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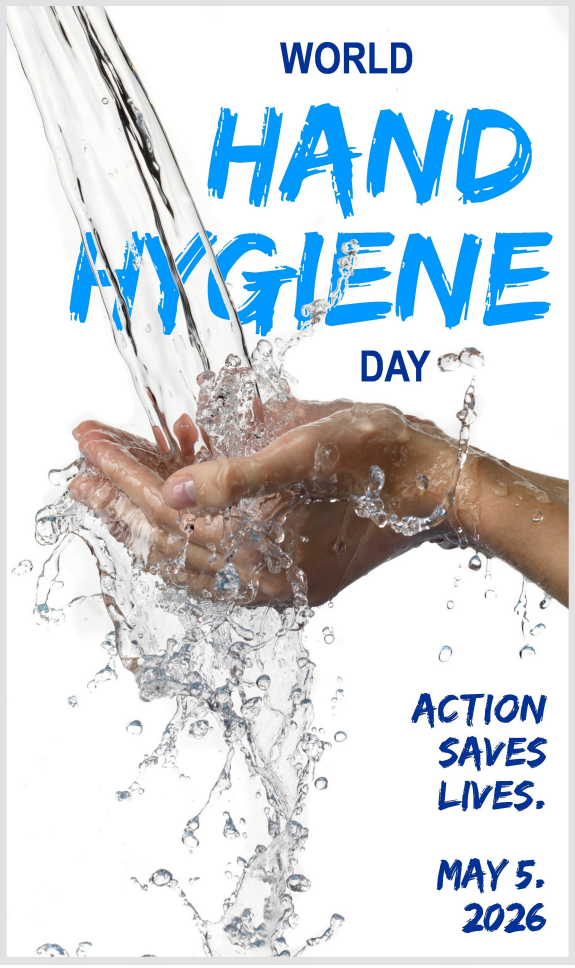

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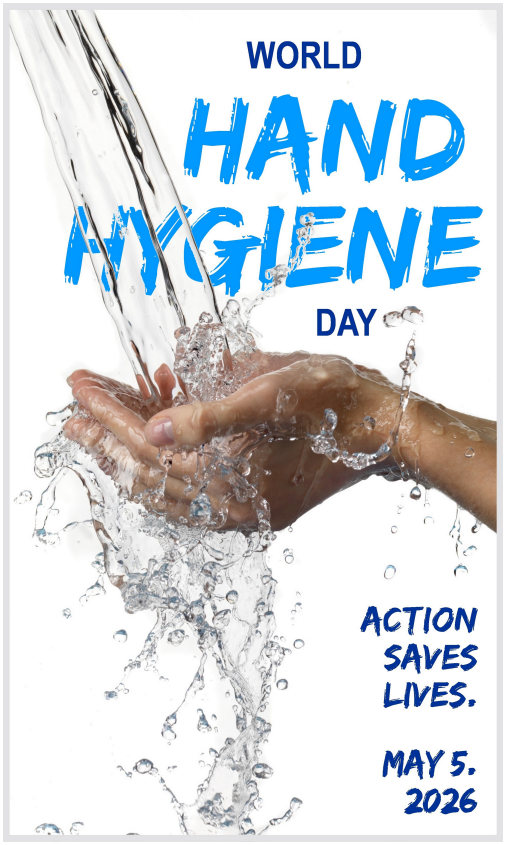

World Hand Hygiene Day is marked on May 5 and is part of the World Health Organization (WHO) campaign, which has been carried out for 18 years under the title "Save lives: Clean your hands". The aim of this campaign is to raise awareness about the importance of hand hygiene in healthcare. According to WHO estimates, proper hand hygiene can prevent up to 50% of healthcare-associated infections, which, apart from increasing treatment costs, also contribute to the development of antimicrobial resistance. This year's World Hand Hygiene Day slogan, "Action saves lives", underscores the importance of continuous education and consistent implementation of hand hygiene in healthcare institutions, as well as its integration into standard operating procedures.

Svetski dan higijene ruku obeležava se 5. maja i deo je kampanje Svetske zdravstvene organizacije (SZO), koja se već 18 godina sprovodi pod nazivom „Sačuvajte živote: Operite svoje ruke". Cilj kampanje je podizanje svesti o značaju higijene ruku u zdravstvenoj zaštiti. Prema procenama SZO, pravilna higijena ruku može sprečiti do 50% infekcija povezanih sa zdravstvenom zaštitom, koje, osim povećanja troškova lečenja, doprinose i razvoju antimikrobne rezistencije. Ovogodišnji slogan svetskog dana higijene ruku „Akcija spasava živote" naglašava važnost stalne edukacije i dosledne primene higijene ruku u zdravstvenim ustanovama i njeno uključivanje u standardne procedure rada.

GENERAL REVIEW

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Host immuno-inflammatory response and intravascular volume replacement therapy in critically ill septic patients – are fluids a double-edged sword?

Imunsko-inflamacijski odgovor domaćina i terapija nadoknadom intravaskularnog volumena kod kritično obolelih sa sepsom – da li su tečnosti mač sa dve oštrice?

Dragan Djordjević, Goran Rondović, Ivo Udovičić, Katarina Mladenović, Snježana Zeba

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Abstract

Intravascular volume replacement fluid therapy plays a pivotal role in the treatment of patients in circulatory shock. The interaction between various lines of therapy and immune response in critically ill septic patients remains a significant clinical challenge with no simple solution. Plasma expanders, crystalloids (such as physiological saline, balanced Ringer's lactate, and Plasma-Lyte solution), and human albumin solution–the only acceptable colloid for critically ill patients–should be considered as necessary potent drugs with significant adverse effects. Sepsis is a very heterogeneous syndrome, with various magnitudes and persistence of inflammatory responses. Bearing in mind that the natural course of sepsis is highly complex, with phases of hyperinflammation and immunosuppression often occurring simultaneously in different locations, it is almost impossible to avoid the adverse effects of crystalloids, which are first-line intravascular volume-replacement solutions. Large-volume replacement therapy or rapid intravenous fluid infusion may induce shedding or partial denudation of the endothelial glycocalyx, thereby propagating tissue injury, leukocyte and platelet adhesion and activation. A compromised glycocalyx leads to detrimental capillary leak syndrome in sepsis. In clinical practice, serum albumin levels may indicate the timing and need to administer intravenous human albumin solution during fluid resuscitation when substantial volumes of crystalloids are required. This is an example of a "glycoprotective" fluid approach, with lower volumes and slower crystalloid infusion rates. The complex interaction among fluid therapy, the endothelium, the glycocalyx, and immune mediators is pathophysiologically relevant and very important for clinicians in therapeutic approaches.

Keywords:
cell membrane; critical illness; drug-related side effects and adverse reactions; infusions, intravenous; isotonic solutions; sepsis.

Apstrakt

Terapija nadoknade intravaskularnog volumena tečnostima igra ključnu ulogu u lečenju bolesnika u cirkulatornom šoku. Interakcija između različitih vidova terapije i imunskog odgovora kod kritično obolelih sa sepsom i dalje predstavlja značajan klinički izazov bez jednostavnog rešenja. Plazma ekspandere, kristaloide (kao što su fiziološki rastvor, balansirani Ringer laktat i Plazma-Lyte rastvor) i rastvore humanog albumina–jedinog prihvatljivog koloida za kritično obolele–treba smatrati neophodnim potentnim lekovima sa značajnim neželjenim efektima. Sepsa je veoma heterogen sindrom, sa različitom jačinom i trajanjem inflamacijskog odgovora. Imajući na umu da je prirodni tok sepse vrlo složen, sa fazama hiperinflamacije i imunosupresije koje se često javljaju istovremeno u različitim delovima organizma, skoro je nemoguće izbeći neželjene efekte kristaloida, koji su terapija prve linije za nadoknadu intravaskularnog volumena. Primena velikog volumena tečnosti ili brza intravenska infuzija rastvora mogu izazvati oštećenje ili parcijalni gubitak endotelnog glikokaliksa, čime se podstiče oštećenje tkiva, adhezija i aktivacija leukocita i trombocita. Oštećeni glikokaliks dovodi do štetnog sindroma kapilarnog curenja kod sepse. U kliničkoj praksi, nivoi serumskog albumina mogu ukazati na vreme i potrebu uključivanja intravenskog rastvora humanog albumina tokom

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reanimacije tečnočšću kada su potrebni veći volumeni kristaloida. Ovo je primer „glikoprotektivnog“ pristupa nadoknadi tečnostima, uz primenu manjih volumena i sporije brzine infuzije kristaloidnih rastvora. Složena interakcija između primene tečnosti, endotela, glikokaliksa i imunskih medijatora je patofiziološki relevantan problem, veoma važan za kliničare u terapijskim pristupima.

Ključne reči:
ćelija, membrana; kritična stanja; lekovi, neželjeni efekti i neželjene reakcije; infuzije, intravenske; rastvori, izotonični; sepsa.

Introduction

Over the past two decades, there have been significant advancements in our understanding of the complex pathogenic underpinnings of sepsis. This idiosyncratic medical condition, characterized as a dysregulated immune response (Ir) to infection, has led to continuous revisions of diagnostic criteria and management protocols, as well as heightened awareness among clinicians. The global burden of sepsis underscores the significance of the disease as a crucial health issue and a predominant factor in mortality and critical illness worldwide [1, 2].

Immune cells and mediators are the most important components of the Ir, but remain insufficiently elucidated to date [3, 4]. The combination of conflicting pro-inflammatory and anti-inflammatory signaling pathways heavily contributes to increased mortality risk as a result of multiple organ failure (MOF). Patients with sepsis typically exhibit a transient pro-inflammatory phase, followed by varying degrees of immunosuppression. The Ir in individual sepsis patients with different types of bacteria in the abdominal sepsis have different cytokine profiles [5, 6]. Moreover, due to the complexity of the Ir, it is rather challenging to determine independent predictors of lethal outcomes early enough to optimize individual therapeutic approaches [7, 8]. Interaction between various modalities of therapy and Ir in patients with sepsis remains a significant challenge with no simple solution [9].

Fluid administration is a very important intervention in the treatment of critically ill sepsis patients. The main goal of this therapy is to restore circulatory blood volume, expand venous return (VR), and ultimately support cardiac function and maintain mean arterial pressure. The resuscitation fluids are broadly divided into two main categories: crystalloid and colloid solutions. Crystalloids are categorized into unbalanced solutions, such as normal (isotonic) saline (0.9% sodium chloride – NaCl), and balanced (buffered) solutions, including Ringer's lactate, i.e., Hartmann's solution, and Plasma-Lyte. Colloid solutions are classified as either natural (albumin) or synthetic (hydroxyethyl starch – HES). The only allowed colloid solution in a critical care setting is human albumin [10].

Pivotal role of intravascular volume in sepsis and septic shock

Simplification of the pathophysiology of circulatory shock leads to four basic types of this detrimental condition: hypovolemic, distributive, cardiogenic, and obstructive. Septic shock is distributive in nature. Intravascular volume is functionally divided into unstressed and stressed components. Approximately two-thirds of the entire intravascular volume is unstressed volume, which does not exert any pressure on the vascular walls. The remaining one-third is stressed volume, which stretches the vessel walls and generates the mean systemic filling pressure (Pmsf). Theoretically, Pmsf is the pressure in the entire circulatory system when the heart stops (the total blood volume is about 5.5 L, but only 1.3–1.4 L is stressed volume) [11]. Unstressed volume serves as a reservoir that can be mobilized through adrenergic stimulation.

At equilibrium, VR is equivalent to cardiac output (CO). The rate of blood flow is determined by the pressure difference between the veins and the right atrium, while the cardiac pump function maintains the right atrial pressure (RAP) at low levels. Thus, the heart maintains the pressure gradient between the upstream Pmsf and the downstream RAP. The VR directly depends on the pressure gradient between the downstream pressure (RAP) and the upstream pressure (Pmsf), divided by the resistance to VR (RVr) [9]. Therefore, VR is defined by the following equation: $VR = (Pmsf – RAP)/RVr$.

The normal value of Pmsf is approximately 7–8 mmHg and directly depends on circulatory volume and venous capacitance. In normal conditions in humans, RAP is an equilibrium between the return function and cardiac function, with a value of 0–2 mmHg. The RAP value is affected by pleural pressure changes during spontaneous breathing and especially during mechanical ventilation. RVr depends on the vein diameter, and it is under direct control of the adrenergic system [10]. Two main factors can modify Pmsf. Fluid administration increases the first factor, i.e., the volume of blood in large veins. The second is the distensible venous reservoir, which can be altered by regulating adrenergic tone (the administration of vasoactive drugs) [12].

Central venous pressure (CVP) reflects RAP as well as right ventricular filling pressure. Importantly, CVP reflects the backpressure on VR and, therefore, on organ perfusion. It is clinically important because high CVP reduces mean organ perfusion pressure, which can be detrimental to critically ill patients [13].

A large venous reservoir might be recruited during acute circulatory failure, as explained before [14]. The main goal of venous expansion is to augment VR and CO in these patients. However, only half of the patients respond to fluid administration. New research indicates that it is also a time-dependent process. In the case of "fluid responders", where the Frank-Starling curve is steep, fluid expansion increases Pmsf more than RAP does, and the pressure

gradient also increases. The test is positive if the increase in stroke volume or CO is 10–15%. CO is clinically expressed as cardiac index, with normal values being 2.5–3.5 L/min/m^2. In the early phase of sepsis, there may be hyperdynamic circulation with a cardiac index > 4 L/min/m^2. In the case of "fluid non-responders", where the Frank-Starling curve is flat, volume expansion increases Pmsf, but venous preload does not increase stroke volume. The increase in end-diastolic ventricular pressure leads to a rise in RAP. Thus, RAP and Pmsf values increase together while their pressure gradient decreases. In this scenario, despite fluid administration, CO will remain unchanged [15].

Fluid resuscitation and de-resuscitation are fundamental postulates in critical care medicine. Resuscitation strives to restore sufficient tissue perfusion and oxygenation, whereas de-resuscitation seeks to eliminate excess fluid to avert consequences such as pulmonary edema, abdominal compartment syndrome, and MOF [16].

Composition of frequently utilized crystalloids

Normal saline, considered a "normal and isotonic" or "physiological" solution, is the most commonly used intravenous (i.v.) solution globally, as opposed to the 0.45% NaCl, which is considered a hypotonic fluid. Normal saline solution contains 154 mmol of both sodium and chloride ions, but in an editorial published in 1970, it was neither described as normal nor physiological [17]. Moreover, it is considered "unbalanced" due to a high concentration of chloride ions [18, 19].

The administration of chloride-rich solutions can lead to hyperchloremic metabolic acidosis with a normal anion gap. Animal studies have shown that high chloride content causes constriction of the afferent arterioles and reduced glomerular filtration rate through the tubulo-glomerular feedback mechanism [20]. A randomized controlled trial comparing the use of "unbalanced" vs. "balanced" solutions did not show worsening of renal function. However, patients who received a larger volume of 0.9% saline had a higher probability of developing acute kidney injury [21].

Moreover, high salt intake can lead to low urinary output, peripheral and organ edema, including pulmonary edema. Today, there is clear evidence that fluid overload is an independent and poor prognostic factor in the treatment of critically ill patients with sepsis. Hyperchloremic metabolic acidosis may adversely affect the immune system through the large production of pro-inflammatory cytokines and oversynthesis of nitric oxide (NO) [22]. It is considered that the use of "balanced" or "buffered" solutions has minimal effect on the immune system and acid-base balance. Furthermore, these solutions contain a low concentration of chloride ions. Examples of these solutions include Ringer's lactate and Plasma-Lyte (which contains acetate and gluconate). Ringer's lactate solution is slightly hypotonic compared to plasma due to partial ionization of solutes in balanced solutions [23]. Individual fluid effects are difficult to delineate because i.v. fluid mixtures are commonly utilized in clinical practice.

Human albumin solutions – the only acceptable colloids for critically ill patients

The main measure of treatment of hypovolemia in critically ill patients is i.v. administration of crystalloid or colloid solutions. In sepsis, increased vascular permeability leads to the leakage of serum albumin into the interstitial space. This process contributes to the onset of hypoalbuminemia and edema formation [24]. Extensive evidence indicates that administration of human albumin reduces net fluid balance compared to crystalloids, with a smaller volume of the administered solution required for hemodynamic stabilization. The effectiveness of albumin solution is better when the serum albumin level is lower. In clinical practice, serum albumin levels may indicate the timing and necessity of incorporating human albumin into fluid resuscitation. There are different albumin solutions in clinical use: hyper-oncotic (20% or 25%, for the treatment of hypoalbuminemia) and iso-oncotic (4% or 5%, for the treatment of hypovolemia). Human albumin is the sole colloid solution that leads to an expansion of intravascular volume and the elevation of plasma oncotic pressure [25].

Crystalloid and colloid solutions are often used together in the treatment of hypovolemia. The duration of effect of i.v. solutions on plasma volume expansion is essential for the sustained enhancement of tissue perfusion. The duration depends on the hydrostatic pressure value, the oncotic pressure value, and the permeability of the vascular wall [26]. Both insufficient and excessive fluid administration can be detrimental; hence, all i.v. solutions are classified as medications. Recent advancements have led to the development of "low-volume resuscitation techniques" and the use of colloid solutions for patients at risk of hypervolemia [27].

Colloid solutions (including synthetic HES, gelatin-based colloids, and dextran) are contraindicated due to safety concerns and insufficient efficacy. In 2022, the European Medicines Agency – EMA, resolved to revoke the marketing license for i.v. solutions containing HES [28]. Human albumin possesses an adequate safety profile, making it the sole recommended colloid solution for volume resuscitation. Balanced crystalloids are the first-line option, with albumin advised as a secure adjunct when crystalloids are inadequate [29].

General adverse effects of fluids

The essential question is then, where does the fluid go? The patient received a bolus of i.v. fluid, but it was completely ineffective because there was no rise in CO. In this scenario of fluid overload, systemic inflammatory responses can be exacerbated, indicated by increased leukocyte adhesion, capillary leakage, and interstitial edema formation, which, in turn, can worsen the overall tissue oxygenation. Sympathetic reaction is decreased, which is induced by low CO. In addition, flow-dependent vasodilatation is reduced due to arteriolar NO production [30].

Sepsis is a very heterogeneous syndrome, with various magnitudes and persistence of inflammatory responses, both pro-inflammatory and anti-inflammatory. The complex in-

terplay among fluids, the endothelium, the glycocalyx, and immune mediators represents a clinically relevant challenge for therapeutic approaches, because what might save one patient could harm another [31].

Components of the sepsis host response include the immune system, endothelial dysfunction, and coagulopathy. Endothelium should be viewed as an organ system for several reasons. It covers virtually all blood vessels (one cell thick); there are 10^{13} cells in total, and the weight is circa 1 kg. The endothelial surface is enormous, spanning around 4,000–7,000 m², with substantial heterogeneity and diverse biological functions, most notably immune recruitment and barrier function. In critical illness, the endothelial phenotype tends to be pro-coagulant. In general, pro-inflammatory cytokines activate endothelial cells, which impair anticoagulant mechanisms. On the other hand, activated monocytes overexpress tissue factor, which combines with tissue factor present on microparticles. The formation of microvascular thrombi is often detrimental [28]. Adhesion molecules play a critical role in mediating firm adhesion of leukocytes to endothelial cells, followed by diapedesis and extravasation. Therefore, there is a pro-adhesive endothelial phenotype in critical illness [32].

For critically ill septic patients, another problem is altered vasomotor tone with elevated levels of endothelial vasodilators (e.g., NO, prostacyclin) and depletion of endothelial vasoconstrictors (e.g., endothelin, thromboxane A_2). This is the basis of vasoplegic septic shock. Disassembly of endothelial cell junctions leads to interstitial edema through increased vascular permeability and capillary leak [33].

Loss of vascular integrity worsens the negative effects of crystalloids used for fluid resuscitation. The natural course of sepsis is very complex, with phases of hyperinflammation and immunosuppression often occurring simultaneously in different locations [34]. It is almost impossible to avoid the adverse effects of crystalloids, which are the first-line solution in intravascular volume replacement therapy.

Volume expansion is one of the most important measures in the treatment of severe acute pancreatitis. In this condition, there is premature activation of digestive enzymes in the pancreas itself and inflammation of peripancreatic fat tissue. In addition, the pancreas secretes a large amount of pro-inflammatory cytokines, resulting in overactive Ir and systemic inflammatory response syndrome. Significant intravascular fluid depletion results in a combination of hypovolemic and distributive circulatory shock, ultimately leading to multiple organ dysfunction syndrome. Although aggressive i.v. fluid therapy offers distinct advantages, excessive fluid administration in the context of severe acute pancreatitis can lead to the development of abdominal hypertension and abdominal compartment syndrome [35].

A syndrome of globally increased permeability can arise from the interplay between severe inflammation (leading to elevated capillary leak) and a positive fluid balance (resulting in tissue edema), ultimately contributing to the development of MOF. Consequently, it is essential to meticulously tailor fluid therapy, as 1 in 5 patients has inadequate i.v. fluid administration, according to the International Fluid Academy. Moreover, i.v. fluids are administered to about 80% of hospitalized patients. As much as 33% of i.v. fluids are classified as “creep fluids”. This term is used for the application of fluids for other purposes (dissolving medications, flushing the system, etc.), which can lead to additional fluid overload. Misuse of i.v. fluids contribute to acute kidney injury, abdominal hypertension, and unnecessary intensive care unit admissions. It is time to treat fluids like the drugs they are, with the same care, stewardship, and precision [36].

The endothelial surface layer and glycocalyx in sepsis

Microvascular dysfunction in infection includes vasodilation, increased procoagulant activity, elevated permeability, and enhanced interactions between circulating cells and endothelium. All these pathological processes can lead to disease progression [37]. New findings indicate that changes in microcirculation begin with damage to the endothelial glycocalyx (EG) [38], and that protecting this structure is important for preserving organ function [39]. The existing guidelines for the treatment of sepsis in adult patients do not specifically address glycocalyx damage [40]; however, several guidelines align with efforts to protect the glycocalyx [41].

The endothelial surface layer (ESL) consists of the EG, together with related chemical particles suspended in a plasma layer. Aggressive administration of “clear” fluids (crystalloids) was previously considered essential to stabilize macrohemodynamic parameters. Nonetheless, microcirculation may not inherently derive advantages from this therapy. Growing evidence suggests that large-volume fluid resuscitation may adversely affect the endothelium by altering, detaching, or discarding the ESL. This structure acts as a protective barrier over the endothelium, and the loss or reduction of the ESL can induce tissue edema and inflammation [42].

The extracellular glycocalyx is a protective, gel-like layer of carbohydrates, up to 3 μm thick, located on the luminal surface of the blood vessel, and serves as a crucial component of cellular signaling and transvascular permeability. It is composed of proteoglycans (proteins anchored to endothelial cell membranes), glycosaminoglycans, and the polysaccharide hyaluronan. High-molecular-weight polysaccharides bind a large amount of water and associate with adsorbed plasma proteins such as albumin and antithrombin [43].

The glycocalyx regulates blood flow, vascular tone, permeability, coagulability, and inflammation. Furthermore, it minimizes endothelial contact with leukocytes and platelets. Glycoproteins contain adhesion molecules such as integrins and selectins [44]. In addition, they are crucial for leukocyte trafficking during inflammation [45]; quite a few of their activities are triggered only after the shedding or thinning of the ESL. The integral part of the ESL consists of proteins, including albumin and anticoagulants, and these proteins are important for preserving the normal structure and permeability of the ESL [46]. EG significantly influences alterations in pressure and blood flow in blood vessels. Proteoglycans are crucial in responding to alterations in vascular wall shear stress or pressure [47]. The identification of these mechanical

stresses results in morphological alterations in endothelial cells and the release of NO [48]. Shear stress may induce a relocation of proteoglycans or an increase in their expression on the cell surface [49]. The shedding (loss of glycocalyx constituents) or disruption of the ESL is a crucial process following tissue injury to promote leukocyte and platelet adhesion [50]. The shedding of ESL is linked to intensified inflammation and augmented vascular permeability.

A compromised glycocalyx leads to capillary leak syndrome in infection and sepsis, characterized by abnormal fluid transfer from the blood vessels to the interstitial space, causing relative hypovolemia, tissue edema, and hypoperfusion. The development of capillary leak syndrome is associated with longer stay in the intensive care unit and prolonged use of vasoactive drugs [51].

The heterogeneity of capillary blood flow may continue in sepsis even when macrohemodynamic indicators, including blood pressure and CO, are normalized [52]. The release of EG components into blood vessels may have downstream implications. Shed components can act as damage-associated molecular patterns or "alarmins", which can further aggravate inflammation. Soluble heparan sulfate molecules are crucial in regulating inflammation, encompassing leukocyte activation, amplifying cytokine production, and promoting endothelial activation. The shedding of the ESL can be identified by many biomarkers, including syndecan-1, heparan sulfate, and hyaluronan. The revealed temporal variations in the release of these biomarkers in individuals with sepsis indicate that hyaluronan levels rise early in therapy, while syndecan-1 levels increase subsequently [53, 54].

Specific effects of fluid therapy on endothelial glycocalyx

In patients requiring volume expansion therapy, partial or total damage to ESL has likely occurred already due to the influence of inflammatory mediators [55]. Considering that modifications to the ESL may already be present in patients with sepsis, there is increasing evidence that the application of "clear" fluids may exacerbate ESL shedding. Numerous clinical trials have established a correlation between the volume of the given fluid and concentrations of EG biomarkers such as hyaluronan [53], syndecan [56], and heparan sulfate [57].

The bolus fluid treatment may directly influence the ESL through hemodilution and the synthesis of natriuretic peptides. Consequently, there has been considerable interest in the development of "glycoprotective" fluid approaches or secondary treatments during the resuscitation phase. Fluid administration is fundamental in the management of acute circulatory failure, with certain exceptions, including cardiogenic shock. This is typically accomplished by providing substantial amounts (exceeding 20 mL/kg) of crystalloid fluid i.v. at a quick rate or as a bolus. That intervention is particularly useful in cases of hypovolemic shock; yet certain types of shock exhibit vasodilation and changes in microcirculation. This encompasses shock states resulting from uncontrolled inflammation, including sepsis and severe trauma. In the above-mentioned shock scenarios, enhancements in macrohemodynamic parameters (CO) may not correlate linearly with changes in microcirculation. This is especially important in the treatment of septic shock, where early vasopressor administration and fluid bolus therapy are recommended simultaneously [58]. In light of the absence of enhancement in microcirculation, it has been proposed that fluid expansion may exacerbate vasodilation. This may lead to the failure of applied vasopressor therapy. A preliminary clinical trial was conducted to assess the feasibility of early restriction of crystalloid fluid therapy in sepsis, addressing concerns that bolus fluids may be detrimental in such cases [59]. The degradation of the glycocalyx in sepsis is significant due to thromboinflammation, and therapies for sepsis and septic shock may worsen endotheliopathy by further damaging the glycocalyx.

Glycocalyx damage, indicated by increased syndecan-1 levels, correlates with the fluid volume required for resuscitation [60]. Hypotensive resuscitation, alongside reduced fluid administration, correlates with a decreased mortality risk in trauma patients [61]. A recent comprehensive review and meta-analysis determined that reduced fluid amounts yield minimal to no variation in all-cause mortality when compared with standard therapy in adult patients with sepsis [62]. Likewise, the research corroborates that reduced i.v. fluid amounts yield minimal to no variation in severe adverse outcomes. While consensus has yet to be reached on the optimal volume, restricted i.v. fluid amounts demonstrate non-inferiority.This is particularly applicable to critically ill septic patients with sufficient hemodynamic monitoring of fluid responsiveness.

Another method to safeguard EG is by reducing the rate of fluid infusion. The rapid fluid-delivery therapeutic regimen aims to rapidly improve macrocirculatory parameters. Nonetheless, hemodynamic incoherence, characterized by a detachment between macrocirculatory variables such as blood pressure and CO and microcirculation, is a perilous complication of circulatory shock [63, 64]. Considering the evidence suggesting that rapid fluid administration (within 10 min) offers no advantage over slower fluid administration (within 20–60 min) [65, 66], larger clinical trials are warranted to investigate the impact of fluid rate on microcirculation and clinical outcomes. EG protection also involves selecting a "clear" fluid type. The use of albumin solution offers advantages for the ESL compared to the exclusive use of "clear" fluids. A recent prospective trial showed an advantage of using human albumin compared to normal saline in sepsis patients with sustained peripheral hypoperfusion [67]. The endothelial coating by EG and its accompanying constituents performs various activities in the body, both when intact and when denuded. The infusion of substantial volumes of fluid disrupts this barrier, potentially leading to tissue edema and inflammation. Evidence suggests that slower administration of fluids or limiting the volume of crystalloid fluids may be advantageous for sepsis patients [68].

ESL is the main element that affects the hydrostatic and oncotic pressure difference between the capillaries and the interstitial space [69]. Volume expansion therapy does not generate the volume distribution anticipated by Starling's origi-

nal concept. Adsorbed plasma proteins and the high-molecular-weight polysaccharides of the glycocalyx enhance its stability, while albumin is the main factor of osmotic pressure. The initial Starling principle asserts that intravascular volume comprises plasma and cellular components. The amended Starling equation further includes glycocalyx volume. The primary Starling forces are the transcapillary pressure gradient and the difference in the plasma-interstitial colloid osmotic pressure (COP). In the changed version, the difference is between the plasma and subglycocalyx COP, rather than between the plasma and interstitial COP. A modification of Starling that includes the glycocalyx model seems to more accurately elucidate the clinical responses observed [70].

Vasoplegia, resulting in a refractory shock state, is characterized by the extensive synthesis of NO and prostaglandin [71]. In addition to fluid administration, early administration of vasoactive drugs is the primary strategy to resolve this condition. Martin et al. [72] showed augmented glycocalyx damage associated with the use of catecholamines in an *in vitro* model. Exogenously applied glycocalyx components, such as hyaluronan, might theoretically repair the glycocalyx structure; however, there is currently no evidence from animal models or clinical studies to support the efficacy of this approach [73].

Sulodexide is a heparan sulfate analog that is impervious to heparanase breakdown. Researchers have documented the safeguarding benefits of sulodexide in a murine sepsis model [74] and in pediatric patients with septic shock. At present, the current guidelines for the treatment of sepsis lack a description of glycocalyx restoration [75, 76].

Conclusion

The optimal amount and speed of fluid administration in critically ill sepsis patients is still a matter of debate. Balanced crystalloids are the first-line option, with human albumin recommended as a safe adjunct when the administration of crystalloid solutions is insufficient. Current studies indicate simultaneous early vasopressor administration and fluid bolus therapy.

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Cone-beam computed tomography–guided precise chemoembolization for hypovascular hepatocellular carcinoma

Precizna hemoembolizacija hipovaskularnog hepatocelularnog karcinoma vođena kompjuterizovanom tomografijom konusnog zraka

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Abstract

Background/Aim. Hypovascular hepatocellular carcinoma (HCC) remains a therapeutic challenge owing to its inadequate arterial supply and limited responsiveness to standard transarterial chemoembolization (TACE). The aim of this study was to assess the clinical efficacy and safety of cone-beam computed tomography (CBCT) three-dimensional (3D) imaging-guided precise TACE vs. conventional digital subtraction angiography (DSA)-guided TACE in the treatment of hypovascular HCC. **Methods.** A retrospective study was performed on patients with hypovascular HCC who underwent TACE at our institution from January 2020 to December 2023. Using propensity score matching (PSM) with a 1 : 1 ratio, 58 patients were allocated to each of the two groups: the CBCT-guided precise TACE group (CBCT group) and the conventional DSA-guided TACE group (DSA group). Matching covariates included age, gender, Child-Pugh grade, tumor size, and tumor number. Short-term efficacy, long-term survival, and safety profiles were compared between the two groups. **Results.** Three months after the procedure, the CBCT group exhibited a markedly higher objective response rate (63.79% vs. 36.21%) and disease control rate (86.21% vs. 68.97%) compared to the DSA group ($p < 0.05$). Regarding long-term survival, the CBCT group exhibited significantly prolonged median progression-free survival (10.80 months vs. 7.10 months) and a higher 1-year progression-free survival rate (65.52% vs. 41.38%) compared to the DSA group ($p < 0.05$). However, no statistically significant differences were observed between the CBCT and DSA groups for median overall survival (22.50 months vs. 19.10 months) or the 1-year overall survival rate (81.03% vs. 72.41%) ($p > 0.05$). The incidence of post-embolization syndrome and severe complications (e.g., liver abscess, hepatic failure) did not differ significantly across the groups ($p > 0.05$). Notably, the elevation in alanine aminotransferase levels on the third postoperative day was considerably lower in the CBCT group than in the DSA group ($p = 0.016$). **Conclusion.** CBCT 3D imaging-guided precise TACE significantly enhances short-term therapeutic efficacy, prolongs progression-free survival, and provides superior hepatoprotection without increasing procedural risks, establishing it as a safe and effective interventional treatment option for hypovascular HCC.

Keywords:
angiography, digital subtraction; carcinoma, hepatocellular; chemoembolization, therapeutic; cone-beam computed tomography; imaging, three-dimensional; treatment outcome.

Apstrakt

Uvod/Cij. Hipovaskularni hepatocelularni karcinom (*hepatocellular carcinoma* – HCC) i dalje predstavlja terapijski izazov zbog nedovoljne arterijske vaskularizacije i slabog odgovora na standardnu transarterijsku hemoembolizaciju (*transarterial chemoembolization* – TACE). Cilj rada bio je da se procene klinička efikasnost i bezbednost precizne TACE vođene trodimenzionalnim (3D) snimanjem pomoću kompjuterizovane tomografije konusnog zraka (*cone-beam computed tomography* – CBCT) u odnosu na konvencionalnu TACE vođenu digitalnom subtrakcionom angiografijom (DSA) u lečenju hipovaskularnog HCC. **Metode.** Retrospektivnom studijom obuhvaćeni su oboleli od hipovaskularnog HCC koji su bili podvrgnuti TACE proceduri u našoj ustanovi u periodu od januara 2020. do decembra 2023. godine. Korišćenjem metode uparivanja na osnovu skora sklonosti (*propensity score matching* – PSM) u odnosu 1 : 1, po 58 bolesnika raspoređena su u svaku od dve grupe: grupu precizne TACE vođene CBCT (grupa CBCT) i grupu konvencionalne TACE vođene DSA (grupa DSA). Kovarijate podudarnosti obuhvatile su životno doba, pol, Child-Pugh stepen, veličinu i broj tumora. Upoređeni su kratkoročna efikasnost, dugoročno

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preživljavanje i bezbednosni profil između ove dve grupe. **Rezultati.** Tri meseca nakon procedure, u grupi CBCT zabeležene su znatno viša stopa objektivnog odgovora (63,79% vs. 36,21%) i stopa kontrole bolesti (86,21% vs. 68,97%) u poređenju sa grupom DSA ($p < 0,05$). U pogledu dugoročnog preživljavanja, grupa CBCT imala je značajno dužu medijanu preživljavanja bez progresije bolesti (10,80 meseci vs. 7,10 meseci) kao i višu jednogodišnju stopu preživljavanja bez progresije bolesti (65,52% vs. 41,38%) u poređenju sa grupom DSA ($p < 0,05$). Međutim, nisu primećene statistički značajne razlike između grupa CBCT i DSA za medijanu ukupnog preživljavanja (22,50 meseci vs. 19,10 meseci), niti za jednogodišnju stopu ukupnog preživljavanja (81,03% vs. 72,41%) ($p > 0,05$). Učestalost postembolizacijskog sindroma i teških komplikacija (npr. apsces jetre, insuficijencija jetre) nije se značajno razlikovala između grupa ($p > 0,05$). Primećeno je da je porast nivoa alanin aminotransferaze trećeg dana posle operacije bio znatno niži u grupi CBCT nego u grupi DSA ($p = 0,016$). **Zaključak.** Precizna TACE vođena 3D CBCT snimanjem značajno poboljšava kratkoročnu terapijsku efikasnost, produžava preživljavanje bez progresije bolesti i obezbeđuje bolju zaštitu jetre bez povećanja rizika od procedure, čime se potvrđuje kao bezbedan i efikasan interventni terapijski izbor za lečenje hipovaskularnog HCC.

Ključne reči:
angiografija, digitalna suptrakcija; karcinom, hepatocelularni; hemoembolizacija, terapijska; kompjuterizovana tomografija konusnog zraka; snimanje, trodimenzionalno; lečenje, ishod.

Introduction

Primary liver cancer (LC) is among the most prevalent malignant neoplasms globally, with consistently elevated morbidity and mortality rates [1]. Transarterial chemoembolization (TACE) is a critical palliative treatment option for most patients with intermediate and advanced stages of LC, forming the foundation of non-surgical therapeutic strategies [2]. However, LC exhibits profound heterogeneity in both biological behavior and imaging manifestations, among which hypovascular hepatocellular carcinoma (HCC) presents a major clinical challenge in treatment. Characterized by the absence of typical tumor staining and abundant neovascularization on conventional digital subtraction angiography (DSA), such tumors often demonstrate arterial-phase enhancement equivalent to or lower than that of the surrounding hepatic parenchyma [3]. The inherent limitations of two-dimensional (2D) DSA imaging hinder clinicians from accurately delineating the true tumor boundaries, infiltration range, and tiny tumor-feeding arteries [4]. Consequently, conventional TACE procedures entail considerable blindness, which is prone to incomplete tumor embolization, residual lesions, and early postoperative recurrence [5]. Meanwhile, the risk of liver function impairment is heightened due to the potential embolization of normal hepatic tissues by embolic agents [6]. In recent years, cone-beam computed tomography (CT) – CBCT technology has emerged as a novel approach to address this predicament [7]. As a three-dimensional (3D) imaging modality integrated into the DSA platform, CBCT enables the rapid acquisition of thin-slice, high soft-tissue resolution 3D images analogous to those obtained *via* multi-detector CT (MDCT) during interventional procedures [8]. A clinical study has demonstrated that CBCT imaging yields a significantly higher detection rate for hepatic tumors–particularly for small and hypovascular lesions–compared with conventional DSA [9]. By fusing CBCT images with preoperative CT or magnetic resonance imaging (MRI) data, clinicians can accurately delineate tumor target volumes in 3D space and clearly identify tumor-feeding vessels that are undetectable on conventional DSA, thereby guiding microcatheter-based superselective catheterization and embolization [10]. Theoretically, this "precision TACE" paradigm can optimize embolization efficacy, improve treatment outcomes, and maximize the preservation of liver function [11]. Nevertheless, systematic clinical research investigating the long-term survival benefits and safety profile of CBCT-guided 3D precision TACE in patients with hypovascular HCC remains insufficient [12].

Based on these considerations, the aim of this study was to conduct a propensity score-matched analysis comparing the short-term efficacy, long-term survival, and safety of CBCT-guided precise TACE vs. conventional DSA-guided TACE in the treatment of hypovascular HCC. This study sought to provide higher-level evidence-based medical support for optimizing clinical interventional therapeutic strategies for this carcinoma subtype.

Methods

The study was approved by the Ethics Committee of the Tongxiang First People's Hospital, Tongxiang, Zhejiang, China (No. 2020/HCC/11/323, from December 20, 2019). Given the retrospective nature of this analysis and the fact that all patients had completed treatment and entered the follow-up phase at the time of protocol approval, the Institutional Review Board granted a waiver of informed consent. The data collection period spanned from January 2020 to December 2023, with patient data from January 2020 to November 2020 included as historical control data. This research performed a retrospective review of clinical data from patients with HCC who obtained TACE at the institution between January 2020 and December 2023, utilizing the hospital's medical record system.

The sum of 58 patients with hypovascular HCC who received CBCT-guided precision TACE were initially enrolled. To control confounding bias, propensity score matching (PSM) was performed. With age, gender, Child-Pugh classification, tumor size, and tumor number as key covariates, 58 patients were successfully matched from 215

cases of hypovascular HCC who underwent conventional DSA-guided TACE during the same period, employing the nearest neighbor matching technique with a calliper value of 0.02 at a 1 : 1 ratio. After matching, the standardized differences of all baseline data between the two groups were less than 10%, indicating good intergroup balance.

Inclusion and exclusion criteria

The inclusion criteria were outlined as follows: patients aged 18 to 75 years; confirmed diagnosis of primary HCC by clinical or pathological examinations [13]; preoperative contrast-enhanced CT or MRI demonstrating hypovascular lesions, defined as tumor parenchymal enhancement < 50% of that of surrounding normal hepatic parenchyma during the arterial phase, with this characteristic consistently present in > 50% of tumor volume; liver function classified as Child-Pugh grade A or B; and Eastern Cooperative Oncology Group performance status score of 0 or 1, indicating that patients could tolerate interventional surgery.

Exclusion criteria included the following: presence of extensive extrahepatic metastasis; complicated with significant impairment of essential organs, including the heart, kidneys, and brain; hypersensitivity to iodine-containing contrast agents used in the study; previous history of local liver radiotherapy, ablation, or systemic therapy; and severe deficiency in clinical or follow-up data.

Equipment and materials

All interventional procedures were performed on a DSA system equipped with a flat-panel detector (Artis zee III, Siemens Healthineers, Germany) with integrated CBCT capability (5 s rotational acquisition, 200° scan angle, 0.5 mm reconstruction slice thickness). The used microcatheters included Progreat™ (Terumo, Japan) or Carnelian® (Tokai Medical Products, Japan), with 0.014-inch hydrophilic microguidewires.

Chemotherapeutic agents consisted of epirubicin (50 mg/m²) or lobaplatin (50 mg/m²), mixed with Lipiodol® Ultra Fluid (Lipiodol, Guerbet, France) in a 1 : 1 volume ratio. The emulsion was prepared using a three-way stopcock with repeated aspiration for no less than 10 min. Embolic materials included gelatin sponge particles (350 μm–560 μm, Hangzhou Alicon, China) or polyvinyl alcohol microspheres (300 μm–500 μm, Boston Scientific, USA), selected according to tumor vascularity.

Study groups

After PSM, 58 patients were included in each group, with well-balanced baseline characteristics. All enrolled patients underwent TACE. They were categorized into two groups according to the intraoperative guiding approach: the CBCT-guided precision TACE group (CBCT group) and the conventional DSA-guided routine TACE group (DSA group). All procedures in both groups were performed by the same team of experienced interventional physicians with senior professional titles, so as to minimize operator variability.

Patients in the CBCT group received a standardized precision embolization protocol. The steps for implementing that protocol are described in the text that follows. Firstly, routine angiography and target area confirmation were performed. Patients were put in supine position. Following standard disinfecting and draping, femoral artery puncture and catheterization were performed using the Seldinger approach. The catheter was placed superselectively into the common hepatic artery or proper hepatic artery for conventional DSA angiography, so as to preliminarily evaluate hepatic blood supply and tumor characteristics. For lesions identified as hypovascular on DSA, the CBCT scanning protocol was immediately initiated. Secondly, 3D imaging and pathway planning was performed. The C-arm machine rotated 180°–240° around the patient's hepatic region, and 3D data were acquired and reconstructed synchronously with contrast agent injection. The reconstructed CBCT images were registered and fused–either automatically or manually–with preoperative contrast-enhanced CT or MRI images. This process enabled accurate 3D delineation of the gross tumor volume (GTV) and the clear identification of tiny tumor-feeding arteries that were not readily visualized on conventional DSA. 3D volume rendering and multi-planar reformation were performed on the workstation. Following identification of tumor-feeding arteries, the optimal working projection was transferred to the DSA system to guide real-time microcatheter superselective cannulation. The third step included superselective embolization and endpoint determination. Under the guidance of 3D imaging, microcatheters were used for superselective catheterization of tumor-feeding arteries. Under real-time fluoroscopic monitoring, chemotherapeutic emulsions (e.g., epirubicin or lobaplatin mixed with lipiodol) were slowly and precisely infused, followed by distal embolization with gelatin sponge particles or polyvinyl alcohol microspheres as appropriate. The embolization endpoint was defined as achieving dense and homogeneous Lipiodol® deposition within the tumor area, as confirmed by intraoperative and postoperative CBCT. The fourth and final step was immediate efficacy verification. Immediately after embolization, a repeat CBCT scan was performed to directly assess the distribution range and homogeneity of Lipiodol® deposition in the tumor from a 3D perspective, so as to verify the completeness of embolization. The detailed procedural workflow of CBCT-guided precise TACE is illustrated in Figure 1.

Patients in the DSA group were treated with 2D DSA imaging guidance alone. Based on the vascular morphology displayed by DSA angiography and personal clinical experience, the operator performed superselective or selective catheterization of suspected tumor-feeding arteries, followed by chemotherapeutic agent infusion and embolization. No CBCT was used for 3D imaging guidance, pathway planning, or immediate postoperative evaluation throughout the procedure in this group.

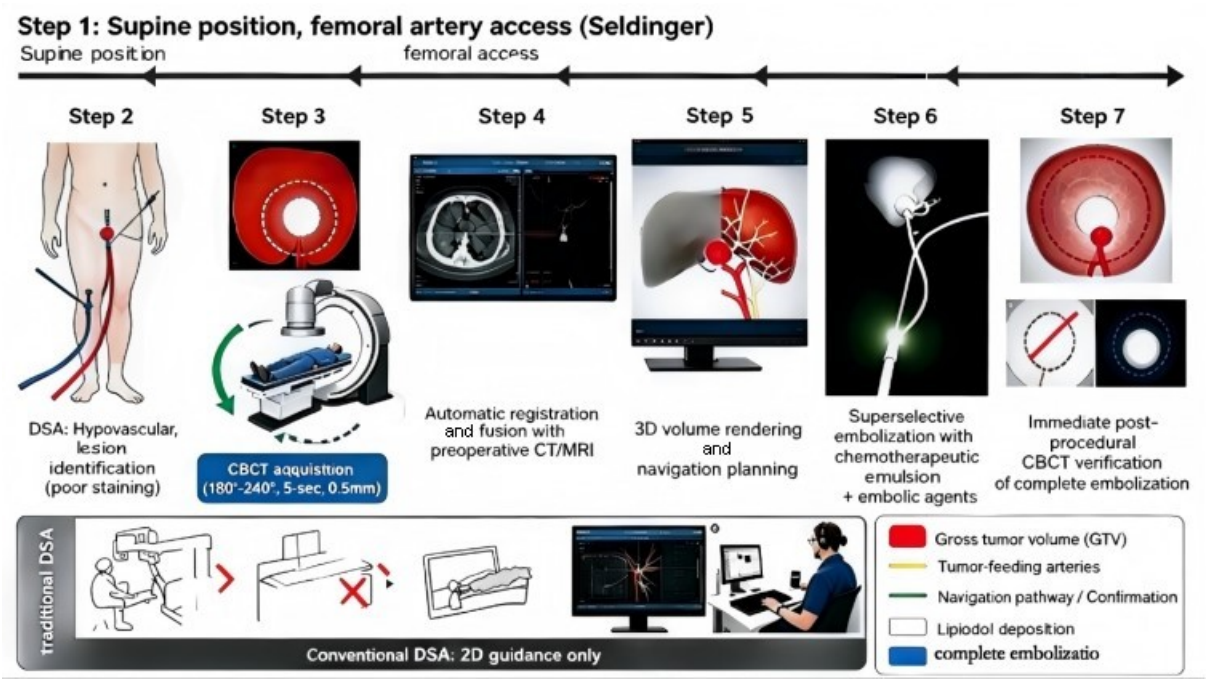

Fig. 1 – Schematic flowchart of cone-beam computed tomography (CBCT)-guided precise transarterial chemoembolization for hypovascular hepatocellular carcinoma.
DSA – digital subtraction angiography; CT – computed tomography; MRI – magnetic resonance imaging; 3D – three-dimensional; 2D – two-dimensional.

TACE treatment in this study was administered on a demand basis. The total intervention cycle was terminated when satisfactory tumor control was achieved, disease progression occurred, or intolerable toxic side effects emerged. All patients underwent regular follow-up after the initial surgery.

Outcome measures

Outcome measures evaluated in this study included short-term efficacy, long-term survival indicators, and safety indicators.

Regarding short-term efficacy, the modified Response Evaluation Criteria in Solid Tumors (mRECIST) were used to assess the outcomes of contrast-enhanced CT or MRI re-examinations 3 months postoperatively. Specifically, the objective response rate (ORR), defined as the percentage of patients attaining complete response (CR) and partial response (PR), and the disease control rate (DCR), defined as the percentage of patients reaching CR, PR, and stable disease, were calculated. CR was defined as complete Lipiodol® deposition throughout the tumor region on post-procedural CT with no residual enhancement on contrast-enhanced imaging at 3 months.

For long-term survival indicators, the study included progression-free survival (PFS) and overall survival (OS). PFS was defined as the time interval from the date of the first TACE treatment to the documentation of radiologically confirmed disease progression or mortality from any cause. Similarly, OS was defined as the duration from the first TACE therapy to mortality from any cause.

Finally, safety indicators focused on the incidence of postoperative complications, including the occurrence rate and severity of post-embolization syndrome (such as fever, pain, nausea, and vomiting) during the acute phase (within 1 week after surgery). Additionally, the dynamic changes in laboratory parameters–namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TBil)–were assessed before surgery, as well as 1, 3, and 7 days postoperatively to evaluate the extent of liver function impairment.

Statistical analysis

All statistical assessments were carried out utilizing SPSS version 26.0. Normally distributed continuous parameters are expressed as mean ± standard deviation and assessed utilizing the independent samples *t*-test. Categorical parameters were expressed as percentages and evaluated *via* the Chi-square test or Fisher's exact test, as applicable. Survival curves are generated utilizing the Kaplan-Meier technique and assessed utilizing the log-rank test for comparison. A two-tailed *p*-value < 0.05 was deemed statistically significant.

Given the retrospective nature of this study, complete raw data for some laboratory parameters could not be extracted from electronic medical records.

Results

No statistically significant differences were observed between the two groups regarding age, sex, Child-Pugh class, tumor size, or tumor number ($p > 0.05$) (Table 1). A

mean tumor diameter of 5.4 cm with hypovascular manifestation was observed in this study.

Treatment and follow-up

Patients received a mean of 2.4 ± 0.8 TACE sessions, with a median inter-treatment interval of 5.3 weeks (range: 4–6 weeks). All patients underwent regular follow-up following the initial procedure.

Given the retrospective study design without prospective documentation of "technical success", we utilized the proportion of cases with clearly evaluable Lipiodol® deposition on immediate post-procedural imaging as a surrogate metric. The resulting image-evaluable rate was 94.83% (55/58) in the CBCT group vs. 72.41% (42/58) in the DSA group, with a statistically significant difference ($p < 0.001$).

Subsequent treatments were evaluated based on available records from our institutional electronic medical record system. During follow-up, approximately 35% of patients in the CBCT group and 40% in the DSA group received subsequent systemic therapy (targeted agents, immunotherapy), while approximately 52% and 59%, respectively, received second-line interventional therapy (radiofrequency ablation, repeat TACE). No significant differences in subsequent treatment patterns were observed between groups. Due to the retrospective nature of this study, some subsequent treatment information was obtained from external institutions, and records were incomplete; therefore, precise case numbers could not be determined.

Comparison of short-term efficacy

The short-term efficacy evaluated using the mRECIST at 3 months after surgery is presented in Table 2. ORR of the CBCT-guided precision TACE group was 63.79% (37/58), greatly surpassing that of the conventional DSA-guided TACE group, 36.21% (21/58), with a statistically significant difference ($p = 0.003$). Regarding the DCR, the CBCT-guided group also exhibited a superior performance (86.21% vs. 68.97%), and the intergroup difference was statistically significant ($p = 0.024$).

Comparison of long-term survival outcomes

A comparison of long-term survival outcomes between the two groups is detailed in Table 3. Survival analysis demonstrated that the median PFS in the CBCT precision TACE group was 10.80 months, which was significantly longer than the 7.10 months observed in the conventional DSA-guided group ($p = 0.013$). In addition, the 1-year PFS rate of the CBCT group (65.52%) was significantly higher than that of the DSA group (41.38%), with a statistically significant difference ($p = 0.024$).

Table 1

Comparison of patients' baseline data according to groups

Group	Age, years	Male, cases	Child-Pugh class A, cases	Maximum tumor diameter, cm	Number of tumors
CBCT (n = 58)	59.12 ± 8.95	40 (68.97)	48 (82.76)	5.35 ± 1.77	1.45 ± 0.62
DSA (n = 58)	58.78 ± 9.41	42 (72.41)	47 (81.03)	5.41 ± 1.82	1.52 ± 0.71
t/χ^2	0.985	0.172	0.000	0.178	0.563
p-value	0.326	0.678	1.000	0.859	0.575

CBCT – cone-beam computed tomography; DSA – digital subtraction angiography; n – number of patients.
All values are given as numbers (percentages) or mean ± standard deviations.

Table 2

Comparison of the efficacy of different TACE modalities between the two groups at 3 months postoperatively

Group	Complete response	Partial response	Stable disease	Progressive disease	Objective response rate	Disease control rate
CBCT (n = 58)	12 (20.69)	25 (43.10)	13 (22.41)	8 (13.79)	37/58 (63.79)	50/58 (86.21)
DSA (n = 58)	5 (8.62)	16 (27.59)	19 (32.76)	18 (31.03)	21/58 (36.21)	40/58 (68.97)
χ^2					9.103	5.126
p-value					0.003	0.024

TACE – transarterial chemoembolization; CBCT – cone-beam computed tomography; DSA – digital subtraction angiography; n – number of patients.
All values are given as numbers (percentages).

Table 3

Comparison of long-term survival outcomes between the two groups

Group	Median progression-free survival, months	1-year progression-free survival rate, %	Median overall survival, months	1-year overall survival rate, %
CBCT (n = 58)	10.80	65.52	22.50	81.03
DSA (n = 58)	7.10	41.38	19.10	72.41
χ^2	2.478	5.124	1.523	1.256
p-value	0.013	0.024	0.128	0.262

CBCT – cone-beam computed tomography; DSA – digital subtraction angiography; n – number of patients.

Figure 2 illustrates an overview of the OS curves among the groups. With respect to OS, although both the median OS and 1-year overall survival rate of the CBCT group exceeded those of the DSA group (median OS: 22.50 months vs. 19.10 months; 1-year OS rate: 81.03% vs. 72.41%), neither of these differences reached statistical significance ($p > 0.05$) (Figure 2).

Safety comparison

Table 4 illustrates an assessment of safety indicators among the two treatment groups. A lack of statistical significance was observed in the incidence of post-embolization syndrome of any grade (e.g., fever, pain, nausea) between the two groups ($p = 0.784$). Concerning serious complications, no statistically significant differences were observed between the CBCT-guided group and DSA group regarding the incidence of liver abscess (3.45% vs. 5.17%), liver failure (1.72% vs. 3.45%), biliary tract injury (1.72% vs. 3.45%), and nontarget embolization (1.72% vs. 1.72%), with all *p*-values exceeding 0.05. Nevertheless, in terms of biochemical indicators for liver function injury, the amplitude of ALT elevation in the CBCT group on the third day after surgery was significantly lower than that in the DSA group ($p = 0.016$). This finding suggests that precise embolization under CBCT guidance results in less damage to normal liver function.

Discussion

The core challenge of TACE in treating hypovascular HCC lies in achieving sufficient and precise distribution of chemoembolic agents within the tumor parenchyma against the background of atypical blood supply characteristics, while minimizing damage to the surrounding liver function [14, 15]. Although the 3D visualization advantage of CBCT theoretically provides a solution to this dilemma, its comprehensive impact on short-term efficacy, long-term survival, and safety in real-world clinical practice still needs to be verified through rigorous clinical research [16]. By conducting a retrospective cohort analysis, this study systematically compared the clinical efficacy of CBCT-guided precision TACE and the conventional DSA-guided TACE in the management of hypovascular HCC. The results demonstrated that the CBCT-guided group exhibited significantly superior short-term efficacy and long-term PFS,

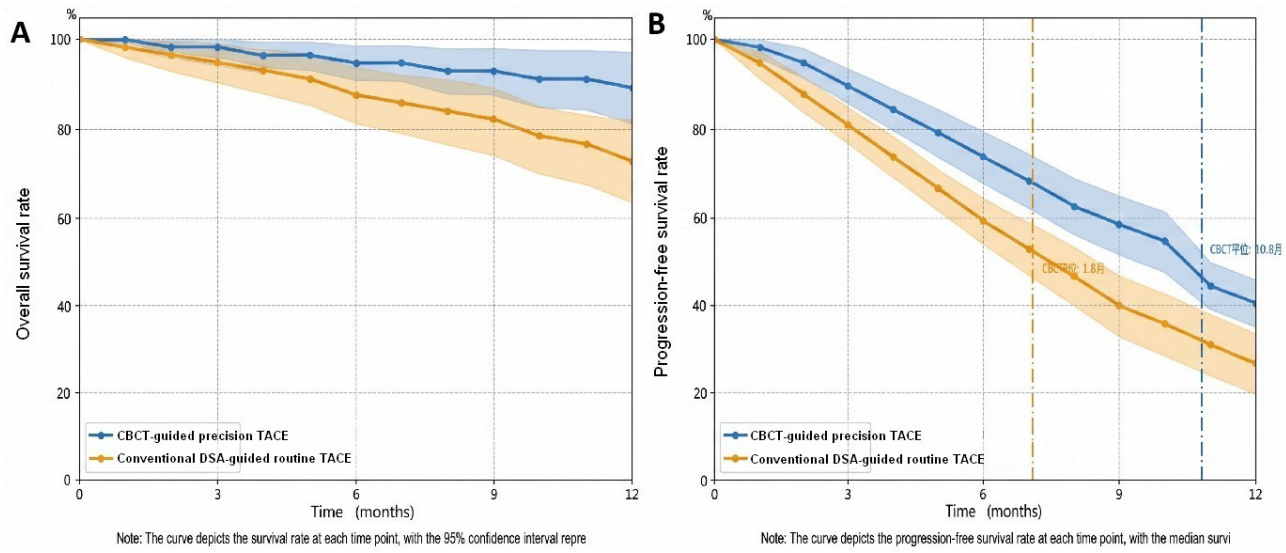

Fig. 2 – Kaplan-Meier curves for overall survival (A) and progression-free survival (B) during one year comparing cone-beam computed tomography (CBCT)-guided and digital subtraction angiography (DSA)-guided transarterial chemoembolisation (TACE).

Table 4

Comparison of postoperative safety profiles between CBCT-guided and conventional DSA-guided TACE groups

Group	Post-embolization syndrome	Liver abscess	Liver failure	Biliary injury	Nontarget embolization	ALT, U/L
CBCT (n = 58)	46/58 (79.31)	2 (3.45)	1 (1.72)	1 (1.72)	1 (1.72)	128.45 ± 70.13
DSA (n = 58)	47/58 (81.03)	3 (5.17)	2 (3.45)	2 (3.45)	1 (1.72)	162.89 ± 79.65
Test statistic	$\chi^2 = 0.075$	Fisher	Fisher	Fisher	Fisher	$t = 2.457$
p-value	0.784	1.000	0.500	1.000	1.000	**0.016**

CBCT – cone-beam computed tomography; DSA – digital subtraction angiography; TACE – transarterial chemoembolization; ALT – alanine aminotransferase; n – number of patients.
All values are given as numbers (percentages) or mean ± standard deviations.
***Note*: Post-embolization syndrome includes fever, pain, nausea/vomiting of any grade within 1 week after the procedure. Severe complications include liver abscess, hepatic failure, biliary tract injury, and nontarget embolization. Values of *p* are derived from the Chi-square test, Fisher's exact test (for categorical variables with expected frequency < 5), or independent samples *t*-test (for ALT). Bold value indicates statistical significance ($p < 0.05$).**

along with notable advantages in liver function preservation. These outcomes are closely associated with the distinct imaging principles and operational precision inherent to the two techniques [17, 18].

In this study, the CBCT-guided group attained markedly superior ORR and DCR at 3 months postoperatively compared with the DSA-guided group. This disparity primarily stems from the fundamental differences between the two imaging modalities [19, 20]. DSA provides 2D vascular images that are plagued by structural overlapping [21]. For hypovascular lesions lacking typical tumor staining characteristics, diagnostic sensitivity is limited, which is prone to missed detection of micro-lesions or misjudgment of the true tumor boundaries [22]. In contrast, CBCT acquires 3D volumetric data through rotational scanning of the C-arm, and the reconstructed tomographic images possess excellent soft tissue resolution, enabling clear visualization of the density differences between the tumor and the surrounding hepatic parenchyma [23]. More importantly, through image fusion and registration with preoperative contrast-enhanced CT or MRI scans, CBCT allows for accurate delineation of GTV in 3D space and identification of micro-tumor-feeding arteries that are otherwise undetectable by conventional DSA [24, 25]. This enhanced "visualization" empowers surgeons to perform true superselective catheterization under guidance, ensuring that chemoembolic agents are more concentrated in the tumor parenchyma, thereby significantly improving the degree of tumor necrosis [26, 27]. This mechanism is directly reflected in the remarkable improvement of short-term efficacy indicators [28].

In terms of long-term survival outcomes, the significant prolongation of median PFS in the CBCT-guided group further confirms the sustained benefits conferred by precision embolization [29]. Complete tumor necrosis and thorough interruption of tumor blood supply fundamentally delay the processes of local recurrence and disease progression [30]. It is noteworthy that although the CBCT-guided group exhibited a trend toward better OS and 1-year OS rate compared with the conventional group, the difference lacked statistical relevance. This observation may be ascribed to multiple factors, including the high inherent heterogeneity of HCC itself, the confounding effects of subsequent treatment modalities (e.g., systemic therapy, second-line interventional procedures) on OS, and the relatively inadequate sample size of this study, which may not possess sufficient statistical power to detect subtle differences in OS. Additionally, for HCC patients, liver function reserve and tumor biological behavior are often more decisive factors influencing long-term prognosis, and the advantages of local treatment may be diluted by other factors during long-term follow-up [31].

Regarding the definition of hypovascular HCC employed in this study, our criterion of "tumor parenchymal enhancement < 50% of surrounding normal hepatic parenchyma" was based on preoperative imaging assessment. While such lesions may encompass some heterogeneous tumors (e.g., with areas of fibrosis or necrosis), this precisely reflects the clinical challenges encountered in TACE treatment [32]. The mean tumor diameter of 5.4 cm with hypovascular manifestation observed in this study may be related to extensive intratumoral fibrosis or necrosis; such lesions indeed present greater challenges in interventional therapy, underscoring the value of CBCT guidance.

The significantly lower ALT elevation observed in the CBCT-guided group (128.45 ± 70.13 U/L vs. 162.89 ± 79.65 U/L, $p = 0.016$) compared with the DSA-guided group provides compelling biochemical evidence for superior hepatoprotection conferred by precise embolization. This finding is mechanistically rooted in the fundamental technical distinctions between the two modalities. In conventional DSA-guided TACE, the inherent limitations of 2D imaging–namely, structural overlap and poor visualization of hypovascular lesions–frequently result in inadvertent embolization of non-target vessels and unintentional deposition of chemoembolic agents in normal hepatic parenchyma. This "anatomical blindness" triggers bystander ischemic injury and chemical hepatitis in healthy liver tissue, manifesting as elevated transaminases and prolonged hepatic dysfunction.

Conversely, CBCT-guided precision TACE operates through a targeted-sparing paradigm. First, pre-procedural 3D mapping enables accurate delineation of the GTV and the identification of tumor-feeding arteries invisible on conventional DSA. Second, real-time navigation directs microcatheter superselective cannulation exclusively to target vessels. Finally, immediate post-procedural verification confirms dense, homogeneous Lipiodol® deposition confined to the tumor, minimizing spillover to the surrounding liver. This "precision-target, precision-treat, precision-verify" workflow ensures that cytotoxic agents and embolic materials are concentrated within the intended therapeutic zone, thereby maximizing tumor necrosis while minimizing collateral damage.

The clinical significance of this hepatoprotective effect extends beyond the immediate postoperative period. For patients with underlying cirrhosis or limited hepatic reserve–the predominant demographic in HCC populations–preservation of functional liver parenchyma is paramount. Lower ALT elevation on day 3 not only indicates reduced acute hepatocellular injury but also predicts faster recovery of liver function, greater tolerance to subsequent treatment cycles, and expanded opportunities for multimodal therapy, including systemic agents, immunotherapy, or repeat locoregional interventions. In this context, CBCT-guided TACE transforms from a mere technical refinement into a strategic therapeutic advantage that optimizes the long-term treatment trajectory for patients with compromised liver function.

Regarding safety profiles, there were no significant differences in the incidence of post-embolization syndrome and serious complications between the two groups, indicating that the CBCT-guided technique itself does not introduce additional surgical risks. However, the ALT levels in the CBCT-guided group on the third postoperative day were much lower than those in the standard DSA group. This

is mainly because during conventional TACE procedures, unclear identification of tumor-feeding arteries on 2D imaging may lead to excessive or inadvertent embolization of non-target vessels. This results in the extravasation and retention of chemoembolic agents in normal liver tissue, triggering more extensive chemical and ischemic liver injury. By accurately identifying tumor-feeding vessels, CBCT-guided precision TACE achieves targeted embolization with high precision. This maximizes the concentration of drugs and embolic agents within the target lesions and effectively reduces damage to normal liver tissue in non-target areas, thus manifesting as a smaller magnitude of postoperative liver enzyme elevation [33]. This advantage is particularly crucial for HCC patients with underlying liver cirrhosis, as superior liver function preservation translates to improved treatment tolerance and expanded opportunities for subsequent therapeutic interventions.

Limitations of the study

This study has several inherent limitations. First, because of its retrospective design, intraoperative "technical success" was not prospectively defined and documented. Instead, we utilized the "image-evaluable rate" as a surrogate metric. Consequently, future studies should employ more standardized definitions of technical success. Second, some subsequent treatment information was obtained from external institutions with incomplete records, which may have influenced the accuracy of the OS analysis. Third, this study employed modified mRECIST criteria for efficacy assessment, but the applicability of these criteria for hypovascular HCC remains limited. Future studies should explore functional imaging modalities, such as diffusion-weighted imaging or positron emission tomography-CT, to establish more appropriate efficacy evaluation systems for hypovascular HCC. Finally, this was a single-center study with a relatively limited sample size. Nevertheless, the results demonstrate that CBCT 3D imaging guidance, through enhanced diagnostic precision and targeting of embolization procedures for hypovascular HCC, effectively improves short-term tumor efficacy, prolongs PFS, and better preserves patient hepatic function. Future larger-sample, prospective randomized controlled studies are warranted to further validate these findings and clarify their ultimate value in improving patient OS.

Conclusion

Cone-beam computed tomography-guided three-dimensional-precision transarterial chemoembolization exhibits enhanced short-term tumor response and significantly extends progression-free survival in patients with hypovascular hepatocellular carcinoma compared to traditional digital subtraction angiography-guided transarterial chemoembolization. This approach allows for precise identification of tumor-feeding arteries, promotes superselective embolization, and provides superior hepatoprotection without elevating operative complications. Despite the absence of a statistically significant improvement in overall survival, the positive effectiveness and safety profile underscore cone-beam computed tomography-guided precision transarterial chemoembolization as a potential interventional approach for this difficult tumor subtype. Comprehensive prospective, multicenter investigations are necessary to further substantiate these results and elucidate their long-term survival advantages.

Conflicts of interest

The authors declare no conflict of interest.

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Association of anticoagulant therapy dosing with laboratory biomarkers and clinical outcomes in critically ill COVID-19 patients in the ICU

Povezanost doziranja antikoagulantne terapije sa laboratorijskim biomarkerima i kliničkim ishodima kod kritično obolelih od COVID-19 u odeljenju intenzivne nege

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Abstract

Background/Aim. In immunothrombotic disorders such as coronavirus disease 2019 (COVID-19), D-dimer levels are frequently elevated, reflecting increased fibrin formation and turnover. Additional biomarkers, such as the neutrophil-to-lymphocyte ratio (NLR) and levels of C-reactive protein (CRP), and lactate dehydrogenase (LDH), are associated with disease severity and outcomes. The aim of the study was to evaluate the impact of two different anticoagulation protocols on serum levels of biomarkers D-dimer, NLR, CRP, and LDH, as well as their prognostic value regarding clinical outcomes in critically ill patients with COVID-19. **Methods.** The retrospective study included critically ill COVID-19 patients, admitted to the Intensive Care Unit (ICU) between April 2020 and December 2021, and compared D-dimer–guided and anti-Xa–guided anticoagulation protocols. Patients were divided into two groups according to the anticoagulant therapy regimen: a group with a protocol guided by anti-Xa values (AXa group – A-XaG) and a group with a protocol dosing according to D-dimer values (D-d group – D-dG). **Results.** A total of 395 patients were analyzed: 137 in A-XaG and 258 in D-dG. The levels of CRP, LDH, and D-dimer were significantly lower in A-XaG compared to D-dG ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively). The univariate analysis identified age [odds ratio (OR): 1.064; $p < 0.001$], LDH (OR: 1.002; $p < 0.001$), CRP (OR: 1.005; $p < 0.001$), and D-dimer (OR: 1.054; $p = 0.020$) as prognostic factors for mortality. The multivariate model analysis revealed that only age > 64 years (OR: 10.215; $p < 0.001$) and LDH > 395 U/L (OR: 5.491; $p = 0.005$) remained independently associated with mortality. **Conclusion.** Anti-Xa–guided anticoagulation was associated with lower inflammatory biomarker levels in ICU COVID-19 patients. While univariate analysis identified age, LDH, CRP, and D-dimer as potential prognostic factors for mortality, only age and LDH remained significant in multivariate modelling, suggesting independent prognostic value in this patient population.

Keywords:
anticoagulants; biomarkers; covid-19; factor xa; heparin; intensive care units; prognosis.

Apstrakt

Uvod/Cilj. Kod imunotrombotskih poremećaja kao što je bolest izazvana korona virusom 2019 (*coronavirus disease* 2019–COVID-19), nivoi D-dimera često su povišeni, što odražava povećano stvaranje i razgradnju fibrina. Dodatni biomarkeri, kao što su odnos neutrofila i limfocita (*neutrophil-to-lymphocyte ratio* – NLR) i nivoi C-reaktivnog proteina (CRP) i laktat dehidrogenaze (LDH), povezani su sa težinom i ishodima bolesti. Cilj rada bio je da se proceni uticaj dva različita antikoagulaciona protokola na nivoe biomarkera D-dimer, NLR, CRP i LDH u serumu, kao i njihova prognostička vrednost u pogledu kliničkih ishoda kod kritično obolelih od COVID-19. **Metode**. Retrospektivnom studijom obuhvaćeni su kritično oboleli od COVID-19, primljeni na Odeljenje intenzivne nege u periodu od aprila 2020. do decembra 2021. godine i upoređeni su protokoli za vođenje antikoagulantne terapije pomoću D-dimera i pomoću anti-Xa inhibitora. Bolesnici su prema režimu antikoagulantne terapije bili podeljeni u dve

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grupe: grupu u kojoj je primenjen protokol vođen anti-Xa vrednostima (AXa grupa – A-XaG) i grupu gde je primenjen protokol sa doziranjem prema D-dimer vrednostima (D-d grupa – D-dG). **Rezultati**. Analizirano je ukupno 395 bolesnika: 137 u A-XaG i 258 u D-dG. Vrednosti CRP, LDH i D-dimera bile su značajno niže u A-XaG u odnosu na D-dG ($p < 0,001$, $p < 0,001$, $p = 0,001$, redom). Univarijantnom analizom su kao prognostički faktori mortaliteta pokazani životno doba [*odds ratio* (OR): 1,064; $p < 0,001$)], LDH (OR: 1,002; $p < 0,001$), CRP (OR: 1,005; $p < 0,001$) i D-dimer (OR: 1,054; $p = 0,020$). Analizom multivarijantnog modela pokazano je da su samo životno doba > 64 godine (OR: 10,215; $p < 0,001$) i LDH > 395 U/L (OR: 5,491; $p = 0,005$) ostali nezavisno povezani sa smrtnim ishodom. **Zaključak.** Antikoagulantna terapija vođena anti-Xa vrednostima bila je povezana sa nižim vrednostima inflamacijskih biomarkera kod obolelih od COVID-19 na Odeljenju intenzivne nege. Iako su univarijantnom analizom kao potencijalni prognostički faktori mortaliteta identifikovani životno doba, LDH, CRP i D-dimer, samo su životno doba i LDH ostali statistički značajni pokazatelji u multivarijantnom modelu, što ukazuje na nezavisnu prognostičku vrednost kod te populacije bolesnika.

Ključne reči:
antikoagulansi; biomarkeri; covid-19; faktor xa; heparin; intenzivna nega, odeljenja; prognoza.

Introduction

Severe coronavirus disease 2019 (COVID-19) with pulmonary involvement is characterized by hypercoagulability and elevated systemic inflammatory activation. Pulmonary vascular injury and microthrombosis are increasingly recognized as central features of this inflammation-coagulation interplay, referred to as immunothrombosis [1, 2]. COVID-19–associated coagulopathy is largely driven by immunothrombosis, in which inflammatory activation of endothelial cells, monocytes, platelets, and leukocytes promotes tissue factor expression, thrombin generation, and fibrin deposition, particularly within the pulmonary microvasculature. In addition, activated neutrophils release neutrophil extracellular traps, which provide a procoagulant surface, further enhancing thrombin generation and platelet activation. Together, these mechanisms contribute to the immunothrombotic phenotype observed in severe COVID-19 cases [3]. Some conceptual models describe severe COVID-19 as a stage-dependent process in which early disease is characterized by high fibrinogen, Von Willebrand factor, and P-selectin levels with normal or only slightly increased D-dimer, whereas disease progression is associated with rapidly rising D-dimer levels, depletion of some coagulation-related biomarkers, and later cytokine storm, indicating poor prognosis [4].

Given this interplay, several biomarkers that can be readily assessed through routine laboratory testing have been identified as indicators of disease progression and mortality [3]. These biomarkers include the neutrophil-to-lymphocyte ratio (NLR) as an independent prognostic factor linked to worse outcomes in COVID-19 patients [5, 6]. Additionally, levels of C-reactive protein (CRP) and lactate dehydrogenase (LDH) have been shown to correlate with the severity of COVID-19, providing further insights into the inflammatory and tissue damage processes [7, 8]. This growing body of evidence underscores the potential of these biomarkers as valuable tools for risk stratification and management in clinical practice [8]. Elevated D-dimer levels predict in-hospital mortality, indicate an increased risk of a procoagulant state, and are associated with an increased risk of thromboembolic complications [9, 10]. Conversely, the safety profiles and effectiveness of various anticoagulation strategies in critically ill patients with COVID-19 remain inadequately understood. This is particularly relevant when considering the multifaceted properties of heparin, highlighting the need for further research [11].

In a previous study conducted in the same cohort of critically ill COVID-19 patients, we demonstrated that anticoagulation tailored according to anti-Xa activity was associated with improved survival compared with D-dimer–guided anticoagulation. Specifically, the anti-Xa–guided strategy was linked to lower observed mortality and a reduced incidence of thromboembolic complications, without an increase in bleeding events. These findings suggested that anti-Xa–guided anticoagulation may offer advantages over D-dimer–based dose adjustment alone [12].

Building on these observations, the present study investigated whether anti-Xa–guided anticoagulation is associated with differences in inflammatory and coagulation biomarker dynamics compared with a D-dimer–guided strategy in critically ill patients with COVID-19, and whether these biomarkers have prognostic value for clinical outcomes.

We hypothesized that anti-Xa–guided anticoagulation, which more accurately reflects the achieved anticoagulant effect of low-molecular-weight heparin (LMWH), would be associated with different inflammatory and coagulation biomarker profiles compared with D-dimer–guided anticoagulation in critically ill COVID-19 patients. We further hypothesized that routinely available admission biomarkers (including NLR, CRP, LDH, and D-dimer) would have prognostic value for clinical outcomes.

Accordingly, the primary aim of the study was to analyze the association of two different anticoagulation protocols with inflammatory biomarker levels. The secondary aim was to assess the prognostic capacity of biomarkers for mortality.

Methods

Study design

This retrospective observational study examined patients with COVID-19, confirmed by laboratory testing, admitted to the Intensive Care Unit (ICU) at the specialized COVID-19 Hospital in Karaburma, Belgrade, Serbia, from April 2020 to

December 2021. The study was approved by the Ethics Committee of the Military Medical Academy (No. 19/2022, from May 25, 2022). This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Data from medical records were analyzed anonymously.

The study included patients aged 18 years and older, of both sexes, who tested positive for COVID-19 and showed signs of respiratory failure.

Patients were excluded from the study if they met any of the following criteria: admission to the ICU for less than 24 hrs, transfer from another ICU setting, or lack of complete anticoagulant therapy data during their treatment course.

Respiratory failure was defined by $SpO_2 < 90\%$ at ICU admission; according to institutional practice, these patients received escalation of respiratory support, including high-flow nasal cannula, noninvasive ventilation, or invasive mechanical ventilation. Patients initially receiving low-flow oxygen support (*via* nasal cannula or oxygen mask) with $SpO_2 < 94\%$, a respiratory rate $\geq$ 25/min, and $pO_2 < 65$ mmHg were also included if they demonstrated rapid clinical and radiological deterioration and subsequently underwent escalation of respiratory support.

Anticoagulation protocols were followed according to local guidelines. Initially, anticoagulation at our institution was guided by D-dimer levels. The threshold of 2 mg/L fibrinogen equivalent units (FEU) reflected the institutional anticoagulation protocol in place at the time of patient enrollment (more than four times the normal upper limit). However, following the admission of 270 patients and in response to the high incidence of thrombotic events and observed variability in heparin responsiveness, the institutional protocol was revised to incorporate anti-Xa–guided dose adjustments. This modification was introduced to assess the achieved anticoagulant effect of LMWH and to guide anticoagulation intensity.

Because the anticoagulation protocols were implemented sequentially during different phases of the pandemic, group allocation was determined by the institutional protocol in place at the time of ICU admission. Accordingly, the study represents a protocol-based observational comparison, not a randomized group allocation. Patient medical records were reviewed, highlighting demographics, treatment interventions, and laboratory and hemostatic parameters. Peripheral venous blood samples were collected for laboratory analysis, using ethylenediaminetetraacetic acid-anticoagulated whole blood for complete blood count assessments *via* the fully automated ADVIA 120 system. Hemostasis parameters were evaluated from citrated plasma obtained after centrifugation of whole blood at 3,000 g for 10 min. Quantification was conducted on a Siemens BCS analyzer under standardized analytical conditions. D-dimer was measured using a latex immunoturbidimetric assay, while LMWH anti-Xa activity was determined using a chromogenic spectrophotometric method in accordance with the manufacturer's instructions. All assays were performed using the same analyzer and methodology throughout the study period, ensuring analytical consistency.

Patients were stratified into two cohorts according to the anticoagulation protocol (Table 1): a D-dimer–guided group (D-dG) and an anti-Xa–guided group (A-XaG).

The first group – D-dG received nadroparin according to the prescribed protocol. Patients weighing 100 kg or less were given nadroparin at a dose of 86 U AXa/kg body weight (BW), administered subcutaneously (sc) once daily. Those exceeding 100 kg were administered 8,550 U AXa daily, provided their D-dimer levels were at or below 2 mg/L FEU. For patients with D-dimer levels higher than 2 mg/L FEU, LMWH was prescribed sc twice daily based on their BW: nadroparin 86 U AXa/kg BW sc twice daily for those weighing $\leq$ 100 kg, and nadroparin 8,550 U AXa twice daily for those over 100 kg (Table 1).

In the D-dimer–guided protocol, patients were stratified according to a threshold of 2 mg/L FEU, which corresponds to approximately 4 times the upper limit of normal in our laboratory. This threshold reflected the institutional anticoagulation protocol in place during the early phase of the pandemic and was selected as a pragmatic marker of increased thrombotic risk based on contemporaneous evidence linking elevated D-dimer levels with poor outcomes in COVID-19. Patients with D-dimer levels $\leq$ 2 mg/L received prophylactic-intensity anticoagulation, whereas those with levels > 2 mg/L received therapeutic-intensity LMWH. Thus, the division of

Table 1

Anti-Xa–guided and D-dimer–guided anticoagulation dosing protocols

Therapeutic protocol	Nadroparin dose
D-dimer	
≤ 2 mg/L FEU	
BW ≤ 100 kg	86 U AXa/kg OD
BW > 100 kg	8,550 U AXa OD
> 2 mg/L FEU	
BW ≤ 100 kg	86 U AXa/kg TD
BW > 100 kg	8,550 U AXa TD
Anti-Xa, U/mL	
< 0.35	increased by 25%
0.36–0.49	increased by 10%
1.1–1.5	decrease by 20%
1.6–2.0	decrease by 30%
> 2.0	decrease by 40% and postpone the next dose until anti-Xa is < 0.5

FEU – fibrinogen equivalent units; BW – body weight; OD – once daily; TD – twice daily.
***Note*: Target anti-Xa level was set between 0.5 U/mL and 1.0 U/mL due to concerns regarding hypercoagulability in COVID-19 patients.**

D-dG reflected prospective routine clinical practice rather than a *post hoc* analytical subdivision. D-dimer was used as a marker of thromboinflammatory activity and risk stratification, not as a diagnostic test for macrothrombosis; patients with clinically suspected or confirmed thromboembolic events were managed according to standard diagnostic and therapeutic pathways independent of protocol allocation.

The second group – A-XaG received weight-adjusted nadroparin with twice a day dosing guided by anti-Xa levels to achieve the desired therapeutic anticoagulation. Anti-Xa peak levels were monitored once the drug concentration stabilized: 4 hrs post-morning dose and after four doses of LMWH treatment. If the anti-Xa level fell below 0.35 U/mL, the dosage was increased by 25%. For levels between 0.36 and 0.49 U/mL, the dosage was raised by 10%. The target anti-Xa level was set between 0.5 U/mL and 1.0 U/mL due to concerns regarding hypercoagulability in COVID-19 patients. If the anti-Xa level exceeded 1.0 U/mL, the dosage was decreased according to the anti-Xa-based protocol (Table 1).

Since nadroparin is available in fixed prefilled syringe strengths, dose adjustments were implemented as clinically practical approximations using the nearest available syringe strength or adjusted injection volume. After each dose adjustment, peak anti-Xa activity was rechecked at steady state (4 hrs after the morning dose) to confirm the change toward the target range and to avoid overdosing.

Additional methodological details have been reported previously [12].

Statistical analysis

The statistical analyses for this study were performed using R software, version 4.3.0. The data are presented using standard descriptive statistics, including means, standard deviations, medians with interquartile ranges, and counts and percentages as appropriate. Data normality was assessed using the Kolmogorov-Smirnov test. Group comparisons of numerical data were performed using a *t*-test or the Mann-Whitney test, depending on the data distribution. Comparisons of numerical data between two measurements were conducted using a paired *t*-test or a Wilcoxon test, depending on the data distribution. Categorical data analysis employed the Chi-squared test or Fisher's exact test. Univariate and multivariate logistic regression analyses were used to estimate the association between a fatal outcome, the dependent variable, and one or more independent variables. Prognostic modelling was performed in the overall cohort to evaluate associations between admission biomarkers and clinical outcomes (survival vs. non-survival); it was not designed to assess prognostic performance separately within each anticoagulation protocol. A receiver operating characteristic (ROC) curve was used to estimate the discriminative ability of inflammation markers and determine the cut-off value for specific variables.

Multiple comparisons were controlled by adjusting all *p*-values using the Benjamini-Hochberg correction to control the false discovery rate. For clarity and transparency, both unadjusted and false discovery rate-adjusted *p*-values were reported. The statistical significance level was set at $p < 0.05$.

Results

Baseline demographic characteristics did not differ significantly between the two groups with respect to age or sex. The mean age was 66.31 years in A-XaG and 68.01 years in D-dG ($p = 0.253$). Comorbidities were generally similar between groups; however, hypertension was more frequent in A-XaG, with borderline significance ($p = 0.050$) (Table 2).

Table 2

Demographic and clinical characteristics of the population according to the two treatment groups

Variable	Group		*p*-value[a]
	A-XaG (n = 137)	D-dG (n = 258)	
Gender			
male	106 (77.0)	193 (74.8)	0.658
female	31 (22.6)	65 (25.5)	
Age, years	66.31 ± 13.39	68.01 ± 14.33	0.253[b]
Comorbidity	108 (78.8)	193 (74.8)	0.441
diabetes mellitus type I	28 (20.4)	54 (21.3)	0.952
diabetes mellitus type II	2 (1.5)	8 (3.2)	0.505
hypertension	84 (61.3)	128 (50.4)	0.050
cardiovascular diseases	27 (19.7)	68 (27.0)	0.141
respiratory diseases	14 (10.2)	18 (7.1)	0.389
malignancy	5 (3.6)	10 (4.0)	1.000
chronic kidney failure	2 (1.5)	10 (4.0)	0.228
other	40 (29.2)	72 (27.9)	0.878
Medications			
Aspirin®, clopidogrel	16 (11.7)	50 (19.4)	0.070
tocilizumab	63 (46.0)	48 (18.6)	< 0.001
dexamethasone	122 (89.1)	182 (70.5)	< 0.001

A-XaG – anti-Xa group; D-dG – D dimer group; n – number of patients.
All values are given as numbers (percentages) or mean ± standard deviation.
***Note*: [a] Chi-squared test; [b] independent *t*-test.**

Table 3

Laboratory parameters for the two treatment groups at admission and the final measurement

Parameter*	Admission	Final measurement†	*p*-value
Leukocytes			
A-XaG	8.44 (6.56–11.40)	9.95 (7.07–14.67)	0.001/**0.003**[c]
D-dG	8.56 (5.74–11.71)	10.82 (7.50–15.14)	< 0.001/< **0.001**[c]
p-value	0.680/**0.711**[b]	0.320/**0.367**[b]	
Neutrophils			
A-XaG	7.50 (5.55–10.45)	8.00 (5.05–13.40)	0.023/**0.037**[c]
D-dG	7.55 (4.80–10.30)	9.65 (5.92–14.00)	< 0.001/< **0.001**[c]
p-value	0.719/**0.719**[b]	0.095/**0.127**[b]	
Lymphocytes			
A-XaG	0.60 (0.40–0.80)	0.70 (0.40–1.20)	< 0.001/< **0.001**[c]
D-dG	0.60 (0.40–0.90)	0.70 (0.40–1.10)	< 0.001/< **0.001**[c]
p-value	0.398/**0.443**[b]	0.238/**0.287**[b]	
Hemoglobin‡			
A-XaG	134.02 ± 18.24	126.61 ± 19.96	< 0.001/< **0.001**[ad]
D-dG	128.06 ± 17.99	122.45 ± 18.59	< 0.001/< **0.001**[d]
p-value	0.002/**0.002**[a]	0.048/**0.069**[a]	
Platelets			
A-XaG	228.00 (167.50–287.00)	213.00 (148.00–283.50)	0.268/**0.318**[c]
D-dG	227.00 (169.00–303.00)	256.50 (169.50–353.50)	0.015/**0.025**[c]
p-value	0.432/**0.465**[b]	0.002/**0.004**[b]	
IL-6			
A-XaG	81.8 (39.6–156.4)		
D-dG	58 (25.2–116.0)		
p-value	0.121/**0.157**[b]		

A-XaG – anti-Xa group; D-dG – D dimer group; IL – interleukin.
***Note*: anti-Xa vs. D-dimer group ([a] independent *t*-test or [b] Mann-Whitney test), admission vs. final measurement ([c] Wilcoxon test/ [d] paired *t*-test); * non-normally distributed data are presented as median (interquartile range) and ‡ normally distributed data are presented as mean ± standard deviation; *p*-values are presented as unadjusted or Benjamini-Hochberg adjusted (adjusted *p*-values are shown in bold); † final measurement refers to the last recorded value prior to death or discharge.**

Reference ranges: leukocytes 4.0–11.0 × 10^9/L, neutrophils 1.9–8.0 × 10^9/L, lymphocytes 0.9–5.2 × 10^9/L, hemoglobin 130–180 g/L, platelets 160–370 × 10^9/L, and IL-6 1.5–7 pg/mL.

Immunomodulatory therapy was administered more frequently in A-XaG compared to D-dG (tocilizumab: 46.0% vs. 18.6%, $p < 0.001$; dexamethasone: 89.1% vs. 70.5%, $p < 0.001$). Antiplatelet therapy was used in 16 (11.7%) patients in A-XaG and 50 (19.4%) patients in D-dG, with no significant difference between groups ($p = 0.070$) (Table 2).

Changes in biomarker levels during follow-up differed between anticoagulation strategies. Table 3 and Figure 1 summarize laboratory parameters for both patient groups at admission and at the final measurement (last measurement at the time of death or discharge from the ICU). At admission, NLR, D-dimer, and LDH values did not differ significantly between groups. At the final measurement, A-XaG demonstrated significantly lower CRP, LDH, and D-dimer levels ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively), with no significant difference in NLR between the groups (Figure 1).

When admission values were compared with final measurements, NLR and D-dimer levels increased significantly in D-dG ($p < 0.001$ for both), while LDH levels did not change significantly ($p = 0.326$). In contrast, CRP levels decreased significantly during follow-up ($p < 0.001$). In A-XaG, LDH and CRP levels decreased significantly over time ($p < 0.001$ for both), while D-dimer and NLR levels remained stable ($p = 0.662$ and $p = 0.376$, respectively) (Figure 1).

After applying the Benjamini-Hochberg correction, all previously significant *p*-values remained significant, except for the hemoglobin levels between groups at the final measurement, which was no longer significant (adjusted $p = 0.069$) (Table 3).

Analysis of mortality risk in the overall studied cohort

Mortality analyses were performed in the overall cohort independent of anticoagulation protocol allocation.

Demographic parameters based on clinical outcomes

The analysis revealed no significant differences in survival rates between male and female cohorts. However, it was noted that the cohort of deceased patients had a significantly higher average age compared to the survivors ($p < 0.001$), as presented in Table 4.

Laboratory parameters at admission and final measurement based on clinical outcomes

The laboratory parameters at admission that were significantly higher in non-survivors included leukocyte count

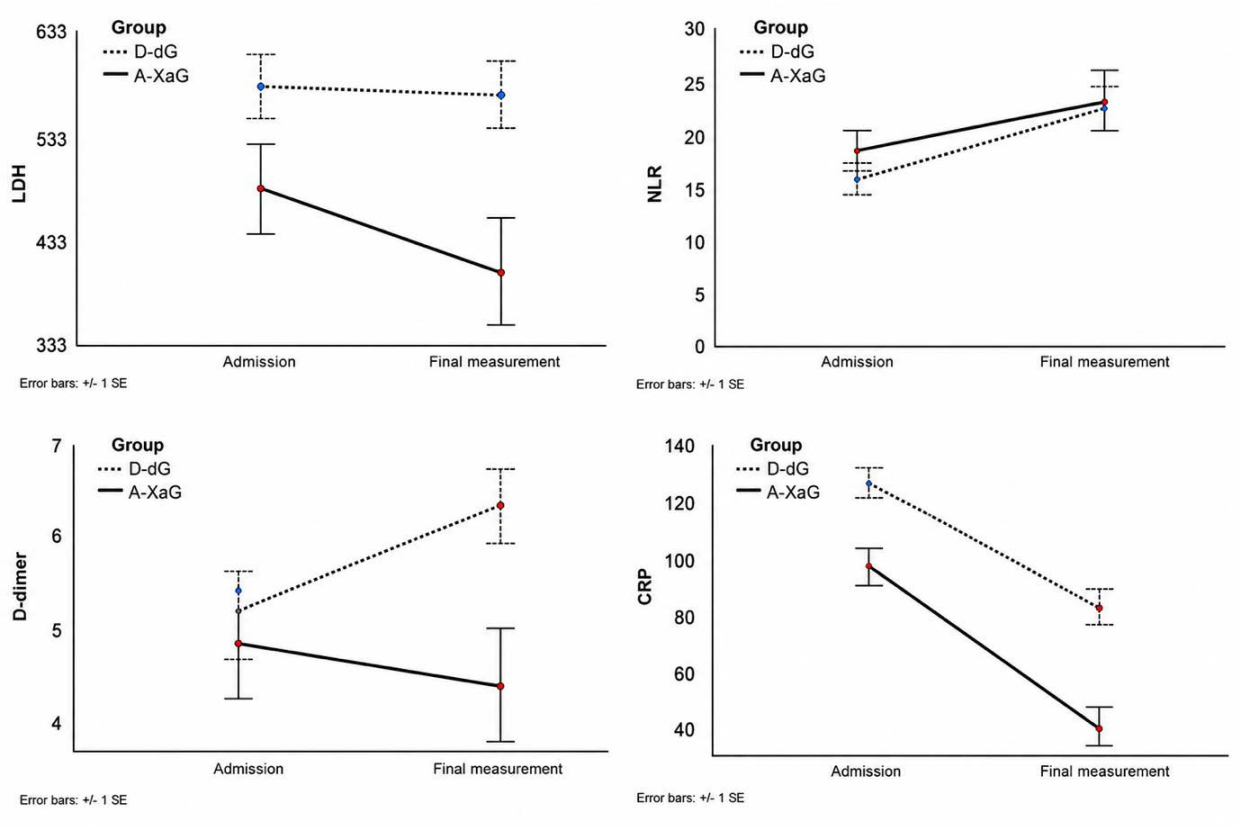


Fig. 1 – Changes in inflammatory and coagulation biomarkers in the D-dimer–guided and anti-Xa–guided anticoagulation groups at admission and at the final measurement (death or Intensive Care Unit discharge). Error bars represent ± 1 standard error (SE) of the mean. A-XaG – anti-Xa group; D-dG – D dimer group; LDH – lactate dehydrogenase; NLR – neutrophil-to-lymphocyte ratio; CRP – C-reactive protein.

Table 4

Demographic parameters based on clinical outcomes

Parameter	Survivors	Non-survivors	*p*-value[a]
Male	161 (78.9)	138 (72.3)	0.122/**0.158**
Female	43 (21.1)	53 (27.7)	
Age	62.39 ± 13.63	72.82 ± 12.33	< 0.001/< **0.001**[b]

All values are given as numbers (percentages) or mean ± standard deviation.
***Note*: [a] Chi-squared test, [b] independent *t*-test; *p*-values are presented as unadjusted or Benjamini-Hochberg adjusted (adjusted *p*-values are shown in bold).**

(p = 0.038), neutrophil count (p = 0.019), NLR (p < 0.001), LDH (p < 0.001), CRP (p = 0.001), D-dimer (p = 0.001), and IL-6 levels (p = 0.001) (Table 5). At the time of the final measurement, non-survivors continued to show significantly higher levels of leukocyte count (p < 0.001), neutrophil count (p < 0.001), NLR (p < 0.001), LDH (p < 0.001), CRP (p < 0.001), and D-dimer levels (p < 0.001) (Table 5).

All previously significant *p*-values remained significant after Benjamini-Hochberg correction, except for the following: leukocyte count between survivors and non-survivors (adjusted p = 0.057), D-dimer levels between admission and final measurements among survivors (adjusted p = 0.070), and LDH values between admission and final measurements among non-survivors (adjusted p = 0.052) (Table 5).

Univariate and multivariate logistic regression analysis for mortality risk assessment

In the univariate analysis, significant prognostic factors for mortality were age [odds ratio (OR): 1.064, 95% confidence interval (CI): 1.045–1.083, p < 0.001], LDH (OR: 1.002, 95% CI: 1.001–1.003, p < 0.001), CRP (OR: 1.005, 95% CI: 1.002–1.008, p < 0.001), and D-dimer (OR: 1.054, 95% CI: 1.009–1.102, p = 0.020) (Table 6). In multivariate model 1, age (OR: 1.085, 95% CI: 1.032–1.140, p = 0.001) and LDH (OR: 1.003, 95% CI: 1.000–1.006, p = 0.024) showed the most consistent independent associations with mortality (Table 6). ROC curve analysis demonstrated good discriminative ability of LDH at admission for a fatal

Table 5

Laboratory parameters based on clinical outcomes

Parameter *	Admission	Final measurement†	*p*-value
Leukocytes			
survivors	8.27 (5.68–10.64)	8.09 (5.98–10.72)	0.703/**0.719** [c]
non–survivors	8.90 (6.54–12.54)	14.27 (10.89–18.99)	< 0.001/< **0.001** [c]
p-value[a]	0.038/**0.057** [b]	< 0.001/< **0.001** [b]	
Neutrophils			
survivors	7.15 (4.50–9.35)	6.25 (4.40–9.10)	0.146/**0.183** [c]
non–survivors	7.82 (5.60–11.50)	13.00 (9.75–17.30)	< 0.001/< **0.001** [c]
p-value[a]	0.019/**0.031** [b]	< 0.001/< **0.001** [b]	
Lymphocytes			
survivors	0.60 (0.50–0.90)	1.00 (0.70–1.40)	< 0.001/**0.001** [c]
non–survivors	0.60 (0.40–0.80)	0.40 (0.30–0.70)	0.001/**0.001** [c]
p-value[a]	0.012/**0.020** [b]	< 0.001/< **0.001** [b]	
NLR			
survivors	9.17 (5.83–16.23)	6.12 (3.39–10.72)	< 0.001/< **0.001** [c]
non–survivors	14.00 (7.17–24.73)	29.00 (17.00–44.03)	< 0.001/< **0.001** [c]
p-value[a]	< 0.001/**0.001**	< 0.001/< **0.001**	
Hemoglobin‡			
survivors	131.39 ± 18.78	128.22 ± 16.44	< 0.001/< **0.001** [ad]
non–survivors	128.80 ± 17.66	119.05 ± 20.91	< 0.001/< **0.001** [d]
p-value[a]	0.123/**0.157**	< 0.001/< **0.001**	
Platelets			
survivors	240.00 (184.00–321.00)	281.00 (199.75–383.00)	< 0.001/< **0.001** [c]
non–survivors	214.00 (159.50–285.50)	185.00 (133.00–256.00)	0.003/**0.006** [c]
p-value[a]	0.003/**0.006** [b]	< 0.001/< **0.001** [b]	
LDH			
survivors	380.50 (282.75–521.75)	293.00 (219.00–379.00)	< 0.001/< **0.001** [c]
non–survivors	526.00 (416.25–742.25)	544.00 (413.00–810.25)	0.034/**0.052** [c]
p-value[a]	< 0.001/< **0.001** [b]	< 0.001/< **0.001** [b]	
CRP			
survivors	90.30 (43.50–148.10)	12.50 (2.82–34.50)	< 0.001/< **0.001** [c]
non–survivors	128.00 (58.87–189.60)	79.30 (28.15–187.10)	0.002/**0.004** [c]
p-value[a]	0.001/**0.001** [b]	< 0.001/< **0.001** [b]	
D-dimer			
survivors	1.64 (0.96–4.07)	1.12 (0.64–3.96)	0.050/**0.070** [c]
non–survivors	2.93 (1.22–8.76)	4.04 (2.20–10.92)	< 0.001/< **0.001** [c]
p-value[a]	0.001/**0.001** [b]	< 0.001/< **0.001** [b]	
IL–6			
survivors	46.79 (19.88–108.87)		
non–survivors	107.25 (58.60–184.98)		
p-value[a]	0.001/**0.003** [b]		

NLR – neutrophil-to-lymphocyte ratio; LDH – lactate dehydrogenase; CRP – C-reactive protein; IL – interleukin.

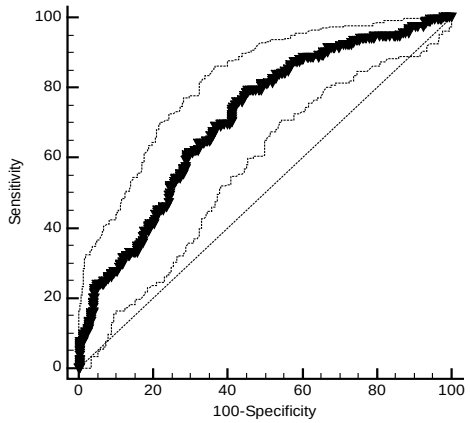
***Note*: survivors vs. non-survivors ([a] independent *t*-test or [b] Mann-Whitney test), admission vs. final measurement ([c] Wilcoxon test/ [d] paired *t*-test); * non-normally distributed data are presented as median (interquartile range); ‡normally distributed data are presented as mean ± standard deviation; *p*-values are presented as unadjusted or Benjamini-Hochberg adjusted (adjusted *p*-values are shown in bold); † final measurement refers to the last recorded value prior to death or discharge.**

Reference ranges: LDH 84–246 U/L, CRP < 10 mg/L, D-dimer 0.22–0.45 mg/L fibrinogen equivalent units (FEU). For other reference ranges, see Table 3.

outcome (area under the curve – AUC: 0.708, $p < 0.001$), with a cut-off value > 395 U/L (Figure 2). Multivariate model 2, built based on LDH cut-off value and age, showed that age > 64 years (OR: 10.215, 95% CI: 3.160–33.021, $p < 0.001$) and LDH > 395 U/L (OR: 5.491, 95% CI: 1.657–18.199, $p = 0.005$) were significantly associated with fatal outcomes (Table 6).

Exploratory subgroup analyses

These analyses were exploratory and are presented for descriptive purposes. Within the D-dimer–guided anticoagulation group, 42 (16.3%) patients had D-dimer levels < 2 mg/L and, therefore, received prophylactic anticoagulation, whereas the majority of patients (83.7%) had D-dimer levels



AUC	0.708
Standard error[a]	0.026
95% CI[b]	0.660–0.753
p-value	< 0.001
Youden index J	0.338
95% CI[a]	0.242–0.412
Associated criterion	> 395
95% CI[a]	> 326–> 441
Sensitivity	79.35
Specificity	54.46

Fig. 2 – Receiver operating characteristic curve analysis of lactate dehydrogenase at admission. AUC – area under the curve; CI – confidence interval.
***Note*: [a] DeLong method, [b] Binomial exact method.**

Table 6

Association of biomarkers at admission with fatal outcomes in logistic regression models

Parameter	Univariate			Multivariate					
				model 1			model 2		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age*	1.064	1.045–1.083	< 0.001	1.085	1.032–1.140	0.001	10.215	3.160–33.021	< 0.001
Sex	1.438	0.906–2.283	0.123	1.186	0.409–3.439	0.754	1.327	0.425–4.144	0.627
Leukocytes	1.034	0.993–1.077	0.109	0.792	0.635–0.987	0.038	0.747	0.582–0.957	0.021
Neutrophils	1.007	0.990–1.024	0.427	1.144	0.935–1.399	0.192	1.182	0.953–1.467	0.128
Lymphocytes	0.947	0.860–1.044	0.272	0.625	0.201–1.940	0.416	0.492	0.147–1.648	0.250
NLR	1.013	1.000–1.026	0.057	1.003	0.920–1.093	0.946	1.007	0.920–1.103	0.880
LDH*	1.002	1.001–1.003	< 0.001	1.003	1.000–1.006	0.024	5.491	1.657–18.199	0.005
CRP	1.005	1.002–1.008	< 0.001	1.000	0.993–1.008	0.950	1.002	0.993–1.010	0.691
D–dimer	1.054	1.009–1.102	0.020	1.020	0.958–1.082	0.533	1.003	1.000–1.006	0.062
IL–6	1.002	1.000–1.004	0.109	1.002	0.999–1.005	0.217	1.007	0.943–1.074	0.845
Constant				0.002		0.005	0.123		0.125

OR – odds ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio; LDH – lactate dehydrogenase; CRP – C-reactive protein; IL – interleukin.
***Note*: * multivariate model 2: age > 64 years, LDH > 395 U/L; Hosmer-Lemeshow test *p* = 0.839.**

≥ 2 mg/L and received therapeutic anticoagulation according to the institutional protocol.

First, patients in D-dG with D-dimer levels ≥ 2 mg/L were compared with patients managed using anti-Xa–guided anticoagulation. In the within-group follow-up analysis, CRP and LDH values decreased significantly in both groups (all $p < 0.001$). In the A-XaG group, NLR decreased significantly ($p < 0.001$), whereas in D-dG, NLR increased significantly ($p < 0.001$). D-dimer levels did not change significantly in A-XaG ($p = 0.662$), but increased significantly in D-dG ($p = 0.005$). In the between-group comparison at the final measurement, CRP values remained significantly higher in D-dG than in A-XaG ($p < 0.001$), while D-dimer was also higher in D-dG, with borderline statistical significance ($p = 0.050$). These biomarker dynamics are presented in Supplementary Table 1. Clinical outcomes in this subgroup also differed between strategies. Mortality was higher in D-dG than in A-XaG [120/216 (55.6%) vs. 55/137 (40.1%), $p = 0.007$], and thromboembolic complications were more frequent in D-dG [24/216 (11.1%) vs. 4/137 (2.9%), $p = 0.005$]. These outcomes are summarized in Supplementary Table 1.

Within the overall cohort, 296 (74.9%) patients received therapeutic anticoagulation alone, whereas 57 (14.4%) patients received therapeutic anticoagulation combined with concomitant antiplatelet therapy. An exploratory comparison was performed between these groups. CRP values decreased significantly during follow-up in both groups, whereas LDH decreased significantly only in the therapeutic anticoagulation-alone group. NLR also changed significantly in both groups, although without a decrease over time. No statistically significant differences were observed between groups in mortality ($p = 0.517$) or thromboembolic complications ($p = 0.063$). Detailed results are presented in Supplementary Table 2.

Discussion

In this cohort of critically ill patients with COVID-19, anti-Xa–guided anticoagulation was associated with lower LDH, CRP, and D-dimer levels compared with D-dimer–guided anticoagulation. These findings suggest that individualized anticoagulation monitoring may be associated with

differences in coagulation and inflammatory biomarker trajectories in severe COVID-19. Because the two anticoagulation strategies were implemented sequentially during different phases of the pandemic, temporal changes in clinical management may also have contributed to observed differences between groups. In addition, age, LDH, CRP, and D-dimer were associated with mortality in this cohort, supporting previous evidence linking these markers to disease severity and adverse outcomes [7–10]. Our findings should be interpreted within the context of limited published data examining the relationship between anticoagulation monitoring strategies and inflammatory biomarker dynamics in critically ill COVID-19 patients.

Our previous study provides a detailed comparative analysis of demographic characteristics, comorbidities, pharmacological treatments, mortality rates, and the incidence of thromboembolic and hemorrhagic complications [12].

Anticoagulation therapy has become an important component of the management of hospitalized patients diagnosed with COVID-19 [13]. However, the precise physiological mechanisms underlying this therapy remain to be explored. Anticoagulation therapy may mitigate coagulopathy by partially reducing thrombin generation and may also be associated with modulation of the inflammatory response associated with lung tissue damage in acute respiratory distress syndrome [14, 15]. Given these considerations, anti-Xa–guided anticoagulation may represent a more individualized approach to anticoagulation management in critically ill COVID-19 patients, particularly in those exhibiting a procoagulant state and concurrent hyperinflammation [16].

NLR has emerged as an important prognostic biomarker in COVID-19, with studies showing that higher NLR values at admission are linked to increased disease severity and mortality [17–20]. In our study, non-survivors had higher NLR levels than survivors. Additionally, CRP, LDH, and D-dimer levels were significantly higher in non-survivors, supporting previous studies linking elevated levels of these biomarkers with increased mortality risk [21–24].

In the univariate analysis, significant prognostic factors for mortality were age, LDH, CRP, and D-dimer. In the multivariate analysis, age and LDH showed the most consistent independent associations with mortality. In our cohort, LDH levels above the ROC-derived cut-off value of 395 U/L were associated with an increased risk of mortality. Consistent with this, another study found that elevated LDH levels are associated with approximately a six-fold increase in the risk of severe disease and a sixteen-fold increase in mortality among COVID-19 patients [25]. Furthermore, Li et al. [26] identified a strong association between LDH levels exceeding 445 U/L and severe cases.

Elevated CRP, LDH, and D-dimer levels are associated with increased disease severity in COVID-19 [27–32]. In our study, A-XaG showed lower CRP, LDH, and D-dimer levels over time compared with D-dG. D-dimer levels increased during follow-up in D-dG, whereas a non-significant decrease was observed in A-XaG. In our previous analysis of the same cohort, the D-dimer–guided strategy was associated with higher mortality and a greater incidence of thromboembolic complications than anti-Xa–guided anticoagulation [12]. Taken together, these observations suggest a possible association between protocol-based anticoagulation strategy and longitudinal changes in coagulation and inflammatory biomarkers and clinical outcomes, supporting further investigation in prospective studies.

An additional exploratory subgroup analysis showed findings broadly consistent with the main study results. In the within-group follow-up analysis, CRP and LDH levels decreased significantly in both groups. In contrast, the NLR decreased in A-XaG but increased in D-dG, whereas D-dimer remained unchanged in A-XaG but increased in D-dG. In the between-group comparison at the final measurement, CRP remained significantly higher in D-dG, while D-dimer was also higher with borderline statistical significance. Mortality and thromboembolic complications were also more frequent in D-dG. However, because these analyses were exploratory, they should be interpreted cautiously.

D-dimer reflects plasmin-mediated degradation of cross-linked fibrin and, therefore, indicates prior fibrin formation and activation of the fibrinolytic system [33]. Plasmin is generated from plasminogen *via* tissue-type and urokinase-type plasminogen activators and is rapidly inhibited by α2-antiplasmin [34]. Accordingly, measurable D-dimer levels require prior formation of cross-linked fibrin followed by sufficient plasmin-mediated fibrinolysis despite endogenous inhibitory mechanisms [35]. Although plasmin-antiplasmin complexes may represent a more direct indicator of plasmin generation, D-dimer reflects downstream fibrin turnover and remains the most widely available laboratory marker in routine clinical practice [33, 35]. Fibrinolytic responses in COVID-19 appear heterogeneous, with reports of both enhanced fibrinolysis and relative hypofibrinolysis or fibrinolysis shutdown, potentially related to endothelial dysfunction and dysregulated plasminogen-plasmin pathways [36, 37]. Accordingly, D-dimer should not be interpreted as a direct measure of thrombus burden, but rather as an indirect marker of fibrin formation and turnover [38]. In our study, D-dimer was used as a clinically accessible marker to guide anticoagulation intensity rather than as a diagnostic screening test for thrombosis.

COVID-19–associated coagulopathy also differs from classical consumptive disseminated intravascular coagulation observed in advanced stages of sepsis [36, 39]. Instead, it shares key features with early sepsis-associated coagulopathy, including inflammation-driven thrombin generation, preserved or elevated fibrinogen levels, and a predominantly hypercoagulable phenotype, sometimes accompanied by a relative shutdown of fibrinolysis [39, 40]. Accordingly, D-dimer may be interpreted as a marker of disease severity rather than as a marker of overt consumptive coagulopathy [36, 38, 39].

Our results should be interpreted in the context of current evidence from large randomized platform trials [Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP)/Accelerating COVID-19 Therapeutic Interventions and Vaccines-4 Acute (ACTIV–4a)/Anti-Thrombotic Therapy to Ameliorate Complications of COVID-19 (ATTACC)], which showed that routine fixed therapeutic-dose heparin did

not improve outcomes in critically ill patients without confirmed thrombosis, supporting recommendations for prophylactic-intensity anticoagulation in this population [41, 42]. In contrast, the anticoagulation strategy in this study was not based on uniform therapeutic dosing but on individualized titration according to measured anti-Xa activity. In the studied cohort, this protocol-based strategy was associated with lower observed mortality and fewer thromboembolic complications, without an increase in bleeding, compared with the D-dimer–guided protocol [12]. These findings raise the possibility that optimization of the achieved anticoagulant effect, rather than routine fixed therapeutic dosing, may warrant further study in selected high-risk critically ill patients. However, given the observational design of our study, these results should be considered hypothesis-generating and require confirmation in prospective randomized trials specifically evaluating anti-Xa–guided strategies.

Although mean nadroparin dose data were not available for analysis, anticoagulation intensity in our study was interpreted primarily according to protocol allocation and the achieved anticoagulant effect, as reflected by anti-Xa activity, rather than according to the administered dose alone. This approach may be relevant in critically ill COVID-19 patients, in whom interindividual variability in LMWH pharmacokinetics and altered heparin responsiveness have been described [43, 44]. In this context, the administered dose may not fully reflect the actual anticoagulant effect, whereas anti-Xa monitoring may better represent the achieved anticoagulation intensity [45, 46]. At the same time, because anti-Xa–guided dose adjustments were performed in all patients with subtherapeutic anti-Xa levels, some patients in this group may have received higher absolute nadroparin doses than those typically used in standard weight-based therapeutic regimens. Similarly, major randomized COVID-19 anticoagulation trials, including the REMAP-CAP/ACTIV-4a/ATTACC platform and the HEP-COVID trial, primarily compared protocol-based anticoagulation strategies [41, 42, 47]. Nevertheless, in the absence of detailed dose data, the present findings should be interpreted with appropriate caution.

Given the retrospective design and the sequential implementation of treatment protocols, residual confounding related to temporal changes in the standard of care during the pandemic cannot be excluded.

Platelet hyperactivation has also been implicated in the pathophysiology of COVID-19–associated immunothrombosis through interactions with activated endothelium, leukocytes, and neutrophil extracellular traps, potentially contributing to microvascular thrombosis [48–50]. However, the present study was designed to evaluate anticoagulation strategies targeting the enzymatic coagulation cascade rather than platelet inhibition. Antiplatelet therapy was not introduced as part of the study protocol; patients already receiving antiplatelet agents prior to admission continued therapy according to standard clinical practice, and no significant difference in antiplatelet use was observed between groups. Future prospective studies integrating antiplatelet and anticoagulant strategies may further clarify the contribution of platelet hyperactivation and the potential benefit of platelet-targeted therapy in selected patients with immunothrombotic states.

Our results are consistent with the hypothesis that anti-Xa–guided anticoagulation may be associated with a more favorable biomarker profile, potentially related in part to pleiotropic effects of heparins beyond their primary anticoagulant properties [51]. In addition to inhibiting thrombin generation, heparins have been investigated as modulators of inflammatory processes in critically ill patients with sepsis [52]. They may interact with chemokines, cytokines, components of the complement system, activated endothelial cells, and macrophages, potentially attenuating inflammatory signaling [53–55]. Evidence from two meta-analyses has suggested potential benefits of heparins in critically ill patients with sepsis, including reduced 28-day mortality [56, 57].

Nevertheless, heparin-induced thrombocytopenia remains an important clinical consideration during prolonged anticoagulation, even though its risk is lower with LMWH than with unfractionated heparin. Current evidence suggests that heparin-induced thrombocytopenia is generally uncommon in patients with COVID-19; however, because its incidence may be higher among critically ill patients, continued vigilance remains warranted [58].

Anti-Xa–guided monitoring may help refine anticoagulant dose titration in critically ill patients, who are particularly vulnerable to variability in therapeutic response. Reflecting the achieved anticoagulant effect may provide a more direct measure of anticoagulation intensity. Further prospective studies are needed to clarify the role of anti-Xa–guided strategies in optimizing anticoagulation management in critically ill populations.

Limitations of the study

This study has several limitations that should be acknowledged. First, the retrospective, single-center, nonrandomized design may introduce bias and limit generalizability. The two anticoagulation protocols were implemented sequentially during different phases of the pandemic, and, therefore, residual temporal confounding related to evolving standards of care cannot be excluded. Second, the unequal group sizes reflect the sequential implementation of anticoagulation protocols across different phases of the pandemic. Although such an imbalance may reduce statistical precision in the smaller group, diagnostic checks did not indicate major violations of model assumptions. Third, detailed nadroparin dose data were unavailable; therefore, anticoagulation intensity was interpreted based on protocol allocation and the achieved anticoagulant effect (anti-Xa activity), rather than the administered dose alone.

Viscoelastic whole-blood coagulation assays, which may provide a more global assessment of coagulation dynamics and hypercoagulability, were not available in the dedicated COVID-19 unit during the study period, thereby limiting assessment of global hemostatic alterations. Additionally, time-resolved analyses linking the duration of respiratory support to longitudinal changes in coagulation biomarkers were not feasible. Evolving institutional

treatment protocols during the pandemic resulted in differences in the use of immunomodulatory therapy between groups, which may represent a potential source of confounding. Accordingly, residual bias related to differences in immunomodulatory therapy and temporal changes in standard of care cannot be excluded. Because of the retrospective design and the structure of the clinical database, the exact interval between the initial and final laboratory measurements could not be reliably reconstructed for each patient. Laboratory testing was performed according to routine ICU monitoring protocols and was not influenced by anticoagulation group allocation. Furthermore, the number of variables included in the multivariate model was limited to those with established clinical relevance to maintain model stability and interpretability. Finally, prognostic modelling was performed on the overall cohort to assess survival vs. non-survival, and was not powered to evaluate protocol-specific prognostic models within each anticoagulation group. Further prospective studies are warranted to clarify the relationship between anticoagulation therapy and inflammatory biomarkers in critically ill populations.

Conclusion

In this cohort of critically ill patients with COVID-19, anti-Xa–guided anticoagulation was associated with lower levels of lactate dehydrogenase, C-reactive protein, and D-dimer, biomarkers linked to disease severity and progression. These findings suggest that anti-Xa monitoring may represent a more individualized approach to anticoagulation management in critically ill patients; however, given the retrospective design and sequential implementation of treatment protocols, this interpretation should be considered hypothesis-generating. In addition, age and lactate dehydrogenase were independently associated with mortality in multivariate analysis, supporting their prognostic value in this population. Further prospective studies are warranted to clarify the role of individualized anticoagulation strategies in critically ill patients with COVID-19.

R E F E R E N C E S

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Supplementary Table 1

Inflammatory biomarkers and clinical outcomes among patients receiving therapeutic anticoagulation according to anti-Xa–guided (n = 137) vs. D-dimer–guided (n = 216) strategies

Parameter*	Admission	Final measurement†	*p*-value (within group)
NLR			
A-XaG	12.33 (7.06–21.63)	9.83 (4.20–30.21)	< 0.001[c]
D-dG	11.30 (6.00–21.89)	15.39 (6.06–31.44)	< 0.001[c]
p-value	0.421[b]	0.870[b]	
CRP			
A-XaG	86.30 (40.85–136.65)	16.60 (3.20–55.30)	< 0.001[c]
D-dG	125.00 (60.43–198.80)	38.00 (9.23–138.48)	< 0.001[c]
p-value	< 0.001[b]	< 0.001[b]	
LDH			
A-XaG	422.00 (317.50–623.50)	348.00 (231.00–482.50)	< 0.001[c]
D-dG	483.50 (329.50–662.25)	432.00 (296.00–679.00)	< 0.001[c]
p-value	0.090[b]	0.114[b]	
D-dimer			
A-XaG	1.35 (0.79–3.61)	1.57 (0.72–3.74)	0.662[c]
D-dG	1.64 (0.90–3.76)	2.83 (1.17–6.73)	0.005[c]
p-value	0.241[b]	0.050[b]	
Clinical outcomes	Groups		*p*-value[d]
	A-XaG	D-dG	
Mortality	55 (40.1)	120 (55.6)	0.007
Thromboembolic complications	4 (2.9)	24 (11.1)	0.005[e]

n – number of patients; NLR – neutrophil-to-lymphocyte ratio; A-XaG – anti-Xa group; D-dG – D dimer group; CRP – C-reactive protein; LDH – lactate dehydrogenase.
***Note*: anti-Xa vs. D-dimer group, analysis restricted to patients receiving therapeutic anticoagulation only ([b] Mann-Whitney test); admission vs. final measurement ([c] Wilcoxon test); [d] Chi-squared test; [e] Fisher's test; [*]non-normally distributed data are presented as median (interquartile range); values for clinical outcomes between groups are given as numbers (percentages); [†] final measurement refers to the last recorded value prior to death or discharge.**

Supplementary Table 2

Inflammatory biomarkers and clinical outcomes among patients receiving therapeutic anticoagulation alone vs. therapeutic anticoagulation combined with concomitant antiplatelet therapy

Parameter*	Admission	Final measurement†	*p*-value (within group)
NLR			
TA	12.18 (6.50–21.36)	12.29 (4.91–30.81)	0.002[c]
TA + antiplatelets	11.00 (6.02–21.98)	16.14 (6.38–33.25)	0.035[c]
p-value	0.937[b]	0.112[b]	
CRP			
TA	106.0 (53.98–178.35)	26.45 (6.20–91.02)	< 0.001[c]
TA + antiplatelets	110.80 (40.95–176.15)	46.00 (7.05–163.85)	0.008[c]
p-value	0.790[b]	0.002[b]	
LDH			
TA	468.50 (321.00–656.50)	399.50 (274.25–604.75)	0.005[c]
TA + antiplatelets	436.00 (333.50–619.00)	365.5 (264.25–539.00)	0.105[c]
p-value	0.605[b]	0.852[b]	
D-dimer			
TA	1.56 (0.80–3.55)	2.28 (0.96–4.52)	0.117[c]
TA + antiplatelets	1.53 (0.99–4.97)	2.43 (1.09–6.74)	0.218[c]
p-value	0.583[b]	0.140[b]	
Clinical outcomes	Therapy		*p*-value[d]
	TA (n = 296)	TA + antiplatelets (n = 57)	
Mortality	144 (48.6)	31 (54.4)	0.517
Thromboembolic complications	27 (9.1)	1 (1.8)	0.063[e]

NLR – neutrophil-to-lymphocyte ratio; CRP – C-reactive protein; LDH – lactate dehydrogenase; TA – therapeutic anticoagulation; n – number of patients.
***Note:* TA vs. TA + antiplatelet therapy ([b] Mann-Whitney test); admission vs. final measurement ([c] Wilcoxon test); [d] Chi-squared test; [e] Fisher's test; [*] non-normally distributed data are presented as median (interquartile range); values for clinical outcomes between TA and TA + antiplatelet therapy are given as numbers (percentages); [†] final measurement refers to the last recorded value prior to death or discharge.**

ORIGINAL ARTICLE

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Diagnostic performance of the McIsaac score for group A streptococcal pharyngitis in children under three years of age

Dijagnostičke karakteristike McIsaac skora za streptokokni faringitis grupe A kod dece mlađe od tri godine

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Abstract

Background/Aim. Group A β-hemolytic streptococcus (GAS) pharyngitis most commonly affects school-aged children. The aim of this study was to assess the effectiveness and clinical applicability of the McIsaac score for diagnosing GAS pharyngitis in children under 3 years of age. **Methods.** The retrospective study included a total of 282 children under 3 years of age diagnosed with acute pharyngitis in a pediatric outpatient setting in Subotica, Serbia, between September 2023 and August 2024. Data on demographic and clinical characteristics were collected, including McIsaac score values. The obtained results were analyzed in relation to rapid antigen detection test (RADT) findings, which were performed on all children. **Results.** Of the total 282 children, 143 (50.7%) were RADT-positive. The McIsaac score demonstrated limited diagnostic accuracy, with sensitivity and specificity values for scores 0–2 of 69.9% and 38.1%, respectively, and for scores 3–4 of 30.7% and 61.9%, respectively. Receiver operating characteristic (ROC) curve analysis yielded an area under the curve (AUC) of 0.561. The absence of cough was the only individual McIsaac criterion significantly associated with a positive RADT result. **Conclusion.** The McIsaac score alone has limited effectiveness in identifying GAS pharyngitis in children under 3 years of age. Given the substantial symptom overlap between bacterial and viral infections in this age group, additional diagnostic methods are necessary to improve diagnostic accuracy.

Keywords:
child, preschool; diagnosis; infant; pharyngitis; rapid diagnostic tests; streptococcus pyogenes.

Apstrakt

Uvod/Cilj. Faringitis izazvan β-hemolitičkim streptokokom grupe A (*group A β-hemolytic streptococcus* – GAS) najčešće pogađa decu školskog uzrasta. Cilj rada bio je da se procene efikasnost i klinička primenljivost McIsaac skora u dijagnostikovanju faringitisa izazvanog GAS-om kod dece mlađe od 3 godine. **Metode.** Retrospektivnom studijom obuhvaćeno je 282 dece mlađe od 3 godine, kojima je postavljena dijagnoza akutni faringitis u pedijatrijskoj ambulanti u Subotici, Srbija, u periodu od septembra 2023. do avgusta 2024. godine. Prikupljeni su podaci o demografskim i kliničkim karakteristikama, uključujući vrednosti McIsaac skora. Dobijeni rezultati analizirani su u odnosu na nalaze brzog antigenskog testa (*rapid antigen detection test* – RADT), koji je urađen svakom detetu. **Rezultati.** Od ukupno 282 dece, 143 (50,7%) bilo je RADT pozitivno. McIsaac skor pokazao je ograničenu dijagnostičku tačnost, sa vrednostima osetljivosti i specifičnosti za rezultate 0–2 od 69,9% i 38,1%, redom, i za rezultate 3–4 od 30,7% i 61,9%, redom. Analizom *receiver operating characteristic* (ROC) krive dobijena je vrednost površine ispod krive (*area under the curve* – AUC) od 0,561. Odsustvo kašlja bio je jedini pojedinačni kriterijum McIsaac skora značajno povezan sa pozitivnim nalazom RADT-a. **Zaključak.** McIsaac skor, sam po sebi, ima ograničenu efikasnost u prepoznavanju GAS izazvanog faringitisa kod dece mlađe od 3 godine. S obzirom na značajno preklapanje simptoma bakterijskih i virusnih infekcija u ovoj uzrasnoj grupi, neophodne su dodatne dijagnostičke metode radi unapređenja tačnosti dijagnoze.

Ključne reči:
deca, predškolska; dijagnoza; odojče; faringitis; dijagnostički testovi, brzi; streptococcus pyogenes.

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Introduction

Group A β-hemolytic streptococcus (GAS) pharyngitis is a prevalent clinical condition in primary pediatric care, most commonly affecting school-aged children. Previous studies have reported that GAS accounts for 15–30% of all pharyngitis cases in this population [1, 2]. However, there is an ongoing debate regarding the optimal strategies for diagnosis, treatment, and avoiding the associated complications of GAS pharyngitis [3]. Accurate diagnosis typically relies on rapid antigen detection tests (RADTs) or throat cultures [4]. Despite the clinical challenges in diagnosing GAS pharyngitis, the necessity of microbiological confirmation varies across different guidelines. The decision to test should take into account clinical signs and symptoms, the patient's age, seasonality, and exposure to individuals with confirmed GAS infection [3–5].

Treatment with antibiotics may shorten the clinical course by up to 16 hrs, reduce transmission, prevent suppurative complications, and reduce the risk of non-suppurative immune-mediated diseases of GAS pharyngitis [6–8].

To aid in the estimation of GAS pharyngitis probability, the Centor and McIsaac scores were developed. The Centor score (ranging from 0 to 4) evaluates four clinical criteria: tonsillar exudate, swollen tender anterior cervical lymph nodes, fever (temperature > 38 °C), and absence of cough [9]. While the Centor score was originally designed for adults, the McIsaac score incorporated additional variables, such as age, to enhance its applicability in pediatric populations, with particular emphasis on tonsillar findings, including exudate or enlargement, as clinically relevant features in pediatric assessment [10]. The McIsaac score (ranging from -1 to 5) assigns points based on clinical findings, with one additional point for patients aged 3–14 years and one point subtracted for those aged 45 years or older.

Diagnosing GAS pharyngitis in children under three years of age is particularly challenging because of the substantial overlap of symptoms with viral upper respiratory infections (URIs). Because clinical manifestations are often nonspecific, relying solely on epidemiological and clinical findings may be insufficient, even for experienced clinicians [11]. In children under three years of age, GAS infections may present atypically, sometimes referred to as streptococcosis, with persistent nasal congestion, low-grade fever, and anterior cervical lymphadenopathy. In infants, symptoms are often nonspecific, manifesting as mild fever, irritability, and reduced feeding [12]. This symptomatic overlap diminishes the effectiveness of the McIsaac score in differentiating bacterial from viral etiologies, thereby increasing the risk of both false-positive and false-negative results.

The aim of this study was to evaluate the effectiveness of the McIsaac score for diagnosing GAS pharyngitis as well as its applicability in children under three years of age.

Methods

A retrospective, single-center cohort study was conducted at the Children's Ambulatory Care Center Subotica, Subotica, Serbia, a primary care facility, from September 1, 2023, to August 31, 2024. The study included children under three years of age diagnosed with acute pharyngitis, regardless of the presence of tonsillar swelling or exudate, fever, cervical lymphadenopathy, or cough. Additional symptoms, such as rhinorrhea, vomiting, abdominal pain, and diarrhea, were recorded but were not included in the scoring criteria. Children exhibiting rales, wheezing, or respiratory distress indicative of lower respiratory tract disease, as well as those diagnosed with otitis media, were excluded. Patients who had received systemic antibiotics within the prior 10 days were also excluded from the study.

The study was approved by the Ethics Committee of the Children's Ambulatory Care Center Subotica (No. 3/2023, from August 07, 2023).

The McIsaac score (ranging from 0 to 4) was calculated using four clinical parameters, each contributing one point: tonsillar swelling or exudate, tender or swollen anterior cervical lymph nodes, fever, and absence of cough [10]. As all participants were younger than three years of age, the age-related component of the original McIsaac score was not applicable. Fever was defined as a temperature > 38 °C. Cervical lymph nodes were classified as abnormal if they were either swollen (> 1 cm in diameter) without tenderness or palpable with tenderness.

All study participants underwent RADT for GAS infection. The tests were administered by trained nurses following the manufacturer's guidelines, and results were promptly reported to the attending physician. Pharyngeal specimens were collected by swabbing both tonsillar surfaces and the posterior pharyngeal wall using a sterile cotton-tipped swab. The QuikRead go® Strep A test (Orion Diagnostica Oy, Espoo, Finland) was utilized for detecting *Streptococcus pyogenes* in throat samples. This test operates in conjunction with the QuikRead go instrument, an automated device for quantitative and qualitative measurements using a turbidimetric method.

The relationship between the McIsaac criteria and RADT results was analyzed.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 29.0.1.1 (IBM Corporation, USA, 2024). Categorical variables were expressed as absolute numbers and percentages and compared using Pearson's Chi-squared test or Fisher's exact test, as appropriate. A p-value < 0.05 was considered statistically significant. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and overall accuracy were calculated for each McIsaac criterion and score among RADT-positive children. RADT results served as the reference standard for these calculations.

Results

A total of 282 patients were enrolled (mean age ± standard deviation: 22.6 ± 6.7 months), of whom 143 (50.7%) tested positive on RADT and 139 (49.3%) tested negative. The study population included 173 (61.4%) males and 109 (38.6%) females, with the youngest RADT-positive

child being 6.5 months old. Age distribution and overall GAS infection prevalence were summarized in Table 1.

No statistically significant correlation was observed between RADT positivity and clinical indicators such as tonsillar swelling/exudates, temperature > 38 °C, or swollen anterior cervical lymph nodes. Among the McIsaac criteria, absence of cough was the only parameter showing a statistically significant association with RADT positivity. McIsaac scores ranging from 1 to 4 did not demonstrate a meaningful correlation with the presence of GAS pharyngitis. Furthermore, no significant relationships were identified between GAS pharyngitis and additional symptoms, including rhinorrhea, vomiting, abdominal pain, or diarrhea (Table 2).

The receiver operating characteristic (ROC) curve for RADT-positive patients demonstrated an area under the curve (AUC) of 0.561. The McIsaac score's validity was further assessed, yielding an asymptotic significance of 0.072 and a 95% confidence interval of 0.494–0.628 (Figure 1).

Table 1

Age distribution of children with acute pharyngitis according to RADT results (n = 282)

Patients	RADT-positive	RADT-negative	Total
Age group, months			
0–11.9	9 (47.4)	10 (52.6)	19 (6.7)
12–23.9	76 (51.4)	72 (48.6)	148 (52.5)
24–35.9	58 (50.4)	57 (49.6)	115 (40.8)
All patients	143 (50.7)	139 (49.3)	282 (100)

RADT – rapid antigen detection test; n – number.
All values are given as numbers (percentages).

Table 2

Clinical indicators and their association with RADT positivity (n = 282)

Clinical manifestations	RADT-positive (n = 143)	RADT-negative (n = 139)	*p*-value
Tonsillar swelling or exudate	64 (50.8)	62 (49.2)	0.886*
Fever (temperature > 38 °C)	121 (50.8)	117 (49.2)	0.601*
Absence of cough	118 (48.2)	127 (51.8)	**0.033***
Cervical node enlargement	9 (56.2)	7 (43.8)	0.648*
Rhinorrhea	55 (52.4)	50 (47.6)	0.497*
Gastrointestinal symptoms (vomiting, abdominal pain, diarrhea)	34 (47.2)	38 (52.8)	0.493*
McIsaac score			
0–1	25 (64.1)	14 (35.9)	0.072*
2	75 (51.0)	72 (49.0)	0.913*
3	41 (45.0)	50 (55.0)	0.190*
4	2 (40.0)	3 (60.0)	0.681**

RADT – rapid antigen detection test; n – number.
All values are given as numbers (percentages).
***Note:* * Pearson's Chi-squared test; ** Fisher's exact test. Values that differ significantly ($p < 0.05$) are marked in bold.**

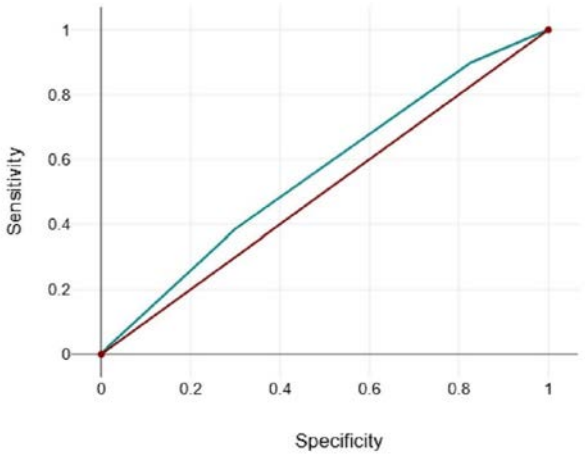

Fig. 1 – Receiver operating characteristic (ROC) curve of McIsaac criteria.
***Note:* Diagonal segments are produced by ties. The blue line represents the ROC curve, while the red line represents the reference diagonal (line of no discrimination, area under the curve – AUC = 0.5).**

Table 3

Evaluation of McIsaac criteria using RADT confirmation

McIsaac criteria and scores	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Ac, %	LR+	LR-
Tonsillar swelling or exudate	45.4	55.0	50.9	49.5	50.1	1.0	0.9
Fever (temperature > 38 °C)	71.3	26.0	48.5	32.4	46.1	0.9	2.0
Absence of cough	82.5	8.6	48.1	32.4	46.0	0.9	2.0
Cervical node enlargement	6.2	94.9	56.2	49.6	50.1	1.2	0.9
McIsaac score							
0–2	69.9	38.1	53.8	55.2	54.3	1.1	0.7
3–4	30.7	61.9	44.8	46.2	45.7	0.7	1.1

RADT – rapid antigen detection test; PPV – positive predictive value; NPV – negative predictive value; Ac – overall accuracy; LR+ – positive likelihood ratio; LR- – negative likelihood ratio.

The diagnostic performance of McIsaac scores compared with RADT results is summarized in Table 3. For McIsaac scores 0–2, sensitivity was 69.9%, specificity 38.1%, PPV 53.8%, NPV 55.2%, LR+ 1.1, LR− 0.7, and overall accuracy 54.3%. In contrast, for McIsaac scores 3–4, sensitivity was 30.7%, specificity 61.9%, PPV 44.8%, NPV 46.2%, LR+ 0.7, LR− 1.1, and overall accuracy 45.7%.

Discussion

In this retrospective study, McIsaac scores did not demonstrate a significant association with RADT positivity in children under three years of age with acute pharyngitis. The predictive validity of the McIsaac score, as assessed by AUC, was low, indicating limited effectiveness in distinguishing between positive and negative cases. Unexpectedly, the highest proportion of RADT-positive cases occurred in children with McIsaac scores of 0–2. For this group, sensitivity was 69.9% and specificity was 38.1%, highlighting the score's limited ability to differentiate between positive and negative cases. Conversely, the lowest proportion was found in children with McIsaac scores of 3 and 4 (sensitivity 30.7%, specificity 61.9%), suggesting poor performance in identifying true positive cases. This unexpected observation may be due to the atypical presentation of GAS pharyngitis in young children, where symptoms frequently resemble those of URIs. It is also possible that co-existing viral infections or underlying immune responses influenced these results. No significant differences in RADT positivity rates were observed across the various McIsaac score groups.

The poor performance of each McIsaac variable warrants further discussion. Previous cohort studies have quantified the frequency of respiratory infections in infants under two years, with an average of 3–6 infections *per* year and a 95th percentile reaching up to 11 infections annually [13, 14]. This equates to approximately one respiratory infection *per* month, with increased prevalence in winter. Given that URIs in children under three typically last 10–15 days, symptoms such as cough are unreliable indicators for distinguishing GAS pharyngitis from viral infections [13]. Nevertheless, our study found an association between GAS pharyngitis and the absence of cough, which aligns with prior research suggesting that cough is more indicative of viral infections, whereas its absence is more specific to GAS pharyngitis [15].

Palpable cervical lymph nodes are frequently observed in young children, with reported prevalence rates of 45–57% due to immune system development [16]. However, younger children may struggle to articulate pain, making tender anterior cervical lymphadenopathy a less reliable diagnostic marker [17]. Similarly, fever, commonly seen in both bacterial and viral infections, does not effectively differentiate etiology or indicate illness severity [18]. A study has shown that hyperpyrexia does not necessarily correlate with a higher probability of bacterial infections over viral ones [19]. In our cohort, elevated temperature had low sensitivity and specificity, reinforcing its limitations as a diagnostic criterion for GAS pharyngitis.

Various clinical scoring systems have been designed to assess GAS pharyngitis risk and guide diagnosis and treatment. A 2021 systematic review of 36 guidelines across 26 countries revealed that most clinical recommendations are based on symptomatology and age-based assessments, such as the Centor and McIsaac scores [20]. A meta-analysis examining 16 distinct scoring methods concluded that no single tool provides sufficient specificity for pediatric cases, emphasizing the need for further testing in high-risk children [21]. A 2024 systematic review of 14 studies (8 on McIsaac and 6 on Centor) concluded that neither score was sufficiently effective for triaging pediatric pharyngitis cases requiring antibiotics [22]. Although some previous studies included a limited number of children under three years of age, these youngest patients were grouped with older children and were not analyzed separately [21, 22]. To the best of our knowledge, this is the first study to specifically evaluate the validity of the McIsaac score exclusively in children under three years of age, thereby addressing an important gap in the existing literature.

Half of the children with acute pharyngitis in our study tested positive for RADTs, suggesting that GAS infections in young children may be more common than previously thought. Early studies with immune response-confirmed diagnoses reported GAS prevalence rates below 10% in this age group [23, 24]. However, a 2010 meta-analysis estimated that up to 26% of children under five years of age with sore throat could have GAS pharyngitis [25]. A large-scale Portuguese study in 2021, including 3,128 children under three, found GAS prevalence rates of 18% in infants under one year, 27% in children under two years, and 34% in those under three years of age [26].

Previous studies highlight the risk of diagnostic errors with RADTs [27, 28]. Stefaniuk et al. [27] reported 14% false-positive and 8% false-negative rates (PPV 83%, NPV 92%), while Azrad et al. [28] observed up to 20% false-positive and 5% false-negative results (specificity 73.3%, sensitivity

80%). These findings underscore the inherent potential for both false-positive and false-negative outcomes. Furthermore, some children in the study may have been asymptomatic GAS carriers rather than experiencing active infections, as epidemiological research from Serbia estimates GAS carriage rates in healthy children to range between 5.5% and 11.4% [29]. Currently, there is no reliable method to differentiate between acute GAS pharyngitis and asymptomatic GAS carriage in children with concurrent viral pharyngitis [30]. Future research could focus on developing molecular or biomarker-based diagnostic tools to distinguish between these conditions, potentially improving the overall accuracy of clinical decisions.

Limitation of the study

The primary limitation of this study is its retrospective nature. However, the McIsaac scores were derived from patient records, and RADTs were independently administered by nurses without access to clinical details, minimizing bias. Furthermore, this research was conducted at a single institution, potentially limiting the generalizability of findings.

Conclusion

Due to the diverse clinical manifestations of pharyngitis caused by group A β-hemolytic streptococcus in early childhood, the McIsaac score alone is insufficient for reliable diagnosis in children under three years of age. Our study demonstrated that the prevalence of infections caused by group A β-hemolytic streptococcus in this age group is higher than previously reported. Although further prospective studies are needed, clinicians should remain vigilant and consider group A β-hemolytic streptococcus pharyngitis even when the symptoms closely resemble viral upper respiratory tract infections.

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PRELIMINARY REPORT

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Clinical and radiographic outcomes of autologous pulp transplantation enhanced with concentrated growth factor in mature necrotic teeth: a clinical study

Klinički i radiografski ishodi autologne transplantacije pulpe unapređene koncentrovanim faktorom rasta kod stalnih nekrotičnih zuba: klinička studija

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Abstract

Background/Aim. Regenerative endodontic therapy in mature teeth remains challenging due to limited stem cell recruitment and apical vascularization. The aim of the prospective pilot clinical study was to evaluate the feasibility and preliminary clinical, radiographic, and functional outcomes of autologous pulp transplantation enhanced with concentrated growth factor (CGF) in mature necrotic permanent teeth. **Methods.** The study included six systemically healthy patients (five females and one male, aged 18–27 years) presenting with single-rooted mature permanent teeth, diagnosed with pulp necrosis and associated periapical lesions. Autologous pulp tissue was harvested from extracted third molars and transplanted into disinfected recipient root canals in combination with CGF prepared from venous blood. Clinical and radiographic evaluations were performed at 3, 6, and 12 months. Periapical healing was assessed using cone-beam computed tomography (CBCT), while pulp sensibility was evaluated using electric pulp testing (EPT). Treatment outcome measures included postoperative symptoms, tooth function, radiographic healing, and recovery of pulp sensibility. **Results.** All treated teeth (6/6) remained asymptomatic and functional throughout the 12-month follow-up period, with no postoperative complications, such as pain, swelling, sinus tract formation, or abnormal mobility. Progressive recovery of pulp sensibility was observed during follow-up, and positive EPT responses (reflecting neural responsiveness) were detected in two cases at 4 months, one case at 6 months, and in all cases by the 12-month evaluation. CBCT analysis at 12 months demonstrated complete resolution of periapical radiolucency and restoration of apical bone architecture in all treated teeth (6/6). **Conclusion.** Within the limitations of this pilot clinical study, CGF-enhanced autologous pulp transplantation was associated with favorable short-term clinical and radiographic outcomes, as well as recovery of pulp sensibility in mature necrotic teeth. These preliminary findings suggest the potential of this biologically based regenerative approach. However, comparative effectiveness remains to be established.

Keywords:
biological factors; dental pulp; regenerative endodontics; stem cells; transplantation, autologous.

Apstrakt

Uvod/Cilj. Regenerativna endodontska terapija kod stalnih zuba predstavlja izazov zbog ograničene migracije matičnih ćelija i nedovoljne apikalne vaskularizacije. Cilj ove prospektivne pilot kliničke studije bio je da se procene izvodljivost i preliminarni klinički, radiografski i funkcionalni ishodi autologne transplantacije pulpe unapređene koncentrovanim faktorom rasta (*concentrated growth factor* – CGF) kod stalnih nekrotičnih zuba. **Metode.** Studijom je obuhvaćeno šest sistemski zdravih pacijenata (pet ženskog i jedan muškog pola, starosti 18–27 godina) sa jednokorenim stalnim zubima, kod kojih je dijagnostikovana nekroza pulpe sa pratećim periapikalnim lezijama. Autologno pulpno tkivo uzeto je iz ekstrahovanih trećih molara i transplantirano u dezinfikovane kanale korena recipijentnih zuba u kombinaciji sa CGF dobijenim iz venske krvi. Kliničke i radiografske procene sprovedene su nakon 3, 6 i 12 meseci. Periapikalno zarastanje procenjivano je pomoću kompjuterizovane tomografije konusnog zraka (*cone-beam computed tomography* – CBCT), dok je osetljivost pulpe procenjivana električnim testiranjem pulpe (*electric pulp testing* – EPT). Mere ishoda lečenja uključivale su postoperativne simptome, funkciju zuba, radiografsko zarastanje i oporavak osetljivosti pulpe. **Rezultati.** Svi lečeni zubi (6/6) ostali su asimptomatski i funkcionalni tokom 12 meseci praćenja, bez postoperativnih komplikacija kao što su bol, otok,

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formiranje sinusnog trakta ili abnormalna pokretljivost. Tokom praćenja zabeležen je progresivan oporavak pulpne osetljivosti, a pozitivni EPT odgovori (koji odražavaju osetljivost nerva) otkriveni su u dva slučaja posle 4 meseca, u jednom slučaju posle 6 meseci i u svim slučajevima do kraja 12. meseca. Primenom CBCT nakon 12 meseci pokazana je potpuna rezolucija periapikalnog rasvetljenja i obnova apikalne koštane strukture u svim lečenim zubima (6/6). **Zaključak.** Uzimajući u obzir ograničenja ove pilot kliničke studije, autologna transplantacija pulpe unapređena primenom CGF bila je povezana sa povoljnijim kratkoročnim kliničkim i radiografskim ishodima, kao i sa oporavakom pulpne osetljivosti kod stalnih nekrotičnih zuba. Ovi preliminarni nalazi ukazuju na potencijal tog biološki zasnovanog regenerativnog pristupa. Međutim, uporedna efikasnost tek treba da bude utvrđena.

Ključne reči:
biološki faktori; zub, pulpa; endodoncija, regenerativna; matične ćelije; transplantacija, autologna.

Introduction

Loss of pulp vitality and periapical infection remain major causes of tooth dysfunction and eventual extraction. Despite substantial advances in conventional endodontic treatment, non-vital teeth continue to be at risk for long-term structural compromise and reinfection. Regenerative endodontic procedures (REPs) were developed to biologically restore the structure and function of the pulp–dentin complex rather than merely obturate the root canal space [1]. This paradigm shift in endodontics aims to reestablish pulp vitality, enhance innate defense mechanisms, and preserve long-term tooth integrity.

Initially introduced for immature permanent teeth, regenerative approaches have recently been extended to mature teeth. Notably, mature teeth are more likely to respond positively to electric pulp testing (EPT) than immature ones (45% vs. 25%), suggesting that mature necrotic teeth may regain pulp sensibility under appropriate biological conditions [2]. Clinical and radiographic evidence indicates that REPs in mature permanent teeth can lead to healing of apical periodontitis and symptom resolution [3, 4]. However, achieving reproducible outcomes in mature necrotic teeth remains challenging due to limited stem cell (SC) migration, reduced apical vascularization, and constrained apical pathways [5].

To address these biological constraints, research has focused on developing biologically driven strategies that promote angiogenesis, neurogenesis, and reinnervation within the canal system. Cell-based regenerative endodontic therapies have been proposed as a promising approach to achieve biologically relevant regeneration [6]. However, the clinical translation of such methods into daily clinical practice is restricted by the need for good manufacturing practice-compliant facilities, SC banking, regulatory constraints, and high costs [7]. Consequently, there is a growing demand for simplified, autologous, and chairside-feasible regenerative approaches.

Pulp tissue transplantation, as a biologically sound alternative, utilizes autologous dental pulp SCs (DPSCs) within their native extracellular matrix, thereby eliminating the need for *ex vivo* cell expansion [8]. Favorable clinical outcomes have been reported when pulp tissue harvested from extracted third molars (TMs) or primary teeth was transplanted into necrotic teeth, demonstrating periapical healing and restored sensibility [9, 10]. These findings highlight pulp transplantation as a clinically applicable cell-based regenerative strategy.

Concentrated growth factor (CGF), the most recent generation of autologous platelet concentrates, represents an additional innovation in regenerative dentistry. CGF provides a dense fibrin scaffold with a sustained release of bioactive molecules such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) [11–13]. These growth factors can enhance cellular proliferation, angiogenesis, and differentiation of human dental pulp cells, thereby accelerating tissue healing and regeneration [12]. Incorporating CGF into pulp transplantation may thus enhance the biological microenvironment for successful revitalization of mature necrotic teeth [14]. Although CGF has demonstrated promising biological and preclinical effects, clinical evidence supporting CGF-assisted pulp transplantation in mature necrotic teeth remains limited, underscoring the need for investigations like the present study.

Therefore, this pilot clinical study aimed to evaluate the feasibility and preliminary clinical and radiographic healing and functional outcomes of autologous pulp transplantation enhanced with CGF in mature necrotic teeth.

Methods

This study was designed as a pilot feasibility clinical investigation rather than a hypothesis-testing trial. The study was approved by the Kahramanmaraş Sutcu Imam University Clinical Research Ethics Committee (No. 2025-KAEK-53) and conducted in accordance with the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (Identifier: NCT07314866). Registration was completed after recruitment had commenced in order to ensure transparency and public accessibility of the study protocol. The study design, inclusion criteria, and intervention protocol were predefined prior to patient enrollment, and no modifications were made after registration. Donor TMs were extracted only when they were already indicated for clinical removal as part of routine dental care; no tooth extraction was performed solely for the purposes of this study. Before participating, all individuals received a comprehensive briefing on the study's objectives, the available treatment options, and the

potential risks, following which written informed consent was obtained. To ensure consistency and standardization of clinical procedures, all were performed by a single endodontist with over 10 years of clinical experience at the Department of Endodontics, Faculty of Dentistry, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Türkiye.

Patient selection and preoperative evaluation

Six systemically healthy patients (five females and one male, aged 18–27 years) were prospectively enrolled and treated according to a predefined clinical protocol at the Kahramanmaraş Sutcu Imam University Endodontics Clinic in 2025. Eligible participants were systemically healthy individuals presenting with single-rooted mature permanent teeth exhibiting clinical and radiographic evidence of pulp necrosis associated with periapical radiolucency.

Teeth were required to have a probing depth of less than 3 mm, no abnormal mobility, and no history of previous endodontic or apical surgery. The study included only those patients for whom autologous donor pulp tissue could be obtained from their own impacted or nonfunctional TMs, already clinically indicated for extraction. No donor tooth was extracted solely for research purposes or for pulp harvesting. Patients were excluded if they had systemic diseases or conditions that could impair healing (e.g., diabetes mellitus, autoimmune disorders, or ongoing immunosuppressive therapy), a history of radiation therapy in the head and neck region, or pregnancy.

Teeth with root fractures, internal or external resorption, severe calcification, or nonrestorable crown destruction were also excluded. Patients with poor oral hygiene, deep periodontal pockets (> 3 mm), or those unable to comply with follow-up visits were not considered eligible.

A formal sample size calculation was not performed, as the study was not intended to test statistical superiority or comparative efficacy. The final sample size (n = 6) reflects consecutive eligible patients meeting strict inclusion criteria during the recruitment period. Recruitment was limited by the requirement for suitable donor TMs and patient consent for autologous pulp harvesting, which inherently restricted the eligible population.

All cases were examined clinically and radiographically using periapical radiographs and cone-beam computed tomography (CBCT) to assess baseline periapical radiolucency, root morphology, and apical patency.

Clinical procedures

All treatments were performed by a single experienced endodontist following a standardized two-visit protocol (Figure 1).

First appointment (canal disinfection)

After anesthesia with articaine hydrochloride and epinephrine (Maxicaine®, Vem Pharma, Türkiye), the teeth were isolated with a rubber dam. Straight-line access was

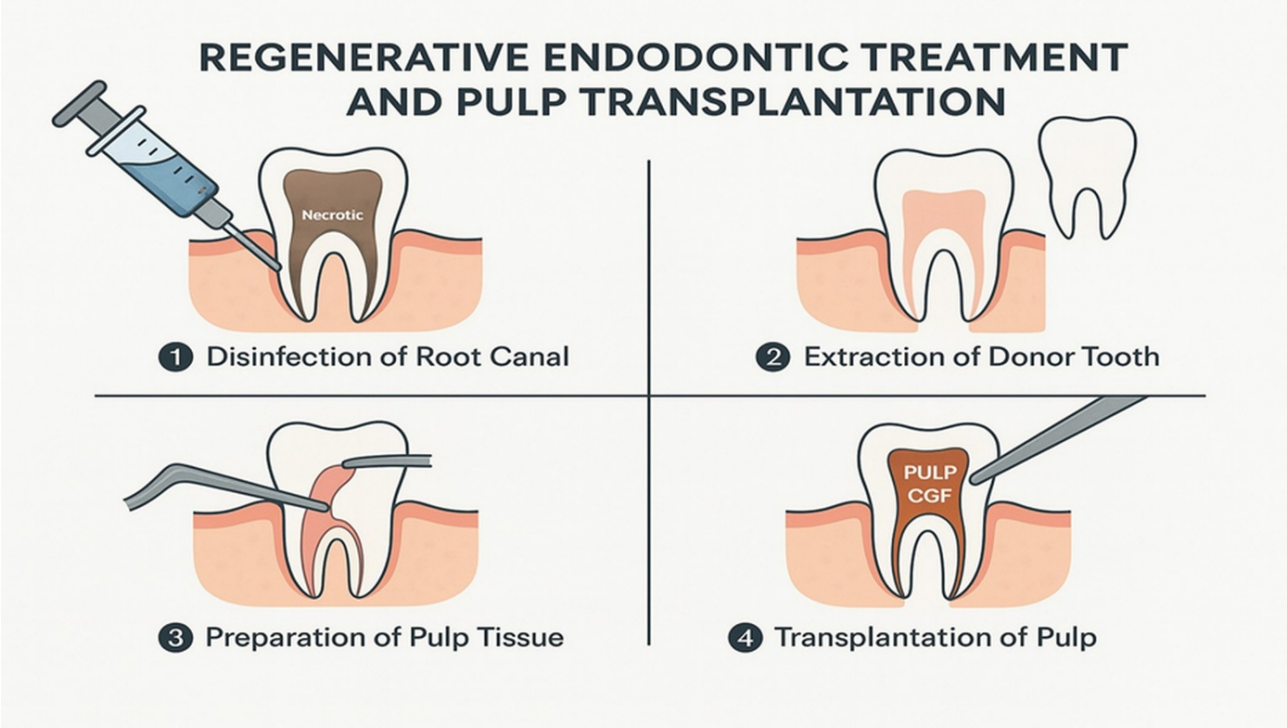

Fig. 1 – Regenerative endodontic treatment and pulp transplantation schematic (AI-assisted illustration). CGF – concentrated growth factor.

created, and the working length was determined using an electronic apex locator (Raypex 6®, VDW, Germany) and confirmed radiographically. Root canals were prepared with stainless-steel K-files (#45–#80) using a step-back technique. However, apical instrumentation was limited to #25–#30 to maintain an apical foramen diameter of approximately 0.25–0.30 mm. During instrumentation, 2 mL of 2.5% sodium hypochlorite (NaOCl) (Microvem®, Türkiye) was used between each file and activated for 5 min using a sonic device (EndoActivator®, Dentsply Sirona, Germany), totaling 20 mL *per* canal. Sonic activation was used to enhance irrigant penetration while minimizing cytotoxic effects. Canals were dried with sterile paper points and medicated with a triple antibiotic paste consisting of cefuroxime, metronidazole, and ciprofloxacin (1 : 1 : 1). Minocycline was intentionally excluded due to its well-documented association with tooth discoloration in REPs [15, 16]. Therefore, cefuroxime was used as an alternative antibiotic to maintain broad-spectrum antimicrobial coverage while reducing the risk of esthetic complications. Temporary restoration was performed using glass-ionomer cement (GC Fuji II LC®, Tokyo, Japan).

Second appointment (pulp transplantation and concentrated growth factor preparation)

After weeks, if no signs of infection were observed, pulp transplantation was initiated. The donor TM was extracted under local anesthesia and stored in sterile saline. The recipient tooth was re-anesthetized with 3% mepivacaine and isolated with a rubber dam.

The temporary material was removed, and the canal was irrigated with 5 mL of sterile saline, 5 mL of 2.5% NaOCl, and 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (MD-Cleanser®, Meta-Biomed, Korea). A #25 K-file was advanced 2 mm beyond the apical foramen to induce controlled bleeding, not to create a scaffold, but to provide the essential oxygenation and nutrient supply required for the survival of the transplanted pulp SCs.

The donor tooth was sectioned under saline cooling, and the pulp tissue was gently removed and trimmed to fit the dimensions of the recipient canal. Immediately after extraction, the donor tooth was stored in sterile saline, and pulp harvesting was performed promptly under continuous saline cooling. The harvested pulp tissue was kept moist and transplanted into the recipient canal without delay. The extraoral period from pulp harvesting to transplantation was limited to approximately 15 min in all cases in order to minimize ischemic injury and preserve tissue viability.

Venous blood was collected into red-top tubes and centrifuged in a MediFuge® (Silfradent, Italy) using the following program: 30 s acceleration; 2 min at 2,700 revolutions *per* minute (rpm) (600 × g); 4 min at 2,400 rpm (400 × g); 4 min at 2,700 rpm (600 × g); 3 min at 3,000 rpm (600 × g); and 36 s deceleration. The middle fibrin layer (CGF) was collected as a soft, elastic gel and placed over the transplanted pulp tissue inside the canal.

A small piece of Spongostan® was placed above the pulp–CGF complex as a matrix for mineral trioxide aggregate (MTA) (ProRoot MTA®, Dentsply Sirona, Germany), which was applied as a 3–4 mm coronal plug positioned 2–3 mm below the cemento-enamel junction. The coronal seal was completed using glass ionomer (Fuji II LC®, GC Corp., Japan) and composite resin (Universal Restorative 200®, 3M ESPE, Germany) (Figure 2).

Outcome measures and definition of success

The primary outcome of this pilot clinical study was radiographic periapical healing at 12 months, assessed by CBCT. Periapical healing was defined as the resolution of periapical radiolucency and restoration of normal apical bone architecture.

Secondary outcomes included the following: recovery of pulp sensibility, assessed by EPT; absence of clinical symptoms, including pain, swelling, sinus tract formation, or abnormal mobility; tooth survival and maintenance of functional integrity throughout the follow-up period.

Treatment success was defined as the presence of complete periapical healing on CBCT combined with the absence of clinical symptoms at 12 months. Recovery of pulp sensibility was considered a secondary functional outcome rather than a mandatory criterion for overall success.

Follow-up and data analysis

Patients were monitored at 3-month intervals for 12 months. Clinical evaluation included assessment of pain, swelling, tenderness, gingival condition, and tooth function. Radiographic healing was assessed by CBCT at 12 months. CBCT scans were obtained using a standardized protocol (90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm). Images were reconstructed in axial, sagittal, and coronal planes for evaluation of periapical healing. CBCT images were evaluated by the treating endodontist with over ten years of clinical experience. Healing was assessed by comparing baseline and 12-month CBCT images and was defined by resolution or marked reduction of periapical radiolucency, re-establishment of trabecular bone pattern within the lesion area, and restoration of apical cortical continuity, when applicable. Images were reviewed in multiplanar reconstructions (axial, sagittal, and coronal planes). Blinded assessment and intra-examiner reliability analysis were not performed, which represents a methodological limitation of this pilot study. Pulp sensibility was tested with EPT at 4, 6, and 12 months.

Given the limited sample size and the exploratory nature of this study, no inferential statistical analyses were performed. Data were analyzed descriptively using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as means ± standard deviations, and categorical variables as percentages.

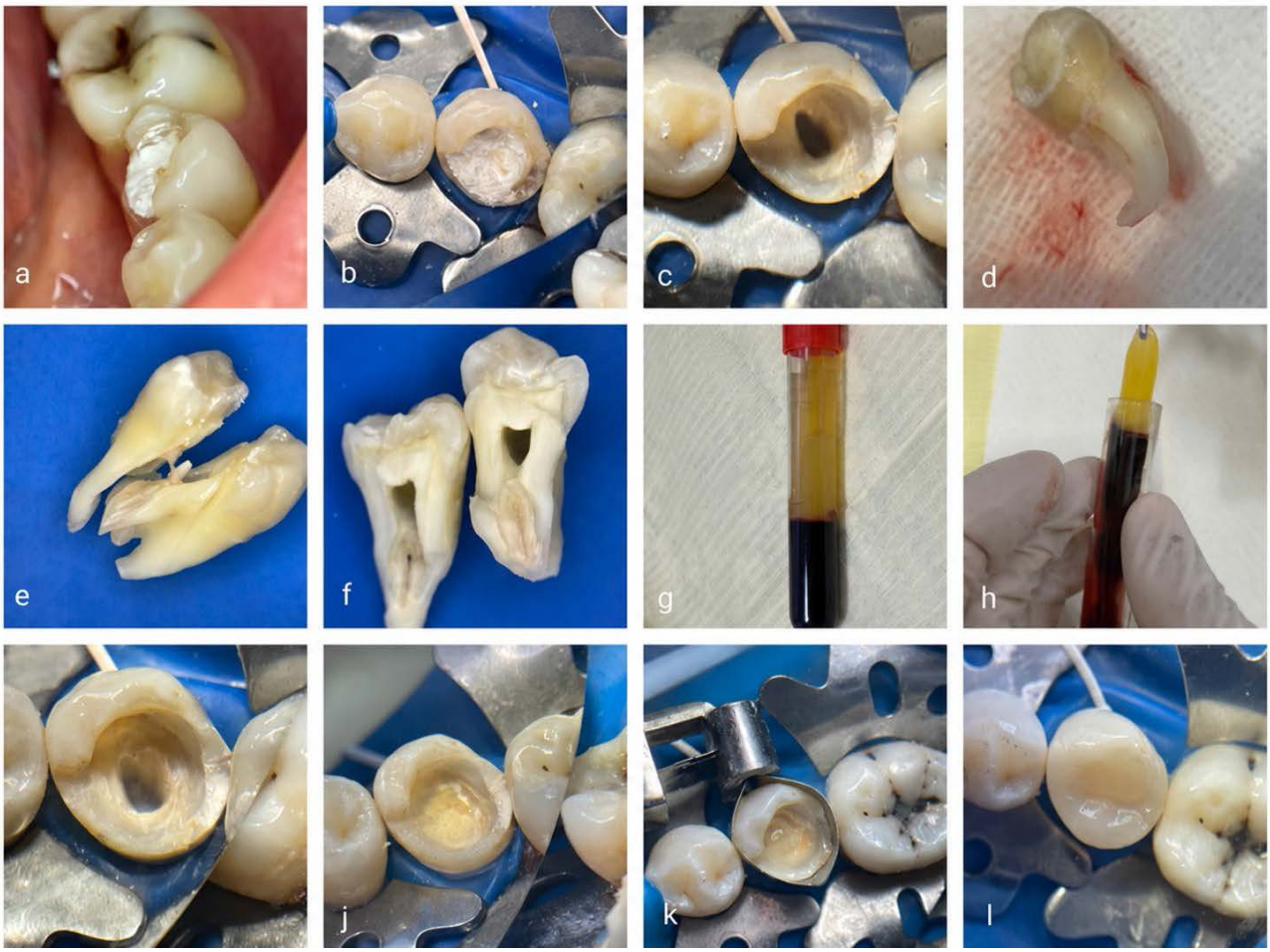

Fig. 2 – Clinical stages of autologous pulp transplantation and regenerative endodontic treatment: a) preoperative intraoral view of the lower left second premolar; b) rubber dam isolation and preparation of the recipient tooth at the second appointment; c) canal preparation for transplantation following removal of the intracanal medicament and irrigation with saline and sodium hypochlorite; d) extraction of the upper left third molar used as the donor tooth; e) sectioning of the donor tooth under saline cooling; f) harvested pulp tissue obtained from the donor tooth; g) concentrated growth factor (CGF) prepared from the patient's venous blood; h) pulp tissue combined with CGF prior to placement into the root canal; i) placement of mineral trioxide aggregate (MTA) as a coronal barrier, 3 mm below the cemento-enamel junction; j) application of a glass ionomer base over the MTA; k) matrix band placement for coronal restoration; and l) final composite resin restoration of the recipient tooth.

Results

Six patients (five females and one male, with a mean age of 21.0 ± 3.7 years) who underwent CGF-enhanced DPSCs were evaluated. All patients were systemically healthy and presented with necrotic permanent teeth associated with periapical radiolucency. Donor pulp tissues were harvested from extracted TMs in all cases. Demographic characteristics, treated teeth, pulp sensibility responses, and radiographic outcomes are summarized in Table 1. In one patient, two adjacent teeth (#22 and #23) were treated during the same clinical session. These teeth are, therefore, presented as separate cases in Table 1. An overview of the clinical procedure is shown in Figure 1.

At baseline, the treated teeth presented with clinical symptoms such as pain, tenderness to percussion, swelling, or discoloration, along with radiographic evidence of periapical bone loss. Following treatment, all patients remained asymptomatic throughout the 12-month follow-up period. No postoperative complications, swelling, sinus tract formation, or abnormal mobility were observed. All teeth remained functional, and coronal restorations were intact at all recall visits.

EPT demonstrated a progressive recovery of pulpal sensibility. Positive EPT responses were observed at 4 months in two cases, at 6 months in one case, and at 12 months in three cases. All EPT-positive teeth were clinically asymptomatic, suggesting recovery of pulp sensibility.

CBCT evaluation at 12 months revealed complete periapical bone healing in all cases (6/6). According to the predefined primary outcome (radiographic healing at 12 months) and success criteria (absence of clinical symptoms and periapical healing), treatment success was achieved in all cases (6/6). Radiographic assessment demonstrated

Table 1

Clinical summary of concentrated growth factor -enhanced pulp transplantation cases

Case No.	Age, years/ Sex	Recipient tooth, #	Donor tooth	Initial symptoms	Preoperative diagnosis	EPT response at 4-6-12 months	CBCT result at 12 months	Notes
1	20/female	35	upper left TM	percussion pain	necrosis, periapical lesion	positive at 12	complete healing	no symptoms during follow-up
2	23/male	22	upper left TM	pain	necrosis, periapical lesion	positive at 6	complete healing	no adverse events
3	27/female	31	upper left TM	swelling, pain, discoloration	calcific metamorphosis, necrosis	positive at 12	complete healing	delayed response, possible trauma effect
4	20/female	21	upper right TM	percussion pain, swelling	necrosis, periapical lesion	positive at 12	complete healing	routine check-ups, no complications
5	18/female	22/23	upper left TM	fractures, mild percussion pain	necrosis, periapical lesions on #22 and #23	positive at 4–6–12	slower but complete healing	gingivectomy and composite lamination may have delayed healing
6	18/female	22/23	upper left TM	fractures, mild percussion pain	necrosis, periapical lesions on #22 and #23	positive at 4–6–12	slower but complete healing	gingivectomy and composite lamination may have delayed healing

EPT– electric pulp testing; CBCT – cone-beam computed tomography; TM – third molar.
Note: **# symbol used to identify a specific tooth number in a patient's mouth.**

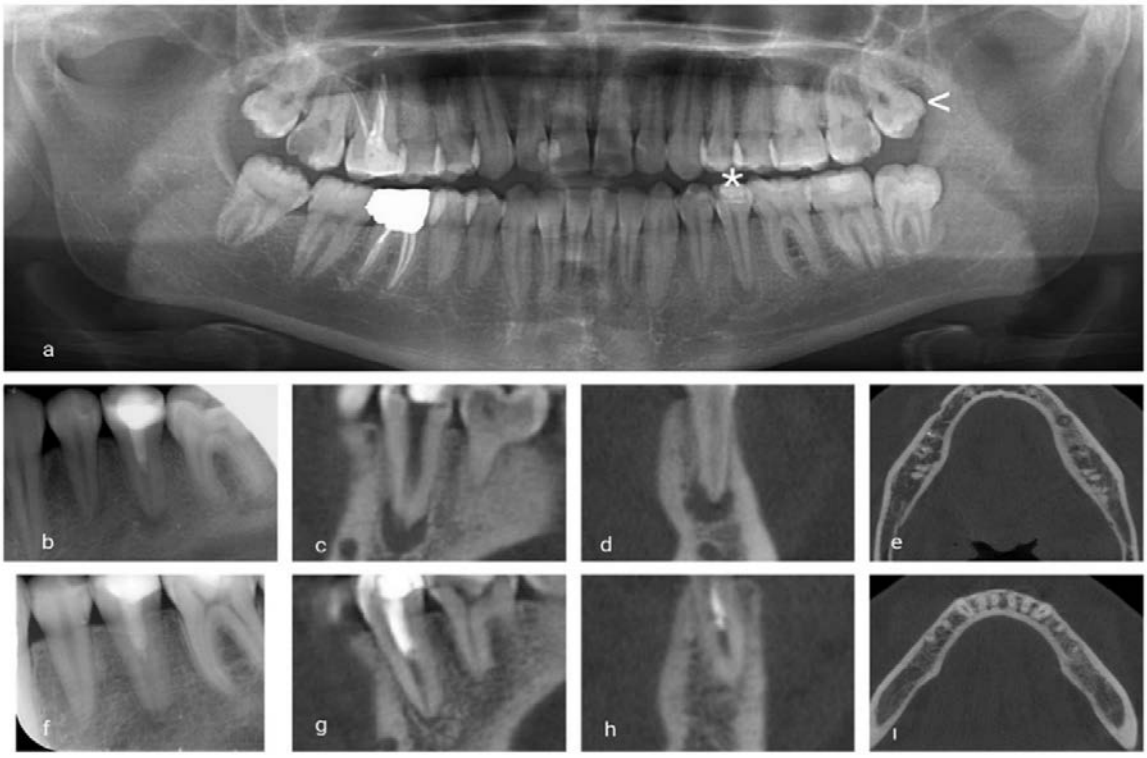

Fig. 3 – Clinical stages and radiographic outcomes of concentrated growth factor-enhanced autologous pulp transplantation (Case 1): a) preoperative panoramic radiograph showing the recipient tooth and donor third molar; b–e) preoperative cone-beam computed tomography (CBCT) images; f–i) CBCT images obtained at the 12-month follow-up demonstrating resolution of periapical radiolucency and bone regeneration. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

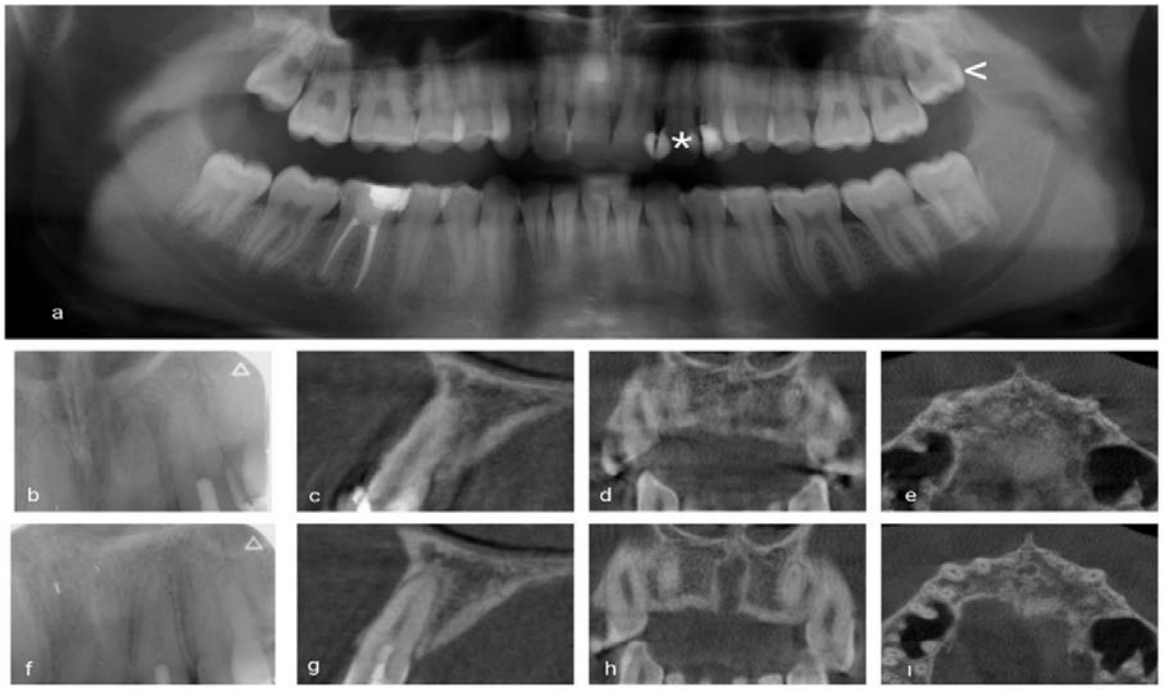

Fig. 4 – Preoperative and 12-month postoperative radiographic and cone-beam computed tomography (CBCT) images of concentrated growth factor-enhanced autologous pulp transplantation (Case 2): a) preoperative panoramic radiograph indicating the recipient tooth and donor third molar; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12-month follow-up showing complete periapical healing. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

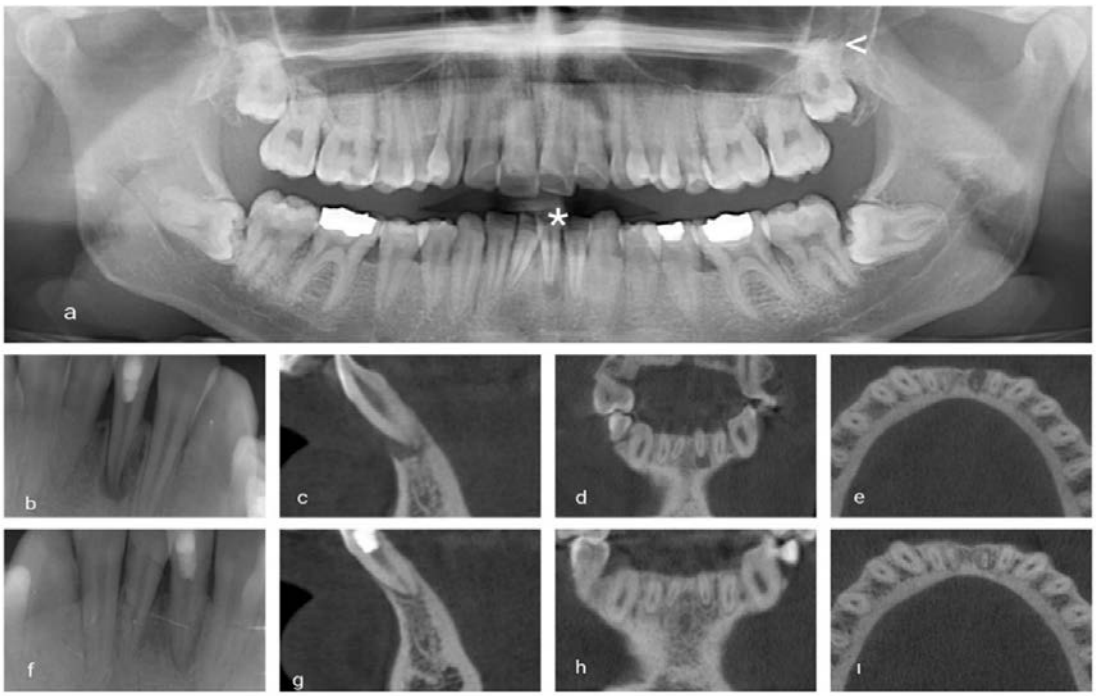

Fig. 5 – Preoperative and 12-month postoperative radiographic and cone-beam computed tomography (CBCT) images of concentrated growth factor-enhanced autologous pulp transplantation (Case 3): a) preoperative panoramic radiograph; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12-month follow-up demonstrating bone regeneration and restoration of apical architecture. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

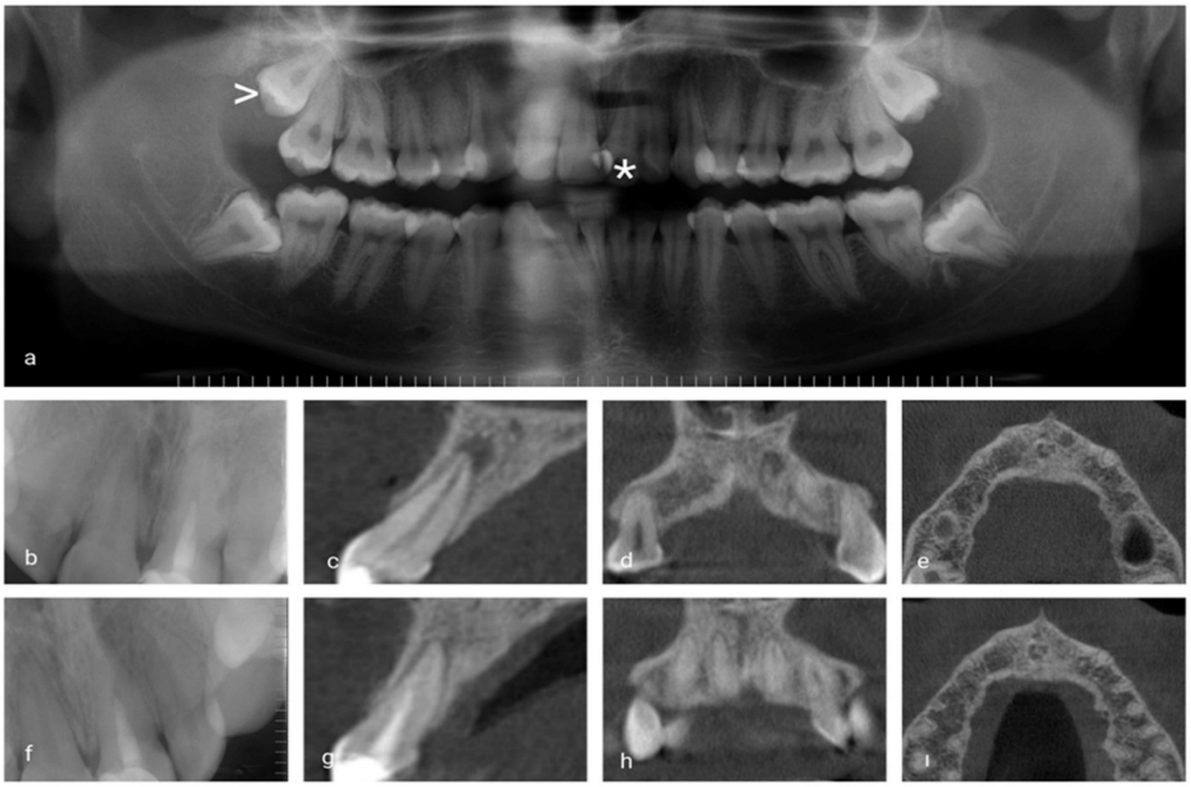

Fig. 6 – Radiographic and cone-beam computed tomography (CBCT) evaluation of concentrated growth factor-enhanced autologous pulp transplantation at baseline and 12-month follow-up (Case 4): a) preoperative panoramic radiograph; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12 months showing resolution of periapical pathology. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

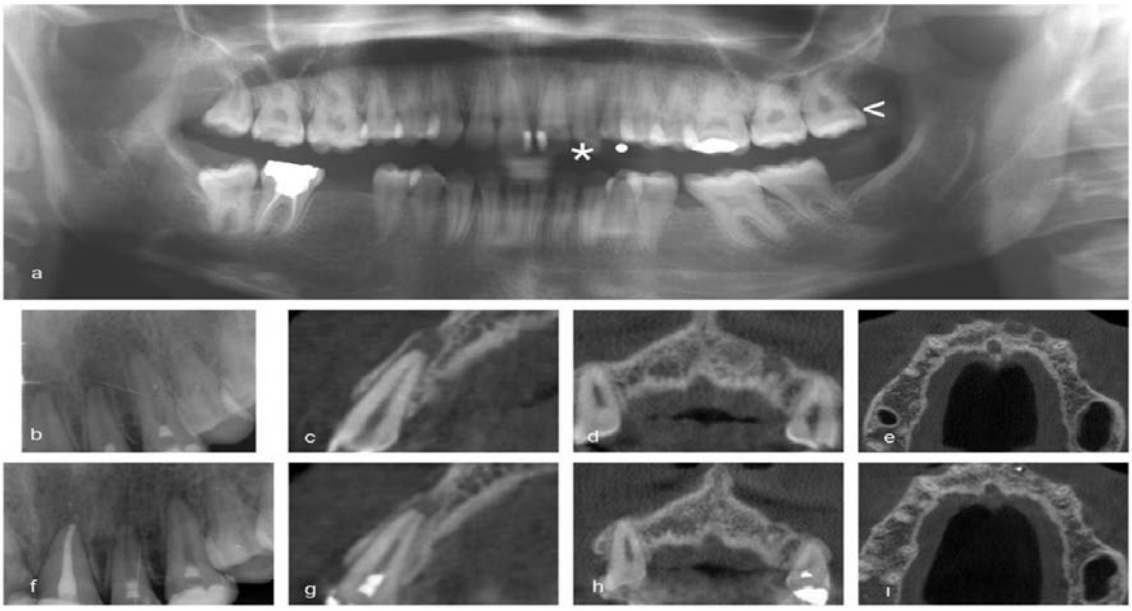

Fig. 7 – Preoperative and 12-month postoperative radiographic and cone-beam computed tomography (CBCT) findings following concentrated growth factor-enhanced autologous pulp transplantation (Case 5): a) preoperative panoramic radiograph identifying the recipient tooth/teeth and donor third molar; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12-month follow-up demonstrating periapical healing. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

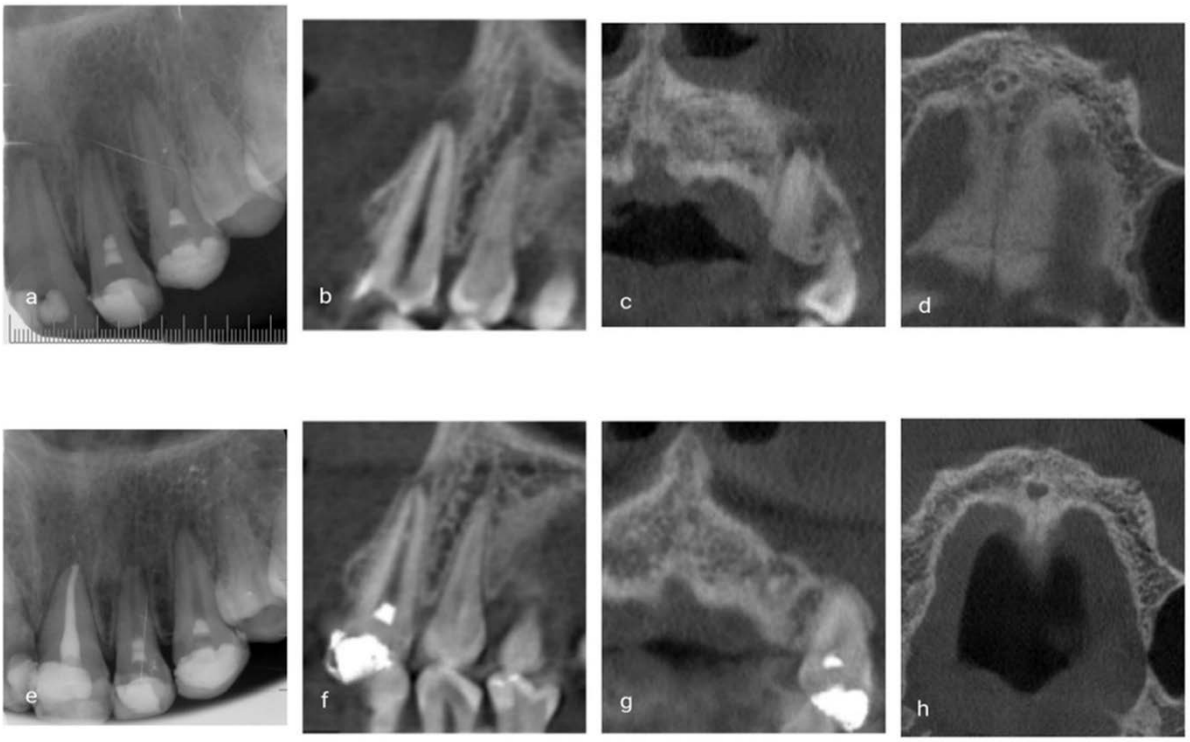

Fig. 8 – Radiographic and cone-beam computed tomography (CBCT) outcomes of concentrated growth factor-enhanced autologous pulp transplantation at baseline and 12-month follow-up (Case 6): a) immediate postoperative periapical radiograph; b–d) preoperative CBCT images (sagittal, coronal, axial); e–h) CBCT images at 12-month follow-up showing complete periapical bone regeneration. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.

resolution of periapical radiolucency, reformation of trabecular bone, and restoration of apical cortical continuity. Slightly slower healing was noted in two teeth that had undergone additional periodontal and restorative procedures (gingivectomy and composite laminate restoration), although complete healing was ultimately achieved. Representative CBCT images are shown in Figures 3–8.

Discussion

Regenerative endodontic treatment in mature teeth represents an innovative approach in contemporary endodontics, aiming not only to eliminate infection but to biologically restore the vitality and function of the pulp–dentin complex. This treatment signifies a shift from traditional endodontic practices, which typically involve root canal therapy without restoration of the biological sensibility of pulp tissue. When an appropriate regenerative strategy is implemented, the dentin–pulp complex in adult teeth may demonstrate recovery of pulp sensibility responses and other functional signs. However, clinical sensibility testing reflects neural responsiveness and does not by itself confirm histologic pulp regeneration or vitality. The concept of reestablishing functional pulp sensibility, rather than merely obturating the root canal, has, therefore, become a central focus in the current era of biologically based endodontics [1].

Empirical evidence indicates that cell-based approaches are more likely to achieve biologically relevant regeneration of the dentin–pulp complex and restore the tooth's original function compared with cell-free strategies [2]. However, the clinical translation of cell-based therapies remains limited by several critical challenges, including the need for SC isolation and expansion, limited access to facilities compliant with good manufacturing practices, and the high costs of SC culture [3]. Although various cell sources–such as autologous dental pulp cells, allogeneic mesenchymal SCs derived from the umbilical cord, and bone marrow–have been investigated [4], their routine integration into daily dental practice is largely impractical due to logistical, ethical, financial, and regulatory constraints. These limitations highlight the growing demand for simplified, autologous, and chairside-feasible regenerative protocols that preserve biological authenticity while remaining clinically applicable.

In the present study, we aimed to overcome the translational limitations of cell-based regenerative therapies by utilizing the intrinsic regenerative potential of autologous DPSCs without *in vitro* expansion. Unlike protocols that require *ex vivo* cell processing, the SC expansion phase was deliberately omitted. A previous study has reported that DPSCs must be transferred to specialized cell processing facilities and cultured for several days to achieve sufficient proliferation [3], a process that increases contamination risk, complicates clinical application, and substantially elevates

treatment costs. In contrast, in the present study, freshly harvested pulp tissue was directly transplanted into the recipient tooth, which served as a naturally protected biological niche. This environment allows transplanted cells to survive, proliferate, and differentiate under physiological conditions, thereby eliminating the need for *ex vivo* manipulation and enhancing clinical feasibility.

To minimize immunological reactions and transplant rejection, DPSCs were obtained exclusively from the patient's own non-functional, ectopic, or malpositioned wisdom teeth requiring extraction. SCs derived from TMs are considered a suitable source for pulp regeneration due to their pronounced neurogenic differentiation potential and neural crest origin [5, 6]. The autologous nature of this approach reduces the risk of immunogenicity and pathogen transmission while eliminating ethical concerns associated with allogeneic or *ex vivo*–expanded cell sources [7]. These biological and immunological advantages render autologous pulp tissue particularly suitable for direct transplantation.

When combined with a biologically active scaffold, such as CGF, this approach may further enhance cell viability, growth factor release, angiogenesis, and neural regeneration [17]. Although wisdom tooth pulp transplantation provides an immediate, fully autologous regenerative strategy, clinical evidence supporting its efficacy–especially when augmented with biological enhancers such as CGF–remains limited. The present study, therefore, contributes valuable clinical and radiographic data supporting a biologically driven regenerative model that integrates living pulp tissue with endogenous growth factors to promote functional regeneration and periapical healing in mature necrotic teeth.

Current guidelines of the American Association of Endodontists (AAE) advocate the use of low-concentration NaOCl (1.5% NaOCl) for canal disinfection during REPs, primarily to minimize cytotoxic effects on apical SCs [18]. Although higher NaOCl concentrations (5.25%–6.00%) demonstrate superior biofilm removal, they have been shown to exert deleterious effects on SC viability and to reduce the availability of dentin-derived growth factors essential for regeneration [8–10]. The adverse biological impact of NaOCl can be significantly attenuated by subsequent irrigation with 17% EDTA, which not only neutralizes residual hypochlorite but also enhances the release of angiogenic and bioactive molecules such as VEGF and TGF-β1 [11–13]. Importantly, AAE Clinical Considerations are largely extrapolated from *in vitro* studies evaluating cytotoxicity to apical papilla SCs rather than from *in vivo* assessments of antimicrobial efficacy [14]. Moreover, current recommendations are primarily tailored to immature teeth, and standardized irrigation protocols for regenerative procedures in mature teeth are lacking. In the present study, a 2.5% NaOCl solution was selected to balance effective canal disinfection with preservation of biologically active dentin components, particularly in the absence of an apical papilla. Based on the favorable clinical and radiographic outcomes, this concentration appeared sufficient for antimicrobial control without compromising periapical SC viability. Adjunctive irrigation with 17% EDTA was used to mitigate potential cytotoxic effects and promote the release of endogenous growth factors. It should also be considered that in mature teeth with closed apices, the likelihood of irrigant extrusion beyond the apical constriction may be reduced compared with immature teeth. Nevertheless, extrusion risk remains dependent on irrigation technique, needle design, and apical preparation size. Therefore, careful irrigation protocols remain essential to ensure biological safety.

A triple antibiotic paste consisting of cefuroxime, ciprofloxacin, and metronidazole was selected as the intracanal medicament. The exclusion of minocycline from the formulation prevented tooth discoloration, and no discoloration was observed in any case. With this intracanal medication protocol, no additional intracanal medicaments were required throughout treatment.

CGF has emerged as a promising adjunct in regenerative endodontic therapy, particularly for mature teeth with necrotic pulp. CGF is free from bovine thrombin and anticoagulants, addressing limitations associated with platelet-rich plasma and platelet-rich fibrin. It contains high concentrations of growth factors, including TGF-β, PDGF, bone morphogenetic protein, and VEGF. CGF modulates the biological behavior of dental SCs, particularly within inflammatory microenvironments, and has been successfully applied in a limited number of endodontic cases [19].

Preclinical studies have demonstrated that CGF enhances the proliferation, migration, and differentiation of DPSCs, supporting pulp-like tissue formation, neovascularization, and neural elements [20, 21]. The clinical findings of the present study align with these observations, as the use of CGF was associated with encouraging periapical healing findings in this small pilot cohort, along with progressive recovery of pulp sensibility.

In regenerative endodontic treatments, thermal and electric pulp tests are commonly used to assess sensibility. A previous study has reported stable sensibility responses for up to 12 months in mature teeth treated with biologically based regenerative protocols [22]. In CGF-assisted pulp transplantation, positive EPT responses have been reported as early as 3–6 months [23], whereas delayed responses are more frequently observed in protocols without CGF [24]. Consistent with these reports, the present study demonstrated progressive recovery of pulp sensibility, with all treated teeth exhibiting positive EPT responses by the 12-month follow-up. Although EPT demonstrated progressive recovery of sensibility, it should be emphasized that sensibility tests assess neural response rather than direct pulpal blood flow. That being said, they cannot conclusively confirm true pulp vitality. More objective vascular assessment methods, such as laser Doppler flowmetry or pulse oximetry, provide direct evaluation of pulpal microcirculation and may offer more reliable confirmation of revascularization. The absence of vascular vitality testing represents a limitation of the present study and should be addressed in future controlled clinical investigations.

Nevertheless, mature teeth present inherent regenerative limitations due to fully developed root structures and restricted apical access. Although induced apical bleeding has been proposed to facilitate mesenchymal SC

recruitment [25], a previous study indicates that apical enlargement alone does not necessarily improve sensibility outcomes [26]. In the present study, apical foramen sizes ranged between 0.25 mm and 0.30 mm. Yet, positive sensibility responses were achieved in all cases, suggesting that apical diameter alone may not be the primary determinant of regenerative success.

Although apical bleeding was intended in most cases, only minimal bleeding was achieved, possibly due to pre-existing periapical pathology. Unlike previous protocols that supplemented regeneration with blood harvested from extraction sockets [26], no additional blood transfusion was performed. Despite this limitation, complete periapical healing and recovery of pulp sensibility were observed in all cases in this pilot series, suggesting that CGF-enhanced pulp transplantation may be less dependent on extensive blood clot formation and more strongly influenced by viable pulp-derived SCs and sustained growth factor release.

These findings provide preliminary clinical observations suggesting that CGF-enhanced pulp transplantation may represent a potential alternative approach to conventional blood-clot-based regenerative techniques by creating a controlled, growth-factor-rich microenvironment. However, definitive conclusions regarding the nature of the regenerated tissue require histological confirmation. Future histologic and molecular studies are necessary to determine whether the regenerated tissue represents true neurovascular pulp or a reparative pulp-like connective tissue. When interpreted in accordance with the predefined primary outcome and success criteria, the present findings indicate favorable short-term radiographic healing and clinical stability, within the limitations of a small pilot clinical design.

The present evaluation suggests that CGF-enhanced DPSCs may be associated with encouraging short-term periapical healing and recovery of pulp sensibility in this pilot cohort of mature necrotic teeth. By combining two autologous biological components–vital pulp tissue and CGF–this approach may represent a biologically based regenerative strategy that could be applicable in clinical practice, although further controlled studies are required.

Limitations of the study

The findings of this pilot clinical study should be interpreted in light of several limitations. First, the sample size was small (n = 6), which limits the generalizability of the results and prevents statistical inference. Second, the study lacked a control or comparison group, making it difficult to directly evaluate the relative effectiveness of CGF-enhanced pulp transplantation compared with other regenerative endodontic approaches. Third, the follow-up period was limited to 12 months, and longer observation periods are required to determine the long-term stability of the regenerative outcomes. In addition, histological confirmation of the regenerated tissue was not possible in this clinical setting; therefore, the nature of the newly formed tissue could not be definitively verified. Finally, the requirement for donor TM for autologous pulp harvesting may introduce selection bias and limit the broader applicability of this technique. Future controlled clinical studies with larger sample sizes and longer follow-up periods are needed to validate these preliminary findings.

Conclusion

Within the limitations of this pilot clinical study, concentrated growth factor-enhanced autologous pulp transplantation appeared to be a feasible regenerative approach for the management of mature necrotic teeth. The preliminary clinical and radiographic outcomes observed in this small cohort suggest potential for periapical healing and recovery of pulp sensibility responses. However, these findings should be interpreted cautiously due to the limited sample size and absence of a control group. Further well-designed randomized controlled clinical trials with larger patient populations and longer follow-up periods are required to confirm the reproducibility, biological outcomes, and long-term clinical effectiveness of this technique.

Conflict of interest

The authors declare no conflict of interest.

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Sudden pulmonary edema induced by phenylephrine misuse: a case report

Iznenadni edem pluća indukovan nepravilnom primenom fenilefrina

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Abstract

Introduction. Phenylephrine, a widely used vasoactive drug in clinical practice, may lead to severe cardiovascular complications when misused. Among these complications, sudden pulmonary edema, though rare, warrants the attention of clinicians. The given case report presents a sudden pulmonary edema caused by the misuse of phenylephrine. **Case report.** A female patient aged 68-years undergoing radical mastectomy for left breast cancer developed severe hypertension and hypoxemia 40 min into the procedure. The patient's medical history included meningioma but no other significant comorbidities. A diagnosis of sudden (acute) pulmonary edema was made. The patient received prompt treatment, including strict perioperative blood pressure control, lung protective ventilation, glucocorticoids, diuretics, coronary-dilation and cardiotonic drugs, postoperative oxygen therapy, and continuous vital sign monitoring. Investigation revealed that phenylephrine had been mistakenly administered intravenously instead of dexamethasone. She recovered and was discharged one week postoperatively. **Conclusion.** This case highlights the risks associated with the inadvertent administration of a high dose of phenylephrine, leading to sudden pulmonary edema. It underscores the importance of vigilance among anesthesiologists, prompt management of complications, and strategies to prevent errors, including enhanced education for resident anesthesiologists, measures to address practitioner fatigue, and improved drug packaging to minimize look-alike errors.

Keywords:
anesthesia, intravenous; anesthesiologists; intensive care units; medical errors; phenylephrine; pulmonary edema.

Apstrakt

Uvod. Fenilefrin, vazoaktivni lek koji se često koristi u kliničkoj praksi, može dovesti do teških kardiovaskularnih komplikacija u slučaju nepravilne primene. Među ovim komplikacijama, iznenadni edem pluća, iako redak, zaslužuje pažnju kliničara. Prikazan je slučaj iznenadnog edema pluća izazvanog pogrešnom upotrebom fenilefrina. **Prikaz bolesnika.** Bolesnica starosti 68 godina, koja je bila podvrgnuta radikalnoj mastektomiji zbog karcinoma leve dojke, razvila je tešku hipertenziju i hipoksemiju 40 minuta nakon početka operacije. U anamnezi je naveden meningeom ali ne i drugi značajni komorbiditeti. Postavljena je dijagnoza iznenadnog (akutnog) edema pluća. Bolesnici je odmah ordinirana terapija, uključujući strogu kontrolu krvnog pritiska perioperativno, protektivnu plućnu ventilaciju, glukokortikoide, diuretike, koronarne dilatatore i kardiotonike, terapiju kiseonikom postoperativno, uz kontinuirano praćenje vitalnih znakova. Istragom je utvrđeno da je umesto deksametazona greškom primenjen fenilefrin intravenski. Bolesnica se oporavila i otpuštena je nedelju dana posle operacije. **Zaključak.** Prikazani slučaj ističe rizike povezane sa nenamernom primenom visoke doze fenilefrina, što dovodi do iznenadnog edema pluća. Time je naglašen značaj postojanja opreznosti među anesteziolozima, brzog rešavanja komplikacija i strategija za sprečavanje grešaka, uključujući bolju edukaciju specijalizanata iz anesteziologije, mera za rešavanje zamora lekara praktičara i poboljšanja pakovanja lekova, u cilju što manjih grešaka zbog njihove sličnosti.

Ključne reči:
anestezija, intravenska; anesteziolozi; intenzivna nega, odeljenja; medicinske greške; fenilefrin; pluća, edem.

Introduction

Phenylephrine is a commonly used vasoactive drug in clinical practice, primarily for hypotension management during the perioperative period and in the intensive care unit. Due to its vasoconstrictive effect, phenylephrine is administered intravenously (i.v.), either as an infusion or in small incremental boluses, to increase systemic vascular

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resistance and maintain blood pressure (BP). It is also commonly used in delicate surgeries with demanding surgical field visualization [1] (e.g., otolaryngological endoscopic procedures, ophthalmic mydriasis) as well as for the treatment of priapism [2]. Improper administration of phenylephrine can result in serious complications, including hypertension, arrhythmias, myocardial infarction, left ventricular failure, and cardiac arrest [3, 4]. Among these, pulmonary edema (PE) is relatively rare.

Previous reports in the literature have primarily described cases of sudden PE caused by improper local administration of phenylephrine, typically with rapid recovery [4, 5]. This report presents a case of severe, sudden PE induced by inadvertent i.v. administration of a high dose of phenylephrine.

The patient data were handled according to the Declaration of Helsinki. Written informed consent was obtained from the patient.

Case report

The patient was a 68-year-old woman (height: 156 cm, weight: 64 kg) scheduled for a radical mastectomy of the left breast due to axillary lymphadenopathy that had been present for 2 weeks. The patient's medical history included meningioma, for which regular observation had been recommended by her neurosurgeon, and a prior cholecystectomy.

Preoperative physical examination and laboratory investigations revealed no abnormalities. Cardiac ultrasound indicated left ventricular diastolic dysfunction, mild regurgitation of the aortic, mitral, and tricuspid valves, and an ejection fraction of 64%. Liver ultrasound revealed fatty liver and liver cysts. Bilateral lower extremity vascular ultrasound showed no thrombus or other abnormalities. Pulmonary computed tomography revealed scattered small nodules and pulmonary fibrosis in both lungs. The electrocardiogram showed sinus rhythm with premature atrial contractions.

Upon entering the operating room, the electrocardiogram, oxygen saturation (SpO_2), and non-invasive BP of the lower extremities were continuously monitored. The monitoring device used was Datex-Ohmeda S/5 CAM. General anesthesia was induced with i.v. injections of etomidate (12 mg), fentanyl (0.3 mg), and rocuronium bromide (40 mg). After the endotracheal intubation, anesthesia was maintained with propofol (4 mg/kg/hr), sevoflurane (0.6 minimum alveolar concentration), and remifentanil (0.2 μg/kg/min). The fraction of inspired oxygen (FiO_2) was maintained at 60%. The tidal volume was set to 400 mL, the respiratory rate was set to 12 breaths *per* minute, and positive end-expiratory pressure was not applied.

Thirty minutes after the start of the surgery, dexamethasone (5 mg) was routinely administered *via* i.v. injection to prevent postoperative nausea and vomiting. Ten minutes after the injection, the patient's BP suddenly increased to 257/134 mmHg, with a heart rate (HR) of 85 beats *per* min (bpm) and SpO_2 of 99%. It was confirmed that BP cuff on the lower limb and i.v. access were functioning normally. Repeated measurements recorded BP of 245/136 mmHg, HR of 84 bpm, and SpO_2 of 98%. When we were about to administer urapidil for BP reduction, the patient's BP (165/82 mmHg) had already started to decrease spontaneously. Given that the cause of BP abnormality remained unclear, we suspended the antihypertensive treatment.

Meanwhile, SpO_2 rapidly dropped to 90%, airway pressure rose to 24 cm H_2O, and crackles were audible bilaterally on lung auscultation. FiO_2 was immediately increased to 100%, with simultaneous manual ventilation. SpO_2 improved to 98% after a few minutes. At the same time, the surgeon observed a strong contraction of the arrector pili muscle in the patient's breast, raising suspicions of a drug-related reaction. Upon inspection of the empty medication bottles, it was discovered that phenylephrine (10 mg, 1 mL) had been mistakenly administered i.v. instead of dexamethasone (5 mg, 1 mL).

To further assess the patient's condition, puncture and catheterization of the right radial artery were performed for invasive arterial pressure monitoring and blood gas analysis. The first recorded invasive arterial BP at that time was 105/72 mmHg. The blood gas results revealed a partial pressure of oxygen (PaO_2) of 82 mmHg, partial pressure of carbon dioxide (PCO_2) of 38.4 mmHg, and PaO_2/FiO_2 < 100. Two minutes later, BP began to drop and was stabilized with epinephrine (3 μg/min). Approximately 50 min later, the patient's hemodynamics stabilized. During this period, urinary catheterization was performed, and methylprednisolone (40 mg), furosemide (10 mg), and inhaled albuterol (100 mg) were administered to improve pulmonary oxygenation.

At the conclusion of the operation, the FiO_2 was reduced to 60%, and the SpO_2 was 100%. Repeat blood gas analysis revealed PaO_2 of 171 mmHg and a PCO_2 of 43.9 mmHg. In the preparation for extubation, we gradually discontinued the anesthetic agents and simultaneously tapered the dose of epinephrine until complete cessation. Simultaneously, bloody secretions were observed in the endotracheal tube, raising suspicion of acute PE. We aborted the extubation procedure. Morphine (5 mg) was administered, and the patient was transferred to the post-anesthesia care unit (PACU) for further observation. Mechanical ventilation was continued in the PACU under sedation, with dobutamine administered to maintain stable circulatory dynamics. The inhaled oxygen concentration was adjusted to 40%. Blood gas analysis conducted 2 hrs later revealed a PaO_2 of 113 mmHg, a PCO_2 of 39.3 mmHg, and no significant change in PaO_2/FiO_2. Bedside echocardiography showed preserved systolic function, with an ejection fraction value of 60%. No further bloody secretions were observed during airway suction, and sedation was discontinued.

Vasoactive drugs were stopped, and the endotracheal tube was removed after the patient regained consciousness and hemodynamic stability. The patient was positioned with the head elevated and observed for 30 min, during which vital signs remained stable. The patient was then transferred to the ward with tabplemental oxygen at a flow rate of 3 L/min.

On the first postoperative day, the patient underwent a series of diagnostic evaluations. High-resolution pulmonary computed tomography revealed exudative lesions in both lungs and bilateral pleural thickening. Laboratory results showed aspartate aminotransferase of 46 U/L [reference range (RR): < 35 U/L], high-sensitivity cardiac troponin I of 0.853 ng/mL (RR: < 0.016 ng/mL), and N-terminal pro B-type natriuretic peptide (NT-ProBNP) of 2,127 pg/mL. Postoperatively, the patient complained of chest tightness and discomfort and was treated with oxygen inhalation at 2 L/min. On the third day postoperatively, re-examination of the laboratory tests showed normal results. One week after the operation, chest tightness was relieved, and the patient was safely discharged from the hospital.

Discussion

This case highlights the occurrence of sudden PE induced by phenylephrine misuse. Phenylephrine is a selective α1 receptor agonist commonly used to increase vascular tone and elevate BP. However, at very high doses, phenylephrine can also elicit β-adrenergic receptor activation, potentially leading to tachycardia [4]. Based on clinical experience, misuse of phenylephrine tends to induce significant bradycardia in patients and may even lead to cardiac arrest in severe cases. However, HR of the patient in this case did not decrease but remained at 85 bpm, and this special clinical manifestation also verified the validity of the conclusions from the aforementioned literature.

Previous reports have documented hypertensive crisis or acute PE following topical instillation [5] or local injection [6] of phenylephrine during pediatric ophthalmic surgeries, as well as in *puerperae* with accidental 2 mg administration [7], all of whom achieved rapid recovery. In contrast, the 10 mg overdose in our case caused more severe myocardial injury and PE, with persistent chest tightness requiring daily oxygen therapy for one week postoperatively, highlighting critical implications for clinical medication safety.

Misuse of phenylephrine caused a sudden increase in peripheral vascular resistance, which induced left ventricular end-diastolic dysfunction and a sharp elevation of left atrial pressure, ultimately leading to acute PE [8]. The study confirmed that rapid BP reduction can alleviate capillary injury, which also explains the rapid resolution of bloody tracheal secretions, indicating that timely identification and standardized management are crucial for avoiding adverse events [9]. The relatively severe condition of the patient in this case was considered to be associated with pre-existing left ventricular diastolic dysfunction, which lowered the threshold for PE [10].

Additionally, the patient might have developed transient left heart failure. This speculation was supported by perioperative hypotension and postoperative elevations of myocardial enzymes, troponin, and NT-proBNP. According to the literature [11], in patients aged 50–75 years, an NT-proBNP level > 900 pg/mL yields a sensitivity > 90% and specificity > 84% for the diagnosis of acute heart failure, which further verifies this inference.

In response to the phenylephrine misuse incident in this case, we promptly implemented corrective measures. Rooted in the similar packaging of the two drugs, the error occurred when the pharmacy misplaced phenylephrine vials into dexamethasone storage boxes. We immediately reported this adverse event to the hospital quality control center to raise awareness among relevant departments and facilitate rectification. Meanwhile, we procured specialized storage boxes for high-alert medications, which are designated for storing vasoactive agents, potassium chloride, and other high-risk drugs, to achieve segregation from regular medications; additionally, we suspended the storage of dexamethasone in standardized boxes, with clinical demand to be met by pharmacy dispensing upon request. Given that the involved staff member was a junior resident physician, not only did we intensify the intensity of pre-service training, but we also added reflective teaching courses based on clinical adverse cases [12]. As confirmed by a previous study [13], reflective training can improve diagnostic accuracy in uncertain and complex scenarios, thereby reducing medical errors. Investigation into the incident revealed that the resident physician had been experiencing fatigue due to prolonged working hours, which contributed to the medication preparation error. In China, the shortage of anesthesiologists and excessively long working hours are the major causes of professional burnout among this cohort [14]. Similarly, an overseas study has also indicated that anesthesiologists worldwide are confronted with unprecedented work pressure and staffing shortages, with professional burnout being an extremely prevalent issue [15]. Therefore, improving the working conditions of anesthesiologists is imperative and urgently requires attention at the national level.

Following the incident, the relevant personnel reported it to the hospital's adverse event management system without delay. Subsequently, the department convened a special meeting and decided to temporarily remove the dexamethasone storage box from the standard anesthesia drug kits. If dexamethasone is indeed required during surgery, medical staff should retrieve it from the operating room pharmacy as needed.

In the meantime, the department customized a batch of dedicated storage boxes for high-alert medications, which are used to store vasoactive agents, potassium chloride, and other high-risk preparations, thus achieving the classified storage and management of general medications and high-alert medications.

In addition, the department has specially added a dedicated training module on such medication error incidents to the standardized training curriculum for resident physicians, aiming to reduce the risk of recurrence of similar events.

Conclusion

The accidental intravenous administration of a high dose of phenylephrine (10 mg) poses a significant risk of sudden pulmonary edema, requiring vigilant attention from an anesthesiologist. While the patient recovered and was discharged, this case underscores critical lessons for clinical

practice and highlights areas for improvement. We hope that this report will serve as a cautionary example for clinicians, emphasizing the importance of vigilance and precision in drug administration. Preventing such incidents necessitates a multifaceted approach, including enhanced post-graduation education for anesthesiologists, proactive measures to address anesthesiologist burnout, and improvements in drug packaging to minimize the likelihood of medication errors.

Conflict of interest

The authors declare no conflict of interest.

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From the ‘Jumpers in Maine’ to the ‘Boy from Paris’: the life of Georges Gilles de la Tourette and the first description of tic disease

Od „Skakača u Mejnu” do „Dečaka iz Pariza“: život Georges Gilles de la Tourette-a i prvi opis bolesti tikova

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Abstract

In 1885, French neuropsychiatrist Dr. Georges Gilles de la Tourette was the first to describe a disease of tics. His success was not unexpected, as he was a student of the renowned neurologist Dr. Jean-Martin Charcot. Georges Gilles de la Tourette was born on October 30, 1857, in Saint-Gervais-les-Trois-Clochers, France, as the eldest of four children. Immediately after graduating in 1881, he went to Paris for further training. His special interests included hysteria, psychotherapy, and hypnotism. By revising an article titled “Experiments with the ‘Jumpers of Maine’ ”, he concluded that a somewhat similar disorder exists in different cultures across the world. He soon described additional signs of the syndrome, including inarticulate sounds, echolalia, and coprolalia, and accentuated the hereditary nature of the disease. Insightful and dedicated yet short-tempered and arrogant, Georges Gilles de la Tourette was an inspiring physician and teacher. He left behind a significant legacy in the study of involuntary movements, particularly through his description of the syndrome that would come to be known as Tourette syndrome. This description of the syndrome is just a portion of his contributions to neurology and psychiatry. His work, often met with both admiration and criticism, reflects the complexities of his personality and the challenges he faced in his professional life.

Keywords:
dyskinesias; history of medicine; history, 19th century; tourette syndrome.

Apstrakt

Godine 1885, francuski neuropsihijatar dr Georges Gilles de la Tourette prvi je opisao bolest tikova. Njegov uspeh nije bio neočekivan, jer je bio učenik čuvenog neurologa dr Jean-Martin Charcot-a. Georges Gilles de la Tourette rođen je 30. oktobra 1857. godine u mestu Sen Žerve le Troa Kloše u Francuskoj, kao najstarije od četvoro dece. Odmah nakon diplomiranja 1881. godine, otišao je u Pariz na dalje usavršavanje. Posebno su ga interesovale histerija, psihoterapija i hipnotizam. Revidirajući članak pod nazivom „Eksperimenti sa ‘Skakačima iz Mejna’ ”, zaključio je da sličan poremećaj postoji u različitim kulturama širom sveta. Ubrzo je opisao dodatne znake sindroma, uključujući neartikulisane zvuke, eholaliju i koprolaliju i naglasio naslednu prirodu bolesti. Pronicljiv i posvećen, ali i nagle naravi i arogantan, Georges Gilles de la Tourette bio je inspirativan lekar i učitelj. Ostavio je značajan trag u proučavanju nevoljnih pokreta, naročito kroz opis sindroma koji će kasnije biti poznat kao Turetov sindrom. Opis ovog sindroma predstavlja samo deo njegovog doprinosa neurologiji i psihijatriji. Njegov rad, često dočekivan i sa divljenjem i sa kritikama, odražava složenost njegove ličnosti i izazove sa kojima se suočavao u profesionalnom životu.

Ključne reči:
diskinezije; istorija medicine; istorija, 19. vek; turetov sindrom.

Introduction

In 1885, Dr. Georges Gilles de la Tourette (Figure 1), a French neuropsychiatrist, was the first to describe *maladie des tics* (a disease of tics), a condition that brought him great fame in medical circles worldwide. His success was not unexpected, as he was a student of the renowned neurologist Dr. Jean-Martin Charcot, often called the “Napoleon of

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Neuroses". Gilles de la Tourette laid the foundations for understanding neuropsychiatric disorders involving tics and compulsive actions [1, 2]. This article focuses on his life and the path to the discovery of the syndrome, which today, in his honor, bears the name Tourette syndrome (TS).

Fig. 1 – Dr. Georges Gilles de la Tourette, portrait. (available from: https://en.wikipedia.org/wiki/Georges_Gilles_de_la_Tourette)

Childhood and youth

Georges Gilles de la Tourette was born on October 30, 1857, in Saint-Gervais-les-Trois-Clochers, France, as the eldest of four children. Significant life events of this physician are shown in Figure 2. His full name was Georges Albert Édouard Brutus Gilles de la Tourette. Although his father was a merchant, several members of his extended family were physicians, which may have influenced his career choice [3, 4]. Not much is known about his early years, except that he was a brilliant yet mischievous student, known for his love of challenges. He managed to complete two years of high school in a single year, which allowed him to enroll in the Faculty of Medicine at Poitiers, France, at a young age of 16 [4]. There was a great deal of excitement that followed a period of *Belle Époque*–a period of peace, enlightenment, optimism, and economic prosperity in France from 1871 to 1914. That surely contributed to the aspirations and ambition of this curious Frenchman to join Parisian academic circles as soon as possible [4, 5].

Career in neuropsychiatry

In 1881, immediately after graduating, Gilles de la Tourette went to Paris for further training [1].

Three years later, he began his internship under the mentorship of one of the greatest neurologists of the time, Jean-Martin Charcot, at the renowned Salpêtrière Hospital in Paris [6, 7]. Persistent and eager not to miss any opportunity, he worked "at a speed that exceeded human capabilities," publishing, teaching, and practicing clinical medicine. His special interests included hysteria, psychotherapy, and hypnotism [3]. Friends described him as a cheerful and lively young man, though with a somewhat arrogant demeanor and an unrefined manner of speaking. He passionately engaged in discussions, often without patience for others' opinions, which led to noisy debates [3]. Gilles de la Tourette also faced criticism, particularly concerning his behavior, relationships with colleagues, and interactions with the outside world. One of his harshest critics was Léon Daudet, a French journalist and writer, who described him as "neither good nor bad, nor industrious nor lazy, neither intelligent nor stupid." He remarked that Gilles de la Tourette had "a hoarse and worn voice, sudden gestures, and a strange gait" [8, 9]. Daudet considered him "a freak who jumps from one interesting topic to another, confusing his teachers" and noted that his behavior worsened over time, becoming less enjoyable [9]. Although Daudet's criticism may have been valid, particularly regarding Gilles de la Tourette's tendency to start multiple projects simultaneously, it also highlights his diverse interests. While studying movement disorders under the supervision of Charcot, Gilles de la Tourette wrote a book on spiritualism, several history texts, and served as a research associate and

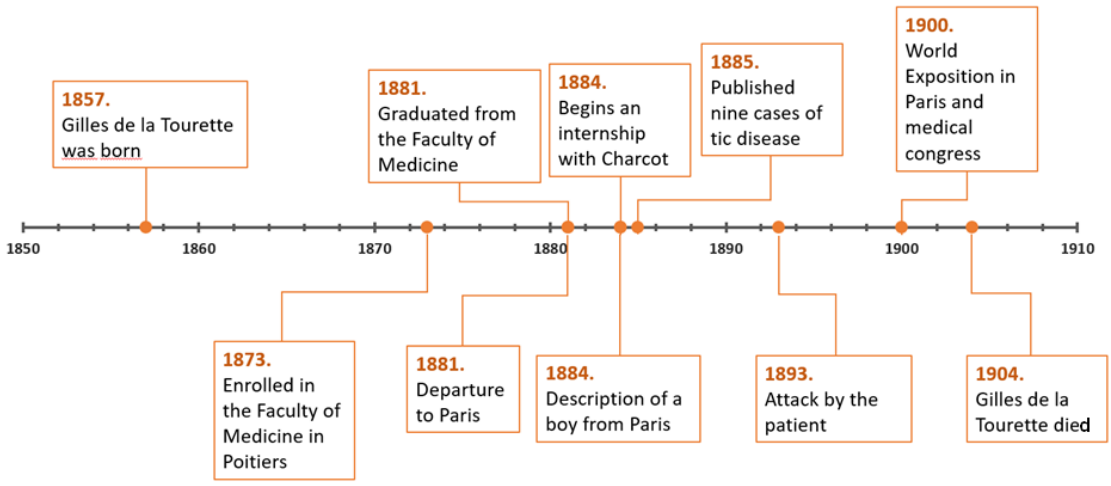

Fig. 2 – Significant events in the life of Gilles de la Tourette.

playwright. He also worked as a critic for a French literary magazine, where he wrote under the pseudonym Paracelsus [4]. Some sources say he even dared to provoke the German Empire, writing about alleged hysteria within its army. This reportedly reached the Chancellor of the German Empire, Otto von Bismarck, who was infuriated by the notion [1].

Description of the syndrome

After Charcot assigned him to classify movement disorders, Georges Gilles de la Tourette revisited an article titled "Experiments with the 'Jumpers of Maine' ", which he had translated into French a few years earlier [4]. It is known today that he translated such articles into French with considerable freedom. The reason for such a free translation is unclear, but in some cases, it seems he did that on purpose [4]. In 'Jumpers of Maine', American psychiatrist Dr. George Miller Beard described people from Maine who, when extremely excited, would "jump, produce unusual sounds, and mimic other people's movements" [10, 11]. This behavior, now recognized as a culturally specific phenomenon known as "startle syndrome" (we know it today as a disease distinct from 'tic disease'), intrigued Gilles de la Tourette, who believed similar cases could be found in other parts of the world beyond the United States.

He began a literature search and came across two additional articles describing seemingly similar disorders, each with names in local languages due to cultural specificity. The first, called *latah*–a Malay term, as the disorder is primarily observed in Southeast Asia, consists of intense reactions to sudden stimuli and compulsive utterance of rude language (coprolalia) [12]. The second disorder, known as *miryachit* (or myriachit in English transcription), was described among patients in Siberia and presented a clinical picture similar to that of the 'Jumpers of Maine' [12, 13].

After studying these works, Georges Gilles de la Tourette concluded that all the descriptions likely referred to the same disorder, which he thought was similar to chorea. In 1884, he decided to document a case of a boy from Paris who appeared to have similar symptoms. This encounter marked Gilles de la Tourette's first personal observation of the syndrome that would later bear his name [4, 14]. The following year, in 1885, he published an article describing nine additional cases, using the term *maladie des tics* (tic disease) to define the disorder. His mentor, Charcot, later honored him by naming it "Gilles de la Tourette syndrome" in recognition of his research [4, 15].

Georges Gilles de la Tourette described additional signs of the syndrome, including inarticulate sounds, echolalia, and coprolalia (without accompanying gestures). He correctly noted that the syndrome typically begins in childhood, predominantly affects males, and does not impair intelligence or sensory functions, which remain within the normal range [4]. Furthermore, the prevailing belief at the time supported his assertion that the disorder is hereditary, although it is now recognized that other factors can also contribute to its onset [16]. Interestingly, just a few years before the world saw the Eiffel Tower at the 1889 World Expo in Paris, the medical community was introduced to the concept of tic disorders.

Events of 1893, downward trajectory, and death

The year 1893 was significant for Georges Gilles de la Tourette, as it was marked by three major personal tragedies. First, he suffered the loss of his son, followed shortly by the death of his esteemed mentor, Jean-Martin Charcot. In the same year, Gilles de la Tourette was also shot by a patient (Figure 3) who claimed to have been hypnotized against her will by one of his colleagues [3]. Although the injuries were not life-threatening, the attack had far-reaching negative consequences. It attracted considerable media attention, resulting in sensationalized newspaper coverage that tarnished Gilles de la Tourette's reputation [3].

Fig. 3 – Illustration of the attack on Gilles de la Tourette. (available from: https://commons.wikimedia.org/wiki/File:Petit-illustre-gdlt.gif)

Nevertheless, after his recovery, he organized a medical congress during the World Exhibition in Paris in 1900, and some sources say he did that with great success [17]. Unfortunately, shortly after the shooting incident, Georges Gilles de la Tourette's health deteriorated significantly. Against his will, he was admitted to a psychiatric hospital in western Switzerland, where he was diagnosed with tertiary syphilis [8, 18]. Some sources suggest he was deceitfully taken there by being told that an important person, in fact a patient, would be waiting for him in Switzerland [7]. Throughout his time in the hospital, his family remained by his side, providing support during this challenging period. His health deteriorated further [3]. Gilles de la Tourette passed away in May 1904 at the age of 47 [3, 6].

From *maladie des tics* to Tourette syndrome: modern neurobiological framing

Although the original synthesis of *maladie des tics* encompassed a broader clinical context, modern neurology draws a clear distinction, separating tic disorders from startle syndromes. Gilles de la Tourette's synthesis included both culture-bound hyperstartle phenomena (the Jumpers of Maine, myriachit, and *latah*) and cases from his own clinical practice that he added to these hyperstartle phenomena, thereby placing *maladie des tics* within a wider clinical framework [4]. Today, TS is defined as a chronic neurodevelopmental disorder characterized by motor tics and vocalizations (phonic tics) that persist for at least one year [19, 20]. The pathophysiology of this syndrome has not yet been fully elucidated. TS is thought to result from a disturbance in corticostriato-thalamo-cortical (mesolimbic) circuits, including motor and limbic/associative loops, with consequent disinhibition of motor and limbic systems [19–22]. From a neurobiological perspective, a recent narrative review suggests an imbalance in neurotransmitter systems–particularly dopaminergic and serotonergic signaling–along with structural and functional changes in neuronal networks involved in motor control [20]. Recent findings broaden the classical neuroanatomical model of this disorder by indicating that, in addition to the basal ganglia, other brain regions such as the prefrontal cortex, motor cortical areas, and the cerebellum may also contribute to the pathophysiology of TS [20, 23]. This supports a broader, network-based rather than a focal model of the disorder.

Furthermore, no single gene or set of genes has been identified that would explain most cases of TS [24]. Multiple loci have been mapped as candidate susceptibility regions, and one of the reported findings is a mutation in the *SLITRK1* gene (13q31.1), which has been linked to dendritic growth [25]. However, this mutation appears to be very rare and has not been found in large series of affected individuals [26]. This supports the notion of a complex and heterogeneous genetic basis of TS. Overall, these observations point to the multifactorial and complex nature of TS, underscoring the need for further research to clarify the neurobiological and genetic basis of this disorder.

Conclusion

Insightful and dedicated, though allegedly short-tempered and arrogant, Georges Gilles de la Tourette was an inspiring physician and teacher. He left behind a significant legacy in the study of involuntary movements, particularly through his description of the syndrome that would come to be known as Tourette syndrome. This work is just a portion of his broader contributions to neurology and psychiatry. Although the work of Georges Gilles de la Tourette was often met with both admiration and criticism, it reflects the complexity of his personality and the challenges he faced in his professional life.

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

SYSTEMATIC LITERATURE REVIEW

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

META-ANALYSIS

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

SYSTEMATIC LITERATURE REVIEW WITH META-ANALYSIS

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

CURRENT TOPIC

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

IN FOCUS

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

CASE REPORTS

CASE REPORT and CASE SERIES (≥4, ≤9)

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

EDITORIAL

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

LETTER TO THE EDITOR

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

RESEARCH LETTER

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

HISTORY OF MEDICINE/STOMATOLOGY/PHARMACY

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

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

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

BOOK REVIEW

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

SCIENTIFIC MEETING REPORT

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

MANUSCRIPT LENGTH

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

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

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

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

MANUSCRIPT PREPARATION

TITLE PAGE

The first page of the manuscript should include the following:

1. Title of the manuscript without abbreviations;
2. Full names of all authors (without academic titles, but with ORCID numbers included for those who have them) with symbols assigned in the following order: ***, †, ‡, §, ‖, ¶, **, ††**… etc.;
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ABSTRACT

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

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

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

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

KEYWORDS

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

STRUCTURE OF THE MAIN TEXT

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

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

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

A Case Report must not have more than five authors.

QUESTIONNAIRES

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

TABLES AND FIGURES

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

Tables

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

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

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ABBREVIATIONS

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

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

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

ACKNOWLEDGEMENTS

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

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Article with more than 6 authors

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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.

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

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

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

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

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

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

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

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

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

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

ОБАВЕЗНА ПРАТЕЋА ДОКУМЕНТА

ИЗЈАВА АУТОРА И АУТОРСТВО

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

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

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

ЕТИЧКА САГЛАСНОСТ

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

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

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

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

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

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

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

ПЛАГИЈАРИЗАМ

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

КОРИШЋЕЊЕ AI

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

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

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

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

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

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

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

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

ТИПОВИ РУКОПИСА

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

ОРИГИНАЛНИ ЧЛАНАК

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

ПРЕТХОДНО САОПШТЕЊЕ

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

КРАТКО САОПШТЕЊЕ

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

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

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

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