lower or absent in the inner membrane (visceral layer). This would account for the increased hypocoagulation and transudation into the hematoma. Soluble tissue plasminogen activator then diffuses into the hematoma, where it converts plasminogen into the active fibrinolytic enzyme plasmin, which further degrades fibrin and fibrinogen. Hyperfibrinolysis caused by increased tissue plasminogen activator may be expected to interfere with the hemostatic mechanism and induce the enlargement of subdural hematoma [12–15].

The NSBV to 1 mm^2 is a determinant statistically significant in positive correlation with hematoma thickness. In this way, the starting assumption that the NSBV to 1 mm^2

has a statistically significant influence on the hematoma thickness is confirmed. This indicates the importance of SBV quantity elevation in the context of hematoma growth. Microscopic findings of fresh erythrocytes marked with chromium-51 were proven in subdural hematoma, and their movement was observed from sinusoids through increased inter-endothelial gaps towards the hematoma [1, 16]. This observation, to some extent, supports the sinusoid-vascular theory of CSDH formation, given that sinusoids are very fragile and porous, due to their atypical morphology. They are so fragile that they are prone to continuous transudation and recurrent bleeding as a result of constant pulsations of the brain parenchyma [17, 18]. The correlation diagram, which shows the relationship between NSBV and CSDH volume, indicates a coefficient of determination (*R*-squared) of 0.1943, which can be considered statistically and clinically relevant. Bearing in mind that the analysis is based on 33 processed cases, and based on clinical experience and insight into epidemiological patterns, it can be assumed that the value of this coefficient would increase if a larger number of respondents were included.

Weigel et al. [10] tested the hypothesis that the CSDH may be considered a member of the angiogenic disease family and that enhanced expression of growth factors may also be involved in the higher vascularization of the parietal membrane and the maintenance of CSDH. They have found 41 times higher concentrations of vascular endothelial growth factor in the hematoma fluid than in the serum levels. Comparably high concentrations have been described by Suzuki et al. [19]. However, Weigel et al. [10] did not find a significant correlation between growth factor concentrations and patient age or computed tomography, which indicated hematoma thickness.

Moskala et al. [20] reported dynamic changes in the cellular and vascular organization of traumatic CSDH capsules that parallel hematoma duration and are expressed in gradual morphological changes in the developing hematoma capsule. This process initially includes angiogenic and aseptic inflammatory reactions, followed by progressive proliferation of fibroblasts and collagen fibril production. As a result of neo-angiogenesis, numerous capillaries are observed mainly in young hematomas, removed between 15 and 21 days after trauma. More numerous capillaries and thin-walled, larger-diameter blood vessels-sinusoids were evidenced in the "older" hematomas (about 40 days after trauma). In the "oldest" hematoma capsules (60 or more days after trauma), they reported that blood vessels were frequently occluded by clots. In our study, univariate multiple regression analysis included gender, age, hematoma osmolality, and hematoma volume in relation to the number of sinusoids. According to the results, hematoma volume was the only statistically significant independent predictor of NSBV. This finding suggests that even a moderate increase in hematoma volume may contribute to continuous sinusoidal damage and perfusion, with consequent release of inflammatory compounds and mediators that initiate and maintain a nonspecific inflammatory response, which further promotes angiogenesis.

As previously pointed out, the increased fragility of these vascular structures reflects their morphological peculiarities. To a significant extent, they resemble sinusoidal-type blood vessels described in brain gliomas, which additionally confirms their tendency to rupture and their role in the pathophysiology of CSDH progression [21, 22].

By analyzing our findings based on the quantification of microvascular structures (sinusoids), we observed that repeated bleeding from fragile sinusoids represents a key mechanism of the progressive increase in CSDH size. The highest density of sinusoids was recorded in the 65–80-year age group, within the range of 30–80 sinusoids/mm^2. This finding coincides with data showing that the highest percentage of patients with hematoma volumes corresponding to a maximum thickness of 50–70 mm (46%) was observed in this age group.

As previously emphasized, the available literature describes in detail the factors that contribute to the fragility of sinusoids, including their histological characteristics and vasoactive molecules released as a result of vascular damage, which indirectly stimulate angiogenesis [1, 10, 21]. According to recent research, angiogenesis in the capsule of CSDH involves multiple complex, interconnected mechanisms. Kim et al. [23] showed that pathological, sinusoidal, and extremely fragile capillaries were formed in the outer membrane of the CSDH, the formation of which is closely related to the branching and terminal branches of the *arteria meningea media* (middle meningeal artery – MMA). Histological analysis of these newly formed vessels indicates that the MMA is the primary source of blood to the neovascularized membrane and that continuous blood flow and recurrent microbleeding occur through this arterial system. This is in accordance with the modern understanding that recurrence of CSDH is often based on a pathological vascular pattern whose primary hemodynamic "trigger" is MMA, which also provides a clear physiological basis for the increasingly widespread use of MMA embolization in the treatment of recurrent hematomas. Given the previously mentioned characteristics of CSDH, as well as the fact that existing surgical treatments are not always effective, leading to recurrences in up to 20% of cases, MMA embolization has emerged as a promising treatment modality with excellent results, although some recommend its use within a research context [6, 7].

Osuka et al. [21] additionally demonstrated that the N-terminal fragment of osteopontin is found in the hematoma fluid, which *via* integrins α9 and β1 activates signaling pathways related to focal adhesion kinase phosphorylation, cytoskeleton reorganization, and proliferation of endothelial cells in the outer membrane. These processes lead to the progressive development of immature, structurally unstable blood vessels, which contribute to further neovascularization and potential re-bleeding. Watanabe et al. [24] documented intense microvascular proliferation within clots in acute subdural hematomas, which provides insight into the early phase of acute to chronic hematoma transformation, a process that involves the formation of new, underdifferentiated vessels with a very similar phenotype to

those in the CSDH outer membrane. Together, these findings clearly demonstrate that CSDH progression represents an active, dynamic biological response based on inflammation, stimulated angiogenesis, and the hemodynamic effects of MMA, with fragile and immature blood vessels playing a key role in recurrent bleeding and further hematoma enlargement.

To the best of our knowledge, there are no such studies in the available literature that have systematically assessed the density of sinusoids *per* mm² in relation to the volume of CSDH. Therefore, we believe that quantitative monitoring of this parameter is of particular importance for confirming and better understanding the angiogenetic mechanisms in the pathophysiology of CSDH. The low number of patients in our study is related to the strict selection criteria, designed only to obtain valid results by achieving between-group homogeneity. While it does limit the external generalization, this is a trade-off to provide more accurate analysis and more reliable comparisons within our specific cohort. Further research in this field is required so that the potential new therapeutic options targeting the cause of the disease may be developed.

Conclusion

The quantity of capillaries and sinusoids in the parietal capsule of chronic subdural hematoma *per* surface unit is a determinant in positive correlation with hematoma thickness, which emphasizes the importance of vascular theory in the development of a hematoma. Even though chronic subdural hematoma is one of the most common neurosurgical diseases, its pathogenesis is still not fully understood. Further research in this field is required so that the potential new therapeutic options targeting the cause of the disease may be developed.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Yadav YR, Parihar V, Namdev H, Bajaj J.* Chronic subdural hematoma. Asian J Neurosurg 2016; 11(4): 330–42. DOI: 10.4103/1793-5482.145102.
2. *Uno M.* Chronic subdural hematoma - evolution of etiology and surgical treatment. Neurol Med Chir (Tokyo) 2023; 63(1): 1–8. DOI: 10.2176/jns-nmc.2022-0207.
3. *Catana D, Koziarz A, Cenic A, Nath S, Singh S, Almenawer S,* et al. Subdural Hematoma Mimickers: A Systematic Review. World Neurosurg 2016; 93: 73–80. DOI: 10.1016/j.wneu.2016.05.084.
4. *Mortazavi MM, Denning M, Yalcin B, Shoja MM, Loukas M, Tubbs RS.* The intracranial bridging veins: a comprehensive review of their history, anatomy, histology, pathology, and neurosurgical implications. Childs Nerv Syst 2013; 29(7): 1073–8. DOI: 10.1007/s00381-013-2054-3.
5. *Petrov A, Ivanov A, Dryagina N, Petrova A, Samochernykh, K, Rozhchenko L.* Angiogenetic Factors in Chronic Subdural Hematoma Development. Diagnostics (Basel) 2022; 12(11): 2787. DOI: 10.3390/diagnostics12112787.
6. *Levitt MR, Hirsch JA, Chen M.* Middle meningeal artery embolization for chronic subdural hematoma: an effective treatment with a bright future. J Neurointerv Surg 2024; 16(4): 329–30. DOI: 10.1136/jnis-2024-021602.
7. *Stubbs DJ, Davies BM, Edlmann E, Ansari A, Bashford TH, Braude P,* et al. Clinical practice guidelines for the care of patients with a chronic subdural haematoma: multidisciplinary recommendations from presentation to recovery. Br J Neurosurg 2024; 1–10. DOI: 10.1080/02688697.2024.2413445.
8. *Edlmann E, Giorgi-Coll S, Whitfield P, Carpenter KLH, Hutchinson PJ.* Pathophysiology of chronic subdural haematoma: inflammation, angiogenesis and implications for pharmacotherapy. J Neuroinflammation 2017; 14(1): 108. DOI: 10.1186/s12974-017-0881-y.
9. *Thomas PAW, Marshman LAG, Rudd D, Moffat C, Mitchell PS.* Growth and Resorption of Chronic Subdural Hematomas: Gardner, Weir, and the Osmotic Hypothesis Revisited. World Neurosurg 2019; 132: e202–7. DOI: 10.1016/j.wneu.2019.08.204.
10. *Weigel R, Schilling L, Krauss JK.* The pathophysiology of chronic subdural hematoma revisited: emphasis on aging processes as key factor. Geroscience 2022; 44(3): 1353–71. DOI: 10.1007/s11357-022-00570-y.
11. *Holl DC, Volovici V, Dirven CMF, Peul WC, van Kooten F, Jellema K,* et al. Pathophysiology and nonsurgical treatment of chronic subdural hematoma: from past to present to future. World Neurosurg 2018; 116: 402–11.e2. DOI: 10.1016/j.wneu.2018.05.037.
12. *Ito H, Yamamoto S, Saito K, Ikeda K, Hisada K.* Quantitative estimation of hemorrhage in chronic subdural hematoma using the 51Cr erythrocyte labeling method. J Neurosurg 1987; 66(6): 862–4. DOI: 10.3171/jns.1987.66.6.0862.
13. *Ito H, Saito K, Yamamoto S, Hasegawa T.* Tissue-type plasminogen activator in the chronic subdural hematoma. Surg Neurol 1988; 30(3): 175–9. DOI: 10.1016/0090-3019(88)90269-8.
14. *Ito H, Yamamoto S, Komai T, Mizukoshi H.* Role of local hyperfibrinolysis in the etiology of chronic subdural hematoma. J Neurosurg 1976; 45(1): 26–31. DOI: 10.3171/jns.1976.45.1.0026.
15. *Ito H, Komai T, Yamamoto S.* Fibrin and fibrinogen degradation products in chronic subdural hematoma. Neurol Med Chit (Tokyo) 1975; 15 pt 1: 51–5. DOI: 10.2176/nmc.15pt1.51.
16. *Idowu OE, Oyeleke SO, Vitowanu JM.* Impact of inflammatory cell ratio, biomarkers, activated partial thromboplastin time and prothrombin time on chronic subdural haematoma severity and outcome. Eur J Trauma Emerg Surg 2022; 48(2): 1085–92. DOI: 10.1007/s00068-021-01665-5.
17. *Nouri A, Gondar R, Schaller K, Meling T.* Chronic Subdural Hematoma (cSDH): A review of the current state of the art. Brain Spine 2021; 1: 100300. DOI: 10.1016/j.bas.2021.100300.
18. *El Rahal A, Beck J, Ahlborn P, Bernasconi C, Marbacher S, Wanderer S,* et al. Incidence, therapy, and outcome in the management of chronic subdural hematoma in Switzerland: a population-based multicenter cohort study. Front Neurol 2023; 14: 1206996. DOI: 10.3389/fneur.2023.1206996.
19. *Suzuki K, Takano S, Nose T, Doi M, Ohashi N.* Increased concentration of vascular endothelial growth factor (VEGF) in chronic subdural hematoma. J Trauma 1999; 46(3): 532–3. DOI: 10.1097/00005373-199903000-00040.
20. *Moskala M, Goscinski I, Kaluza J, Polak J, Krupa M, Adamek D,* et al. Morphological aspects of the traumatic chronic subdural hematoma capsule: SEM studies. Microsc Microanal 2007; 13(3): 211–9. DOI: 10.1017/S1431927607070286.
21. *Osuka K, Ohmichi Y, Ohmichi M, Honma S, Suzuki C, Aoyama M,* et al. Angiogenesis in the outer membrane of chronic subdural hematomas through thrombin-cleaved osteopontin and the in-

tegrin α9 and integrin β1 signaling pathways. Biomedicines 2023; 11(5): 1440. DOI: 10.3390/biomedicines11051440.

22. *Wei W, Yang T, Liu X, Li L, Fan Y.* Gliomagenesis following chronic subdural hematoma: A case report. Exp Ther Med 2025; 30(1): 139. DOI: 10.3892/etm.2025.12889.
23. *Kim H, Choi Y, Lee Y, Won JK, Lee SH, Suh M,* et al. Neovascularization in outer membrane of chronic subdural hematoma: a rationale for middle meningeal artery embolization. J Korean Neurosurg Soc 2024; 67(2): 146–57. DOI: 10.3340/jkns.2023.0105.
24. *Watanabe A, Tsutsumi S, Nonaka S, Ishii H.* Microvascular proliferation in the clots: The key finding of acute subdural hematoma transforming into chronic subdural hematoma? Surg Neurol Int 2021; 12: 601. DOI: 10.25259/SNI_1103_2021.

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Analysis of the effect of high-frequency electromagnetic radiation on electroencephalography wave frequencies

Analiza uticaja visokofrekventnog elektromagnetnog zračenja na frekvenciju talasa na elektroencefalografiji

Boban Biševac*†, Stevo Lukić*†, Mina Stojković*

***University Clinical Center Niš, Clinic for Neurology, Niš, Serbia; †University of Niš, Faculty of Medicine, Niš, Serbia**

Abstract

Background/Aim. Interest in the effects of electromagnetic fields on the human organism has grown significantly with the advent of digital mobile communication systems, which employ pulsed high-frequency electromagnetic fields. In standby mode, a mobile phone does not emit significant signal power, while during active communication, the intensity of the electromagnetic field may reach values of up to 250 mW. The aim of this study was to examine whether exposure to high-frequency electromagnetic fields affects the frequency of electroencephalography (EEG) waves. **Methods.** The study included 60 participants (30 males and 30 females). Each participant underwent two consecutive EEG recordings, each lasting approximately 20 min. The first EEG recording was performed at rest, without exposure to an electromagnetic field generator. This was followed by a second EEG recording while using a mobile phone for 10 minutes on one ear, then a break of about 2 minutes was made and the recording was repeated on the opposite ear, also for 10 minutes. A standard mobile phone was used as the source of the high-frequency electromagnetic field. **Results.** The analysis of EEG wave frequencies revealed no statistically significant differences in either sex before and after mobile phone exposure in the alpha, beta, or delta frequency bands. A change in the theta frequency band in female participants following mobile phone exposure was localized to the right hemisphere. **Conclusion.** Methodological limitations are the most likely reason for the absence of recorded changes in the majority of participants. The observed effects may be sufficiently subtle or infrequent to evade detection by standard EEG recordings. Therefore, the lack of observed changes cannot be interpreted as evidence that high-frequency electromagnetic fields have no effect on EEG activity.

Keywords:
cell phone; electroencephalography; electromagnetic fields; health; risk assessment.

Apstrakt

Uvod/Cilj. Interesovanje za uticaje elektromagnetnih polja na ljudski organizam značajno je poraslo sa pojavom digitalnih mobilnih komunikacionih sistema, koji koriste pulsirajuća visokofrekventna elektromagnetna polja. U stanju mirovanja mobilni telefon ne emituje značajnu snagu signala, dok tokom aktivne komunikacije intenzitet elektromagnetnog polja može dostići vrednosti do 250 mW. Cilj rada bio je da se ispita da li izlaganje visokofrekventnim elektromagnetnim poljima utiče na frekvenciju talasa na elektroencefalografiji (EEG). **Metode.** Istraživanje je obuhvatilo ukupno 60 ispitanika (30 osoba muškog pola i 30 osoba ženskog pola). Svakom ispitaniku su urađena dva uzastopna EEG snimanja u trajanju od oko 20 min. Prvo EEG snimanje obavljeno je u stanju mirovanja bez upotrebe generatora elektromagnetnog polja. Nakon toga je usledilo drugo EEG snimanje tokom korišćenja mobilnog telefona u trajanju od 10 min na jednom uvu, potom je načinjena pauza od oko 2 min i snimanje je ponovljeno na suprotnom uvu, takođe u trajanju od 10 min. Kao izvor visokofrekventnog elektromagnetnog polja korišćen je standardni mobilni telefon. **Rezultati.** Analiza frekvencija talasa na EEG nije pokazala statistički značajne razlike kod oba pola pre i posle izlaganja mobilnom telefonu u alfa, beta i delta frekventnim opsezima. Promena u teta frekventnom opsegu kod ženskih ispitanica nakon izlaganja mobilnom telefonu bila je lokalizovana u desnoj hemisferi. **Zaključak.** Metodološka ograničenja su najverovatniji razlog za odsustvo zabeleženih promena kod većine ispitanika. Uočeni efekti mogu biti toliko suptilni ili sporadični da standardna EEG snimanja ne uspevaju da ih detektuju. Iz tog razloga, izostanak uočenih promena ne može se tumačiti kao dokaz da visokofrekventna elektromagnetna polja ne utiču na EEG aktivnost.

Ključne reči:
mobilni telefon; elektroencefalografija; elektromagnentna polja; zdravlje; rizik, procena.

Correspondence to: Boban Biševac, University Clinical Center Niš, Clinic for Neurology, Bulevar dr Zorana Đinđića 48, 18 000 Niš, Serbia. E-mail: bbisevac@yahoo.com

Introduction

Interest in studying the effects of electromagnetic fields (EMFs) on the human organism has increased significantly with the introduction of digital mobile radiotelephone systems. These communication systems use high-frequency pulsed EMFs in the lower microwave range. Since the power density of Global System for Mobile Communications signals is generally insufficient to induce thermal effects, research attention has been directed toward possible non-thermal mechanisms of action. Over several decades of investigations into the interaction of low-intensity microwave radiation with biological systems, only a limited number of effects have been identified as reproducible and physiologically relevant, in addition to those whose mechanisms of action are most likely of thermal origin [1–3].

Mobile phones use wireless communication technologies in which signal transmission typically occurs within the 900–1,800 MHz frequency range, with the signal modulated at a pulse frequency of 217 Hz. Under standby conditions, when the user is neither making nor receiving a call, the emitted power is negligible. However, during active communication, the output power of the pulsed EMF may reach a maximum value of 250 mW. There is concern that such pulsed EMFs may penetrate neuronal structures and directly affect cell membrane function, a notion supported by the results of numerous studies indicating alterations in certain physiological processes [4, 5].

In studies focused on assessing the effects of mobile phone use in humans, electroencephalography (EEG) is most commonly employed because of its high sensitivity to immediate changes in neuronal function. Although previous EEG studies have not provided consistent evidence for specific effects, the observed inconsistencies are likely due to methodological limitations. Therefore, there is a clear need for the development of appropriate experimental models that would allow a reliable demonstration of the potential impact of exposure to active mobile phones on neuronal cell function [6].

The insufficiently clarified reasons for the contradictory findings in studies examining the influence of mobile phones on electroencephalographic activity may be attributed to methodological variations in the application of EEG techniques, as well as to differences in the duration of exposure to mobile phones [7]. Detection of subtle changes in brain electrical activity induced by high-frequency electromagnetic waves requires the use of non-conventional analytical approaches that go beyond the scope of classical visual EEG analysis. In this context, the introduction of digital EEG has enabled the effective application of quantitative EEG methods, primarily the analysis of amplitude and frequency, as well as the mapping of the obtained results [8].

The aim of this study was to determine whether exposure to high-frequency electromagnetic radiation emitted by a mobile phone affects the bioelectrical activity of the brain as recorded by EEG, or rather to assess whether exposure leads to increased variability in EEG wave frequencies.

Methods

The study included 60 participants (30 males and 30 females), aged between 20 and 30 years. The monitored parameters were changes in EEG frequency before and after exposure to an active mobile phone, analyzed separately for each side of exposure.

All participants were free of any neurological or psychiatric disorders and had not used any medications or psychoactive substances for at least one month prior to the beginning of EEG recordings. Alcohol and caffeine intake were prohibited for a period of four days prior to EEG recording and up to the time of examination. Each participant served as their own control. The EEG recordings obtained prior to exposure to electromagnetic radiation were compared with those obtained after exposure.

Upon arrival at the laboratory, demographic data were collected, after which participants were connected to the EEG system and positioned in the recording booth. All experimental procedures were conducted in a sound-attenuated electrophysiological laboratory. In all cases, testing was performed in the morning, between 9:00 a.m. and 12:00 p.m.

The EEG recordings were performed with participants in the supine position, in a relaxed yet alert wakeful state, with eyes closed. Monopolar EEG derivations were obtained using silver/silver chloride (Ag/AgCl) surface electrodes positioned according to the international 10–20 system on a 32-channel EEG device, and the signals were analyzed using a bipolar longitudinal montage. Electrode impedance was maintained below 5 kΩ, and the scalp was thoroughly cleansed with alcohol prior to electrode placement. Artifacts related to eye or body movements were automatically excluded by rejecting epochs in which the amplitude of any channel exceeded predefined voltage thresholds. A 50 Hz notch filter was applied to reduce power line interference.

Each participant underwent two consecutive EEG recordings, each lasting approximately 20 min, separated by a break of about 5 min. The first EEG recording was obtained at rest, without exposure to an EMF generator. After a pause of approximately 3 min, the EEG recording was repeated during mobile phone use for 10 min with the phone placed at one ear, followed by a break of approximately 2 min, after which the recording was repeated with the phone placed at the opposite ear, also for a duration of 10 min.

High-frequency EMF exposure was generated using a standard mobile phone operating as a receiver and source of electromagnetic radiation (approximately 900 MHz EMF, 217 Hz pulse repetition rate, 0.577 μs pulse width; estimated average power output 3–4 mW; actual emissions during the experiment were not directly measured). The phone was positioned approximately 2 cm radially from the participant's head, midway between the occipital midline (Oz) and parietal midline (Pz) electrodes.

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Priština/Kosovska Mitrovica, Serbia (No. 489/1, from September 20, 2011).

Statistical analysis

For statistical analysis, the MedCalc software was used. Descriptive statistical measures included the arithmetic mean with a 95% confidence interval, standard deviation (SD), and minimum and maximum values. Statistical procedures for group comparisons comprised exploratory descriptive methods (Student's *t*-test) and confirmatory methods (analysis of variance and *t*-test).

Results

Mean values and variability of EEG frequencies in male participants at the right ear before and after mobile phone exposure are presented in Table 1. The mean ± SD of alpha-wave frequency before exposure was 11.1 ± 1.2 Hz and 11.4 ± 1.5 Hz after exposure, with no statistically significant difference (t = 1.524; p = 0.138). The mean ± SD of beta-wave frequency before exposure was 20.8 ± 4.4 Hz and 22.3 ± 6.1 Hz after exposure; there was also no statistically significant difference (t = 1.445; p = 0.159). The mean ± SD of theta-wave frequency before exposure was 5.7 ± 1.0 Hz and 5.6 ± 1.1 Hz after exposure, with no statistically significant difference (t = 0.223; p = 0.825). The mean ± SD of delta-wave frequency before exposure was 2.6 ± 0.8 Hz and 2.5 ± 0.8 Hz after exposure; there was no statistically significant difference (t = 0.397; p = 0.695).

Mean values and variability of EEG frequencies in male participants at the left ear before and after exposure are presented in Table 2. The mean ± SD of alpha-wave frequency before exposure was 10.7 ± 1.2 Hz and 10.8 ± 1.3 Hz after exposure, without a statistically significant difference (t = 0.260; p = 0.797). The mean ± SD of beta-wave frequency before exposure was 19.3 ± 3.8 Hz and 19.9 ± 3.5 Hz after exposure, with no statistically significant difference (t = 0.161; p = 0.873). The mean ± SD of theta-wave frequency before exposure was 5.7 ± 1.2 Hz and 5.9 ± 1.2 Hz after exposure, again without statistical significance (t = 0.644; p = 0.524). The mean ± SD of delta-wave frequency before exposure was 2.7 ± 0.7 Hz and remained 2.7 ± 0.7 Hz after exposure, with no statistically significant difference (t = 0.339; p = 0.737).

Table 1

Mean values and variability of frequency in male participants at the right ear before and after exposure to a mobile phone

Waves	Mean ± SD	Median	Min–Max	*p*
Alpha				
before	11.1 ± 1.2	11.0	(8.7–13.8)	0.138
after	11.4 ± 1.5	11.1	(8.7–14.0)	
Beta				
before	20.8 ± 4.4	20.2	(15.6–31.3)	0.159
after	22.3 ± 6.1	20.8	(14.6–33.6)	
Theta				
before	5.7 ± 1.0	5.7	(4.1–7.6)	0.825
after	5.6 ± 1.1	5.5	(4.1–7.8)	
Delta				
before	2.6 ± 0.8	2.2	(1.0–3.9)	0.695
after	2.6 ± 0.8	2.6	(1.1–3.9)	

SD – standard deviation; Min – minimum; Max – maximum.

Table 2

Mean values and variability of frequency in male participants at the left ear before and after exposure to a mobile phone

Waves	Mean ± SD	Median	Min–Max	*p*
Alpha				
before	10.7 ± 1.2	10.4	(8.8–13.1)	0.797
after	10.8 ± 1.3	10.6	(8.8–13.8)	
Beta				
before	19.3 ± 3.8	19.8	(15.5–30.1)	0.873
after	19.9 ± 3.5	19.0	(15.7–29.9)	
Theta				
before	5.7 ± 1.2	5.8	(4.1–7.9)	0.524
after	5.9 ± 1.2	5.8	(4.1–7.9)	
Delta				
before	2.7 ± 0.7	2.3	(1.1–3.9)	0.737
after	2.7 ± 0.7	2.7	(1.8–3.9)	

SD – standard deviation; Min – minimum; Max – maximum.

Frequency variability in female participants when the right ear was exposed is shown in Table 3. The mean ± SD of alpha-wave frequency before exposure was 10.9 ± 1.3 Hz and 11.3 ± 1.5 Hz after exposure, with no statistically significant difference (t = 1.240; p = 0.225). The mean ± SD of beta-wave frequency before exposure was 20.8 ± 4.4 Hz and 20.6 ± 4.8 Hz after exposure; again, there was no statistical significance (t = 0.245; p = 0.808). The mean ± SD of theta-wave frequency before exposure was 6.0 ± 1.0 Hz and increased to 6.3 ± 0.9 Hz after exposure, representing a statistically significant difference (t = 2.347; p = 0.026). The mean ± SD of delta-wave frequency before exposure was 3.1 ± 0.6 Hz and remained 3.1 ± 0.7 Hz after exposure, with no statistically significant difference (t = 0.087; p = 0.931).

Frequency variability in female participants at the left ear before and after exposure is presented in Table 4. The mean ± SD of alpha-wave frequency before exposure was 11.3 ± 1.2 Hz and 11.4 ± 1.6 Hz after exposure, with no statistically significant difference (t = 0.230; p = 0.820). The mean ± SD of beta-wave frequency before exposure was 19.9 ± 3.8 Hz and remained 19.9 ± 3.5 Hz after exposure, without statistical significance (t = 0.188; p = 0.852). The mean ± SD of theta-wave frequency before exposure was 6.4 ± 1.1 Hz and decreased to 5.7 ± 1.2 Hz after exposure, indicating a statistically significant difference (t = 2.637; p = 0.013). The mean ± SD of delta-wave frequency before exposure was 2.6 ± 0.7 Hz and remained 2.6 ± 0.7 Hz after exposure, with no statistically significant difference (t = 0.152; p = 0.880).

Discussion

In this study, the observed changes primarily involved alterations in the spectrum of baseline electroencephalographic activity, predominantly within the alpha and theta rhythms. An increase in alpha spectral power was noted, accompanied by changes in slower activity, which were mainly manifested within the theta spectrum.

Table 3

Mean values and variability of frequency in female participants at the right ear before and after exposure to a mobile phone

Waves	Mean ± SD	Median	Min–Max	*p*
Alpha				
before	10.9 ± 1.3	11.0	(8.8–13.1)	0.225
after	11.3 ± 1.5	11.2	(8.7–13.6)	
Beta				
before	20.8 ± 4.4	19.5	(14.9–31.9)	0.808
after	20.6 ± 4.8	19.8	(14.9–31.8)	
Theta				
before	6.0 ± 1.0	6.0	(4.0–7.8)	**0.026**
after	6.3 ± 0.9	6.3	(5.0–7.8)	
Delta				
before	3.1 ± 0.6	3.1	(2.0–3.9)	0.931
after	3.1 ± 0.7	3.2	(1.1–3.9)	

SD – standard deviation; Min – minimum; Max – maximum.
The bold value is statistically significant ($p < 0.05$).

Table 4

Mean values and variability of frequency in female participants at the left ear before and after exposure to a mobile phone

Waves	Mean ± SD	Median	Min–Max	*p*
Alpha				
before	11.3 ± 1.2	11.4	(8.9–13.5)	0.820
after	11.4 ± 1.6	11.0	(8.9–13.9)	
Beta				
before	19.9 ± 3.8	19.8	(15.5–30.1)	0.852
after	19.9 ± 3.5	19.0	(15.7–29.9)	
Theta				
before	6.4 ± 1.1	6.7	(4.1–7.9)	**0.013**
after	5.7 ± 1.2	5.7	(4.1–7.8)	
Delta				
before	2.6 ± 0.7	2.4	(1.1–3.9)	0.880
after	2.6 ± 0.7	2.8	(1.1–3.9)	

SD – standard deviation; Min – minimum; Max – maximum.
The bold value is statistically significant ($p < 0.05$).

The *in vivo* study conducted in humans indicates that electroencephalographic activity in the awake state, following exposure to radiofrequency fields emitted by mobile phones, demonstrates a delayed increase in spectral power density, particularly in the alpha frequency band [9]. In the cited study, the potential effects of EMFs on human brain activity were evaluated through changes in EEG signals. Healthy volunteers were exposed to EMFs emitted by a mobile phone, and the exposure resulted in a statistically significant increase in EEG power in the alpha and beta frequency bands. In our study, exposure to the mobile phone was limited to 10 min, and EEG changes were monitored during exposure, whereas in the aforementioned study, it was observed that mobile phone activity led to an increase in EEG power in certain frequency bands with a temporal delay of approximately 15 min after cessation of exposure.

Similar findings were reported by Takashima et al. [10], who described a reduction in high-frequency EEG bands accompanied by a simultaneous increase in low-frequency components. In contrast, some studies failed to identify consistent changes in EEG spectral power following exposure to continuous microwave fields [11].

Croft et al. [7, 12] reported that exposure to an active mobile phone induces changes in resting-state electroencephalographic activity, manifested as a reduction in activity within the 1–4 Hz frequency range and an increase in activity within the 8–12 Hz range. Although these authors observed EEG changes in the awake state, they did not detect an increase in delta activity, which was the only finding registered in our frequency analysis. This discrepancy may be explained by methodological differences between studies. Croft et al. [7, 12] discussed the possible origins of inconsistencies in results regarding the effects of mobile phones on the human brain. They concluded that prolonged sitting on a chair may have induced drowsiness in participants, thereby altering baseline activity. This suggests that differences in results among studies may arise from methodological variations, such as the duration of exposure to an active mobile phone, and that the effects of EMF exposure from mobile phones on EEG activity are time-dependent. These authors also concluded that active mobile phones affect neuronal function in humans in relation to the duration of exposure.

In the study by Preece et al. [13], a limited number of studies examining the effects of magnetic radiation on cognitive and perceptual processing in humans were analyzed. In this context, time-independent changes in the delta band may be interpreted as a relatively direct response to mobile phone exposure, whereas a time-dependent increase in alpha activity may reflect a more indirect effect of exposure. Specifically, studies with shorter exposure durations do not often detect changes associated with mobile phone effects.

In our study, in addition to changes in the frequency spectrum, similarly to the studies by Kramarenko and Tan [14] and Zhang et al. [15], an increase in the amplitude of alpha activity and a decrease in the amplitude of beta activity were observed. The frequency spectrum shifted toward increased alpha power and slow-wave activity; however, these changes did not reach statistical significance.

A study analyzing results published between 1995 and 2023 investigating EMF effects on EEG has most consistently reported changes in the alpha band, while findings in other frequency ranges remain heterogeneous [16]. When effects were observed, they primarily involved increases in alpha and beta activity. Short-term exposure has also been associated with alterations in alpha power, generally without evidence of adverse health consequences. Overall inconsistencies highlight the need for methodological standardization and a clearer definition of EEG outcome parameters [17–20].

In the present study, we focused specifically on EEG wave frequency, a comparatively underexplored parameter. Unlike spectral power, frequency may more sensitively reflect subtle changes in neuronal synchronization and oscillatory network dynamics induced by high-frequency, pulse-modulated EMFs [7, 12]. Such effects could manifest as minor frequency shifts without substantial alterations in signal power, potentially indicating discrete modifications in cortical excitability.

There is a lack of clear evidence regarding EEG changes during or following mobile phone exposure in conjunction with functional magnetic resonance imaging findings in the same participants, and these associations are typically interpreted indirectly rather than through formal real-time EEG-functional magnetic resonance imaging correlation analyses [21].

Study limitations

In the present study, the specific absorption rate was not directly measured, and no individual dosimetric assessment of exposure was conducted during the experimental protocol. The experimental configuration was designed to replicate typical patterns of everyday mobile phone use. In this context, the dynamic variability of the device's output power constitutes a methodological limitation, as it may influence the precise estimation of individual energy absorption during the experiment. Therefore, the study should be interpreted as an approximation of real-life mobile phone use rather than a strictly controlled dosimetric exposure model.

Conclusion

Analysis of electroencephalography frequency activity in male and female participants before and after exposure to the electromagnetic field emitted by a mobile phone did not reveal statistically significant differences in the variability of alpha, beta, or delta wave frequencies. The only parameter demonstrating statistical significance was a change in theta frequency following mobile phone exposure in female participants, observed in the dominant hemisphere. Although a statistically significant change in theta-band frequency was observed in female participants, localized to the right hemisphere, this finding was not accompanied by consistent effects in other frequency bands, nor was it detected in both sexes or bilaterally. Therefore, this iso-

lated theta-band result most likely represents a potential chance finding. Given that the specific absorption rate was not measured in the present study, and that the experimental configuration was designed to reproduce typical patterns of everyday mobile phone use, the investigation should be interpreted as an approximation of real-life exposure rather than a strictly controlled dosimetric model.

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R E F E R E N C E S

1. *Adey WR.* Joint actions of environmental nonionizing electromagnetic fields and chemical pollution in cancer promotion. Environ Health Perspect 1990; 86: 297–305. DOI: 10.1289/ehp.9086297.
2. *Arber SL, Lin JC.* Microwave induced changes in nerve cells: effects of modulation and temperature. Bioelectromagnetics 1985; 6(3): 257–70. DOI:10.1002/bem.2250060306.
3. *Bawin SM, Kaczmarek LK, Adey WR.* Effects of modulated VHF fields on the central nervous system. Ann N Y Acad Sci 1975; 247: 74–81. DOI: 10.1111/j.1749-6632.1975.tb35984.x.
4. *Adey WR, Bawin SM.* Brain interactions with weak electric and magnetic fields. Neurosci Res Prog Bull 1977; 15(1): 1–129.
5. *Hermann DM, Hossmann KA.* Neurological effects of microwave exposure related to mobile communication. J Neurol Sci 1997; 152(1): 1–14. DOI: 10.1016/s0022-510x(97)00140-8.
6. *Eulitz C, Ullsperger P, Freude G, Elbert T.* Mobile phones modulate response patterns of human brain activity. Neuroreport 1998; 9(14): 3229–32. DOI: 10.1097/00001756-199810050-00018.
7. *Croft RJ, Chandler JS, Burgess AP, Barry RJ, Williams JD, Clarke AR.* Acute mobile phone operation affects neural function in humans. Clinical Neurophy 2002; 113(10): 1623–32. DOI: 10.1016/s1388-2457(02)00215-8.
8. *Duffy FH.* Brain electrical activity mapping: ideas and answer. In: *Duffy FH,* editor. Topographic mapping of brain electrical activity. Boston: Butter-worths; 1986. p. 401–98.
9. *Reiser H, Dimpfel W, Schober F.* The influence of electromagnetic fields on human brain activity. Eur J Med Res 1995; 1(1): 27–32.
10. *Takashima S, Onaral B, Schwan HP.* Effects of modulated RF energy on the EEG of mammalian brain. Radiat Environ Biophys 1979; 16(1): 15–27. DOI: 10.1007/BF01326893.
11. *Mitchell DS, Switzer WG, Bronaugh EL.* Hyperactivity and disruption of operant behavior in rats after multiple exposures to microwave radiation. Radio Sci 1977; 12: 263–71. DOI: 10.1029/RS012i06Sp00263.
12. *Croft RJ, Leung S, McKenzie RJ, Loughran SP, Iskra S, Hamblin DL,* et al. Effects of 2G and 3G mobile phones on human alpha rhythms: Resting EEG in adolescents, young adults, and the elderly. Bioelectromagnetics 2010; 31(6): 434–44. DOI: 10.1002/bem.20583.
13. *Preece AW, Iwi G, Davies-Smith A, Wesnes K, Butler S, Lim E,* et al. Effect of a 915 MHz simulated mobile phone signal on cognitive function in man. Int J Radiat Biol 1999; 75(4): 447–56. DOI: 10.1080/095530099140375.
14. *Kramarenko VA, Tan U.* Effects of high-frequency electromagnetic fields on human EEG: A brain mapping study. Int J Neurosci 2003; 113(7): 1007–19. DOI: 10.1080/00207450390220330.
15. *Zhang J, Sumich A, Wang GY.* Acute effects of radiofrequency electromagnetic field emitted by mobile phone on brain function. Bioelectromagnetics 2017; 38(5): 329–38. DOI: 10.1002/bem.22052.
16. *Prins AC, Baas K, van der Meer JN, Jacobs M, Nederveen AJ.* The effect of mobile phone electromagnetic fields on the human resting state wake EEG and event-related potential: A systematic review and meta-analysis. Bioelectromagnetics 2025; 46(1): e22531. DOI:10.1002/bem.22531.
17. *Henz D, Schöllhorn WI, Poeggeler B.* Mobile phone chips reduce increases in EEG brain activity induced by mobile phone-emitted electromagnetic fields. Front Neurosci 2018; 12: 190. DOI: 10.3389/fnins.2018.00190.
18. *Vecsei Z, Knakker B, Juhász P, Thuróczy G, Trunk A, Hernádi I.* Short-term radiofrequency exposure from new generation mobile phones reduces EEG alpha power with no effects on cognitive performance. Sci Rep 2018; 8(1): 18010. DOI: 10.1038/s41598-018-36353-9.
19. *Van der Meer JN, Eisma YB, Meester R, Jacobs M, Nederveen AJ.* Effects of mobile phone electromagnetic fields on brain waves in healthy volunteers. Sci Rep 2023; 13(1): 21758. DOI: 10.1038/s41598-023-48561-z.
20. *Danker-Hopfe H, Eggert T, Dorn H, Sauter C.* Effects of RF-EMF on the human resting-state EEG—the inconsistencies in the consistency. Part 1: Non-exposure-related limitations of comparability between studies. Bioelectromagentics 2019; 40(5): 291–19. DOI: 10.1002/bem.22194.
21. *Wei Y, Yang J, Chen Z, Wu T, Lv B.* Modulation of resting-state brain functional connectivity by exposure to acute fourth-generation long-term evolution electromagnetic field: An fMRI study. Bioelectromagnetics 2019; 40(1): 42–51. DOI: 10.1002/bem.22165.

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CASE REPORT

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Trifid and tetrafid renal sinus: a rare anatomical variation present in a pediatric patient

Trodelni i četvorodelni bubrežni sinus: retka anatomska varijacija prisutna kod pedijatrijskog pacijenta

Edis Çolak

University of Health Sciences, Dr. Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, Department of Radiology, Izmir, Türkiye

Abstract

Introduction. The anatomical variations of the renal sinus appear as hyperechoic areas on ultrasonography, which may be misinterpreted as true hyperechoic masses like renal angiomyolipomas. **Case report.** A 10-year-old girl was referred to a tertiary children's hospital due to suspected bilateral renal angiomyolipomas. Upon admission, laboratory test results were within normal reference ranges. Ultrasonographic examination revealed that both kidneys had a normal position and size. On the sagittal ultrasound scan of the right kidney, three columnar masses extending across the sinus were observed, creating the appearance of three hyperechoic zones, indicating a trifid renal sinus. On the sagittal ultrasound scan of the left kidney, multiple columnar masses extending across the sinus were observed, creating the appearance of four hyperechoic zones, identified as a tetrafid renal sinus. The findings were interpreted as a rare benign anatomical variation with no signs of pathology, and additional radiological imaging was not required. **Conclusion.** Accurate identification of these variations is crucial to prevent patients from undergoing unnecessary biopsies and additional imaging.

Keywords:
anatomic variation; diagnosis; kidney; pediatrics; ultrasonography.

Apstrakt

Uvod. Anatomske varijacije bubrežnog sinusa se na ultrazvuku pojavljuju kao hiperehogena područja koja se mogu pogrešno protumačiti kao prave hiperehogene mase, poput bubrežnih angiomiolipoma. **Prikaz bolesnika.** Desetogodišnja devojčica upućena je u dečiju bolnicu tercijarnog nivoa zbog sumnje na bilateralne angiomiolipome bubrega. Prilikom prijema, rezultati laboratorijskih analiza bili su u granicama normalnih vrednosti. Ultrazvučnim pregledom utvrđeno je da su oba bubrega imala normalan položaj i veličinu. Na sagitalnom ultrazvučnom snimku desnog bubrega uočene su tri cilindrične mase koje se pružaju kroz sinus i daju izgled tri hiperehogene zone što ukazuje na trodelni bubrežni sinus. Na sagitalnom snimku levog bubrega prikazano je prisustvo višestrukih cilindričnih masa koje se pružaju kroz sinus i stvaraju izgled četiri hiperehogene zone identifikovane kao četvorodelni bubrežni sinus. Nalaz je protumačen kao retka benigna anatomska varijacija bez patoloških promena, te dodatna radiološka snimanja nisu bila potrebna. **Zaključak.** Precizna identifikacija ovih varijacija je važna kako bi se sprečilo da bolesnici budu podvrgnuti nepotrebnoj biopsiji i dodatnom snimanju.

Ključne reči:
anatomija, varijacije; dijagnoza; bubreg; pedijatrija; ultrasonografija.

Introduction

The renal sinus is a fatty area on the inner side of the kidney. It encompasses adipose tissue, fibrous material, the collection system, renal blood vessels, and lymphatic structures [1, 2]. The renal sinus appears as an echogenic oval zone on ultrasound imaging [1–3]. The most common variations of the renal sinus include a bifid renal sinus, a parenchymal junctional defect, a parenchymal interjunctional line, and a hypertrophied column of Bertin.

A junctional parenchymal defect appears as a triangular echogenic cortical irregularity in the upper pole of the kidney. A parenchymal interjunctional line appears as an echogenic line crossing the kidney from the antero-superior outline to the renal sinus. The presence of a hypertrophied column of Bertin is attributed to the indentation

Correspondence to: Edis Çolak, University of Health Sciences, Dr. Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, Department of Radiology, Sezer Doğan 11, 35 210 Izmir, Türkiye. E-mail: edisezgicolak@gmail.com

of renal cortical tissue in the renal sinus. In a bifid renal sinus, an intrasinus columnar mass crosses the sinus, creating the appearance of two separate hyperechoic zones [4–6]. Familiarity with these variations is important for distinguishing them from renal hyperechoic lesions, such as angiomyolipomas, lipomas, lymphomas, extramedullary hematopoiesis, and hematomas [7–11]. These insights are crucial to prevent unnecessary additional imaging, biopsies, and follow-up procedures.

We present the first case report of a rare right trifid and a left tetrafid renal sinus variation in a pediatric patient.

Case report

A 10-year-old girl was referred to our tertiary children's hospital because of the suspicion of bilateral renal angiomyolipomas identified through ultrasound evaluation at the regional hospital. Her past medical history was otherwise unremarkable. On admission, the results of the laboratory examination (complete blood cell count, kidney function tests, urine analysis) appeared to be within normal limits. The abdominal real-time ultrasound examination was performed using a Mindray R9 ultrasound machine (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) equipped with a convex 6-1 MHz probe and color Doppler imaging. A comprehensive evaluation of the kidneys was performed using longitudinal, transverse, and oblique imaging approaches. Both kidneys were placed in their normal anatomical positions, with the right kidney measuring 11.2 × 2.8 × 2.5 cm and the left kidney measuring 10.5 × 3.8 × 2.7 cm.

On sagittal and oblique scans, the right kidney was presented by three intrasinus columnar masses crossing the sinus and giving an appearance of three hyperechoic zones indicating a trifid renal sinus (Figure 1). The examination of the left kidney revealed multiple columnar masses crossing the sinus, producing the appearance of four hyperechoic structures, identified as a tetrafid renal sinus (Figure 2). Ultrasound image analysis showed that the columnar masses were surrounded by echogenic renal sinus fat, which partially traversed the sinus rather than completely dividing it. The thickness of the intrasinus columnar masses ranged from 3.7 mm to 12 mm (mean value 7.9 mm) in the right kidney and from 4.5 mm to 9.3 mm in the left kidney (mean value 6.9 mm). No evidence of renal calculi, solid-cystic masses, or hydronephrosis was found in the analysis.

A radiological diagnosis identified an incidental finding of a right trifid and left tetrafid renal sinus, highlighting a unique radiological anatomical variation. Since the variant was well characterized by ultrasonography and there were no clinical or laboratory findings suggestive of pathology, further imaging with magnetic resonance or computed tomography urography was considered unnecessary. This case highlights the diagnostic value of ultrasound in characterizing benign renal sinus variants, avoiding additional cost and radiation exposure.

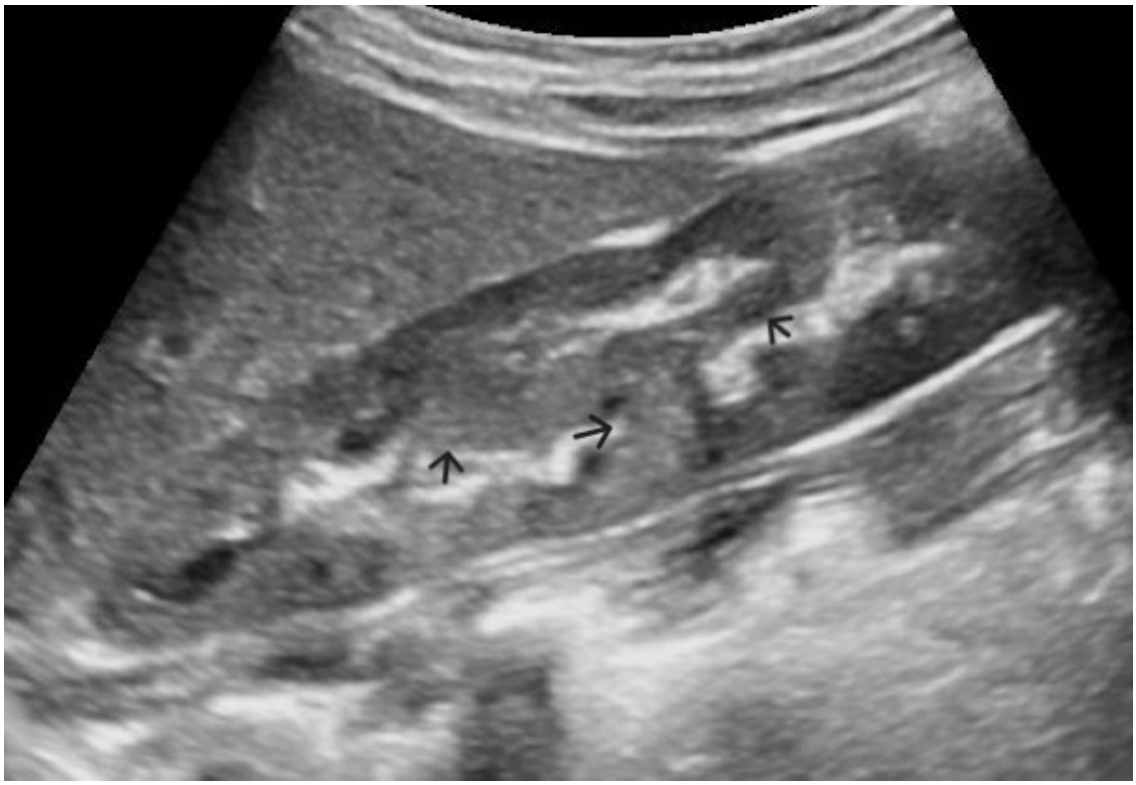

Fig. 1 – Ultrasound image of the right kidney (sagittal scan) shows three columnar masses (arrows) crossing the sinus and giving an appearance of a trifid renal sinus.

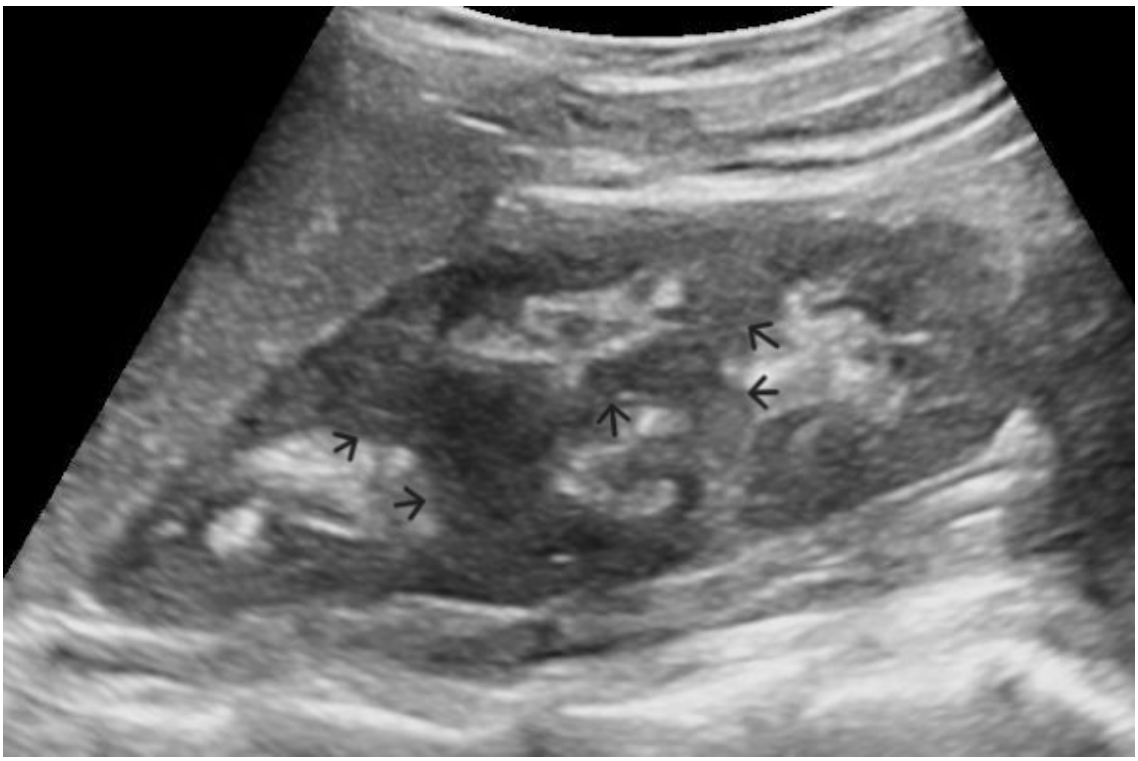

Fig. 2 – Ultrasound image of the left kidney (sagittal scan) shows multiple columnar masses (arrows) crossing the sinus and giving an appearance of a tetrafid renal sinus.

Discussion

The ultrasonographic assessment of our patient indicated the presence of a right trifid renal sinus, resulting in the appearance of three hyperechoic areas, and a left tetrafid renal sinus, characterized by the formation of four hyperechoic areas.

The study conducted by Dalla Palma et al. [12] examined the prevalence of renal sinus anomalies in a cohort of 50 children (0–9 years) and 200 adults (20–89 years). In the pediatric group, a parenchymal junctional defect was identified in 16 (32%) right kidneys and 8 (16%) left kidneys, a parenchymal interjunctional line in 26 (52%) right and 11 (22%) left kidneys, and a bifid renal sinus in 17 (34%) right and 8 (16%) left kidneys. A bifid renal sinus was observed bilaterally in 3 (6%) children, whereas no trifid or tetrafid renal sinus was detected in this group. In the adult group (n = 200), a parenchymal junctional defect was detected in 29 (14.5%) right kidneys and 16 (8%) left kidneys, a parenchymal interjunctional line in 38 (19%) right and 17 (8.5%) left kidneys, and a bifid renal sinus in 118 (59%) right and 68 (34%) left kidneys. Bilateral bifid renal sinuses were observed in 63 (31.5%) adults. In addition, a trifid renal sinus was identified in 16 (8%) right and 6 (3%) left kidneys, and a tetrafid renal sinus in 14 (7%) right and 8 (4%) left kidneys among the adult population. These findings demonstrate that the visualization of intrasinusal cortical columns increases with age and is more frequent on the right side in both populations.

Dalla Palma et al. [12] reported that the thickness of the intrasinus columnar masses varied between 4 and 12 mm with an average of 8.6 mm on the right side, and between 4 and 18 mm with an average of 10 mm on the left side. In our patient, the thickness of the intrasinus columnar masses varied from 3.7 mm to 12 mm, with an average of 7.9 mm in the right kidney, and from 4.5 mm to 9.3 mm, with an average of 6.9 mm in the left kidney.

Similar to our findings, Kumar et al. [4] reported a rare case of a trifid renal pelvis in an adult with a solitary kidney, emphasizing the clinical importance of recognizing such anatomical variants to avoid unnecessary invasive investigations.

Conclusion

We reported the first case of a rare anatomical variation of the renal sinus presented as a right trifid and a left tetrafid renal sinus in a pediatric patient. Awareness of the differences in kidney anatomy is important to avoid unnecessary additional imaging and biopsy examinations.

REFERENCES

1. *Hansen KL, Nielsen MB, Ewertsen C.* Ultrasonography of the Kidney: A Pictorial Review. Diagnostics (Basel) 2015; 6(1): 2. DOI: 10.3390/diagnostics6010002.
2. *Morales-Tisnés T, Lopez-Rippe J, Martínez-Correa S, Sultan LR, Otero HJ, Viteri B.* Renal Ultrasonography in Children: Principles and Emerging Techniques. Curr Treat Options Pediatr 2024; 10(4): 257–64. DOI: 10.1007/s40746-024-00315-3.
3. *Meyer S, Fuchs D, Meier M.* Ultrasound and Photoacoustic Imaging of the Kidney: Basic Concepts and Protocols. Methods

Mol Biol 2021; 2216: 109–30. DOI: 10.1007/978-1-0716-0978-1_7.

4. *Kumar GA, Nerli RB, Devaraju S, Hiremath MB.* A trifid pelvis in a patient with a solitary kidney with LUTS: An unusual presentation. J Sci Soc 2013; 40(3): 177–9. DOI: 10.4103/0974-5009.120062
5. *Ilounoh CE, Eteng RE, Leahy A.* Renal Mass Mimicking Prominent Columns of Bertin: A Case Series. J Diagn Med Sonogr 2023; 39(5): 533–8. DOI: 10.1177/87564793231176054
6. *Krishna S, Schieda N, Flood TA, Shanbhogue AK, Ramanathan S, Siegelman E.* Magnetic resonance imaging (MRI) of the renal sinus. Abdom Radiol (NY) 2018; 43(11): 3082–100. DOI: 10.1007/s00261-018-1593-1.
7. *Gayer G, Zissin R.* The renal sinus--transitional cell carcinoma and its mimickers on computed tomography. Semin Ultrasound CT MR 2014; 35(3): 308–19. DOI: 10.1053/j.sult.2014.02.004.
8. *Tsai MC, Lu HH, Chang YC, Huang YC, Fu LS.* Automatic Screening of Pediatric Renal Ultrasound Abnormalities: Deep Learning and Transfer Learning Approach. JMIR Med Inform 2022; 10(11): e40878. DOI:10.2196/40878
9. *Aldughiem A.* Imaging Diagnosis of Major Kidney and Urinary Tract Disorders in Children. Medicina (Kaunas) 2025; 61(4): 696. DOI: 10.3390/medicina61040696.
10. *Lyske J, Mathew RP, Hutchinson C, Patel V, Low G.* Multimodality imaging review of focal renal lesions. Egypt J Radiol Nucl Med 2021; 52: 14. DOI: 10.1186/s43055-020-00391-z
11. *Bhatt S, MacLennan G, Dogra V.* Renal pseudotumors. AJR Am J Roentgenol 2007; 188(5): 1380–7. DOI: 10.2214/AJR.06.0920.
12. *Dalla Palma L, Bazzocchi M, Cressa C, Tommasini G.* Radiological anatomy of the kidney revisited. Br J Radiol 1990; 63(753): 680–90. DOI: 10.1259/0007-1285-63-753-680.

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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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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.

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

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Volume with a Supplement

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

Issue with a Supplement

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

Volume with Part (Pt)

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

Issue with Part (Pt)

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

Issue with no Volume

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

No Volume or Issue

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

Pagination with Roman numerals

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

Book

Printed Book

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

Book in electronic format

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

Chapter

In an edited book

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

In an edited electronic (online) book

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

Website

Homepage

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

Part of a website

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

Conference Proceedings

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

Article from Conference Proceedings

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

Dissertation

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

Other published articles

News article

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

Holy Bible

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

Dictionaries and similar references

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

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

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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 (ºC).

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

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

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

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

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ОБАВЕЗНА ПРАТЕЋА ДОКУМЕНТА

ИЗЈАВА АУТОРА И АУТОРСТВО

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

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

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

ЕТИЧКА САГЛАСНОСТ

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

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

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

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

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

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

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

ПЛАГИЈАРИЗАМ

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

КОРИШЋЕЊЕ AI

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

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

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

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

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

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

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

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

ТИПОВИ РУКОПИСА

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

ОРИГИНАЛНИ ЧЛАНАК

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

ПРЕТХОДНО САОПШТЕЊЕ

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

КРАТКО САОПШТЕЊЕ

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

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

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

МЕТА-АНАЛИЗА

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

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

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

АКТУЕЛНА ТЕМА

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

У ФОКУСУ

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

КАЗУИСТИКА

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

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

УВОДНИК

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

ПИСМО УРЕДНИКУ

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

ИСТРАЖИВАЧКО ПИСМО

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

ИСТОРИЈА МЕДИЦИНЕ/СТОМАТОЛОГИЈЕ/ФАРМАЦИЈЕ

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

КЛИНИЧКО ИСТРАЖИВАЊЕ

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

ПРИКАЗ КЊИГЕ

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

ИЗВЕШТАЈ СА НАУЧНОГ ИЛИ СТРУЧНОГ СКУПА

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

ОБИМ РУКОПИСА

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

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

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

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

ПРИПРЕМА РАДА

НАСЛОВНА СТРАНА

На првој страници рукописа треба навести следеће:

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

АПСТРАКТ

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

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

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

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

КЉУЧНЕ РЕЧИ

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

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

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

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

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

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

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

ПРИЛОЗИ

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

Табеле

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

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

Слике (илустрације)

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

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

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

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

СКРАЋЕНИЦЕ

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

ДЕЦИМАЛНИ БРОЈЕВИ

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

ЈЕДИНИЦЕ МЕРА

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

ЗАХВАЛНИЦА

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

СТАТИСТИЧКА АНАЛИЗА

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

ЛИТЕРАТУРА

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

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

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

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

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

Примери цитирања:

Чланак са 1 до 6 аутора

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

Чланак са више од 6 аутора

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

Волумен са суплементом

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

Свеска са суплементом

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

Волумен са делом (Pt)

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

Свеска са делом

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

Свеска без волумена

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

Без волумена и свеске

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

Пагинација римским бројевима

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

Књига

Штампана књига

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

Књига у електронском формату

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

Поглавље

У едитованој књизи

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

У едитованој електронској (online) књизи

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

Веб страница

Интернет страница

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

Део интернет странице

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

Зборник радова са конгреса

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

Рад из зборника

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

Дисертација

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

Остали објављени чланци

Новински чланак

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

Свето писмо

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

Речници и сличне референце

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

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

НАКНАДЕ ЗА ОБЈАВЉИВАЊЕ ЧЛАНКА

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

Уплата није гаранција да ће рад бити објављен у ВСП.

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