NICOLAE TESTEMITANU
STATE UNIVERSITY
OF MEDICINE AND PHARMACY
OF THE REPUBLIC OF MOLDOVA

MJHS Moldovan Journal of Health Sciences

REVISTA DE ȘTIINȚE ALE SĂNĂTĂȚII DIN MOLDOVA

Vol. 13
Issue 2,
June 2026

Category B

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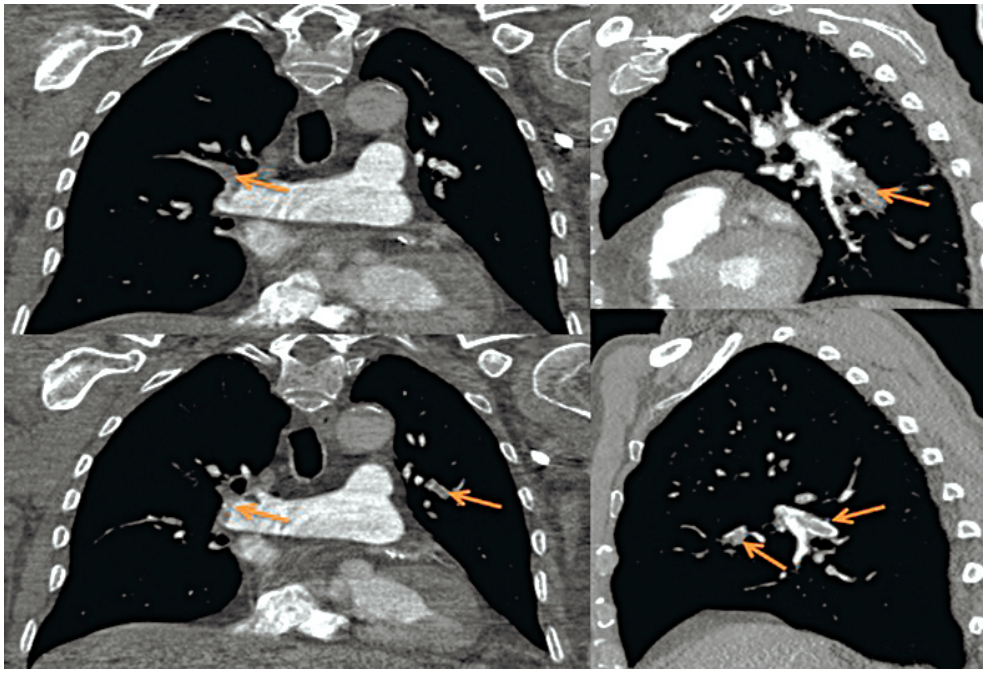

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ISSN: 2345-1467
e-ISSN: 1857-4696

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MJHS
Moldovan Journal of Health Sciences
Revista de Ştiinţe ale Sănătăţii din Moldova
2026;13(2)

CONTENT

Moldovan Journal of Health Sciences
Revista de Ştiinţe ale Sănătăţii din Moldova

Ediţie în limba engleză

Fondator:
Instituţia Publică Universitatea de Stat de Medicină şi Farmacie „Nicolae Testemiţanu" din Republica Moldova

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Editat: Editura „Lexon-Prim"
Tiraj: 200 ex.

Înregistrată la Ministerul Justiţiei al Republicii Moldova (nr. 250 din 01.08.2014).

Categoria B acordată de Agenţia Naţională de Asigurare a Calităţii în Educaţie şi Cercetare (decizia nr. 2 din 04.11.2022)

English edition

Founder:
Public Institution *Nicolae Testemitanu* State University of Medicine and Pharmacy from Republic of Moldova

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EDITORIAL BOARD

https://doi.org/10.52645/MJHS.2026.2.01

UDC: 616.12-008.46+616.1/.8

RESEARCH ARTICLE

The spectrum of comorbidities in patients with heart failure with preserved ejection fraction

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ABSTRACT

Introduction. Heart failure with preserved ejection fraction (HFpEF) accounts for nearly half of all heart failure cases and is frequently associated with cardiovascular and metabolic comorbidities. The phenotype of HFpEF patients is heterogeneous, and the impact of comorbidities on prognosis, exercise capacity, and functional status remains insufficiently elucidated.

Objective. The study aimed to characterize the clinical, functional, and comorbidity profiles of patients with HFpEF and to assess their influence on functional status, prognosis, and treatment response.

Materials and methods. This was an observational, cross-sectional study including 206 patients with HFpEF (LVEF ≥50%) consecutively recruited from the General Cardiology Department of the Institute of Cardiology, aged ≥18 years, with an echocardiographically confirmed diagnosis. Demographic and anthropometric data, HFpEF etiology, hemodynamic biomarkers (NT-proBNP), functional status (NYHA), cardiovascular and non-cardiovascular comorbidities, and history of revascularization procedures (PCI, coronary bypass) were collected. Statistical analysis included descriptive statistics for continuous variables (mean ± SD, median, IQR), categorical variables (frequencies and percentages), and parametric/nonparametric tests for correlations and subgroup analyses, with statistical significance set at $P < 0.05$.

Results. The study population showed a typical overweight/obese profile, with arterial hypertension and chronic coronary artery disease as predominant mechanisms. Cardiovascular and metabolic comorbidities influenced exercise capacity, functional status, and treatment response, identifying distinct phenotypic subgroups with differential prognostic impact. Elevated NT-proBNP levels reflected increased ventricular filling pressures and functional heterogeneity, underscoring the need for individualized management.

Conclusions. HFpEF is associated with a complex clinical profile dominated by hypertension, coronary artery disease, and metabolic comorbidities. Detailed assessment of comorbidities and biomarkers allows patient phenotyping and personalized therapeutic management. A multidisciplinary approach is essential for optimizing prognosis, exercise capacity, and quality of life in patients with HFpEF.

Keywords: HFpEF, comorbidities, exercise capacity, NT-proBNP, phenotype, prognosis.

Cite this article: Cabac-Pogorevici I, Scaleţchi A, Revenco V. The spectrum of comorbidities in patients with heart failure with preserved ejection fraction. Mold J Health Sci. 2026;13(2):3-9. https:doi.org/10.52645/MJHS.2026.2.01

Manuscript received: 17.12.2025

Accepted for publication: 18.03.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Although comorbidities in patients with heart failure with preserved ejection fraction (HFpEF) are recognized as key factors in the development and progression of heart failure, the exact mechanisms through which these comorbidities influence disease phenotype, prognosis, and therapeutic response remain insufficiently elucidated. Existing data derive from heterogeneous populations and lack a standardized approach to patient phenotyping accord-

ing to the complexity and clustering of comorbidities. Currently, there is no validated and practical clinical framework that integrates the comorbidity profile into the risk stratification and management of patients with HFpEF.

The research hypothesis

The presence and combination of cardiac and non-cardiac comorbidities contribute to the heterogeneity of heart failure with preserved ejection fraction (HFpEF), leading to significant differences in exercise capacity, functional status, and long-term prognosis, suggesting the need for a personalized, phenotype-based approach in patient evaluation and treatment.

The novelty added by the manuscript to the already published scientific literature

This study offers an integrated evaluation of comorbidity burden and clustering in patients with HFpEF, identifying distinct patterns associated with clinical phenotype and outcomes. By moving beyond single-comorbidity analyses, our findings provide new insights into HFpEF heterogeneity and support a more personalized, comorbidity-oriented approach to risk stratification and management.

Introduction

Heart failure (HF) is a complex clinical syndrome defined by the heart's inability to maintain an adequate cardiac output to meet the body's metabolic demands, in the presence of elevated filling pressures [1]. The modern classification of HF is based on the left ventricular ejection fraction (LVEF), an echocardiographic indicator of systolic function: heart failure with reduced ejection fraction (HFrEF, ≤40%), heart failure with mildly reduced ejection fraction (HFmrEF, 41–49%), and heart failure with preserved ejection fraction (HFpEF, ≥50%) [1, 2]. HFpEF accounts for approximately half of all HF cases and represents a major diagnostic and therapeutic challenge. The condition predominantly affects women, especially after menopause, due to increased ventricular stiffness, hormonal changes, endothelial dysfunction, and the accumulation of metabolic risk factors [3]. Although patients with HFpEF may have a normal ejection fraction, they typically exhibit elevated filling pressures, diastolic dysfunction, concentric hypertrophy, and reduced ventricular compliance, which limit exercise capacity and cause the characteristic symptoms [4].

A key aspect in the management of HFpEF is the presence of cardiovascular comorbidities, which contribute to the onset and progression of cardiac dysfunction through hemodynamic, inflammatory, and neurohormonal mechanisms. Among these, arterial hypertension, atrial fibrillation, and coronary artery disease are the most significant and frequently encountered [5-7].

Arterial hypertension (HTN) is the most prevalent comorbidity in HFpEF, being identified in over 80% of patients [5, 8]. Chronic exposure to pressure overload leads to concentric left ventricular hypertrophy, myocardial stiffness, impaired active relaxation, and increased diastolic filling pressures [8, 9]. Moreover, endothelial dysfunction and altered vascular reactivity contribute to increased afterload, creating a vicious cycle between arterial and ventricular stiffness [9]. These changes explain why strict blood pressure control plays a crucial role in preventing HFpEF progression and improving symptoms.

Atrial fibrillation (AF) is present in 30–40% of patients with HFpEF and has a bidirectional relationship with diastolic dysfunction [7]. On one hand, the loss of atrial contraction and an inappropriate ventricular rate reduce diastolic filling and cardiac output; on the other hand, elevated filling pressures and atrial remodeling secondary to diastolic dysfunction promote the development and maintenance of AF [6, 10]. The presence of AF is associated with reduced exercise capacity, lower Kansas City Cardiomyopathy Questionnaire (KCCQ) scores, and higher mortality, particularly in elderly patients [10].

Coronary artery disease (CAD) is highly prevalent in HFpEF and manifests in two major forms: obstructive, characterized by significant stenosis or occlusion of the epicardial coronary arteries, and non-obstructive, in which microvascular mechanisms and endothelial dysfunction predominate [11, 12]. The obstructive form leads to chronic myocardial ischemia, ventricular remodeling, and interstitial fibrosis, contributing to increased stiffness and impaired diastolic relaxation [12]. Conversely, non-obstructive CAD, present in a substantial proportion of HFpEF patients (up to 50%), is associated with coronary microvascular dysfunction (CMD), characterized by reduced coronary flow reserve, endothelial inflammation, and oxidative stress [13]. These microvascular alterations impair myocardial perfusion during exercise, increase diastolic stiffness, and limit functional capacity [13, 14]. Microvascular endothelial dysfunction, through reduced nitric oxide (NO) bioavailability and activation of the renin–angiotensin system, promotes chronic inflammation and interstitial remodeling characteristic of HFpEF [14]. Thus, CAD – regardless of its obstructive or non-obstructive nature – represents a central determinant of the ischemic phenotype in HFpEF, influencing both prognosis and treatment response.

Obesity, metabolic syndrome, sleep apnea, and type 2 diabetes mellitus are key elements in the pathogenesis of HFpEF, forming the so-called "cardiometabolic phenotype" [9, 15, 16]. Epicardial adipose tissue (EAT) acts as an active endocrine organ, releasing proinflammatory cytokines (IL-6, TNF-α) that induce local inflammation, oxidative stress, and myocardial fibrosis [9, 15, 17]. Diabetes further contributes through endothelial dysfunction, vascular stiffening, myocardial protein glycosylation, and activation of systemic inflammatory pathways [9]. These processes create a profibrotic and prooxidative milieu that amplifies diastolic dysfunction and reduces the energetic efficiency of cardiomyocytes. Moreover, obesity alters thoracic mechanics, in-

creases intrathoracic pressure, and exacerbates pulmonary congestion, leading to exercise intolerance [9, 15, 16].

Chronic kidney disease (CKD) is another major comorbidity, present in over one-third of HFpEF patients, and is associated with significantly increased mortality [5]. Renal dysfunction activates neurohormonal systems – the Renin-Angiotensin-Aldosterone System (RAAS) and the sympathetic nervous system (SNS) – leading to sodium retention, vasoconstriction, and cardiac remodeling [5, 6]. Cardiorenal syndrome type 2 describes the progressive deterioration of renal function secondary to chronic cardiac dysfunction, perpetuating hemodynamic decompensation and systemic inflammation [5].

The heterogeneity of HFpEF patients arises from the complex interaction between cardiometabolic comorbidities, systemic inflammation, and endothelial dysfunction. Recent studies using cluster or machine learning analyses have identified distinct phenotypic subgroups combining various patterns of HTN, CAD, AF, obesity, and CKD, each with different pathogenic mechanisms and risks for mortality and functional impairment [13-15].

Modern, holistic approaches provide an integrated perspective on these complex interactions, linking molecular, clinical, and functional dimensions of comorbidities in HFpEF [14, 16]. This holistic view enhances understanding of clinical variability and supports the development of personalized treatment strategies.

Therefore, detailed analysis of cardiovascular, metabolic, and renal comorbidities in patients with HFpEF is essential for identifying key prognostic determinants and defining targeted therapeutic strategies. The present study aims to characterize these complex interactions, evaluate the impact of comorbidities on functional status and prognosis, and contribute to the development of an integrated management model for HFpEF.

Materials and methods

This study represents a cross-sectional observational analysis of patients with heart failure with preserved ejection fraction (HFpEF), defined by a left ventricular ejection fraction (LVEF) ≥50%, the presence of signs and symptoms of heart failure, and objective evidence of structural and/or functional cardiac abnormalities consistent with left ventricular diastolic dysfunction and/or elevated left ventricular filling pressures, including elevated natriuretic peptide levels [1]. The main objective was to characterize the clinical, functional, and comorbidity phenotype of patients and to assess the impact of these factors on hemodynamic biomarkers and functional status.

The study cohort included 206 consecutive patients diagnosed with HFpEF, recruited from the General Cardiology Department of the Institute of Cardiology. Inclusion criteria were: age ≥18 years, diagnosis of HFpEF according to the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure [1], availability for complete clinical and paraclinical assessments, and written informed consent. Exclusion criteria included acute heart failure, significant loss of clinical data, presence of severe comorbidities that could interfere with functional evaluation, or inability to participate in standardized testing.

For each patient, demographic data (age, sex), anthropometric data (weight, height, body mass index – BMI), and the medical history of cardiovascular and non-cardiovascular diseases were recorded. BMI was calculated as weight (kg)/height (m^2). Sex distribution was coded according to the dataset as follows: 1 = male, 2 = female.

The etiology of HFpEF was classified as follows: documented coronary artery disease, undocumented coronary disease, arterial hypertension, significant valvular disease, tachycardia-induced, idiopathic dilated cardiomyopathy, hereditary/genetic, alcoholic, infectious, pulmonary hypertension without left heart disease, and other rare causes.

Functional class was assessed using the New York Heart Association (NYHA) classification. The biomarker NT-proBNP was measured to evaluate hemodynamic stress and ventricular filling pressures, using standardized laboratory techniques.

Cardiovascular comorbidities included arterial hypertension, atrial fibrillation, myocardial infarction, coronary artery disease, prior revascularization procedures, valvular disease, peripheral vascular disease, stroke/transient ischemic attack, and venous thromboembolism. Non-cardiac comorbidities included diabetes mellitus, chronic obstructive pulmonary disease (COPD), thyroid dysfunction, hepatic dysfunction, sleep apnea, depression, cognitive impairment, active malignancy, and rheumatoid arthritis.

All patients underwent standardized clinical, paraclinical, and functional evaluations, including anthropometric measurements, functional testing (NYHA), NT-proBNP assessment, and detailed medical history collection. No experimental therapeutic interventions were applied, as the study was purely observational.

Data were centralized and validated for consistency and completeness. Continuous variables were described as mean ± standard deviation (SD), median, and interquartile range (IQR). Categorical variables were expressed as frequencies and percentages. The distribution of variables was tested for normality using the Shapiro–Wilk test. Group comparisons were performed using parametric tests (independent t-test) or nonparametric tests (Mann–Whitney U), as appropriate. Relationships between comorbidities, biomarkers, and functional status were analyzed using linear and logistic regression models, with statistical significance set at $P < 0.05$.

All patients provided written informed consent, and the study protocol was approved by the institutional ethics committee (Approval No. 25/1, May 13, 2025). The study was conducted in accordance with the principles of the Declaration of Helsinki on biomedical research involving human subjects.

Results

The study cohort included 206 patients with HFpEF. The mean age was 65.5 years (SD 9.6), with a median of 66.0 and an interquartile range (IQR) of 59.0–71.0; the age range was 39–94 years, reflecting a typical age profile for heart failure with preserved ejection fraction. Body mass index (BMI)

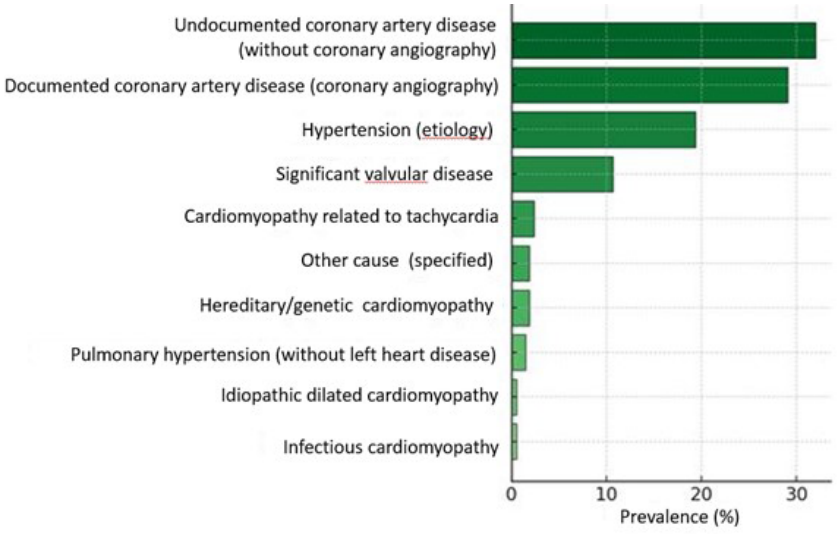

Fig. 1 Distribution of HFpEF etiologies.

***Note**: The figure illustrates the prevalence (%) of etiological conditions identified in patients with heart failure with preserved ejection fraction (HFpEF). Data are presented as percentages of the overall study population. The figure presents descriptive data only; no inferential statistical tests were applied.*

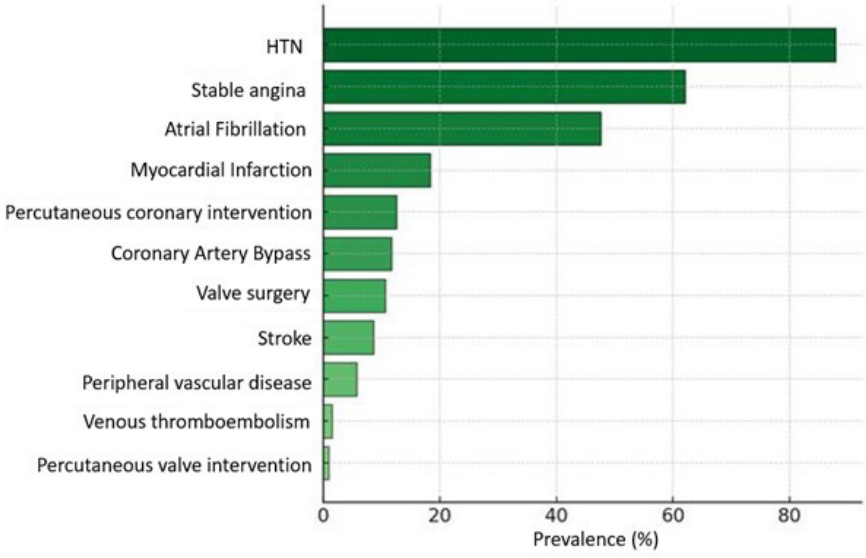

Fig. 2 Prevalence of cardiovascular comorbidities in patients with HFpEF

***Note:** Bars indicate the percentage (%) of patients presenting each comorbidity or with a history of cardiovascular procedures. HTN = hypertension; HFpEF = heart failure with preserved ejection fraction; PCI = percutaneous coronary intervention. Data are presented as percentages of the study population. Descriptive statistics are shown; no inferential statistical tests were applied.*

confirmed a phenotype marked by overweight/obesity: mean BMI 29.7 kg/m^2 (SD 5.1), median 29.22, IQR 26.23–32.74, with extremes ranging from 19.44–54.53 kg/m^2. Sex distribution, according to dataset coding, was approximately 44.2% male (91/206) and 55.8% female (115/206), showing a slightly female-predominant structure consistent with HFpEF epidemiology.

The etiologic profile was dominated by chronic coronary artery disease and pressure overload (Figure 1). "Undocumented coronary disease (no coronary angiography)" accounted for 32.0% (66/206), followed by "documented coronary disease" 29.1% (60/206), and "arterial hypertension (etiology)" 19.4% (40/206). "Significant valvular disease" occurred in 10.7% (22/206), while other causes (tachycardia-induced, hereditary/genetic, alcoholic, infectious, pulmonary hypertension without left heart disease, idiopathic dilated cardiomyopathy, other rare causes) individually accounted for ≤2.4%.

This distribution reinforces a phenotype in which "pressure–overload" mechanisms and chronic coronary disease are central, while acute myocardial necrosis is less frequent than in reduced ejection fraction phenotypes.

The hemodynamic stress biomarker showed an expected HFpEF profile. NT-proBNP had a mean of 1,388 pg/mL (SD 2,111), median 863.5 pg/mL, and IQR 562.5–1,454.5 pg/mL (n analyzed = 190, range 50–18,802 pg/mL), indicating elevated filling pressures and significant heterogeneity of load. Functionally, the NYHA distribution (Table 1) was centered on class III, with 88.8% (183/206) in class III and 11.2% (23/206) in class II, reflecting moderate functional limitation in the majority of HFpEF patients.

Table 1. Functional class (NYHA) distribution in HFpEF patients

NYHA class	N	%
II	23	11.2%
III	183	88.8%

***Note:** Data are presented as absolute numbers (N) and percentages (%). NYHA = New York Heart Association; HFpEF = heart failure with preserved ejection fraction. This table presents descriptive statistics only; no inferential statistical tests were applied.*

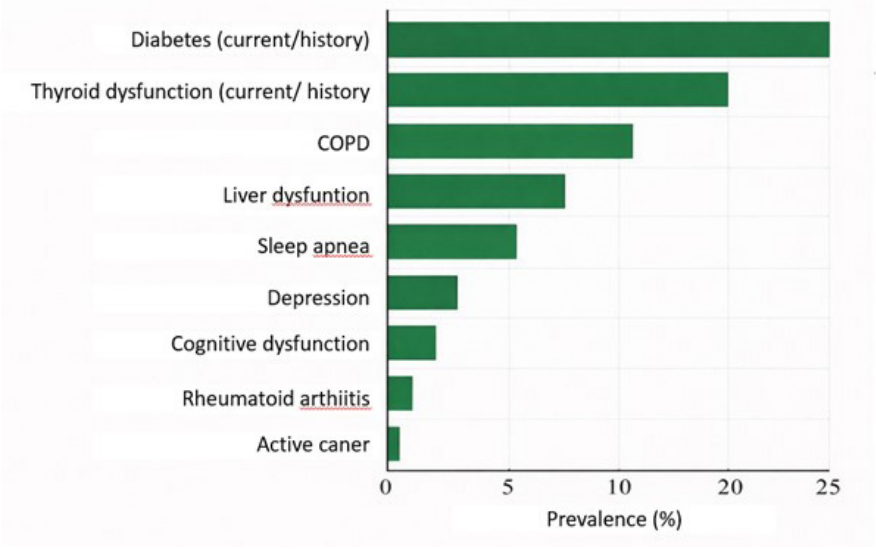

Fig. 3 Prevalence of non-cardiovascular comorbidities in patients with HFpEF

Note: *Data are presented as percentages of the total study population. Abbreviations: HFpEF = heart failure with preserved ejection fraction; COPD = chronic obstructive pulmonary disease. The figure presents descriptive statistics only; no inferential statistical tests were applied.*

Cardiovascular comorbidities reflected the pathophysiology of the phenotype (Figure 2). Arterial hypertension was quasi-ubiquitous at 87.9% (181/206), confirming the role of pressure overload in maintaining symptoms and decompensation. Chronic coronary artery disease was frequent (62.1%, stable angina 128/206), whereas myocardial infarction was less common (18.4%, 38/206), distinguishing HFpEF from the reduced ejection fraction phenotype. The burden of atrial fibrillation remained substantial at 47.6% (98/206), with direct implications for thromboembolic protection and rate/rhythm control. A history of revascularization was present in a relevant subgroup: PCI 12.6% (26/206) and bypass 11.7% (24/206), with treated valvular disease contributing additionally (surgery 10.7%, 22/206; percutaneous intervention 1.0%, 2/206). The extent of extra-coronary atherosclerosis was moderate, with peripheral vascular disease 5.8% (12/206), stroke/TIA 8.7% (18/206), and venous thromboembolism 1.5% (3/206); these values support the need for multi-territorial secondary prevention without altering the dominant pressure–overload profile.

Non-cardiovascular comorbidities completed a moderately metabolic–endocrine profile, with less systemic congestion than in HFrEF (Figure 3). Diabetes (current/history) was present in 26.2% (54/206), representing a transversal risk factor complicating vascular stiffness and diastolic function, justifying preferential use of cardiometabolic therapies with event-related benefits. COPD occurred in 9.2% (19/206), affecting dyspnea and exercise tolerance, requiring careful differentiation from congestion. Thyroid dysfunction (current/history) was reported in 9.2% (19/206), consistent with higher prevalence in patients with LVEF ≥40% and potentially affecting heart rate, arrhythmias, and symptom perception. Hepatic dysfunction was relatively rare at 3.9% (8/206), suggesting less systemic congestion than in reduced EF. Other comorbidities were infrequent: sleep apnea 2.4% (5/206), depression 1.9% (4/206), cognitive dysfunction 1.0% (2/206), active malignancy 0.5% (1/206), rheumatoid arthritis 0.5% (1/206), but remain relevant for adherence, quality of life, and care planning.

Overall, our typical HFpEF patient is middle-aged to elderly, slightly more often female, with a BMI ≈30 kg/m^2, predominantly hypertensive, chronic coronary disease in over half, atrial fibrillation in nearly half, diabetes in about a quarter, and NT-proBNP values indicative of elevated filling pressures. This profile calls for aggressive blood pressure control, systematic AF management (anticoagulation, rate/rhythm control), ischemic secondary prevention where indicated, cardiometabolic optimization (including weight loss and cardiovascular-beneficial therapies), screening for thyroid dysfunction and sleep apnea, and consistent lifestyle and adherence interventions.

Study limitations include the relatively small sample size and observational design, which precludes establishing causality. Additionally, biological variability of NT-proBNP and the absence of advanced imaging or functional assessments (e.g., strain echocardiography, invasive filling pressure measurements) may influence the interpretation of correlations between comorbidities and cardiac function.

Discussion

This study provides a detailed phenotypic characterization of HFpEF patients, emphasizing the central role of comorbidities in shaping functional status and clinical outcomes. The predominance of older women with elevated BMI highlights the typical demographic and metabolic profile associated with HFpEF, consistent with prior reports. Our findings reinforce the concept that chronic coronary disease and hypertension are key drivers of disease pathophysiology, contributing to diastolic dysfunction, elevated filling pressures, and exercise limitation.

The observed heterogeneity in NT-proBNP levels and functional NYHA classes underscores the complexity of HFpEF, where biomarker variability and multiple comorbidities influence symptom burden and prognosis.

Cardiovascular comorbidities such as atrial fibrillation, coronary artery disease, and hypertension, alongside

non-cardiovascular conditions including diabetes, COPD, and thyroid dysfunction, further modulate the clinical phenotype. These results align with recent cluster and phenotyping studies, emphasizing the importance of individualized evaluation and management.

Our study highlights practical implications for HFpEF management. Multidisciplinary approaches targeting blood pressure control, atrial fibrillation management, cardiometabolic optimization, and secondary prevention of ischemic events are critical. Screening for thyroid dysfunction, sleep apnea, and implementing lifestyle interventions can further improve functional capacity and quality of life. The identification of comorbidity clusters may guide precision therapy and optimize response to interventions, including aerobic exercise and pharmacologic strategies.

Study limitations include the relatively small sample size and the observational design, which preclude establishing causality. Furthermore, biological variability of NT-proBNP and the absence of advanced imaging or functional assessments (e.g., strain echocardiography, invasive measurements of ventricular filling pressures) may have influenced the interpretation of correlations between comorbidities and cardiac function. Selection bias is also possible, as patients were recruited from a single tertiary center.

Conclusions

This study shows that HFpEF is a complex and diverse condition influenced by many comorbidities. It highlights the need for a comprehensive, phenotype-based approach to care that combines comorbidity assessment, clinical evaluation, and biomarker analysis to personalize treatment. Identifying groups of comorbidities helps clinicians choose more targeted interventions and improve outcomes through a multidisciplinary approach, including cardiometabolic management, lifestyle changes, and screening for non-cardiac diseases. The findings support the move toward precision medicine in HFpEF, promoting individualized strategies to improve treatment response. Although causality cannot be confirmed, the study offers a useful framework for future research on phenotype-guided therapies and emphasizes the importance of personalized and integrated care to improve prognosis.

Competing interests

None declared.

Authors' contributions

The study was conceived and designed by C-PI. Data collection and analysis were performed by C-PI, SA, and RV. Drafting of the manuscript was done by C-PI and SA. All authors critically reviewed and approved the final version of the manuscript.

Ethics approval

The study protocol was approved by the institutional ethics committee (Approval No. 25/1, May 13, 2025). The study was conducted in accordance with the principles of the Declaration of Helsinki on biomedical research involving human subjects.

Informed consent

Obtained.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. McDonagh TA, Metra M, Adamo M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599-3726. doi: 10.1093/eurheartj/ehab368.
2. Li L, Li Z, Xu P, Huang L, Peng X, Yue J, Ge N. Comorbidities and incidence of heart failure with preserved ejection fraction: a systematic review and meta-analysis of cohort studies. BMJ Open. 2025;15(7):e093306. doi: 10.1136/bmjopen-2024-093306.
3. Ambardekar AV, Thielen SC. Comorbidities in heart failure: expect and embrace the complexity. J Am Coll Cardiol Heart Fail. 2023;11(11):1504-1506. doi: 10.1016/j.jchf.2023.06.025.
4. Wong B, Dodd JD, Gallagher J, Dyer B, Ryan C, McDonald K, Ledwidge M. Sex-related pathophysiological mechanisms may be present before symptoms of HFpEF develop. ESC Heart Fail. 2025;12(3):2387-2390. doi: 10.1002/ehf2.15228.
5. Morgen CS, Haase CL, Oral TK, Schnecke V, Varbo A, Borlaug BA. Obesity, cardiorenal comorbidities, and risk of hospitalization in patients with heart failure with preserved ejection fraction. Mayo Clin Proc. 2023 Oct;98(10):1458-1468. doi: 10.1016/j.mayocp.2023.07.008.
6. Szlagor M, Dybiec J, Młynarska E, Rysz J, Franczyk B. Chronic kidney disease as a comorbidity in heart failure. Int J Mol Sci. 2023;24(3):2988. doi: 10.3390/ijms24032988.
7. Kallistratos E, Konstantinidis D, Triantafillidi H, Grassos Ch, Maragkoudakis S, Bostanitis I, et al. Epidemiology and clinical characteristics of heart failure with preserved ejection fraction in hypertensive patients over 60 years old (HFpEF-Hypertension). J Hypertens. 2025 May;43(Suppl 1):e157. doi: 10.1097/01.hjh.0001116764.68064.09.
8. Hobbach AJ, Brix TJ, Weyer-Elberich V, Varghese J, Reinecke H, Linke WA. Obesity and comorbidities in HFpEF: a retrospective cohort analysis in a University Hospital Setting. J Clin Med. 2025 May 12;14(10):3348. doi: 10.3390/jcm14103348.
9. Savic L, Mrdovic I, Asanin M, Stankovic S, Lasica R, Matic D, Simic D, Krljanac G. Prognostic impact of non-cardiac comorbidities on long-term prognosis in patients with reduced and preserved ejection fraction following acute myocardial infarction. J Pers Med. 2023;13(7):1110. doi: 10.3390/jpm13071110.
10. Martens P, Augusto SN Jr, Finet JE, Tang WHW. Distinct impact of noncardiac comorbidities on exercise capacity and functional status in chronic heart fail-

ure. JACC Heart Fail. 2023;11(10):1365-1376. doi: 10.1016/j.jchf.2023.05.018.
11. Ono R, Falcão LM. Primary heart failure with preserved ejection fraction: the intertwined pathophysiology and treatment of some of the most relevant phenotypes. Rev Port Cardiol. 2025 Jul;44(7):445-456. doi: 10.1016/j.repc.2025.02.007.
12. Savarese G, Settergren C, Schrage B, Thorvaldsen T, Löfman I, Sartipy U, et al. Comorbidities, quality of life and cause-specific outcomes in heart failure across the ejection fraction spectrum: a blueprint for clinical trial design. Int J Cardiol. 2020;313:76-82. doi: 10.1016/j.ijcard.2020.04.068.
13. Menghoum N, Badii MC, Leroy M, Parra M, Roy C, Lejeune S, et al. Exploring the impact of metabolic comorbidities on epicardial adipose tissue in heart failure with preserved ejection fraction. Cardiovasc Diabetol. 2025;24(1):134. doi: 10.1186/s12933-025-02688-7.
14. Zatsarinnyy A, Miyerbekov Y, Uvaliyeva S, Sugurbayev M, Musrepov N, Rakisheva A. Pathophysiology and phenotypes of heart failure: current concepts and future directions. Heart Fail J India. 2025;3(2):124. doi: 10.4103/HFJI.HFJI_19_25.
15. Lanzer JD, Valdeolivas A, Pepin M, Hund H, Backs J, Frey N, et al. A network medicine approach to study comorbidities in heart failure with preserved ejection fraction. BMC Med. 2023 Jul 24;21(1):267. doi: 10.1186/s12916-023-02922-7.
16. Cabac-Pogorevici I, Revenco V. Heart failure and central sleep apnea in the era of implantable recorders. Anatol J Cardiol. 2021;25(4):216-224. doi: 10.5152/AnatolJCardiol.2021.63668.
17. Jitari I, Savca D, Ochişor V, Revenco V, Cabac-Pogorevici I. Abordarea individualizată în insuficiența cardiacă cu fracţia de ejecţie păstrată – de la practica clinică la inteligenţă artificială [The individualized approach in heart failure with preserved ejection fraction. From clinical practice to Artificial Intelligence]. Bull Acad Sci Mold. Med Sci (Chisinau). 2024;78(1):205-211. Romanian. doi: 10.52692/1857-0011.2024.1-78.22.

https://doi.org/10.52645/MJHS.2026.2.02

UDC: 616.131-005.6/.7-07

RESEARCH ARTICLE

Correlation between inflammatory hematological indices with severity of pulmonary thromboembolism

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ABSTRACT

Introduction. Pulmonary thromboembolism (PTE) is a major cardiovascular emergency associated with significant mortality. Systemic inflammation contributes to the pathogenesis of thrombosis and to disease severity, and hematological indices derived from the complete blood count, such as the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), have been proposed as prognostic predictors.

Materials and methods. A prospective observational study was conducted on a cohort of 182 consecutively investigated patients at *Holy Trinity* Municipal Clinical Hospital and the Institute of Cardiology. The diagnosis of PTE was confirmed by CT pulmonary angiography. Clinical data, inflammatory hematological indices, echocardiographic parameters, and severity scores were analyzed during the course of inpatient care. The prognostic value of NLR and PLR was assessed using ROC curve analysis.

Results. Pulmonary thromboembolism was confirmed in 153 patients (84.1%, 95% CI [78.7, 89.4]). Elevated NLR was identified in 82 patients (45.1%; 95% CI [37.8, 52.3]), and elevated PLR in 89 patients (48.9%; 95% CI [41.6, 56.2]). Overall mortality was 17.0% (95% CI [11.6, 22.5]) (31 patients). Elevated NLR was present in 26 (14.3; 95% CI [9.2, 19.4]) of deceased patients ($p<0.00001$), while elevated PLR was present in 22 (12.1%; 95% CI [7.4, 16.8]) of deceased patients ($p = 0.012$). ROC analysis demonstrated that NLR has a very good predictive ability for mortality (AUC = 0.799), whereas PLR has good predictive ability (AUC = 0.715). Additionally, NLR was significantly correlated with severity according to the PESI score (AUC = 0.614; $p = 0.0048$). Echocardiography revealed right ventricular dysfunction in 80.2% (95% CI [74.4, 86.0]) of patients and reduced TAPSE in 57.7% (95% CI [50.5, 64.9]) of patients.

Conclusions. The neutrophil-to-lymphocyte ratio is an important prognostic marker of severity and mortality in pulmonary thromboembolism, with a predictive value superior to that of the platelet-to-lymphocyte ratio. Integrating inflammatory hematological indices with clinical scores and imaging assessment may improve risk stratification and the management of patients with pulmonary thromboembolism.

Keywords: neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, pulmonary thromboembolism, mortality, prognosis.

Cite this article: Ranga D, Talmaci C, Matcovschi S, Caproș N, Grib L, Cealan A. Correlation between inflammatory hematological indices with severity of pulmonary thromboembolism. Mold J Health Sci. 2026;13(2):10-14. https://doi.org/10.52645/MJHS.2026.2.02

Manuscript received: 09.03.2025

Accepted for publication: 21.05.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Interest in easily accessible inflammatory hematological indices, such as the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), has increased considerably in the evaluation of pulmonary thromboembolism. However, the correlation between these hematological indices and disease severity, as well as their clinical relevance, remains insufficiently clarified, particularly in the population of the Republic of Moldova.

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The research hypothesis

Inflammatory hematological indices derived from the complete blood count, namely NLR and PLR, are associated with disease severity and mortality in patients with pulmonary thromboembolism and may serve as prognostic predictors.

The novelty added by the manuscript to the already published scientific literature

This study provides original data on the prognostic value of the hematological indices NLR and PLR in pulmonary thromboembolism and demonstrates their association with disease severity and mortality, which remain insufficiently investigated in the population of the Republic of Moldova.

Introduction

Pulmonary thromboembolism (PTE) is a major cardiovascular condition associated with high mortality, particularly in patients with hemodynamic instability [1]. Systemic inflammation plays a key role in the pathogenesis of thrombosis and disease progression, and inflammatory hematological indices derived from the complete blood count, such as the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), have been identified as predictors of disease severity and mortality [2], although evidence for PLR remains limited, with some studies failing to demonstrate an independent prognostic role [3]. The purpose of the study was to evaluate the correlation of NLR and PLR with CT pulmonary angiography findings, echocardiographic parameters, Pulmonary Embolism Severity Index (PESI) severity score, and mortality in patients with pulmonary thromboembolism.

Materials and methods

This prospective observational study was conducted on a cohort of 182 consecutive patients admitted with clinical suspicion of PTE to two hospital institutions between 2021 and 2025: *Holy Trinity* Municipal Clinical Hospital and the Institute of Cardiology.

Participant Selection. The study included adult patients (≥18 years) with clinical suspicion of acute PTE. The diagnosis was confirmed by CT pulmonary angiography (CTPA), which is considered the standard of care for this condition and allows exclusion of relevant differential diagnoses [1, 4].

For each patient, demographic data (age, sex), clinical presentation at admission, comorbidities, laboratory parameters, and imaging findings were collected. Laboratory analyses included a complete blood count, from which the NLR and PLR were calculated, as well as D-dimer and NT-proBNP levels, Wells score, revised Geneva score. All patients underwent echocardiographic evaluation, including assessment of right ventricular function parameters such as right ventricular diameter and tricuspid annular plane systolic excursion (TAPSE), and TAPSE/PSAP index. Disease severity was assessed using the Pulmonary Embolism Severity Index (PESI) score. Patients were followed up for clinical outcomes and mortality during the course of inpatient care. Exclusion criteria were: patients with symptoms attributable to other acute conditions, such as acute or chronic coronary syndrome, dissecting aortic aneurysm, acute abdomen, or acute heart or respiratory failure, as well as patients who used drugs that may affect laboratory parameters (steroids, antibiotics, cytotoxic agents, immunosuppressants, antiplatelet drugs, anticoagulants, epinephrine).

Statistical analysis. Categorical variables were summarized as frequencies and percentages. The predictive value of NLR and PLR for mortality was assessed using Receiver Operating Characteristic (ROC) curve analysis, calculating the area under the curve (AUC), sensitivity, and specificity. A p-value <0.05 was considered statistically significant.

Results

The prospective study included a total of 182 patients with clinical suspicion of PTE, of whom 99 were men (54.4%; 95% CI [47.2, 61.6]) and 83 were women (45.6%; 95% CI [38.4, 52.8]). Age distribution showed a predominance of elderly patients, with the most frequent age group being 60–69 years, comprising 64 cases (35.2%; 95% CI [28.2, 42.1]), followed by the 70–79 years group with 48 patients (26.4%; 95% CI [20.0, 32.8]), 50–59 years with 27 patients (14.8%; 95% CI [9.7, 20.0]), patients over 80 years with 16 cases (8.8%; 95% CI [4.7, 12.9]), 40–49 years with 18 cases (9.9%; 95% CI [5.6, 14.2]), and patients under 40 years, who accounted for 9 cases (4.9%; 95% CI [1.8, 8.1]).

Clinically, dyspnea was the main symptom present in 181 patients (99.5%; 95% CI [98.4, 100]). Other manifestations included cough in 68 patients (37.4%; 95% CI [30.3, 44.4]), fever in 37 patients (20.3%; 95% CI [14.5, 26.2]), syncope or presyncope in 10 patients (5.5%; 95% CI [2.2, 8.8]) and hemoptysis in 4 patients (2.2%; 95% CI [0.1, 4.3]).

Concomitant pathologies were common, the most frequent being angina pectoris in 149 patients (81.9%; 95% CI [76.3, 87.5]), arterial hypertension in 141 patients (77.5%; 95% CI [71.4, 83.5]), pneumonia in 65 patients (35.7%; 95% CI [28.8, 42.7]), arrhythmias in 60 patients (33.0%; 95% CI [26.1, 39.8]), diabetes mellitus in 34 patients (18.7%; 95% CI [13.0, 24.3]), and chronic obstructive pulmonary disease in 32 patients (17.6%; 95% CI [12.1, 23.1]).

Laboratory parameters revealed elevated D-dimer levels in 181 patients (99.5%), increased NT-proBNP in 33.0% of cases (95% CI [26.1, 39.8]), and elevated C-reactive protein in 149 patients (81.9%; 95% CI [76.3, 87.5]). The neutrophil-to-lymphocyte ratio (NLR) was elevated in 82 patients (45.1%; 95% CI [37.8, 52.3]), and the platelet-to-lymphocyte ratio (PLR) was elevated in 89 patients (48.9%; 95% CI [41.6, 56.2]). The overall mortality was 17.0% (95% CI [11.6, 22.5]), corresponding to 31 patients. Among these, 26 patients (14.3%; 95% CI [9.2, 19.4]) had elevated NLR, and 22 patients (12.1%; 95% CI [7.4, 16.8]) had elevated PLR, demonstrating a significant association between inflammatory hematological indices and mortality.

According to the Wells score, intermediate clinical probability was present in 129 patients (70.9%; 95% CI [64.3, 77.5]), and high probability in 52 patients (28.6%; 95% CI [22.0, 35.1]). The revised Geneva score indicated intermediate probability in 118 patients (64.8%; 95% CI [57.9, 71.8]) and high probability in 52 patients (28.6%; 95% CI [22.0, 35.1]).

Echocardiographic evaluation revealed significant right ventricular alterations. Right ventricular dysfunction was present in 146 patients (80.2%; 95% CI [74.4, 86.0]), and TAPSE was reduced in 105 patients (57.7%; 95% CI [50.5, 64.9]). Assessment of right ventriculo-arterial coupling, expressed as the TAPSE/PASP ratio, showed normal values in 41 patients (22.5%; 95% CI [16.5, 28.6]), mildly reduced values in 44 patients (24.2%; 95% CI [18.0, 30.4]), and reduced values in 97 patients (53.3%; 95% CI [46.0, 60.5]), indicating severe right ventricular dysfunction in more than half of the patients.

The diagnosis of PTE was confirmed by CTPA in 153 patients (84.1%; 95% CI [78.7, 89.4]) while in 29 patients (15.9%; 95% CI [10.6, 21.3]) the diagnosis was not confirmed.

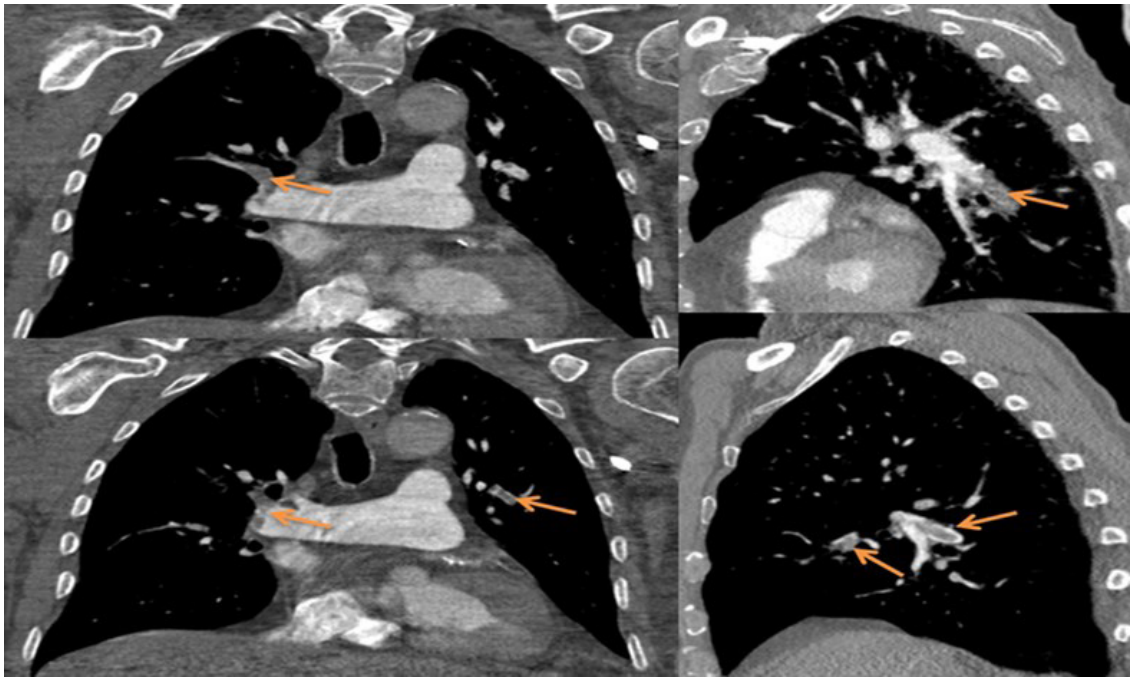

Fig. 1 Chest and mediastinal CT pulmonary angiography.

Note: *After intravenous contrast administration, filling defects are observed in the right middle and lower lobe pulmonary arteries, associated with areas of pulmonary consolidation containing air bronchograms, suggestive of pulmonary infarction with abscess formation on the right. A right-sided pleural effusion with air-fluid levels is also present, consistent with pleural empyema. Pulmonary hypertension is indicated by a pulmonary trunk diameter of 3.2 cm.*

Among patients with confirmed PTE (Figure 1), bilateral filling defects were identified in 123 patients (67.6%; 95% CI [60.8, 74.4]), central-peripheral location in 114 patients (62.6%; 95% CI [55.6, 69.7]), peripheral location in 38 patients (20.9%; 95% CI [15.0, 26.8]), involvement of the right pulmonary artery in 22 patients (12.1%; 95% CI [7.4, 16.8]), and the left pulmonary artery in 8 patients (4.4%; 95% CI [1.4, 7.4]). Signs of right ventricular dilatation on CTPA were present in 77 patients (42.3%; 95% CI [35.1, 49.5]).

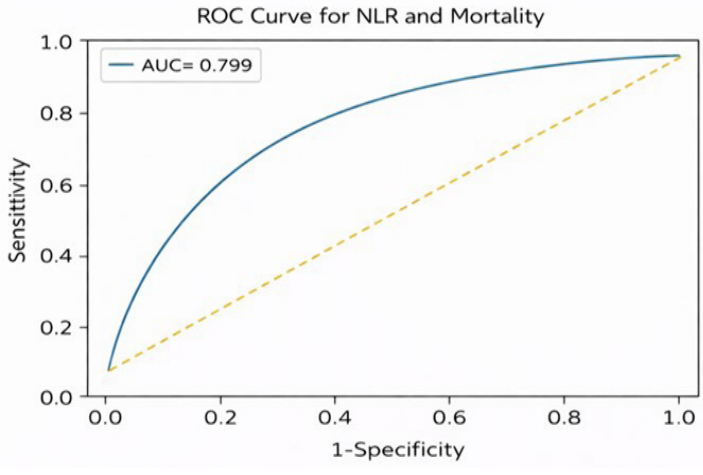

Fig. 2. ROC curve for NLR predicting mortality.

Note: *Receiver operating characteristic (ROC) curve demonstrating the predictive performance of the neutrophil-to-lymphocyte ratio (NLR) for mortality in patients with pulmonary embolism. The area under the curve (AUC) was 0.799. The dashed line represents the line of no discrimination.*

ROC curve analysis demonstrated that the neutrophil-to-lymphocyte ratio (NLR) had the highest predictive value for mortality, with an AUC of 0.799, indicating very good prognostic performance (Figure 2).

The platelet-to-lymphocyte ratio (PLR) showed good predictive value, with an AUC of 0.715 (Figure 3), and NLR correlated with disease severity assessed by the PESI score, with an AUC of 0.614 (Figure 4).

Assessment of disease severity using the PESI score showed a predominance of patients with intermediate and high risk, comprising 104 patients (57.1%; 95% CI [50.0, 64.3]).

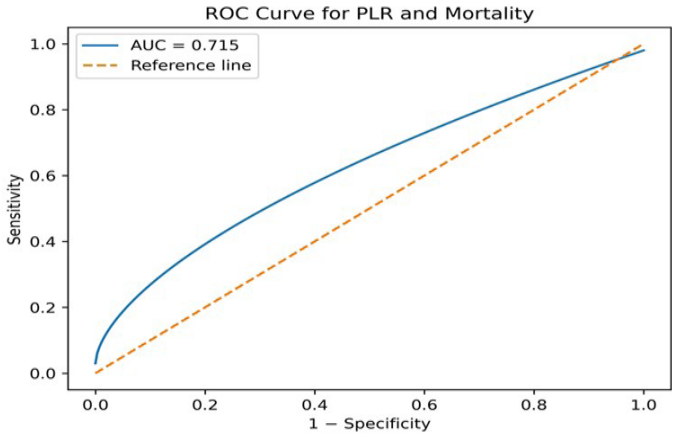

Fig. 3 ROC curve for PLR predicting mortality.

Note: *Receiver operating characteristic (ROC) curve demonstrating the predictive performance of the platelet-to-lymphocyte ratio (PLR) for mortality in patients with pulmonary embolism. The area under the curve (AUC) was 0.715. The dashed line represents the reference line.*

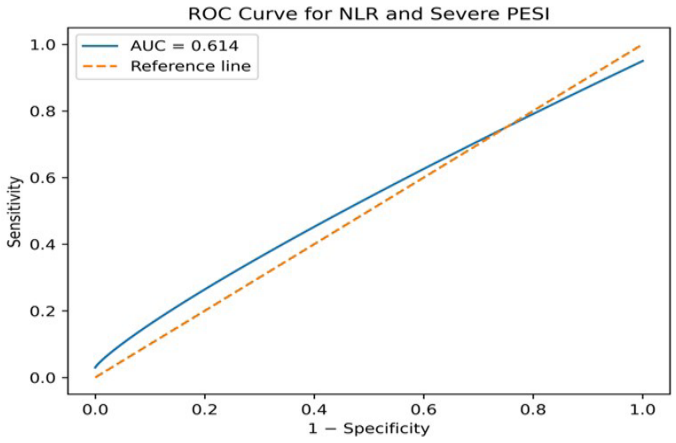

Fig. 4 ROC curve for NLR predicting severe PESI.

Note: *Receiver operating characteristic (ROC) curve showing the predictive value of the neutrophil-to-lymphocyte ratio (NLR) for severe pulmonary embolism assessed by the Pulmonary Embolism Severity Index (PESI). The area under the curve (AUC) was 0.614. The dashed line represents the reference line (line of no discrimination).*

Discussion

In our study, the diagnosis of pulmonary thromboembolism was confirmed by CT pulmonary angiography, which remains the *gold standard* for identifying arterial filling defects and assessing disease severity, with high sensitivity and specificity, as previously reported, including in studies conducted at our institution [1].

The results of our study demonstrated that the NLR is an important predictor of mortality in patients with pulmonary thromboembolism, with an AUC of 0.799. These findings are consistent with those reported by Tang et al., who found similar predictive value for NLR, confirming the role of systemic inflammation in disease progression [3]. Elevated NLR reflects activation of the inflammatory response and has been associated with right ventricular dysfunction and poor prognosis, data consistent with the findings of Kowsika R. et al [2].

Elevated NLR in acute PE was associated with a worse short-term and long-term prognosis and with a longer duration of hospitalization in a study by Efros O. et al [5]. In a meta-analysis by Wang Q, was revealed that NLR and PLR are promising biomarkers in predicting prognosis in acute PE patients and recommended NLR and PLR be used routinely in the PE prognostic assessment [6].

Severity assessed by the PESI score correlated with elevated NLR values, confirming the results reported by Teodoru M. et al., who showed that inflammatory hematological indices can improve risk stratification in patients with pulmonary thromboembolism [7].

Conclusions

The neutrophil-to-lymphocyte ratio is an important prognostic marker of severity and mortality in pulmonary thromboembolism, with a predictive value superior to that of the platelet-to-lymphocyte ratio. Integrating inflammatory hematological indices with clinical scores and imaging assessment may improve risk stratification and the management of patients with pulmonary thromboembolism.

Competing interests

None declared.

Authors' contributions

DR conceived the study design and performed the statistical analysis. CT participated in diagnosing and treating patients with pulmonary thromboembolism. SM critically reviewed the manuscript and contributed to its intellectual content. NC participated in the study design and critically reviewed the manuscript. LG provided clinical expertise and consultation for the management of most patients. CA radiologist, performed CT, pulmonary angiography in the majority of patients. All authors have read and approved the final version of the manuscript.

Ethics approval

The study protocol was reviewed and approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of

Moldova (Approval No. 2/25; March 30, 2023). All procedures were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Patient consent

Obtained.

Acknowledgements and funding

No external funding.

Provenance and peer review

Not commissioned, externally peer reviewed.

References

1. Konstantinides SV, Meyer G, Becattini C, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). Eur Heart J. 2020;41(4):543-603. doi: 10.1093/eurheartj/ehz405.
2. Kowsika R, Muthukumaran L, Sridhar R et al. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as biomarkers for risk stratification and predictors of 90-day mortality in acute pulmonary embolism. South East Eur J Pub Health. 2025;26(Suppl 1):6009-6018.
3. Tang S, Hu Y. Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio as predictors of mortality in acute pulmonary embolism: a systematic review and meta-analysis. Pak J Med Sci. 2024 Jul;40(6):1274-1279. doi: 10.12669/pjms.40.6.8802.
4. Ranga D, Caproş N, Cealan A, Sirbu I, Talmaci C, Matcovschi S. Diagnosing pulmonary embolism with computed tomography pulmonary angiography. In: Sontea V, Tiginyanu I, Railean S, editors. IFMBE-2023: 6th International Conference on Nanotechnologies and Biomedical Engineering, 20-23 September 2023, Chişinău, Republic Moldova: Abstract book. Chişinău: Springer; 2024. Vol.92, p. 333-342. ISBN 978-303142781-7. https://doi.org/10.1007/978-3-031-42782-4_36.
5. Efros O, Beit Halevi T, Meisel E, et al. The prognostic role of neutrophil-to-lymphocyte ratio in patients hospitalized with acute pulmonary embolism. J Clin Med. 2021 Sep 8;10(18):4058. doi: 10.3390/jcm10184058.
6. Wang Q, Ma J, Jiang Z, Ming L. Et al. Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in acute pulmonary embolism: a systematic review and meta-analysis. Int Angiol. 2018 Feb;37(1):4-11. doi: 10.23736/S0392-9590.17.03848-2.
7. Teodoru M, Negrea MO, Cozgarea A, et al. Enhancing pulmonary embolism mortality risk stratification using machine learning: the role of the neutrophil-to-lymphocyte ratio. J Clin Med. 2024 Feb 20;13(5):1191. doi: 10.3390/jcm13051191.

https://doi.org/10.52645/MJHS.2026.2.03

UDC: 616.24-002.5-092-078

RESEARCH ARTICLE

Evaluation of oxidant and antioxidant system markers in patients with pulmonary tuberculosis before and after hospital treatment

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ABSTRACT

Introduction. Pulmonary tuberculosis remains a major cause of morbidity and mortality worldwide. According to data published by the World Health Organization in 2024, a total of 8.2 million people were newly diagnosed with TB in 2023, compared with 7.5 million in 2022, 7.1 million in 2019, and markedly higher than the 5.8 million and 6.4 million in 2020 and 2021, respectively.

Materials and methods. The prospective study involved 59 participants before and after treatment: 11 women (18.6%) and 48 men (81.4%). The participants were divided into 2 groups: group L_1 – patients with tuberculosis before treatment, and group L_2 – patients with tuberculosis after treatment. Serum levels of nitric oxide, malondialdehyde, glutathione reductase, and total antioxidant activity were measured using a spectrophotometric method.

Results. In the study, we demonstrated that nitric oxide and malondialdehyde serum concentrations were non-significantly higher in the L_2 group compared with the L_1 group. Glutathione reductase activity showed a significant decrease in antioxidant activity in the L_2 group, indicating reduced antioxidant capacity. Total antioxidant activity showed a non-significant decrease in the L_1 group compared with the L_2 group.

Conclusions. The results of the research demonstrated that the administered anti-tuberculosis treatment increased nitric oxide and malondialdehyde levels, and reduced glutathione reductase and total antioxidant activity. This phenomenon indicates the persistence of oxidative stress even after treatment. The levels of nitric oxide, malondialdehyde, glutathione reductase, and total antioxidant activity in patients with pulmonary tuberculosis may serve as biomarkers for monitoring disease progression.

Keywords: tuberculosis, oxidative stress, antioxidant, nitric oxide, malondialdehyde, glutathione reductase.

Cite this article: Reabiseva M, Pantea V, Visnevschi A. Evaluation of oxidant and antioxidant system markers in serum of patients with pulmonary tuberculosis. Mold J Health Sci. 2026;13(2): 15-19. https://doi.org/10.52645/MJHS.2026.2.03.

Manuscript received: 19.06.2025

Accepted for publication: 17.02.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The study demonstrated the involvement of pro- and antioxidant systems in the pathogenesis of pulmonary tuberculosis before and after hospital treatment, although this role is still not fully elucidated.

The research hypothesis

Pulmonary tuberculosis is associated with the amplification of oxidative stress and the reduction of antioxidant activity.

The novelty added by the manuscript to the already published scientific literature

For the first time, data will be obtained on laboratory manifestations of pro- and antioxidant activity in patients with pulmonary tuberculosis before and after hospital treatment. The study ad-

dressed an important issue, as applied research on the role of antioxidant status and oxidative stress markers in the course of tuberculosis, both before and after treatment, may prove useful in assessing disease progression and predicting future prognosis.

Introduction

Tuberculosis (TB) remains a major public health problem, with approximately 10 million new cases and 1.8 million deaths recorded each year worldwide.

According to data provided by the World Health Organization (WHO), the Republic of Moldova is among the 18 countries in the European region where tuberculosis control is a priority, as well as one of the 27 countries globally with a high incidence of multidrug-resistant tuberculosis (MDR-TB). According to WHO Pulmonary Tuberculosis Report 2024, 8.2 million people were newly diagnosed with TB in 2023, up from 7.5 million in 2022 and 7.1 million in 2019, and well above the 5.8 million and 6.4 million cases reported in 2020 and 2021, respectively [1]. These data indicate that TB prevention and control efforts worldwide remain insufficiently effective.

Mycobacterium tuberculosis can induce the production of reactive oxygen species (ROS) by activating both mononuclear and polymorphonuclear phagocytes, which possess antimicrobial activity. The increased level of free radical production, although intended to combat the pathogen, also has the potential to damage lung tissue. Normally, such tissue damage is limited by the host's enhanced antioxidant defense systems [1, 2]. However, weak antioxidant defenses have also been reported, which may expose the lung tissue of patients with pulmonary tuberculosis to oxidative damage [3, 4].

The oxidant-antioxidant balance is essential for maintaining lung function. Both an increase and a decrease in antioxidants can disrupt the physiological oxidant-antioxidant balance in favor of oxidants, leading to lung injury. Recent research suggests that oxygen and its related species (oxidants) may contribute to the pathogenesis of a considerable number of lung diseases. Increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurs in pulmonary tuberculosis and causes the phagocyte respiratory burst. Research results confirm that increased levels of circulating free radical activity are found in the pathogenesis of active pulmonary tuberculosis and, therefore, play an important role in the resulting fibrosis [5].

Mycobacterium tuberculosis is an intracellular pathogen that grows and replicates in host macrophages. It is well known that macrophages undergo a respiratory burst upon contact with this microorganism. These cells can generate huge amounts of reactive oxygen species (ROS), and ROS induce lipid peroxidation (LP), a chain reaction that affects unsaturated fatty acids located mainly in cell membranes, generating the end product malondialdehyde (MDA), which is responsible for some of the damaging effects of free radicals on DNA and cell membranes [6].

Lipid peroxidation products diffuse from the site of inflammation into the circulation and can be measured in blood samples. MDA is a three-carbon marker of oxidative stress–a low-molecular-weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids in biological membranes. The determination of MDA is used to monitor lipid peroxidation in biological samples. Although the concentrations of plasma antioxidant components can be measured individually, this process is time-consuming and involves additional costs. A more comprehensive assessment of the body's redox status can be achieved by measuring total antioxidant activity (TAA).

Recent studies further indicate that circulating markers of oxidative stress, such as nitric oxide (NO), are elevated in patients with pulmonary tuberculosis, whereas antioxidant parameters such as glutathione reductase (GR) activity are decreased. This imbalance between oxidants and antioxidants may contribute to the development of pulmonary function abnormalities and disease progression [7].

Materials and methods

The prospective observational study was conducted between 2019 and 2024 at the *Chiril Draganiuc* Institute of Pneumology in Chişinău and was approved by decision No. 31 of 18.05.2019, issued by the Research Ethics Committee of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy.

A total of 59 patients with newly diagnosed pulmonary tuberculosis were enrolled. The study population included 11 women (18.6%) and 48 men (81.4%). The participants were divided into 2 groups: group L_1 – patients with TB before treatment, and group L_2 – patients with TB after inpatient treatment, according to individualized plans and assessed after one month of hospitalization. The levels of MDA, NO, TAA, and GR were measured in blood serum using a spectrophotometric method adapted for use with a Synergy H1 Hybrid Reader (BioTek Instruments, USA) and multimodal plates.

Nitric oxide dosage was performed according to the method described by Metelskaya V. and Gumanova N. [8]. The principle of the method involves deproteinization of the biological material, reduction of nitrates to nitrites, treatment of the supernatant with Griess reagent, and subsequent measurement of the optical density of the reaction product.

Determination of malondialdehyde, the final product of lipid peroxidation, was carried out according to the procedure described by Galaktionova L. and co-authors [9]. The method is based on the spectrophotometric identification of the colored trimethine complex formed by the interaction of thiobarbituric acid with MDA. The MDA concentration in

the analyzed sample is directly proportional to the color intensity, and the final result was expressed in μM/L in the studied biological serum.

Total antioxidant activity was assessed using the methodology described by Zhang M., adapted to the Synergy H1 Hybrid Reader multimodal plate assay (BioTek Instruments, USA) [10].

The activity of glutathione reductase (EC 1.6.4.2) was determined by the method described by Vlasova S. and co-authors [11], based on the Warburg optical test. The principle of the method is the measurement of NADPH consumption, used by GR to reduce GSSG, observed by the decrease in absorbance at 340 nm.

R Studio and Python programs were used for data analysis. For each parameter, the mean, standard deviation (SD), and the confidence interval (CI) were calculated. To compare the two groups, the non-parametric Mann-Whitney U test was applied, and the statistical significance of the differences was assessed using the p-value (considered significant at $p < 0.05$).

Sample and data collection procedure. All eligible study participants completed a questionnaire designed to obtain demographic information such as age and gender. A volume of 10 ml of venous blood was collected from the antecubital vein of the arm of each consenting participant. The test tubes for sample collection were labeled with a unique identification code for each participant. After 15 minutes–the time required for blood coagulation–the serum was immediately separated by centrifugation at 3000 rpm for 10 minutes. Subsequently, the obtained serum was transferred into sterile Eppendorf tubes. The serum samples were then preserved by freezing at a temperature of minus 40°C until the time of analysis.

Results

Based on demographic characteristics, a similar gender distribution in pulmonary tuberculosis before and after treatment was found in both groups, with a predominance of males – 48 participants (81.4%) compared to 11 females (18.6%), $p = 0.094$. This comparable distribution between the groups allowed for a relevant comparison.

The age difference between the two groups was found to be statistically significant (Wilcoxon rank-sum test = 2452.5, $p < 0.001$). In order to evaluate the practical significance of this finding, the rank biserial correlation coefficient was calculated, indicating a moderate effect size with a value of 0.41 (95% CI: 0.22–0.57). Therefore, the age difference, with higher values in the L_1 group, is not only statistically significant but also clinically relevant, potentially impacting the values of the analyzed indicators.

Serum malondialdehyde (MDA) concentrations were higher in the L_2 group (24.0 ± 8.3 μM/L) compared with the L_1 group, where values were 18.6 ± 5.2 μM/L, but the difference was not statistically significant ($p = 0.40$). Serum nitric oxide (NO) levels were significantly higher in the L_2 group, with a mean of 107.4 ± 99.7 μM/L versus 92.6 ± 43.5 μM/L ($p = 0.010$) in the L_1 group (Table 1).

Table 1. Pro-oxidant system indicators

Group	L_1n = 59	95% CI	L_2n = 59	95% CI	p
MDA (malondialdehyde), μM/L	18.6 ± 5.2	17–20	24.0 ± 8.3	22–26	= 0.40
NO (nitric oxide), μM/L	92.6 ± 43.5	81–104	107.4 ± 99.7	81–133	= 0.010*

Note: *The data presented include mean (standard deviation), 95% confidence interval, and statistically significant difference with the control group; L_1 – patients with TB before treatment; L_2 – patients with TB after inpatient treatment; CI – confidence interval; * – p<0.05; ** – p<0.01; *** – p<0.001; MDA – malondialdehyde; NO – nitric oxide.*

Serum total antioxidant activity (TAA) showed a slight, non-significant decrease in the L_2 group (3.6 ± 0.5 u/c) compared with the L_1 group (4.0 ± 1.0 u/c, $p > 0.90$). Conversely, serum glutathione reductase (GR) activity was significantly reduced in the L_2 group (298.2 ± 169.2 nM/s·L) compared with the L_1 group (704.6 ± 1,529.7 nM/s·L, $p < 0.001$) (Table 2).

Table 2. Antioxidant system indicators

Group	L_1n = 59	95% CI	L_2n = 59	95% CI	p
TAA (total antioxidant activity), u/c	4.0 ± 1.0	3.7–4.3	3.6 ± 0.5	3.7–4.0	>0.9
GR (glutathione reductase) nM/s·L	704.6 ± 1,529.7	306–1,103	298.2 ± 169.2	254–342	<0.001*

Note: *The data presented include mean (standard deviation), 95% confidence interval, and statistically significant difference with the control group; L_1 – patients with TB before treatment; L_2 – patients with TB after inpatient treatment; CI - confidence interval; * – p<0.05; ** – p<0.01; *** – p<0.001; TAA – total antioxidant activity; GR – glutathione reductase.*

Discussion

The study analyzed oxidative and antioxidative parameters in patients diagnosed with pulmonary tuberculosis before and after hospital treatment. Oxidative stress occurs as a result of increased production of reactive oxygen species during the respiratory burst, concomitantly with a decrease in the effects of antioxidants [12, 13]. This imbalance disrupts normal lung function and host immune responses, contributing to the development of the disease through the body's inability to effectively eliminate oxidative stress and causing pulmonary dysfunction [13, 14]. Elevated MDA and NO values in the serum of patients after anti-tuberculosis treatment indicate a high level of lipid peroxidation and an imbalance of redox homeostasis [15]. According to data from the specialized literature, the high levels of MDA and NO persist even after the end of treatment. Circulating antioxidants, such as total TAA and GR mentioned in this study, appear to be insufficient to counterbalance oxidative stress in pulmonary tuberculosis, and the accumulation of free radicals in lung tissue leads to the attack of cell membrane lipids and lipid peroxidation [13, 16]. Thus, the existence of a deficiency in the antioxidant system at the alveolar level in pulmonary tuberculosis is confirmed. A defining feature of the evolution of this condition is the association between hypoxia, oxidative stress, and inflammation.

Regarding the distribution of patients by gender and age, there was a predominance of men compared with women. In both studied groups, the predominance of patients aged between 18 and 55 created optimal conditions for comparability of the results obtained.

The results of the study highlight the important role of glutathione reductase in both innate and cellular immunity against tuberculosis infection. Oxidative stress related to inflammation has been implicated in the pathogenesis of fibrosis and lung dysfunction in patients with pulmonary tuberculosis [17,18]. The specialized literature reports that several circulating markers of free radical activity are elevated in patients with pulmonary tuberculosis, and some of these markers remain elevated even after the completion of anti-tuberculosis therapy, indicating ongoing oxidative stress that may contribute to the decrease in GR levels [19].

Finally, nutritional status directly influences the health and proper functioning of all body systems, including the immune system, which plays an essential role in protecting against infectious diseases, such as pulmonary tuberculosis. Considering that cellular immunity is the body's main line of defense against tuberculosis, malnutrition becomes a significant risk factor for the onset and progression of this disease.

Conclusions

As a result of the analysis of oxidative system biomarkers, such as NO and MDA, it was found that after the administration of anti-tuberculosis treatment, the levels of NO and MDA increased, while the values of TAA and GR decreased, indicating the persistence of oxidative stress even after treatment. These biomarkers provide important information both before and after the initiation of standard therapy. They are necessary for evaluating treatment efficacy, as well as for the development of new therapeutic strategies that include antioxidants, and can also be used to monitor the evolution of the disease and lung lesions.

The presence of elevated oxidative biomarkers and decreased antioxidant parameters after anti-tuberculosis treatment indicates that oxidative stress remains active in the body, which may negatively influence pulmonary recovery. Monitoring these markers may be essential for optimizing therapy and may pave the way for the integration of antioxidants into tuberculosis treatment strategies in the future.

Competing interests

None declared.

Authors' contributions

All the authors participated in the study design and contributed to drafting the manuscript. The authors critically reviewed the work and approved the final version of the manuscript.

Ethics approval

The research project was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes No. 31 from 18.05.2019).

Patient consent

Obtained.

Acknowledgements and funding

No external funding.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Yang H, Ruan X, Li W, Xiong J, Zheng Y. Global, regional, and national burden of tuberculosis and attributable risk factors for 204 countries and territories, 1990-2021: a systematic analysis for the Global Burden of Diseases 2021 study. BMC Public Health. 2024 Nov 11;24(1):3111. doi: 10.1186/s12889-024-20664-w.
2. Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. Am J Clin Nutr. 2003;78(1):117-122. doi: 10.1093/ajcn/78.1.117.
3. Jack CI, Jackson MJ, Hind CR. Circulating markers of free radical activity in patients with pulmonary tuberculosis. Tuber Lung Dis. 1994 Apr;75(2):132-7. doi: 10.1016/0962-8479(94)90042-6.
4. Salunkhe V, Rai P, Ingale P. Oxidative DNA damage and deranged lipid profile in patients with pulmonary tuberculosis: across-sectional study. Natl J Lab Med. 2024;13(3):8-10. https://doi.org/10.7860/NJLM/2024/67091.2858.
5. Wadhwa R, Seghal N, Naresh G, et al. Oxidative stress and immunological complexities in multidrug-resistant tuberculosis. In: Maurya P, Dua K, editors. Role of oxidative stress in pathophysiology of diseases. Singapore: Springer; 2020. p. 107-124. https://doi.org/10.1007/978-981-15-1568-2_7.
6. Batta A. Markers of oxidative stress evaluation in people living with pulmonary tuberculosis. Int J Health Sci Res. 2024;14(3):81-86. https://doi.org/10.52403/ijhsr.20240314.
7. Rai RR, Phadke MS. Plasma oxidant antioxidant status in different respiratory disorder. Indian J Clin Biochem. 2006;21:161-164. http://dx.doi.org/10.1007/BF02912934.
8. Metelskaya VA, Gumanova NG. Skrining-metod opredeleniia urovnia metabolitov oksida azota v syvorotke krovi cheloveka [Screening-method for nitric oxide metabolites determination in human serum]. Klin Lab Diagn. 2005; (6): 15-18. Russian.
9. Galaktionova LP, Molchanov AV, El'chaninova SA, Varshavskii BIa. Sostoianie perekisnogo okisleniia u bol'nykh s iazvennoi bolezni iuzheludkaidvenadtsatiperstnoikishki[The state of lipid peroxidation in patients with gastric ulcer and duodenal ulcer]. Klin Lab Diagn. 1998;(6):10-14. Russian.

10. Zhang M, Liu N, Liu H. Determination of the total mass of antioxidant substances and antioxidant capacity per unit mass in serum using redox titration. Bioinorg Chem Appl. 2014;2014:928595. doi: 10.1155/2014/928595.
11. Vlasova SN, Shabunina EI, Pereslegina IA. Aktivnost' glutationzavisimykh fermentov éritrotsitov pri khronicheskikh zabolevaniiakh pecheni u deteĭ [The activity of the glutathione-dependent enzymes of erythrocytes in chronic liver diseases in children]. Lab Delo. 1990;(8):19-22. Russian.
12. Shastri MD, Shukla SD, Chong WC, Dua K, Peterson GM, et. al. Role of oxidative stress in the pathology and management of human tuberculosis. Oxid Med Cell Longev. 2018;2018:7695364. doi: 10.1155/2018/7695364.
13. Meca AD, Turcu-Stiolica A, Stanciulescu EC, Andrei AM, Nitu FM, et al. Variations of serum oxidative stress biomarkers under first-line antituberculosis treatment: a pilot study. J Pers Med. 2021;11(2):112. doi: 10.3390/jpm11020112.
14. Vidhya R, Rathnakumar K, Balu V, Pugalendi KV. Oxidative stress, antioxidant status and lipid profile in pulmonary tuberculosis patients before and after anti-tubercular therapy. Indian J Tuberc. 2019;66(3):375-381. doi: 10.1016/j.ijtb.2018.11.002.
15. Suresh DR, Annam V. Lipid peroxidation and total antioxidant capacity in health and disease– pathophysiology and markers: an overview. Int J Med Sci Public Health. 2013;2(3):478-479. doi: 10.5455/ijmsph.2013.250420136.
16. Liang T, Chen J, Xu G, Zhang Z, Xue J, et al. Ferroptosis-related gene SOCS1, a marker for tuberculosis diagnosis and treatment, involves in macrophage polarization and facilitates bone destruction in tuberculosis. Tuberculosis (Edinb). 2022;132:102140. doi: 10.1016/j.tube.2021.102140.
17. Kwiatkowska S, Piasecka G, Zieba M, Piotrowski W, Nowak D. Increased serum concentrations of conjugated diens and malondialdehyde in patients with pulmonary tuberculosis. Respir Med. 1999;93(4): 272-6. doi: 10.1016/s0954-6111(99)90024-0.
18. Amaral E, Vinhaes C, Oliveira-de-Souza D,Nogueria B,Akrami KM, Andrade BB. The interplay between systemic inflammation, oxidative stress, and tissue remodeling in tuberculosis. Antioxid Redox Signal. 2021;34(6):471-485. doi: 10.1089/ars.2020.8124.
19. Reyes-Lazcano ME, Rosete-Olvera D, Guzmán-Beltrán S. Uso de antioxidants en pacientes con tuberculosis [Use of antioxidants in patients with tuberculosis]. Neumol Cir Torax. 2023;82(3):174-182. doi: 10.35366/116816. Spanish.

https://doi.org/10.52645/MJHS.2026.2.04

UDC: 616.61-036.12-06:[616.98:578.834.1]

RESEARCH ARTICLE

Impact of COVID-19 on chronic kidney disease progression: a prospective cohort study

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ABSTRACT

Introduction. Chronic kidney disease and COVID-19 are both associated with significant morbidity. Patients with chronic kidney disease are at risk for severe COVID-19, and SARS-CoV-2 infection may accelerate CKD progression. This study aimed to compare renal outcomes in CKD patients with and without prior COVID-19 and to identify predictors of progression.

Materials and methods. We conducted a prospective cohort study of 280 pre-dialysis CKD patients (stages G2–G5), followed for 12 months. Of these, 140 had a history of COVID-19 (post-COVID group), and 140 had no such history (control group). Baseline assessments included renal function (eGFR, creatinine, urea), inflammatory markers (CRP, ferritin, LDH), hematologic indices (hemoglobin, leukocytes, platelets), and SF-36 quality of life scores. CKD progression was defined as a ≥30% eGFR decline or the initiation of dialysis. Analyses included group comparisons, correlations, logistic regression, and ROC curves.

Results. Baseline characteristics and mean eGFR (~60 mL/min/1.73 m^2) were similar across groups. CRP and ferritin levels were elevated in both groups without significant differences. Post-COVID patients reported lower vitality and higher social functioning on SF-36 (both $p < 0.001$). After 12 months, the post-COVID group showed greater eGFR decline (–3.1 vs –1.2 mL/min) and a higher progression rate (28% vs 15%, $p < 0.01$). Multivariable analysis identified prior COVID-19 (adjusted OR ≈2.3, 95% CI: 1.3–4.0) and low baseline hemoglobin as independent predictors of progression; CRP and ferritin were not predictive. LDH showed a modest association. Hemoglobin alone predicted progression with an AUC of 0.78; the combined model (COVID status + hemoglobin) yielded an AUC of 0.85.

Conclusions. CKD patients with prior COVID-19 experienced a faster renal function decline over one year than those without COVID-19. Persistent anemia and elevated LDH were also associated with increased progression risk. These findings emphasize the importance of post-COVID renal monitoring and early intervention in CKD patients to prevent deterioration.

Keywords: chronic kidney disease, COVID-19, disease progression, inflammation, quality of life.

Cite this article: Răzlog T, Russu E, Groza C, Groppa L. Impact of COVID-19 on chronic kidney disease progression: a prospective cohort study. Mold J Health Sci. 2026;13(2):20-27. https://doi.org/10.52645/MJHS.2026.2.04.

Manuscript received: 21.12.2025

Accepted for publication: 9.02.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

It remains unclear whether COVID-19 independently accelerates renal decline in patients with pre-existing CKD, beyond the effects of baseline kidney function and comorbidities. Existing studies are mostly retrospective and lack prospective comparative data. Additionally, the prognostic value of post-COVID biomarkers such as hemoglobin, LDH, CRP, and ferritin has not been well established.

The research hypothesis

We hypothesized that CKD patients with prior COVID-19 experience a significantly faster decline in renal function compared to CKD patients without COVID-19. We further hypothesized that

markers of anemia and tissue injury – rather than classical inflammatory markers – would predict CKD progression in the post-COVID population.

The novelty added by manuscript to the already published scientific literature

This study provides prospective evidence that prior COVID-19 is an independent accelerator of CKD progression, nearly doubling the risk of significant renal decline within one year. The manuscript identifies hemoglobin and LDH as key predictors of progression, while CRP and ferritin show no prognostic value – highlighting a shift toward chronic rather than acute inflammatory mechanisms. It is also one of the first studies from Eastern Europe to quantify post-COVID renal vulnerability in a well-matched CKD cohort.

Introduction

Chronic kidney disease (CKD) is a prevalent global health problem, affecting an estimated 13.4% of the global population (over 800 million people worldwide) and contributing significantly to mortality [1]. CKD is often termed a "silent epidemic" due to its insidious progression, high cardiovascular mortality, and substantial public health burden [1, 2]. COVID-19, caused by SARS-CoV-2, has emerged as a multisystem disease; although initially recognized as a respiratory illness, it is now known to cause widespread organ damage, including acute kidney injury (AKI). The kidney's high expression of ACE2 (the SARS-CoV-2 entry receptor) makes it particularly vulnerable to COVID-19-associated injury. Clinical reports indicate a high frequency of AKI in severe COVID-19, along with direct viral effects on renal tubular and glomerular cells leading to structural damage.

There is a bidirectional, syndemic interaction between CKD and COVID-19. CKD patients, especially those with advanced stages, are at a markedly increased risk for severe COVID-19 outcomes due to chronic uremic immunosuppression, systemic inflammation, and comorbidities. Indeed, CKD has been identified as an independent risk factor for COVID-19 severity and mortality. Conversely, surviving a COVID-19 infection may adversely affect kidney health. SARS-CoV-2 infection can trigger AKI and may accelerate the trajectory of chronic kidney damage, even in individuals without prior renal disease. An episode of COVID-19-related AKI can become a "reset point", leading to faster-than-expected CKD progression or transition to end-stage renal disease (ESRD) than expected from the prior course. In practical terms, a CKD patient who contracts COVID-19 might reach advanced CKD stages or dialysis faster than a similar patient who never had COVID.

Emerging evidence suggests that COVID-19 survivors can experience persistent subclinical renal impairment. One cohort study found that at 6 months post-COVID, 35% of patients had a reduced estimated glomerular filtration rate (eGFR <90 mL/min/1.73 m^2) despite normal kidney function during the acute infection. Notably, 13% of patients with no AKI in the hospital still showed a significant decline in eGFR by 6 months. These findings imply that even relatively mild COVID-19 can have lasting renal sequelae. Longer-term studies indicate a substantial proportion of COVID-19 survivors have persistent abnormalities such as reduced eGFR, proteinuria, or hematuria at 6–12 months. COVID-19 has thus been recognized as an emergent risk factor for kidney disease, adding to traditional factors like diabetes and hypertension.

Despite these concerns, data on the impact of COVID-19 on the progression of pre-existing CKD are limited, particularly in Eastern European populations with a high CKD burden. Most studies have been retrospective and have focused on AKI incidence or short-term outcomes. There is a need for a prospective analysis of how COVID-19 may influence the CKD trajectory and which patients are most vulnerable to renal function decline after infection. We hypothesized that CKD patients who recovered from COVID-19 would experience a faster decline in renal function compared to CKD patients with no COVID history, and that markers of inflammation or tissue injury might predict this decline.

This study aimed to evaluate the long-term impact of COVID-19 on CKD progression and to identify clinical and biological predictors of adverse renal outcomes. Specifically, we compared a cohort of CKD patients with prior COVID-19 to a matched cohort of CKD patients without COVID-19, in terms of renal function decline, the incidence of CKD stage progression or dialysis, and other health outcomes. We also explored associations between baseline inflammatory markers (CRP, ferritin, LDH), hematologic parameters, and progression risk. By providing prospective data in a previously under-studied population, our study seeks to inform post-COVID care strategies for CKD patients and guide early interventions to improve their prognosis.

Materials and methods

This prospective observational cohort study enrolled 280 adult patients with chronic kidney disease not on dialysis. Patients were recruited from nephrology clinics and inpatient services at *Timofei Moşneaga* Republican Clinical Hospital (Chişinău, Moldova) between 2023 and 2024. Inclusion criteria were: age 18–70 years, confirmed CKD (pre-dialysis, defined by evidence of chronic renal disease and/or eGFR <60 mL/min/1.73 m^2 for ≥3 months), and written informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional ethics committee (favorable opinion of the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, no. 6 of 18.05.2023). All participants provided informed consent.

We excluded individuals receiving renal replacement therapy (dialysis) or with a history of kidney transplant, as well as those with active malignancy or tuberculosis, or who declined participation. After applying criteria, the study population consisted of 280 eligible CKD patients. These were divided into two equal groups based on COVID-19 exposure:

- *Post-COVID group (exposed cohort):* 140 CKD patients who had recovered from a documented COVID-19 infection prior to or during the study period. COVID-19 was confirmed by clinical diagnosis and/or positive laboratory tests (e.g., PCR). Only patients evaluated after clinical recovery from acute COVID-19 were included, with a median of ~3 months from infection to enrollment. We recorded the date and severity of the COVID-19 episode for each (mild, moderate, or severe; whether hospitalization or ICU care was needed).
- *CKD control group (unexposed cohort):* 140 CKD patients with no history of COVID-19 (by patient report and available testing). These patients served as the comparison group to isolate the effect of COVID-19 on CKD evolution.

The two groups were frequency-matched on key characteristics. They were similar in age and sex distribution and had comparable distributions of CKD etiology (diabetic nephropathy, hypertensive nephrosclerosis, glomerulonephritis, etc.). Both groups predominantly comprised patients in middle to advanced CKD stages (G2–G5 ND), with no significant differences in baseline CKD stage distribution (median stage 3 in each). Comorbid conditions such as diabetes mellitus (overall ~26%) and hypertension (~82%) were common and similarly distributed, aside from a non-significant trend toward higher diabetes prevalence in the post-COVID group (31% vs 21%, $p \approx 0.06$). All patients continued to receive standard CKD care (e.g., blood pressure control, renin–angiotensin system blockers, anemia management) as per clinical guidelines.

At baseline (study entry), all patients underwent a comprehensive evaluation including clinical history, physical examination, laboratory tests, and questionnaire administration. Baseline evaluations for the post-COVID group were performed after recovery from acute infection (no patient had active COVID-19 at time of assessment).

Baseline characteristics: mean age 58.7 ± 12.4 years (post-COVID) and 56.9 ± 13.1 (control); males 55.0% vs 50.7%; diabetes prevalence 31% vs 21% ($p \approx 0.06$); hypertension ~82% in both groups. Median CKD stage was 3 in both cohorts. All patients received standard nephrology care.

Baseline evaluations included clinical history, physical exam, SF-36 questionnaire, and laboratory tests.

- *Renal function:* Serum creatinine, urea, eGFR (CKD-EPI 2021), urinalysis, proteinuria.
- *Hematology:* Hemoglobin, leukocyte and platelet counts.
- *Inflammation/tissue injury:* C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH).
- *Metabolic labs:* Electrolytes, uric acid, liver enzymes, D-dimer, total protein, and albumin.
- *Quality of life:* SF-36 assessed 8 domains (scored 0-100), including vitality and physical function.

Patients were followed for 12 months. Outcomes included CKD progression (≥30% eGFR decline or dialysis), changes in eGFR, dialysis initiation, and mortality.

Statistical analysis. We used SPSS v25 and R v4.1. Continuous variables were compared using *t*-tests or Mann-Whitney *U*; categorical variables via χ^2 tests. Correlations (Pearson or Spearman) evaluated associations between biomarkers and eGFR change. Multivariable logistic regression identified independent predictors of CKD progression. ROC curves assessed predictive value (AUC). Statistical significance was set at $p < 0.05$.

Results

A total of 280 CKD patients were analyzed (140 post-COVID and 140 controls). Baseline demographic and clinical characteristics are shown in Table 1. The two groups were comparable in age (58.7 vs. 56.9 years), sex distribution (55.0% vs. 50.7% male), and comorbidity burden, including hypertension (>80%) and diabetes (~26%, with a non-significant trend toward higher prevalence in the post-COVID group). Baseline BMI and blood pressure were similar.

Renal function at study entry did not differ significantly: mean serum creatinine (2.23 vs. 2.38 mg/dL) and eGFR (57 vs. 65 mL/min/1.73 m^2, $p = 0.19$) were comparable. CKD stages were evenly distributed, with a median stage of 3 in both cohorts. Rates of proteinuria, microscopic hematuria, and leukocyturia were also similar.

Inflammatory markers showed wide interindividual variation, characteristic of CKD. Although mean CRP was numerically higher in the post-COVID group, median values were nearly identical (1.8 vs. 1.7 mg/L), and the difference was not statistically significant. Ferritin levels, ESR, and other inflammatory parameters likewise showed no meaningful group differences. Hemoglobin values averaged 11.4–11.5 g/dL across groups, consistent with CKD-related anemia.

LDH levels were mildly elevated in both groups, with a non-significant trend toward higher levels in controls (195 vs. 183 U/L). Albumin was modestly reduced (~3.5 g/dL) in both cohorts.

SF-36 results indicated similar physical functioning between the groups, while post-COVID patients reported significantly lower vitality (greater fatigue) and higher social functioning scores.

Overall, baseline clinical and laboratory characteristics were well balanced between groups, ensuring comparable starting conditions for assessing subsequent renal outcomes.

Table 1. Baseline characteristics of CKD patients with and without prior COVID-19

Characteristic	Post-COVID CKD (n = 140)	CKD Control (n = 140)	*p*-value
Age, years	58.7 ± 12.4	56.9 ± 13.1	0.32
Male sex, %	55.0%	50.7%	0.48
Body mass index, kg/m^2	28.3 ± 5.7	27.8 ± 5.4	0.45
Diabetes mellitus, %	31.4%	21.4%	0.06
Hypertension, %	84.3%	80.0%	0.34
Coronary artery disease, %	18.6%	16.4%	0.62
Renal function			
Serum creatinine, mg/dL	2.23 ± 1.84	2.38 ± 1.73	0.53
eGFR, mL/min/1.73 m^2	56.9 ± 33.4	64.7 ± 62.8	0.19
CKD stage (median)	3 (G3b predominant)	3 (G3a-G4 predominant)	0.59
Proteinuria ≥0.3 g/day, %	58.6%	51.4%	0.21
Hematuria (micro) %	20.0%	22.9%	0.58
Inflammation & labs			
C-reactive protein, mg/L	13.96 ± 42.5 (median 1.8)	4.25 ± 8.25 (median 1.7)	0.51[1]
Ferritin, µg/L	116 ± 141	139 ± 180	0.23
Lactate dehydrogenase, U/L	183 ± 51	195 ± 66	0.10
Hemoglobin, g/dL	11.4 ± 1.8	11.5 ± 1.9	0.74
White blood cells, ×10^9/L	7.9 ± 2.5	7.6 ± 2.2	0.30
Platelet count, ×10^9/L	262 ± 78	254 ± 69	0.55
Albumin, g/dL	3.50 ± 0.42	3.55 ± 0.45	0.68
SF-36 Physical Function[1]	47.4 ± 25.0	40.4 ± 38.0	0.07
SF-36 Vitality (Energy)[1]	44.1 ± 16.9	56.5 ± 19.0	<0.001
SF-36 Social Function[1]	59.0 ± 22.0	43.8 ± 25.6	<0.001

***Note**: [1]CRP values were highly skewed; medians are given in parentheses. SF-36 domain scores are scaled 0–100 (higher = better function). Abbreviations: eGFR = estimated glomerular filtration rate (CKD-EPI formula); CKD stage per KDIGO G categories; SF-36 = 36-item Short Form health survey. Values expressed as mean ± SD, median [IQR], or %*

Quality of life (SF-36) results. All patients completed the SF-36 at baseline. Overall quality of life was reduced in both groups, as expected in CKD. As shown in Table 1, Physical Functioning was slightly higher in the post-COVID group (47.4 vs. 40.4), though the difference was not statistically significant. Most physical and emotional role limitations were similar between the groups.

Two domains showed significant differences. Vitality scores were lower in the post-COVID group (44.1 vs. 56.5, $p < 0.001$), indicating more pronounced fatigue, which likely reflected persistent post-COVID symptoms. In contrast, Social Functioning was higher among post-COVID patients (59.0 vs. 43.8, $p < 0.001$), suggesting better perceived social engagement. A possible explanation is that recovery from COVID-19 may have reinforced social connectedness or coping behaviors.

Mental Health scores (~59), Bodily Pain (~38), and General Health perception (~40) were comparable. Both groups rated their overall health as relatively poor, consistent with the CKD burden.

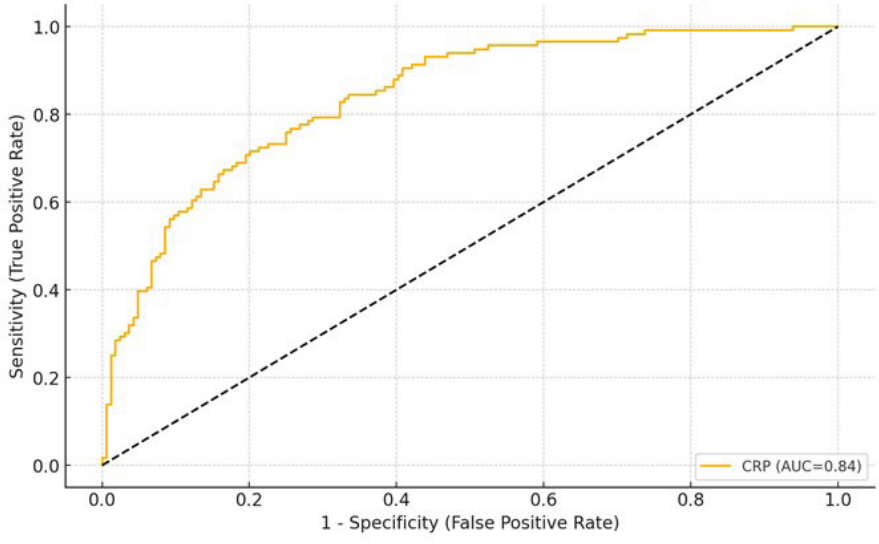

Fig. 1 CKD progression rates at 12 months in post-COVID vs control groups.

***Note**: Bar heights represent the percentage of patients who progressed (≥30% eGFR decline or dialysis start). A significantly higher proportion of the post-COVID CKD group progressed (28.0%) compared to the CKD control group (15.0%, p = 0.002).*

In summary, post-COVID CKD patients reported greater fatigue but better social functioning compared to controls, highlighting the complex and multidimensional impact of COVID-19 on patient-reported outcomes.

Follow-up and renal outcomes. All patients were followed for a median of 12 months (minimum 12, maximum 15 months). During this period, no patients were lost to follow-up. We monitored for changes in renal function and the occurrence of our defined outcome of CKD progression. The results are presented in Table 2 and illustrated in Figure 1.

By the end of follow-up, the post-COVID cohort experienced a faster decline in kidney function compared to controls. The mean change in eGFR from baseline was –3.1 ± 6.5 mL/min/1.73 m^2 in the post-COVID group versus –1.2 ± 5.8 mL/min/1.73 m^2 in controls (a more than twofold greater decline; $p = 0.01$). Specifically, 62% of post-COVID patients had some drop in eGFR (any decline >0), compared to 45% of controls (where many control patients remained stable or even had slight improvements due to interventions or variability). Figure 1 illustrates the proportion of patients meeting our progression criteria in each group: 28% of the post-COVID group vs 15% of controls reached the composite endpoint of ≥30% eGFR decline or need for dialysis within one year (Figure 1, $p = 0.002$).

In absolute terms, 39 out of 140 post-COVID patients vs. 18 out of 140 controls had a ≥30% decline in eGFR over 12 months ($p = 0.001$). Moreover, renal failure requiring dialysis occurred more frequently in the post-COVID group: 10 patients (7.1%) initiated chronic dialysis during follow-up compared to 2 patients (1.4%) in the control group ($p = 0.03$). The dialysis cases in the post-COVID group predominantly had severe baseline CKD (eGFR <20 mL/min/1.73 m^2) and subsequently experienced COVID-19-associated AKI that never fully recovered, whereas the two control cases were due to the natural progression of CKD. Additionally, three post-COVID patients (2.1%) versus one control (0.7%) experienced rapid progression to an eGFR <15 mL/min/1.73 m^2 without initiating dialysis by 12 months (being managed conservatively or awaiting access creation). When combining dialysis initiation with these late-stage cases, the difference remained significant. No patient in either cohort received a transplant during the study.

We also tracked other outcomes. All-cause mortality during follow-up was low, reflecting the relatively short observation period: 4 deaths occurred in the post-COVID group (2.9%) versus 3 in controls (2.1%, $p = 0.70$). Causes of death were cardiovascular in most cases (myocardial infarction or stroke), with one COVID-group patient dying of sepsis and one control of unknown sudden death. Although mortality was slightly higher in the post-COVID group, the difference was not statistically significant. Notably, two of the post-COVID deaths occurred in patients who had progressed to dialysis; this aligns with literature showing worse outcomes in CKD patients post-COVID, but our study size was not powered for mortality analysis.

Regarding quality of life over time, we did not perform a second SF-36 assessment at 12 months for all patients, therefore longitudinal QoL changes are not reported quantitatively. However, during clinical visits many post-COVID patients subjectively reported improvement in fatigue over time. By contrast, objective renal outcomes clearly diverged between the groups as described.

Table 2. Renal function outcomes at 12-month follow-up

Outcome (12 months)	Post-COVID CKD (n = 140)	CKD Control (n = 140)	*p*-value
eGFR change from baseline (mL/min/1.73 m^2)	–3.1 ± 6.5	–1.2 ± 5.8	0.010 **
≥30% decline in eGFR, n (%)	39 (27.9%)	18 (12.9%)	0.001 **
Initiation of dialysis, n (%)	10 (7.1%)	2 (1.4%)	0.017 *
Composite progression (either of above)	42 (30.0%)	20 (14.3%)	0.001 **
All-cause mortality, n (%)	4 (2.9%)	3 (2.1%)	0.70
Δ Serum creatinine, mg/dL (baseline→12 m)	+0.43 ± 1.10	+0.15 ± 0.96	0.07
Δ CKD stage (median)	+0.5	±0	–

*Note: statistical significance: p < 0.05 *, p < 0.01 **. Δ indicates change; a positive Δ creatinine means an increase (worsening). "Composite progression" denotes meeting the primary endpoint (≥30% eGFR drop or dialysis start). Median CKD stage change: post-COVID group median progressed by half a stage (e.g. many G3a→G3b), whereas median control remained the same stage.*

As seen in Table 2, CKD progression was significantly more common among those with prior COVID-19. The unadjusted odds ratio (OR) for CKD progression in the post-COVID group was approximately 2.5 (95% CI ~1.4–4.4) relative to controls. This finding supports our hypothesis that COVID-19 has a measurable adverse effect on the CKD trajectory. It is consistent with recently published retrospective data, which reported that CKD patients with COVID-19 had about 3.7-fold higher odds of rapid eGFR decline compared to those without COVID. Our prospective design strengthens the causal inference that COVID-19 may accelerate underlying kidney disease.

Factors associated with CKD progression. We next evaluated baseline predictors of CKD progression. In univariate analysis, progressors were more frequently from the post-COVID group (67%, $p < 0.01$) and had lower baseline eGFR (35 vs. 62 mL/min, $p < 0.001$), lower hemoglobin (10.8 vs. 11.7 g/dL, $p = 0.004$), and higher LDH (210 vs. 182 U/L, $p = 0.01$). CRP, ferritin, and other inflammatory markers did not differ between progressors and non-progressors, suggesting that post-recovery inflammation was not a major determinant of decline.

Correlation analysis showed that hemoglobin correlated inversely with eGFR decline (r = –0.25, $p < 0.001$), and LDH

showed a weaker association (r = –0.14, p = 0.02). CRP and ferritin showed no meaningful correlations. ESR correlated moderately with decline (r≈–0.20, p < 0.01) and strongly with anemia, reflecting chronic inflammatory burden.

Multivariable logistic regression identified three independent predictors of CKD progression: prior COVID-19, lower hemoglobin, and higher LDH (Table 3). COVID-19 history remained a significant risk factor (aOR 2.28, p = 0.008). Each 1 g/dL decrease in hemoglobin increased progression risk by ~40% (aOR 1.38, p = 0.005), while LDH also predicted progression (aOR 1.20 per +50 U/L, p = 0.027). CRP, ferritin, diabetes, age, and baseline eGFR were not independent predictors.

These findings indicate that CKD patients with prior COVID-19, anemia, and elevated LDH are at a substantially higher risk for accelerated renal decline. Traditional risk factors such as diabetes showed no independent effect, likely because the impact of COVID-19 and anemia dominated short-term outcomes.

Table 3. Multivariate logistic regression for predictors of CKD progression (N = 280)

Predictor	Adjusted OR (95% CI)	p-value
Prior COVID-19 (yes vs no)	2.28 (1.24–4.21)	0.008 **
Hemoglobin (per –1 g/dL)	1.38 (1.10–1.73)	0.005 **
LDH (per +50 U/L)	1.20 (1.02–1.42)	0.027 *
Baseline eGFR (per +5 mL/min)	0.96 (0.89–1.04)	0.30
Diabetes (yes vs no)	1.32 (0.70–2.50)	0.39
CRP (per +10 mg/L)	1.05 (0.92–1.21)	0.47
Ferritin (per +50 µg/L)	0.98 (0.88–1.08)	0.65
Age (per +10 years)	1.11 (0.85–1.45)	0.45
Sex (male vs female)	0.89 (0.50–1.58)	0.69

Note: Model χ^2 = 29.5, p < 0.001. OR = odds ratio; CI = confidence interval. OR >1 indicates higher odds of progression. Bold indicates p < 0.05. (Baseline eGFR and diabetes were not significant here, possibly due to collinearity and cohort matching).

To illustrate the predictive value of the model, we plotted ROC curves. The baseline hemoglobin level alone yielded an AUC of 0.78 for predicting progression, suggesting fair discrimination. Adding COVID-19 status improved the AUC to ~0.81. A full model with COVID status + hemoglobin + LDH achieved an AUC of 0.85. In contrast, baseline CRP had minimal predictive power (AUC = 0.58, Figure 2), performing little better than chance. This reinforces that acute inflammatory markers measured after recovery are not useful prognostic indicators in this setting, whereas markers of chronic disease severity (anemia) and the exposure to COVID-19 are more critical.

Notably, the finding that anemia is a strong predictor aligns with the idea that low hemoglobin signifies advanced CKD and systemic inflammation/malnutrition, which contribute to faster decline. Anemia can also worsen renal hypoxia, accelerating nephron loss. The importance of prior COVID-19 as an independent predictor, even when controlling for baseline CKD severity, suggests a direct effect of the infection on kidney disease progression. This could be mediated by residual damage (e.g., from AKI)

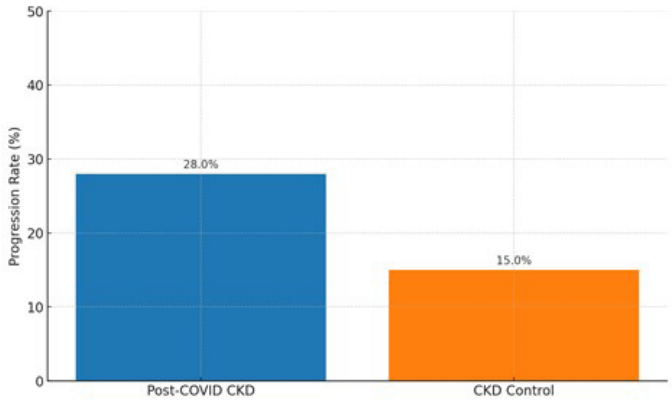

Fig. 2 ROC curve for baseline CRP level predicting CKD progression.

Note: *The curve (orange line) shows the trade-off between sensitivity and specificity for various CRP cut-offs. The area under the ROC curve (AUC) was 0.58, indicating poor discriminative ability of CRP alone for predicting progression in this cohort (no better than chance). The diagonal dashed line represents the line of no discrimination (AUC = 0.50).*

and/or persistent inflammatory or immune dysregulation after the infection.

Post-hoc analysis: COVID-19 severity and renal outcomes. Within the post-COVID group, we conducted exploratory analyses to see if the severity of the acute COVID-19 episode was associated with the degree of CKD progression. Patients were stratified by their COVID-19 severity: 28 had mild (outpatient) cases, 82 had moderate cases (hospitalized with pneumonia but no ICU), and 30 had severe cases (required ICU or high-flow oxygen). We found a significant gradient: 50% of those with severe COVID progressed, compared to 30% with moderate and 14% with mild disease (χ^2 trend p = 0.002). Those with severe COVID had a mean eGFR decline of -5.8 mL/min, vs. -2.7 for moderate and –0.5 for mild (ANOVA p = 0.01). This aligns with external data from large cohorts: for example, a US Veterans study showed that patients who required ICU for COVID had an additional 5-7 mL/min/year loss of eGFR beyond normal decline, while even non-hospitalized COVID patients had an extra ~3 mL/min/year decline. In our cohort, severe COVID often coincided with AKI; among post-COVID patients who had COVID-related AKI (n = 22), the progression rate was 55% versus 24% in those without AKI (p = 0.003). These observations, although from a subgroup, suggest that the acute insult severity correlates with long-term CKD impact – a finding echoed by other studies.

It is important to note that even some patients with mild COVID (no initial AKI) experienced CKD progression, consistent with reports that even mild COVID-19 can leave residual renal sequelae in susceptible individuals. We did not find a significant correlation between time since COVID infection and progression in this one-year window (most infections were within 6-18 months prior to baseline), though longer follow-up is needed to see if these effects attenuate or persist.

Discussion

This prospective study demonstrates that CKD patients with prior COVID-19 experienced a significantly faster renal decline than CKD patients without COVID-19, with nearly one-third meeting the criteria for progression within one year. Prior COVID-19 remained an independent predictor of progression even after adjustment for baseline renal function, supporting the hypothesis that SARS-CoV-2 may accelerate CKD deterioration [2]. These results align with earlier research indicating higher risks of rapid eGFR decline among CKD patients who contract COVID-19 [2, 3] and with long-term cohort data showing increased adverse renal outcomes in COVID-19 survivors [3].

A likely mechanism is COVID-19-related AKI, which can lead to irreversible nephron loss or maladaptive fibrosis. In our cohort, patients with previous AKI – especially severe cases requiring ICU care – had the highest progression rates [4], consistent with evidence that COVID-19-associated AKI increases the risk of sustained CKD worsening. SARS-CoV-2 may injure renal tubular and endothelial cells directly, and autopsy studies have confirmed viral particles and inflammatory injury in kidney tissue [4, 5]. Systemic inflammation, coagulopathy, and hemodynamic instability during the acute infection may further accelerate CKD progression [5, 6].

Inflammation is a known driver of CKD decline [5, 6], yet traditional inflammatory markers (CRP, ferritin) did not predict outcomes in our cohort, likely because acute inflammation had resolved by baseline [6, 7]. ESR, however, correlated with decline, suggesting that the chronic inflammatory burden remains a relevant pathway [6, 7]. LDH, another marker linked to acute and long-COVID states, independently predicted progression, possibly indicating persistent subclinical organ injury [6, 7].

Anemia emerged as one of the strongest predictors of progression, consistent with prior studies [8]. Low hemoglobin both reflects the disease severity and contributes to renal hypoxia, creating a cycle of worsening kidney injury. These findings underscore the importance of optimal anemia management, particularly in post-COVID CKD patients [8, 9].

Quality-of-life data showed greater fatigue among post-COVID patients, consistent with post-COVID syndrome [10, 11], which may indirectly influence disease trajectory by limiting functional capacity. Higher social functioning scores may reflect behavioral or psychological changes following recovery from severe illness [11].

Clinically, CKD patients with a history of COVID-19 – especially severe disease – should receive intensified renal monitoring. More frequent assessment of eGFR and proteinuria may allow earlier detection of decline and timely interventions [12, 13]. Kidney-protective strategies and optimization of comorbidity management may be particularly important in this group [12, 13]. SGLT2 inhibitors could be beneficial post-COVID, a hypothesis requiring further study [14]. Preventive measures, including vaccination, remain essential.

From a pathophysiological standpoint, our findings support the concept that COVID-19 acts as a "second hit," accelerating CKD progression through persistent metabolic and inflammatory effects [15]. Reports of post-COVID glomerulopathies, although rare, reinforce the need for vigilance [16, 17].

The study's limitations include its one-year follow-up, single-center design, and limited sample size for subgroup analyses. Despite these, strengths include complete follow-up, rigorous matching of cohorts, and detailed phenotyping [17, 18]. These results generate important hypotheses for future research, particularly regarding anemia correction and LDH-based risk stratification [19].

In conclusion, COVID-19 exposure should be recognized as a significant prognostic factor in CKD. Incorporating COVID-19 history into risk stratification and follow-up pathways may help prevent premature progression to ESRD [19, 20]. The interaction between COVID-19 and CKD illustrates how acute infectious diseases can substantially alter the course of chronic conditions, contributing to a broader syndemic burden.

Conclusions

This study demonstrates that prior COVID-19 infection is a significant accelerator of chronic kidney disease progression. CKD patients who recovered from COVID-19 showed nearly a twofold risk of major renal function decline within one year compared to those without COVID-19, even after adjusting for baseline renal status.

COVID-19 history, low hemoglobin, and elevated LDH were the strongest independent predictors of progression, highlighting the roles of persistent tissue injury and anemia in post-COVID renal vulnerability. Classical inflammatory markers such as CRP and ferritin were not predictive, suggesting that chronic – not acute – inflammation is more relevant for long-term renal decline.

Clinically, CKD patients with prior COVID-19 – especially those with a history of severe disease or COVID-related AKI – require closer monitoring, more frequent assessment of eGFR and proteinuria, and early optimization of renoprotective therapy. Attention to anemia correction may be particularly important in this population.

Overall, COVID-19 should be considered a key prognostic factor in CKD management. Integrating COVID-19 history into risk stratification algorithms may help prevent premature progression to end-stage renal disease.

Competing interests

None declared.

Authors' contribution

TR and LG conceived and designed the study. TR, ER, and CG collected clinical data. RE and CG performed statistical analysis. TR drafted the manuscript. LG and ER critically revised the manuscript. All authors approved the final version.

Informed consent for publication

Obtained.

Ethics approval and informed consent

The study involving human participants was reviewed and approved by the Research Ethics Committee of *Nico-*

lae Testemițanu State University of Medicine and Pharmacy (favorable opinion no.6 of 18.05.2023). Patient consent was waived due to the retrospective nature of the study and use of de-identified data. All methods were carried out in accordance with relevant guidelines and regulations, and the study conforms to the principles of the Declaration of Helsinki.

Acknowledgements and funding

No external funding.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Coresh J, Byrd-Holt D, Astor BC, et al. Chronic kidney disease awareness, prevalence, and trends among U.S. adults, 1999 to 2000. J Am Soc Nephrol. 2005;16(1):180-188. doi: 10.1681/ASN.2004070539.
2. Couturier A, Ferlicot S, Chevalier K, et al. Indirect effects of severe acute respiratory syndrome coronavirus 2 on the kidney in coronavirus disease patients. Clin Kidney J. 2020;13(3):347-353. doi: 10.1093/ckj/sfaa088.
3. Del Vecchio, L, Balafa O, Dounousi E, et al. COVID-19 and cardiovascular disease in patients with chronic kidney disease. Nephrol Dial Transplant. 2024;39(2)177-189. https://doi.org/10.1093/ndt/gfad170.
4. Dellepiane S, Vaid A, Jaladanki SK, et al. Acute kidney injury in patients hospitalized with COVID-19 in New York City: temporal trends from march 2020 to april 2021. Kidney Med. 2021;3(5):877-879. doi: 10.1016/j.xkme.2021.06.008.
5. Farouk SS, Fiaccadori E, Cravedi P, Campbell KN. COVID-19 and the kidney: what we think we know so far and what we don't. J Nephrol. 2020;33(6):1213-1218. doi: 10.1007/s40620-020-00789-y.
6. Eligulashvili, A, Gordon M, Lee JS, et al. Long-term outcomes of hospitalized patients with SARS-CoV-2/COVID-19 with and without neurological involvement: 3-year follow-up assessment. PLoS Med. 2024;21(4):e1004263. https://doi.org/10.1371/journal.pmed.1004263.
7. Fisher, M, Neugarten J, Bellin E, et al. AKI in hospitalized patients with and without COVID-19: a comparison study. J Am Soc Nephrol. 2020;31(9):2145-2157. https://doi.org/10.1681/ASN.2020040509.
8. Flythe JE, Assimon MM, Tugman MJ, et al. Characteristics and outcomes of individuals with pre-existing kidney disease and COVID-19 admitted to intensive care units in the United States. Am J Kidney Dis. 2021;77(2):190-203.e1. doi: 10.1053/j.ajkd.2020.09.003.
9. Gok, M, Cetinkaya H, Kandemir T, et al. Chronic kidney disease predicts poor outcomes of COVID-19 patients. Int Urol Nephrol. 2021;53(9):1891-1898. https://doi.org/10.1007/s11255-020-02758-7.
10. Graham EL, Clark JR, Orban ZS, et al. Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 "long haulers. Ann Clin Trans Neurol. 2021;8(5):1073-1085. doi: 10.1002/acn3.51350.
11. Groza C, Russu E, Rotaru L, Razlog T, Calancea V, Groppa L. Evaluarea influenţei psihosociale asupra calităţii vieţii pacienţilor cu boala cronică de rinichi = Assessing the psychosocial influence on the quality of life of patients with chronic kidney disease. Mold J Health Sci. 2024;11(2 Suppl 1):58. Romanian, English.
12. Răzlog T, Russu E, Ceban E, Groza C, Alexa Z, Groppa L. Factorii de progresie ai bolii cronice de rinichi la pacienţii cu COVID-19 = Factors of progression of chronic kidney disease in COVID-19 patients. Mold J Health Sci. 2024;11(2 Suppl 1):59. Romanian, English.
13. Răzlog T, Russu E, Alexa Z, Ceban E, Groza C, Groppa L. Manifestations of kidney involvement in COVID-19 patients and progression to chronic kidney disease. Mold J Health Sci. 2024;11(1):11-18. https://doi.org/10.52645/MJHS.2024.1.02
14. Khatri, M, Charytan DM, Parnia S, et al. Outcomes among hospitalized chronic kidney disease patients with COVID-19. Kidney 360. 2021;2(7):1107-1114. https://doi.org/10.34067/KID.0006852020.
15. Gupta S, Hayek SS, Wang W, et al. Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. JAMA Int Med. 2020;180(11):1436-1447. doi: 10.1001/jamainternmed.2020.3596.
16. Henry BM, Lippi G. Chronic kidney disease is associated with severe coronavirus disease 2019 (COVID-19) infection. Int Urol Nephrol. 2020;52(6):1193-1194. doi: 10.1007/s11255-020-02451-9.
17. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021;397(10270):220-232. doi: 10.1016/S0140-6736(20)32656-8.
18. Long JD, Strohbehn I, Sawtell R, Bhattacharyya R, Sise ME. COVID-19 survival and its impact on chronic kidney disease. Transl Res. 2022;241:70-82. https://doi.org/10.1016/j.trsl.2021.11.003.
19. Ng JH, Hirsch JS, Hazzan A, et al. Outcomes among patients hospitalized with COVID-19 and acute kidney injury. Am J Kidney Dis. 2021;77(2):204-215.e1. doi: 10.1053/j.ajkd.2020.09.002.
20. Valeri AM, Robbins-Juarez SY, Stevens JS, et al. Presentation and outcomes of patients with ESKD and COVID-19. J Am Soc Nephrol. 2020;31(7):1409-1415. doi: 10.1681/ASN.2020040470.

https://doi.org/10.52645/MJHS.2026.2.05

UDC: 616.831-001

RESEARCH ARTICLE

Clinical assessment of risk factors in traumatic brain injury

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ABSTRACT

Introduction. Traumatic brain injury remains a leading global health concern with significant social and economic impact. The main causes include traffic accidents, falls, and violence, especially affecting young adults. In the Republic of Moldova, TBI incidence is rising, particularly during the prehospital phase. TBI involves both primary and secondary brain injuries, the latter often resulting from hypoxia, hypotension, or hyperglycemia. These secondary insults critically influence outcomes and are associated with high mortality. Effective prehospital management – focused on stabilizing oxygenation and hemodynamics – is essential in reducing neurological deterioration. Emergency teams play a key role in preventing secondary injury and improving survival.

Material and methods. This study, conducted from 2020 to 2024, analyzed 486 patients with acute traumatic brain injury (TBI) assessed in both prehospital and emergency department settings. It aimed to evaluate injury severity and prognosis using clinical tools and structured observation forms, developed specifically for this research.

Results. Significant correlations were found between increased age, low systolic blood pressure, prehospital hypoxia, and both TBI severity and mortality ($p < 0.0001$). While hyperglycemia was not significantly associated with injury severity, it showed a moderate negative correlation with mortality ($p < 0.01$). Findings emphasize the importance of early monitoring and stabilization of vital signs in the prehospital phase to improve TBI outcomes.

Conclusions. This study emphasizes the importance of systematic prehospital monitoring and management of physiological parameters to mitigate secondary brain injury and improve patient prognosis. Early intervention targeting hypoxia and hypotension remains vital in the acute management of TBI.

Keywords: traumatic brain injury (TBI), prehospital care, emergency medicine, injury severity, prognosis, clinical assessment, secondary brain injury, hemodynamic stabilization, hypoxia.

Cite this article: Mocanu N, Rezneac L, Catanoi L, Malacinschi-Codreanu T. Clinical assessment of risk factors in traumatic brain injury. Mold J Health Sci. 2026;13(2):28-33. https://doi.org/10.52645/MJHS.2026.2.05.

Manuscript received: 18.07.2025

Accepted for publication: 18.02.2026

Published: 15.06.2026

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

This study highlights key risk factors in TBI outcomes; it remains unclear how specific prehospital interventions directly impact long-term neurological recovery and whether standardized early protocols could further reduce mortality across diverse settings.

The research hypothesis

Early identification and management of physiological risk factors – such as hypoxia, hypotension, and hyperglycemia – during the prehospital and emergency stages can significantly reduce the severity and mortality from traumatic brain injury.

The novelty added by the manuscript to the already published scientific literature

This study provides new evidence from the Republic of Moldova

on the impact of prehospital physiological parameters on TBI outcomes, using structured clinical tools tailored for both prehospital and emergency settings. It emphasizes the predictive value of early hypoxia and hypotension in a regional context where such data were previously limited.

Introduction

Traumatic brain injury (TBI) remains a major global public health concern in the 21st century, with substantial social and economic ramifications. Epidemiological data reveal a variable incidence across different regions, ranging from 2.18 to 8.65 cases per 1,000 inhabitants, with a markedly higher prevalence among males. In countries such as the United States, the United Kingdom, China, Finland, and Sweden, the hospitalization rate due to TBI is approximately 2‰ [1].

The leading causes of TBI are road traffic accidents, falls, and interpersonal violence, accounting for 80–90% of all cases [2]. Central nervous system injuries represent 30–40% of all trauma cases and are the primary contributors to post-traumatic disability (25–30%) [3]. Among individuals in the active age group (18–35 years), TBI is the foremost cause of trauma-related mortality [1, 3].

In the Republic of Moldova, recent trends indicate a rise in the number of TBI cases managed during the prehospital phase, increasing from 16,443 cases in 2021 to 17,493 in 2022. Within the national trauma profile, TBI ranks second, following musculoskeletal injuries.

Pathophysiologically, TBI involves both primary brain injuries – classified as focal or diffuse – and secondary injuries that develop subsequent to the initial insult. These secondary lesions are triggered by systemic factors such as hypoxia, hyperglycemia, and hypotension, as well as intracranial factors, including cerebral edema, elevated intracranial pressure, and ischemia. Among these, cerebral ischemia is considered the most critical secondary insult, observed in approximately 90% of TBI-related deaths [4].

Secondary brain injuries exacerbate neurological deterioration through complex molecular and cellular mechanisms, potentially leading to irreversible damage. For instance, hypoxia contributes to neuronal death and cognitive impairment, while even transient episodes of hypotension may significantly impair cerebral perfusion and worsen neurological outcomes [5]. Within this context, effective prehospital management of TBI is paramount, aiming to mitigate secondary brain injury by maintaining adequate cerebral perfusion. Prompt correction of hypoxia and hemodynamic stabilization are essential interventions that directly influence clinical outcomes [6].

Emergency medical teams operating in the prehospital environment play a vital role in the initial stabilization of TBI patients. Continuous monitoring of vital signs and timely, targeted interventions are critical in preventing further neurological deterioration and improving prognosis [7].

Material and methods

This prospective, observational study was conducted between January 2, 2020, and January 2, 2024, within the Department of Emergency Medicine at *Nicolae Testemițanu* State University of Medicine and Pharmacy, in collaboration with the Institute of Emergency Medicine and the National Center for Prehospital Emergency Medical Assistance.

A total of 486 patients diagnosed with acute traumatic brain injury were enrolled, based on predefined inclusion criteria: age ≥ 18 years, confirmed TBI diagnosis, and availability of complete clinical data from both the prehospital and emergency department stages. Patients with incomplete documentation or pre-existing neurological disorders were excluded. The study focused on the acute phase of TBI, assessing each patient both in the prehospital setting and upon arrival at the Emergency Medicine Department.

Standardized clinical and paraclinical assessments were carried out using structured observation forms specifically developed for this research. Key parameters included Glasgow Coma Scale (GCS) scores, systolic blood pressure, oxygen saturation, blood glucose levels, and computed tomography (CT) findings. Data on interventions and clinical outcomes were also collected.

Statistical analyses were performed using SPSS version [2019]. Descriptive statistics were used to summarize demographic and clinical characteristics. Inferential statistics – including Pearson's correlation and logistic regression – were applied to assess the associations between physiological variables and both injury severity and patient outcomes. Statistical significance was defined as $p < 0.05$.

The study received ethical approval from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes no. 38, dated 02.04.2013). Written informed consent was obtained from all participants or their legal representatives.

Results and discussions

Statistical analysis of the patient cohort included in the study revealed a mean age of 51.29 ± 18.34 years, with age limits ranging from 18 to 84 years. Regarding sex distribution, a predominance was observed among male patients, who accounted for 62.9% of all cases, while female patients represented 37.1%. The predominance of traumatic brain injuries among male patients in the study cohort yielded a male-to-female ratio of 1.7:1, a finding consistent with existing literature. This significant gender difference is frequently attributed to increased male exposure to risk factors such as physically demanding occupations, high-risk behaviors, greater involvement in road traffic accidents, physical altercations, and contact sports with a high traumatic potential.

The severity of traumatic brain injury (TBI) was assessed using the Glasgow Coma Scale (GCS), with the following distribution: 79.6% of patients presented with mild

TBI (GCS 13–15), 12.3% with moderate TBI (GCS 9–12), and 8.0% with severe TBI (GCS ≤ 8).

Regarding the environment of residence, the majority of patients originated from rural areas (71.2%), compared to 28.8% from urban settings. The individual patient assessment form incorporated several descriptive variables related to the context of the injury, including environmental setting (urban/rural), time of occurrence (day/night, season), presence or absence of alcohol intoxication, patient age, and the exact location of the incident (residence, public road, workplace, etc.).

The analysis of the circumstances in which traumatic brain injuries (TBI) occurred in the study cohort revealed that the main etiological mechanisms were falls (both same-level and from height), recorded in 44.65% of cases. These were followed by physical assaults, which accounted for 25.72% of injuries. The third most frequent cause was road traffic accidents, resulting in TBI in 91 patients (18.72% of the total).

Table 1. Demographic and clinical characteristics of the study group (n = 486)

Feature	Number of patients	Value
Age (years), Mean ± SD	-	51,29 ± 18,34
Sex		
– Male	306	62,9%
– Female	180	37,1%
TBI severity (GCS)		
– Mild (GCS 13–15)	387	79,6%
– Moderate (GCS 9–12)	60	12,3%
– Severe (GCS ≤ 8)	39	8,0%
Residence		
– Urban	140	28,8%
– Rural	346	71,2%

Note: Data are presented as mean ± SD or n (%). TBI = Traumatic Brain Injury; GCS = Glasgow Coma Scale. Statistical analysis was performed using chi-square and t-tests; $p < 0.05$ was considered significant.

The distribution of causes according to TBI severity showed the following trends.

In cases of mild TBI, the leading causes were:

- Falls (same-level and from height) – 47.55% (n = 184),
- Physical assaults – 28.42% (n = 110),
- Road traffic accidents – 16.54% (n = 64).

For moderate TBI, the most common causes were:

- Falls – 60.00% (n = 36),
- Road traffic accidents – 21.67% (n = 13),
- Physical assaults – 18.33% (n = 11).

Regarding severe TBI, the predominant causes were:

- Falls – 53.84% (n = 21),
- Road traffic accidents – 35.9% (n = 14),
- Physical assaults – 10.26% (n = 4).

The coexistence of traumatic brain injury (TBI) with other types of bodily trauma significantly influences both clinical severity and the patient's prognostic outcomes. Analysis of the study cohort revealed that out of the 486 included patients, 289 individuals (59.46%) sustained isolated TBI, without any associated injuries.

The distribution of these isolated cases according to TBI severity was as follows:

- Mild TBI: 278 patients (96.19%)
- Moderate TBI: 7 patients (2.42%)
- Severe TBI: 4 patients (1.39%)

These data indicate a predominance of mild forms in isolated TBI cases, suggesting a correlation between the absence of multiple traumas and a more favorable clinical prognosis. Moreover, isolated TBI allows for a more rapid and focused therapeutic approach, with a reduced risk of systemic complications.

Special attention was given to systemic-origin secondary cerebral insults – commonly referred to in the literature by the acronym "ACSOS" (Aggressions Cérébrales Secondaires d'Origine Systémique) – which play a decisive role in determining the prognosis of TBI patients. Based on this, specific recommendations were formulated to optimize interventions during the prehospital phase and within the Emergency Medicine Department / Emergency Admission Units.

While most studies in the literature focus on patients with severe TBI, the present research included individuals with a broad spectrum of clinical presentations – mild, moderate, and severe – which may account for certain discrepancies observed in comparison with previously published findings.

The results of the correlation analysis between patient age and mortality rate following TBI are presented in Table 2.

Table 2. Correlation matrix between age, TBI severity, and mortality rate (n = 486)

Variables	Statistical Indicators	Age	TBI Severity	Mortality Rate
Age	Pearson Correlation	1	0.030	0.097*
	Sig (2-tailed)		0.513	0.033
	N	486	486	486
TBI Severity	Pearson Correlation	0.030	1	0.594**
	Sig (2-tailed)	0.513		0,000
	N	486	486	486
Mortality Rate	Pearson Correlation	0.097*	0.030	1
	Sig (2-tailed)	0.033	0.513	
	N	486	486	486

Note: Pearson correlation coefficients are shown. * - Correlation is significant at the 0.05 level (2-tailed); ** - Correlation is significant at the 0.01 level (2-tailed). Sample size: n = 486; TBI = Traumatic Brain Injury. Statistical analysis was performed using chi-square and t-tests; $p < 0.05$ was considered significant.

The Pearson correlation analysis highlights the relationships between patient age, the severity of traumatic brain injury (TBI), and the mortality rate.

The results indicate a statistically significant positive correlation between age and mortality rate ($r = 0.097$, $p = 0.033$), suggesting that older age is associated with a higher risk of death among TBI patients. Although the correlation coefficient is modest, its statistical significance underscores the importance of age as a prognostic factor. In contrast, the

correlation between age and TBI severity was not statistically significant (r = 0.030, p = 0.513), indicating that TBI severity does not substantially vary with age in the analyzed cohort.

A strong and statistically significant positive correlation was found between TBI severity and mortality rate (r = 0.594, p < 0.001), demonstrating that patients with more severe brain injuries have a substantially higher risk of death. This finding is consistent with existing literature and confirms the crucial impact of injury severity on survival outcomes.

In addition, the study investigated correlations between systolic blood pressure (SBP) values, TBI severity, and mortality rate. Statistical analysis revealed significant negative correlations between prehospital SBP and TBI severity (r = –0.254, p = 0.0001), as well as between SBP and mortality rate (r = –0.138, p = 0.002), as shown in Table 3.

Table 3. Correlation between systolic blood pressure and TBI severity and mortality in TBI patients

Variables	Statistical Indicators	SBP	TBI Severity	Mortality Rate
Age	Pearson Correlation	1	-.254**	-.138**
	Sig (2-tailed)		0.000	0.002
	N	486	486	486
TBI Severity	Pearson Correlation	-.254**	1	0.594**
	Sig (2-tailed)	0.000		0.000
	N	486	486	486
Mortality Rate	Pearson Correlation	-.138**	0.594**	1
	Sig (2-tailed)	0.002	0.000	
	N	486	486	486

Note: Pearson correlation coefficients are shown. * - Correlation is significant at the 0.05 level (2-tailed); ** - Correlation is significant at the 0.01 level (2-tailed). Sample size: n = 486; SBP – systolic blood pressure; TBI - Traumatic Brain Injury. Statistical analysis was performed using chi-square and t-tests; p < 0.05 was considered significant.

These findings highlight that decreased systolic blood pressure (SBP) values are associated with both increased TBI severity and higher mortality, emphasizing the critical role of early hemodynamic stabilization in the prehospital management of TBI patients.

Compared to existing literature – where studies often include smaller patient samples, lack prehospital data, or focus primarily on general trauma [8] – this study offers a valuable contribution by clearly demonstrating the relationship between prehospital physiological parameters and the clinical course of TBI patients. The results underline the importance of prompt monitoring and correction of hypotension to reduce the risk of complications and death.

The correlation between mean arterial pressure (MAP) and both TBI severity and mortality rate is presented in Table 4.

Statistical analysis revealed significant negative correlations between MAP and both TBI severity and mortality. Specifically, a moderate negative correlation was observed between MAP and TBI severity (r = –0.195, p < 0.0001), as well as a slightly stronger negative correlation between MAP and mortality rate (r = –0.213, p < 0.0001).

Table 4. Correlation between mean arterial pressure and TBI severity and mortality in TBI patients

Variables	Statistical Indicators	MAP	TBI Severity	Mortality Rate
Age	Pearson Correlation	1	-.195**	-.213**
	Sig (2-tailed)		0.000	0.000
	N	486	486	486
TBI Severity	Pearson Correlation	-.195**	1	0.594**
	Sig (2-tailed)	0.000		0.000
	N	486	486	486
Mortality Rate	Pearson Correlation	-.213**	0.594**	1
	Sig (2-tailed)	0.000	0.000	
	N	486	486	486

Note: Pearson correlation coefficients are shown. * - Correlation is significant at the 0.05 level (2-tailed); ** - Correlation is significant at the 0.01 level (2-tailed). Sample size: n = 486; SBP – systolic blood pressure; TBI - Traumatic Brain Injury. Statistical analysis was performed using chi-square and t-tests; p < 0.05 was considered significant.

These results suggest that as the value of the analyzed variable increases, both the severity of trauma and the likelihood of death decrease, indicating a potential protective or favorable predictive role in the clinical course of TBI patients.

The correlation analysis between prehospital hypoxia and indicators of TBI severity revealed statistically significant associations.

Table 5. Correlation between hypoxia and TBI severity and mortality in TBI patients

Variables	Statistical Indicators	Hypoxia	TBI Severity	Mortality Rate
Age	Pearson Correlation	1	.324**	.264**
	Sig (2-tailed)		0.000	0.000
	N	486	438	438
TBI Severity	Pearson Correlation	.324**	1	0.594**
	Sig (2-tailed)	0.000		0.000
	N	438	486	438
Mortality Rate	Pearson Correlation	.264**	0.594**	1
	Sig (2-tailed)	0.000	0.000	
	N	438	438	486

Note: Pearson correlation coefficients are shown. * - Correlation is significant at the 0.05 level (2-tailed); ** - Correlation is significant at the 0.01 level (2-tailed). Sample size: n = 486; TBI - Traumatic Brain Injury. Statistical analysis was performed using chi-square and t-tests; p < 0.05 was considered significant.

More specifically, a moderate positive correlation was identified between the presence of hypoxia in the prehospital phase and TBI severity (r = 0.324, p < 0.0001), as well as a positive correlation between hypoxia and the mortality

rate among TBI patients (r = 0.264, p < 0.0001), as shown in Table 5.

These findings suggest that prehospital hypoxia may serve as a relevant predictive factor for both the deterioration of neurological status and the vital prognosis of patients with TBI.

The findings support the importance of early intervention in the prehospital phase, as adequate monitoring and assessment of oxygenation – and the prevention of hypoxic episodes – have been associated in the literature with significantly improved outcomes in patients with traumatic brain injury (TBI) [9]. Thus, addressing hypoxia from the prehospital stage may not only reduce the severity of neurological damage but also positively influence survival rates.

Another systemic factor involved in secondary cerebral injury and analyzed in the present study was hyperglycemia. Traumatic brain injury induces an acute stress response mediated by the activation of the sympatho-adrenal-medullary axis, leading to increased plasma levels of cortisol, glucagon, insulin, catecholamines, glucose, lactate, and free fatty acids (Shi et al., 2016 [10]; Bosarge et al., 2015 [11]). In this context, blood glucose may serve as a relevant biomarker of post-traumatic metabolic stress.

However, hyperglycemia is not merely an epiphenomenon – it may actively exacerbate brain injury by increasing oxidative stress (through free radical production), inducing apoptosis, and promoting tissue lactic acidosis (Galgano et al., 2017 [12]). While therapeutic efforts primarily target the prevention and management of primary brain injury, secondary injuries such as hyperglycemia are often underestimated, contributing to the so-called "second hit phenomenon" (Galgano et al., 2017 [12]), which negatively impacts patient outcomes.

The correlation between hyperglycemia, TBI severity, and TBI-related mortality is presented in Table 6.

Table 6. Correlation between hyperglycemia and TBI severity and mortality in TBI patients

Variables	Statistical Indicators	Hyperglycemia	TBI Severity	Mortality Rate
Age	Pearson Correlation	1	-0.088 *	-.213**
	Sig (2-tailed)		0.060	0.000
	N	486	456	456
TBI Severity	Pearson Correlation	-0.088 *	1	0.594**
	Sig (2-tailed)	0.060		0.000
	N	456	486	438
Mortality Rate	Pearson Correlation	-.213**	0.594**	1
	Sig (2-tailed)	0.000	0.000	
	N	456	438	486

Note: Pearson correlation coefficients are shown. * - Correlation is significant at the 0.05 level (2-tailed); ** - Correlation is significant at the 0.01 level (2-tailed). Sample size: n = 486; TBI - Traumatic Brain Injury. Statistical analysis was performed using chi-square and t-tests; p < 0.05 was considered significant.

Pearson correlation analysis indicates a weak negative association between hyperglycemia and TBI severity (r = –0.088), with a marginal level of significance (p = 0.060), suggesting a possible inverse trend but without clear statistical significance. In contrast, a moderate and statistically significant negative correlation was observed between hyperglycemia and mortality rate (r = –0.213, p < 0.01), indicating that higher blood glucose levels might be associated with lower mortality—a counterintuitive finding that may warrant further investigation. The strongest association was found between TBI severity and mortality rate (r = 0.594, p < 0.01), reflecting that greater injury severity is significantly correlated with a higher likelihood of death.

Conclusions

The results obtained in this study highlighted that, in the prehospital phase, certain factors such as patient age, systolic blood pressure values, and the presence of hypoxia play a major role, directly influencing both the severity of traumatic brain injury (TBI) and mortality rate. This significant impact was confirmed by relevant statistical correlations (p < 0.0001). Regarding hyperglycemia, it was not found to be significantly associated with TBI severity (p = 0.06) but showed a significant correlation with patient mortality (p < 0.0001), suggesting a potential prognostic role in assessing vital risk.

Competing interests

None declared.

Authors' contributions

NM – substantial contribution to the conception and design of the work, substantial contribution to the acquisition of data, substantial contribution to the analysis and interpretation of data, taking responsibility and being accountable for all aspects of the work. NC – substantial contribution to the conception and design of the work, substantial contribution to the acquisition of data, substantial contribution to the analysis and interpretation of data, drafting the article, taking responsibility for and being accountable for all aspects of the work. LR – critically reviewing the article for important intellectual content, taking responsibility for and being accountable for all aspects of the work. TMC – drafting the article. All authors approved the final version of the manuscript.

Acknowledgements and funding

The study had no external funding.

Ethics approval

The research was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes no. 38, dated 02.04.2013).

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Gerritsen H, Samim M, Peters H, et al. Incidence, course and risk factors of head injury: a retrospective cohort study. BMJ Open. 2018;8(5):e020364. doi: 10.1136/bmjopen-2017-020364.
2. James S, Castle C, Dingels Z, Fox J, et al. Global injury morbidity and mortality from 1990 to 2017: results from the global burden of disease study 2017. Injury Prev. 2020;26:i96-i114. doi: 10.1136/injuryprev-2019-043494.
3. Ciobanu G, Kusturov V, Mishin I. Vozmozhnosti uluchsheniia rezul'tatov lecheniia postradavshikh s politravmoi [Possibilities for improving treatment results for victims of polytrauma]. Bull Acad Sci Mold. Med Sci. 2011;(2):234-239. Russian.
4. Ciobanu G. Agresiuni cerebrale secundare de origine sistemică [Secondary cerebral aggressions of systemic origin]. Bull Acad Sci Mold. Med Sci. 2009;(2):311-324. Romanian.
5. Ciobanu G. Agresiunile cerebrale secundare de origine sistemică la politraumatizaţi [Systemic secondary cerebral aggressions at polytraumatised patients]. Arta Medica (Chisinau). 2009;(1 Suppl):29-34. Romanian.
6. Stiell IG, Clement CM, Rowe BH, et al. Comparison of the Canadian CT head rule and the New Orleans criteria in patients with minor head injury. JAMA. 2005;294(12):1511-1518. doi: 10.1001/jama.294.12.1511.
7. Cătanoi N, Rezneac L, Mocanu N. Protocols for the diagnosis of urgent neurological pathologies in the pre-hospital stage. Arch Balk Med Union. 2025;60(1):140-147. https://doi.org/10.31688/ABMU.2025.60.1.15
8. Cojocari V, Ciobanu G, Scurtov N. The impact of pre-hospital arterial hypo- and hypertension on clinical severity and prognosis of patients with traumatic brain injury. Curier Med (Chisinau). 2013;56(3):290-293.
9. Spaite DW, Hu C, Bobrow BJ, Chikani V, Barnhart B, Gaither JB, et al. The effect of combined out-of-hospital hypotension and hypoxia. Ann Emerg Med. 2017;69(1):62-72. doi: 10.1016/j.annemergmed.2016.08.007.
10. Shi J, Dong B, Mao Y, et al. Traumatic brain injury and hyperglycemia, a potentially modifiable risk factor: review. Oncotarget. 2016;7(43):71052-71061. doi: 10.18632/oncotarget.11958.
11. Bosarge PL, Shoultz TH, Griffin RL, Kerby JD. Stress-induced hyperglycemia is associated with higher mortality in severe traumatic brain injury. J Trauma Acute Care Surg. 2015;79(2):289-294. doi: 10.1097/TA.0000000000000716.
12. Galgano M, Toshkezi G, Qiu X, Russell T, Chin L, Zhao L. Traumatic brain injury: current treatment strategies and future endeavors. Cell Transplant. 2017;26(7):1118-1130. doi: 10.1177/0963689717714102.

https://doi.org/10.52645/MJHS.2026.2.06

UDC: 616.314.26-089.23

RESEARCH ARTICLE

Clinical application of wide and extra-wide archwires in the treatment of dentoalveolar malocclusions

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ABSTRACT

Introduction. Aesthetic and functional considerations have always been the main concerns in the orthodontic treatment of dentoalveolar malocclusions. The objective of this study was to assess the effectiveness of the use of wide and extra-wide archwires in reducing treatment time, compared with conventional archwire therapy.

Materials and methods. A retrospective cohort study was performed. A total of 180 patients aged between 14 and 36 years old were enrolled and divided into two groups: a classical treatment group, including standard NiTi/SS archwires (n=100), and an alternative treatment group with wide and extra-wide CuNiTi/TA/TMA/SS archwires (n=80). The treatment period, estimated in months, was considered the primary outcome. As secondary outcomes, inter-canine, inter-premolar, and inter-molar widths were estimated.

Results. The duration of treatment in the Alternative group was 20.2 ± 4.4 months, with a median of 19.0 months (range: 12–38 months), while the Classic group showed a mean treatment duration of 28.9 ± 5.2 months, a median of 30.0 months (range: 14–39 months), with a mean difference of 8.7 months. Statistical analysis revealed a significant difference in treatment duration between the two protocols (Mann–Whitney U = 863, $p < 0.001$), with a large effect size ($r_{rb} = -0.78$; 95% CI: −0.84 to −0.71), indicating practical relevance.

Regarding secondary outcomes, transverse maxillary measurements, including inter-canine, inter-premolar, and inter-molar widths, showed similar changes before and after treatment in both groups. The available data did not provide sufficient evidence to reject the null hypothesis about the differences between the groups for these parameters, with no clinically meaningful differences.

Conclusions. The application of wide and extra-wide archwires represents an effective orthodontic approach associated with reduced treatment duration, while preserving satisfactory occlusal stability and ensuring a favorable level of patient comfort.

Keywords: orthodontic expansion, wide archwires, dentoalveolar malocclusion, orthodontic treatment, clinical efficiency.

Cite this article: Jarovlea-Bejenari M, Solomon O, Arnaut O. Clinical application of wide and extra-wide archwires in the treatment of dentoalveolar malocclusions. Mold J Health Sci. 2026;13(2):34-40. https://doi.org/10.52645/MJHS.2026.2.06.

Manuscript received: 26.01.2026

Accepted for publication: 25.05.2026

Published: 15.06.2026

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

While wide and extra-wide archwire systems have gained widespread application in contemporary orthodontics, there is limited and inconsistent quantitative evidence to show their effect on treatment efficiency, transverse dentoalveolar outcomes, and post-treatment stability compared to conventional archwire protocols.

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The research hypothesis

Wide and extra-wide archwire protocols facilitate transverse dentoalveolar expansion on par with conventional archwire sequences while significantly reducing overall orthodontic treatment duration, without increasing the risk of post-treatment relapse.

The novelty added by the manuscript to the already published scientific literature

This study offers objective cohort-based evidence that wide and extra-wide archwire protocols markedly reduce the duration of treatment while achieving equivalent transverse outcomes and stability. Our findings elucidate the biomechanical and clinical performance of wide archwire systems and help to justify their adoption in modern orthodontic practice as a standard protocol.

Introduction

The efficiency of orthodontic therapy is largely dependent on the mechanical response and biomechanical properties of the archwires used during treatment [1, 2]. The evolution of orthodontic techniques over the past decades has been strongly influenced by the search for optimal force systems capable of achieving precise tooth movement in a shorter time and with improved patient comfort [3, 4]. Traditional wire progressions, such as those proposed by Tweed (1946) and Ricketts (1982), rely on a sequence of archwires with progressively increasing rigidity and dimensions [5, 6]. Although this conventional approach ensures controlled movement and stability, it often requires an extended treatment duration due to multiple wire changes and the biological adaptation time of the periodontal structures [7].

In contrast, modern orthodontics has shifted towards the development of low-friction systems and wide archwire designs, which aim to simplify mechanics and reduce treatment time. Damon (2004) and McLaughlin (2011) have introduced self-ligating brackets and preformed wide arch forms that promote more physiological expansion of the dental arches and better accommodation of the tongue space [8, 9]. These innovations are claimed to minimize the need for extractions, improve facial aesthetics, and enhance stability by promoting a more balanced arch form [10-12].

However, despite the widespread clinical adoption of wide and extra-wide archwire systems, there remains a need for objective and quantitative evidence to support their claimed advantages over standard archwire forms. The biomechanical effects, degree of arch expansion, and overall treatment efficiency achieved through such systems remain only partially understood and variably reported in the literature [13-15]. Factors such as initial malocclusion type, periodontal condition, and patient-specific anatomical variations can significantly influence treatment outcomes [16].

Therefore, the present study aims to evaluate the efficiency of orthodontic expansion methods using wide and extra-wide archwires in the treatment of dentoalveolar malocclusions. By comparing these modern systems with conventional archwire progressions, this research seeks to provide clinically relevant data on treatment duration, degree of transverse expansion, and stability of achieved results [17-19]. The outcomes will contribute to the orthodontic research community by validating – or potentially refuting – the claims made regarding the superiority of wide and extra-wide archwire systems in enhancing treatment efficiency and biomechanical harmony [20, 21].

Materials and methods

Study design. This observational cohort study was carried out between 2020 and 2025 at the *Ilarion Postolachi* Department of Orthopedic Stomatology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova, and the Galadent Prim Clinic. The research protocol was reviewed and approved by the Research Ethics Committee of Nicolae Testemițanu State University of Medicine and Pharmacy (Minutes No. 47/2020, dated March 15, 2020). Written informed consent was obtained from all participants prior to their inclusion in the study. Patients in the control group (n=100) were treated using the conventional fixed appliance protocol with standard NiTi/SS archwire sequences, whereas subjects in the alternative group (n=80) underwent orthodontic treatment employing wide and extra-wide CuNiTi/TA/TMA/SS archwires.

The study inclusion criteria comprised patients presenting dentomaxillary anomalies associated with dental space deficiency. Exclusion criteria included a history of previous orthodontic or maxillofacial treatment, congenital craniofacial malformations, and facial asymmetries requiring surgical management. Diagnostic assessment was based on comprehensive clinical and photographic examination, digital biometric analysis using Medit Link and NemoCast software, as well as radiographic evaluation performed according to the Tweed, Ricketts, and Roth-Jarabak cephalometric methods [22].

Treatment Protocol: All patients were treated with a standard fixed multibracket appliance (0.022-inch slot), following a sequential archwire system using NiTi, CuNiTi, and TMA wires [23].

- Stage 1: Initial alignment and leveling – superelastic NiTi archwires (0.012–0.014 inch) (Fig. 1)
- Stage 2: Controlled transverse expansion – CuNi-Ti archwires (0.016 × 0.022 inch and 0.017 × 0.025 inch) (Fig. 2)
- Stage 3: Finishing and consolidation – TMA archwires (0.019 × 0.025 inch) (Fig. 3, 4)

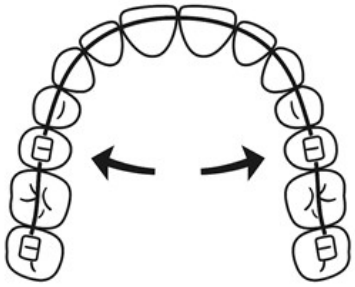

Fig. 1 Schematic representation of transverse dentoalveolar expansion with a CuNiTi archwire (active phase).

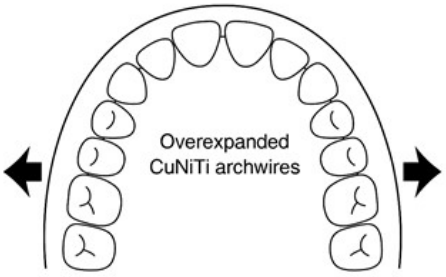

Fig. 2 Controlled transverse expiation using CuNiTi archwires (0.016 × 0.022 inch and 0.017 × 0.025 inch): schematic representation of lateral expansion forces.

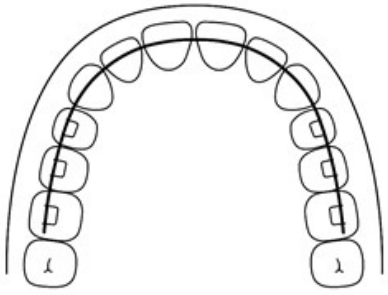

Fig. 3 Finishing and consolidation – TMA archwires (0.019 × 0.025 inch).

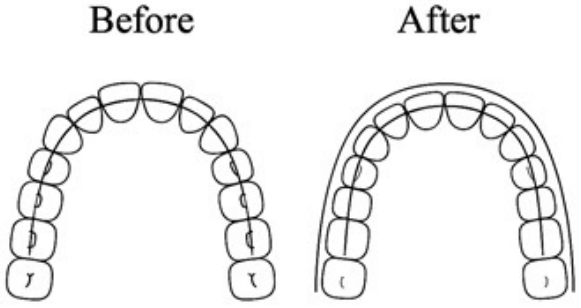

Fig. 4 Before and after finishing.

Statistical analysis was performed using the RStudio open-source software. The mean with a 95% confidence interval, median, standard deviation, interquartile range, and minimal and maximal values were considered for descriptive statistics of numerical variables. For categorical parameters, absolute and relative frequencies with a 95% confidence interval were estimated. Comparative evaluation between the groups was performed using the Mann-Whitney test (visualization through boxplots and jitter plots), completed by a rank biserial correlation analysis (r_{rb}) for practical significance estimation in numerical variables. Pearson's Chi-squared test with Monte Carlo simulation was applied for association hypothesis testing for categorical variables.

Results

The study included a total of 180 participants, divided into the Alternative group (n = 80) and the Classic group (n = 100). The overall mean age was 18.6 ± 5.1 years, with no statistically significant age difference between the two treatment groups (p = 0.20). Age distribution was comparable between groups, with similar medians and interquartile ranges.

A statistically significant difference was observed in gender distribution between the two groups ($\chi^2 = 5.2, p = 0.032$). Females predominated in the overall sample (71.1%), with a higher proportion in the Classic group (78.0%) compared to the Alternative group (62.5%). No significant differences were found regarding settlement (urban vs. rural) between groups ($p = 0.10$).

The distribution of constitutional craniofacial types (brachyfacial, dolichocephalic, and mesocephalic) was similar between groups, with the brachyfacial type being the most prevalent (approximately 59%), and no statistically significant differences were detected ($p > 0.9$).

Regarding Angle's classification, Class I malocclusion was the most common in the overall sample (60.6%). Although a higher proportion of Class I was observed in the Alternative group and a higher proportion of Class II in the Classic group, these differences were not statistically significant ($p = 0.069$).

Analysis of occlusal relationships revealed no statistically significant differences between groups for: RMR (Right Molar Relationship) ($p > 0.9$), RML (Left Molar Relationship) ($p = 0.50$), RCR (Right Canine Relationship) ($p = 0.40$), and RCL (Left Canine Relationship) ($p = 0.30$).

Across all participants, Class I molar and canine relationships were the most prevalent on both sides, followed by Class II, while Class III relationships were relatively rare.

Overall, aside from gender distribution, the Alternative and Classic groups were comparable with respect to de-

mographic characteristics, craniofacial morphology, Angle classification, and molar and canine sagittal relationships, indicating good baseline homogeneity between groups (Table 1).

Transverse expansion outcomes. The analysis of transverse maxillary dimensions (Table 2) demonstrated a high degree of similarity between the Alternative and Classic groups at both the initial and final evaluations. At baseline, the initial intercanine width (DMSICT**)** showed comparable mean values and distributions in the two groups, indicating similar transverse maxillary morphology in the anterior region. Likewise, the initial inter-first premolar (DMSIPIT), inter-second premolar (DMSIPIIT), and intermolar widths (DMSIMT) did not differ significantly between groups (all $p > 0.05$).

At the anterior maxillary level, the intercanine transverse dimension (DMSICT) showed identical mean values in both groups (34.0 ± 3.0 mm in the Alternative group and 34.0 ± 2.9 mm in the Classic group), with similar median values (34 mm) and overlapping ranges (27.0–41.0 mm and 28.0–40.0 mm, respectively). Likewise, the first premolar transverse width (DMSFCT) demonstrated comparable mean values between the Alternative group (35.8 ± 2.8 mm) and the Classic group (36.0 ± 2.6 mm), with overlapping confidence intervals and no statistically significant difference ($p = 0.7$). Measurements at the premolar and intermediate regions further confirmed this pattern. The DMSIPIT parameter showed almost identical mean values in the Alternative and Classic groups (36.1 ± 3.2 mm vs. 36.0 ± 2.8 mm), while the DMSFPIT parameter recorded mean transverse widths of 39.0 ± 2.9 mm and 39.0 ± 3.1 mm, respectively. For both variables, medians and interquartile ranges were highly similar, and statistical testing revealed no significant intergroup differences ($p > 0.8$). At the intermediate posterior level, DMSIPIIT and DMSFPIIT measurements remained consistent between groups, with mean values ranging from 40.7 to 40.9 mm and from 43.7 to 43.8 mm, respectively. The confidence intervals overlapped extensively, and no statistically significant differences were detected ($p > 0.9$). In the posterior maxillary region, intermolar transverse dimensions (DMSIMT and DMSFMT) were also comparable. Mean DMSIMT values were 49.1 ± 4.1 mm in the Alternative group and 49.8 ± 3.7 mm in the Classic group, while DMSFMT values were identical in both groups (51.8 ± 3.8 mm). The Wilcoxon rank-sum test confirmed the absence of statistically significant differences between groups for these parameters ($p = 0.2$ and $p = 0.8$, respectively).

Overall, the absence of statistically significant differences across all transverse maxillary measurements indicates that the Alternative and Classic groups were homogeneous with respect to transverse maxillary morphology. This homogeneity supports the validity of intergroup comparisons and suggests that observed outcomes are unlikely to be influenced by baseline transverse maxillary discrepancies.

Table 1. Demographic and Clinical Characteristics of the Study Populations

Group	Overall N = 180[1]	95% CI[2]	Alternative N = 80[1]	95% CI[2]	Classic N = 100[1]	95% CI[2]	Statistic Test[3]	*p*-value[3]
Age	18.6 (5.1) 17.0 (6.0) 14.0 36.0	18, 19	18.5 (5.5) 16.0 (7.0) 14.0 36.0	17, 20	18.8 (4.8) 17.0 (6.3) 14.0 36.0	18, 20	3,583	0.2
Gender							5.2	0.032
F	128 (71.1%)	64%, 78%	50 (62.5%)	52%, 73%	78 (78.0%)	70%, 86%		
M	52 (28.9%)	22%, 36%	30 (37.5%)	27%, 48%	22 (22.0%)	14%, 30%		
Settlement							3.0	0.10
Town	94 (52.2%)	45%, 60%	36 (45.0%)	34%, 56%	58 (58.0%)	48%, 68%		
Village	86 (47.8%)	40%, 55%	44 (55.0%)	44%, 66%	42 (42.0%)	32%, 52%		
Constitutional type							0.17	>0.9
Brachyfacial	106 (58.9%)	52%, 66%	47 (58.8%)	48%, 70%	59 (59.0%)	49%, 69%		
Dolichocephalic	52 (28.9%)	22%, 36%	24 (30.0%)	20%, 40%	28 (28.0%)	19%, 37%		
Mesocephalic	22 (12.2%)	7.4%, 17%	9 (11.3%)	4.3%, 18%	13 (13.0%)	6.4%, 20%		
Angle							5.4	0.069
cl. I	109 (60.6%)	53%, 68%	56 (70.0%)	60%, 80%	53 (53.0%)	43%, 63%		
cl. II	53 (29.4%)	23%, 36%	18 (22.5%)	13%, 32%	35 (35.0%)	26%, 44%		
cl. III	18 (10.0%)	5.6%, 14%	6 (7.5%)	1.7%, 13%	12 (12.0%)	5.6%, 18%		

Table 1. Demographic and Clinical Characteristics of the Study Populations

Group	Overall N = 180[1]	95% CI[2]	Alternative N = 80[1]	95% CI[2]	Classic N = 100[1]	95% CI[2]	Statistic Test[3]	*p*-value[3]
RMR							0.04	>0.9
cl. I	102 (56.7%)	49%, 64%	46 (57.5%)	47%, 68%	56 (56.0%)	46%, 66%		
cl. II	62 (34.4%)	28%, 41%	27 (33.8%)	23%, 44%	35 (35.0%)	26%, 44%		
cl. III	16 (8.9%)	4.7%, 13%	7 (8.8%)	2.6%, 15%	9 (9.0%)	3.4%, 15%		
RML							1.5	0.5
cl. I	104 (57.8%)	51%, 65%	47 (58.8%)	48%, 70%	57 (57.0%)	47%, 67%		
cl. II	58 (32.2%)	25%, 39%	23 (28.8%)	19%, 39%	35 (35.0%)	26%, 44%		
cl. III	18 (10.0%)	5.6%, 14%	10 (12.5%)	5.3%, 20%	8 (8.0%)	2.7%, 13%		
RCR							2.2	0.4
cl. I	76 (42.2%)	35%, 49%	29 (36.3%)	26%, 47%	47 (47.0%)	37%, 57%		
cl. II	97 (53.9%)	47%, 61%	48 (60.0%)	49%, 71%	49 (49.0%)	39%, 59%		
cl. III	7 (3.9%)	1.1%, 6.7%	3 (3.8%)	0.00%, 7.9%	4 (4.0%)	0.16%, 7.8%		
RCL							2.4	0.3
cl. I	58 (32.2%)	25%, 39%	21 (26.3%)	17%, 36%	37 (37.0%)	28%, 46%		
cl. II	106 (58.9%)	52%, 66%	51 (63.8%)	53%, 74%	55 (55.0%)	45%, 65%		
cl. III	16 (8.9%)	4.7%, 13%	8 (10.0%)	3.4%, 17%	8 (8.0%)	2.7%, 13%		

Note: [1]Mean (SD), Median (IQR), Min Max; n (%), [2]CI = Confidence Interval, [3]Wilcoxon rank sum test; Pearson's Chi-squared test with simulated *p*-value (based on 10^5 replicates)

Table 2. Maxillary Transverse Arch Measurement Data

Group	Overall N = 180[1]	95% CI[2]	Alternative N = 80[1]	95% CI[2]	Classic N = 100[1]	95% CI[2]	Statistic Test[3]	*p*-value[3]
DMSICT	34.0 (2.9) 34.0 (4.0) 27.0 41.0	34, 34	34.0 (3.0) 34.0 (4.0) 27.0 41.0	33, 35	34.0 (2.9) 34.0 (4.0) 28.0 40.0	33, 35	3,984	>0.9
DMSFCT	35.9 (2.7) 35.8 (2.6) 28.0 45.0	36, 36	35.8 (2.8) 35.5 (2.0) 28.0 43.0	35, 36	36.0 (2.6) 36.0 (3.0) 31.0 45.0	36, 37	3,886	0.7
DMSIPIT	36.1 (3.0) 36.0 (4.0) 29.0 45.0	36, 36	36.1 (3.2) 36.0 (4.0) 29.0 45.0	35, 37	36.0 (2.8) 36.0 (3.3) 29.0 45.0	35, 37	4,049	0.9
DMSFPIT	39.0 (3.0) 39.0 (4.0) 31.0 50.0	39, 39	39.0 (2.9) 39.0 (3.6) 33.0 49.0	38, 40	39.0 (3.1) 39.0 (4.0) 31.0 50.0	38, 40	3,921	0.8
DMSIPIIT	40.8 (3.4) 41.0 (5.0) 31.0 50.0	40, 41	40.7 (3.6) 40.0 (5.0) 31.0 48.0	40, 42	40.9 (3.2) 41.0 (4.5) 33.0 50.0	40, 41	3,997	>0.9
DMSFPIIT	43.7 (3.1) 44.0 (3.5) 35.0 55.0	43, 44	43.8 (3.2) 44.0 (3.9) 37.5 55.0	43, 45	43.7 (3.1) 44.0 (3.0) 35.0 52.0	43, 44	4,040	>0.9
DMSIMT	49.5 (3.9) 50.0 (5.0) 40.5 60.0	49, 50	49.1 (4.1) 50.0 (5.0) 40.5 60.0	48, 50	49.8 (3.7) 50.0 (5.0) 41.0 59.0	49, 51	3,599	0.2
DMSFMT	51.8 (3.8) 52.0 (5.0) 42.0 61.0	51, 52	51.8 (3.8) 52.0 (5.0) 42.0 59.0	51, 53	51.8 (3.8) 51.5 (5.0) 42.0 61.0	51, 53	4,100	0.8

Note: [1]n (%); Mean (SD), Median (IQR), Min Max, [2]CI = Confidence Interval, [3]Wilcoxon rank sum test

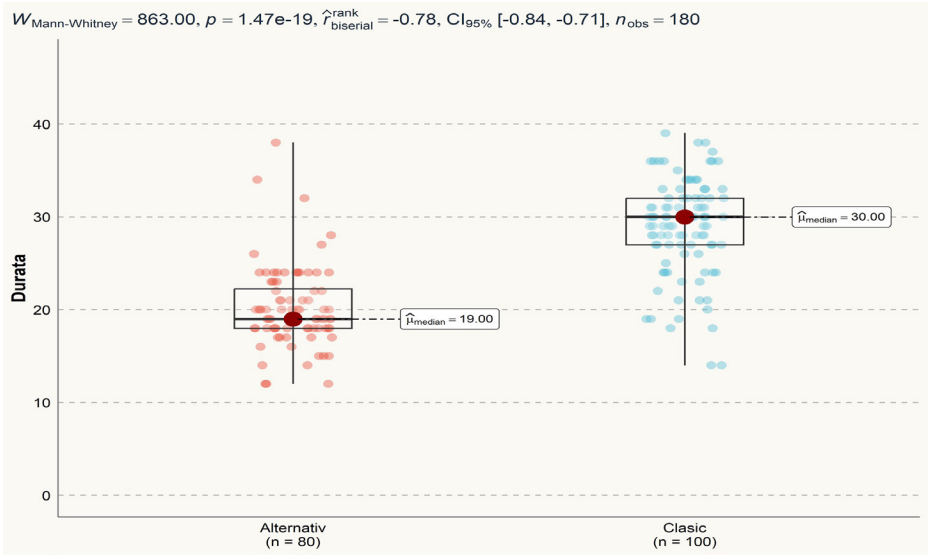

Fig. 5 Graphical representation of treatment duration (months) between the Alternative and Classic groups

Note: Boxes represent the interquartile range (IQR), the horizontal line within each box indicates the median value, and whiskers denote the minimum and maximum recorded treatment durations. W represents the Mann–Whitney U test statistic; p denotes the probability value indicating statistical significance; r_{rb} indicates the rank-biserial correlation coefficient (effect size); $CI_{95\%}$ represents the 95% confidence interval of the effect size; and n_{obs} denotes the total number of observations included in the analysis.

Treatment duration. Treatment duration differed markedly between protocols. In the overall cohort, the mean duration was 25.0 ± 6.5 months (median 24.0 months; range 12–39 months). Patients treated with the Alternative method completed therapy in 20.2 ± 4.4 months (median 19.0 months; range 12–38 months), whereas those in the Classic group required 28.9 ± 5.2 months (median 30.0 months; range 14–39 months). This difference of 8.7 months was both statistically significant ($p < 0.001$) and clinically relevant. An inverse and strong correlation was observed between treatment duration and the magnitude of transverse expansion ($r = –0.69$), indicating that greater controlled widening of the dental arches was associated with shorter overall treatment time (Fig. 5). No increase in post-treatment relapse incidence was detected in the Alternative group, suggesting that the more rapid expansion did not compromise occlusal stability.

Discussion

This study evaluated the efficiency of wide and extra-wide archwires in orthodontic expansion therapy and demonstrated that their use results in significantly greater transverse dentoalveolar widening and substantially shorter treatment durations compared with a conventional archwire protocol. Because the two groups were similar at baseline in terms of age, occlusal characteristics, and cephalometric parameters, the observed differences are most plausibly attributable to the archwire strategy rather than to sample imbalance. The transverse dimensions at the canine, premolar, and molar levels were comparable between the two groups under similar clinical conditions; however, the Alternative protocol achieved these equivalent transverse outcomes within a shorter overall treatment duration. These findings are consistent with previous reports that low-friction, wide-arch systems can enhance transverse development and reduce the need for extractions while preserving facial aesthetics and stability. The systematic increase in DMSICT/DMSFCT and DMSIPIT/DMSFPIT, along with the relatively narrow dispersion of post-treatment values, suggests that expansion was controlled rather than excessive. A major clinical implication of the present work is the significant reduction in treatment time – approximately 8–9 months shorter with the wide archwire protocol. This result is in line with studies reporting slight to moderate decreases in treatment duration when low-friction systems or expanded archforms are used. Importantly, in our sample, this acceleration did not appear to compromise post-treatment stability, as no increase in relapse incidence was observed in the Alternative group during the documented follow-up period. The nearly normal distributions of DMSIPIT and DMSFPIT further support the predictability of the biomechanical response. Mild outliers in the final measurements did not translate into clinical instability and may reflect individual anatomical variability. Recent three-dimensional studies emphasize the value of digital models for assessing arch form and symmetry, and similar approaches could be applied in future prospective trials to quantify volumetric changes and periodontal responses to wide archwire expansion. This study has limitations. Its retrospective design may introduce selection bias, and the significant difference in gender distribution between groups could serve as an uncontrolled confounder. In addition, although transverse and cephalometric measurements were detailed, skeletal maturation stages and long-term (>5 years) retention outcomes were not systematically evaluated. Future research should include prospective randomized trials using three-dimensional imaging, standardized retention protocols, and multivariate analysis to control simultaneously for demographic, skeletal, and occlusal variables. Despite these limitations, the present findings provide robust clinical evidence that wide and extra-wide archwires can be safely incorporated into contemporary orthodontic practice to improve efficiency. When carefully selected and monitored, such mechanics offer predictable transverse gains and meaningful reductions in treatment duration without compromising occlusal stability.

Conclusions

The Alternative protocol enhances treatment efficiency without compromising transverse outcomes or post-treatment stability, while wide archwire mechanics represent a reliable approach for managing dentoalveolar crowding in contemporary orthodontic practice; however, further prospective studies are required to confirm these findings.

Competing interests

None declared.

Authors' contributions

MJV conceived and designed the study, collected the data, performed the analysis and interpretation of the data, drafted the manuscript, and takes responsibility for the integrity of the work as a whole. OS contributed to drafting the manuscript and critically reviewed it for important intellectual content. OA supervised the study process, contributed to the study design and methodology validation, performed the statistical analysis, and provided critical revision of the manuscript.

Ethics approval

The research project was approved by the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes no. 47/2020 dated March 15, 2020).

Patient consent

Obtained.

Acknowledgements and funding

No external funding.

Provenance and peer review

Not commissioned, externally peer reviewed.

References

1. Burstone CJ. Biomechanics of deep overbite correction. Semin Orthod. 2001;7(1):26-33. doi: 10.1053/sodo.2001.21059.
2. Melsen B, editor. Adult orthodontics. Oxford: Wiley-Blackwell; 2012.
3. Graber LW, Vanarsdall RL Jr, Vig KWL, Huang GJ. Orthodontics: current principles and techniques. 6th ed. St. Louis: Elsevier; 2017. 1016 p.
4. Proffit WR, Fields HW, Larson BE, Sarver DM. Contemporary orthodontics. 6th ed. Philadelphia: Elsevier; 2019. 729 p.
5. Ricketts RM. Bioprogressive therapy as an answer to orthodontic needs. Part I. Am J Orthod. 19976;70(3):241-268. doi: 10.1016/0002-9416(76)90332-8.
6. Tweed CH. The Frankfort-mandibular plane angle in orthodontic diagnosis, classification, treatment planning and prognosis. Am J Orthod. 1946;32(4):175-230. doi: 10.1016/0096-6347(46)90001-4.
7. Leon-Valencia J, Alarcon JA, Martin C. Assessment of dental arch width expansion effectiveness using a novel hybrid aligner with virtual brackets and nickel-titanium archwires: a prospective clinical study. Appl Sci. 2025;15(1):39. doi: 10.3390/app15010039.
8. Damon DH. The Damon low-friction bracket: a biologically compatible straight-wire system. J Clin Orthod. 1998;32(11):670-680.
9. McLaughlin RP, Bennett JC, Trevisi HJ. Systemized orthodontic treatment mechanics. Edinburgh: Mosby; 2001. 324 p.
10. Nanda R. Biomechanics and esthetic strategies in clinical orthodontics. St. Louis: Elsevier Saunders; 2005. 385 p.
11. Pandis N, Miles PG. Treatment efficiency with self-ligating brackets: the clinical evidence. Semin Orthod. 2010;16(4):258-265. doi: 10.1053/j.sodo.2010.06.004.
12. Trifan V, Lupan I, Trifan D, Calfa S. Morbiditatea prin anomaliile dento-maxilare în Republica Moldova [Morbidity by dental-maxillary anomalies the Republic of Moldova]. Stomatol Med (Chisinau). 2015;(1):47-52. Romanian.
13. Dehbi H, Azaroual MF, Zaoui F, Halimi A, Benyahia H. Efficacité thérapeutique des brackets auto-ligaturants: revue systématique. Int Orthod. 2017;15(3):297-311. doi: 10.1016/j.ortho.2017.06.010.
14. Fleming PS, Johal A. Self-ligating brackets in orthodontics: a systematic review. Angle Orthod. 2010;80(3):575-584. doi: 10.2319/081009-454.1.
15. Tachi A, Tochigi K, Saze N, Arai K. Impact of the prefabricated forms of NiTi archwires on orthodontic forces delivered to the mandibular dental arch. Prog Orthod. 2021;22(1):41. doi: 10.1186/s40510-021-00385-1.
16. Allan D, Woods MG. Arch-dimensional changes in non-extraction cases with finishing wires of a particular material, size and arch form. Aust Orthod J. 2015;31(1):26-36.
17. Mahmood B, Hassan B. Three-dimensional analysis of dental arch form and dimensions in a sample of Kurdish students in Hawler Medical University. Erbil Dent J. 2023;6(1). doi: 10.15218/edj.2023.01.
18. Mateu ME, Benítez-Rogé S, Calabrese D, Lumi M, Iglesias M, Méndez P, et al. Prospective clinical study of transverse development with self-ligating brackets. Acta Odontol Latinoam. 2020;33(2):112-116.
19. Nguyen M, Al-Moghrabi D, Tomlinson L, Azami N, Dolce C, Abu Arqub S. Early orthodontic treatment practices and perceptions: a cross-sectional survey of AAO members. J Orthod. 2025;52(3):285-294. doi: 10.1177/14653125251358832.
20. Arora MA, Shah A, Somani D. Self-ligating versus conventional brackets: a narrative review. Cureus. 2025;17(3):e81499. doi: 10.7759/cureus.81499.
21. Serrano-Velasco D, Martín-Vacas A, Giovannini G, Paz-Cortés MM, Aragoneses JM. Accuracy analysis of digital models from intraoral scanners and 3D-printed casts in children and teenagers. Children (Basel). 2024;11(9):1082. doi: 10.3390/children11091082.
22. Andrews LF. The straight-wire appliance. Br J Orthod. 1979;6(3):125-143. doi: 10.1179/bjo.6.3.125.

https://doi.org/10.52645/MJHS.2026.2.07

UDC:615.014.4

RESEARCH ARTICLE

The influence of stress factors on dioxoindolinone stability

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ABSTRACT

Introduction. Stability studies for pharmaceutical products represent a primary stage in the development and manufacture of a new medicinal product, being a fundamental condition that guarantees its quality and efficacy. The research was initiated with the aim of the determining the stability of Dioxoindolinone under stress conditions in order to find out the factors that can induce possible changes in the molecular structure of the Dioxoindolinone, which consequently can lead to a partial or total diminution of the therapeutic effect.

Materials and methods. In the experimental research, Dioxoindolinone was used, synthesized in the Organic Synthesis Laboratory of the Institute of Chemistry, at USM, (purity 99.9%). The following apparatus was used: analytical balance (*OHAUS DV215 CD*, Switzerland); spectrophotometer (*Shimadzu UV-1800*, Japan); a pair of quartz cuvettes with a layer thickness of 10 mm; ultraviolet lamp chamber (UV with CN-6 filter, France) for exposure to 254 nm and 365 nm radiation; thermostat (*TC-80M-2*, Ukraine) set at 60 ± 1°C; ultrasonic bath (*Sapfir*, St. Petersburg); reagents: analytical grade reagents – 0.1 M hydrochloric acid (HCl) (ChemLab, Belgium); 0.1 M sodium hydroxide (NaOH) (ChemLab, Belgium); 3% hydrogen peroxide solution (H_2O_2) (CentroChem, Poland); ethanol (96%) (CentroChem, Poland).

Results. The influence of stress factors, such as oxidants, acids, bases, humidity, high temperatures and UV irradiation on the stability of Dioxoindolinone was studied. Under conditions of oxidative, hydrolytic, thermal, acid-base, photolytic stress by the UV-Vis spectrophotometric method it was determined that the substance is stable to humidity and in the acidic environment. Dioxoindolinone degrades under the influence of oxidant, it was found to be unstable in the basic environment (a change in concentration was observed). The insignificant influence of UV light and high temperature was demonstrated.

Conclusions. The influence of stress factors on the stability of Dioxoindolinone was studied. The results obtained will be used to establish optimal storage conditions that will be introduced in the Quality Standardization Specification for Dioxoindolinone.

Keywords: stability, Dioxoindolinone, forced degradation.

Cite this article: Ştefaneţ T. The influence of stress factors on dioxoindolinone stability. Mold J Health Sci. 2026;13(2):41-47. https://doi.org/10.52645/MJHS.2026.2.07.

Manuscript received: 18.02.2026

Accepted for publication: 21.05.2026

Published: 15.06.2026

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Dioxoindolinone has significant pharmacological potential as an MAO inhibitor. This substance is being investigated experimentally in order to develop the Quality Specification. To date, no stability study of Dioxoindolinone has been performed.

The research hypothesis

The stability of medicinal substances is an important and mandatory factor in determining the quality of the medicine, and for establishing the optimal storage conditions that will be included in the Quality Specification for Dioxoindolinone.

The novelty added by the manuscript to the already published scientific literature

For the first time, a study of the stability of Dioxoindolinone to the action of stress factors was carried out.

Introduction

Neurodegenerative and mental disorders, such as Parkinson's disease and major depressive disorder, present a considerable health issue, affecting hundreds of millions globally [1]. Monoamine oxidase (MAO) inhibitors remain important therapeutic agents in the treatment of these diseases [2]. The development of new drugs with improved safety and stability characteristics is thus of notable pharmaceutical interest. At the Organic Synthesis Laboratory, Institute of Chemistry, Moldova State University, a newly synthesized compound Dioxoindolinone (1-(2-oxo-propyl)-spiro[[1,3]dioxolane-2,3'-indolin]-2'-one) was obtained [3]. The substance has demonstrated significant pharmacological potential as an MAO inhibitor [4]. Therefore, establishing its stability profile represents a critical step for further pharmaceutical development and rational dosage form design.

In addition to efficacy, purity, and safety, stability is an important factor in ensuring the quality of a drug [5].

The quality, therapeutic efficacy and safety of the drug during storage directly depend on its ability to maintain its properties within the limits established by regulatory/normative documentation over a certain period of time under appropriate storage and transportation conditions, i.e., stability. Based on the results of the stability study, the shelf life is determined, the materials used for primary and secondary packaging and its type are selected, and the storage conditions of the drug are determined, which are indicated in regulatory/normative documents [6]. The main method for establishing and confirming the shelf life is considered to be real-time testing, which is carried out under conditions as close as possible to the expected storage conditions of commercial products. To determine the stability of new drug substances, forced degradation methods are usually applied. The main objective of these studies is to find out some fundamental properties of the substances, such as the nature and direction of degradation reactions, the identification (based on the data obtained) of the most important degraded products and the selection of the most appropriate analytical techniques to determine the active substance and its degradation products in the presence of each other. At the same time, the results of these tests can highlight how not only the substances but also, sometimes, the pharmaceutical forms, can cope with short-term, but extremely critical conditions, such as those during transportation [7-9].

However, data concerning the stability and degradation behavior of newly synthesized Dioxoindolinone are presently missing. The absence of such information constrains further pharmaceutical development and formulations studies.

The objective of the present study was to investigate the stability profile of Dioxoindolinone under stress conditions (acidic, alkaline, thermal, oxidative, humid and photolytic exposure) to identify possible degradation pathways and degradation products, and to formulate a stability-indicating analytical procedure appropriate for future quality monitoring of the substance.

Material and methods

The study was conducted at the Drug Development Center within *Nicolae Testemițanu* State University of Medicine and Pharmacy. The stability evaluation of Dioxoindolinone was performed in accordance with ICH regulations and guidelines [10, 11].

For the stability studies under stress conditions, three experimental synthesis batches (01, 02, 03) of Dioxoindolinone, obtained at the Organic Synthesis Laboratory, Institute of Chemistry, Moldova State University, were used. An internal reference standard of 1-(2-oxo-propyl)-spiro[[1,3] dioxolane-2,3'-indolin]-2'-one-substance, purified by recrystallization with a purity of 99.98%, was also employed.

The following apparatus was used: analytical balance (*OHAUS DV215 CD*, Switzerland); spectrophotometer (*Shimadzu UV-1800*, Japan) and 10 mm quartz cuvettes; ultraviolet lamp chamber (UV with CN-6 filter, France) for exposure to 254 nm and 365 nm radiation; thermostat (*TC-80M-2*, Ukraine) set at 60 ± 1°C; ultrasonic bath (*Sapfir*, St. Petersburg). The following analytical grade reagents were used: 0.1 M hydrochloric acid (HCl) (ChemLab, Belgium); 0.1 M sodium hydroxide (NaOH) (ChemLab, Belgium); 3% hydrogen peroxide solution (H_2O_2) (CentroChem, Poland); ethanol (96%) (CentroChem, Poland).

To assess the stability of Dioxoindolinone, the spectrophotometric method, previously developed and validated [12], was used.

Preparation of the standard solution: 0.05 g Dioxoindolinone internal standard (exact mass) was dissolved in 20 ml of ethanol (96%) in an ultrasonic bath for 1 min and diluted to 50 ml with the same solvent in a volumetric flask. 1.5 ml of this solution was further diluted to 50 ml in a volumetric flask with the same solvent, and absorbance was measured at 257 ± 1 nm, using ethanol (96%) as the reference solution.

Preparation of the sample solution. 0.05 g of the Dioxoindolinone substance (exact mass) was dissolved in 20 ml of ethanol (96%) in an ultrasonic bath for 1 min and diluted to 50 ml with the same solvent in a volumetric flask (solution A).

The study of forced degradation.

The sample solution was subjected to oxidative degradation and hydrolysis in acidic and basic environments for 24 hours, with three UV-Vis spectrophotometric analyses: at 0, 3 and 24 hours. The absorbances of the test solutions were measured at 257 ± 1 nm, using ethanol (96%) as the reference solution. The concentration was calculated in relation to the absorbance of the standard solution.

Preparation of the sample solution for oxidative degradation (acid hydrolysis, basic hydrolysis). 1 ml of solution A was transferred into three stoppered test tubes, to which 1 ml of 3% hydrogen peroxide solution (1 ml 0.1 M hydrochloric acid; 1 ml 0.1 M sodium hydroxide) was added, respectively. From each test tube, 0.75 ml of the treated solution was diluted to 25 ml in a volumetric flask with ethanol (96%), and absorbance was measured at 257 ± 1 nm, using ethanol (96%) as the reference solution. The first test tube was analyzed immediately; the second test tube after 3 hours and the third one after 24 hours.

To test the stability to physical stress factors, the substance samples were exposed to high humidity (80% RH) by keeping them for 2 weeks in a desiccator above water. Other samples were exposed to ultraviolet radiation (in the UV chamber) for 48 hours and to high temperature (60 °C in the thermostat for 2 weeks). Subsequently, quantitative analysis was performed by the UV-Vis spectrophotometric method to evaluate the variations in the stability of Dioxoindolinone under the action of these stress factors [13].

Statistical analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics) 10.5 software.

Results and discussion

To evaluate the degradation processes under stress, the assay variations of Dioxoindolinone were followed. For the assay, the absorption spectra of the standard solution and of the sample solutions were recorded before the application of the stress conditions (Fig. 1).

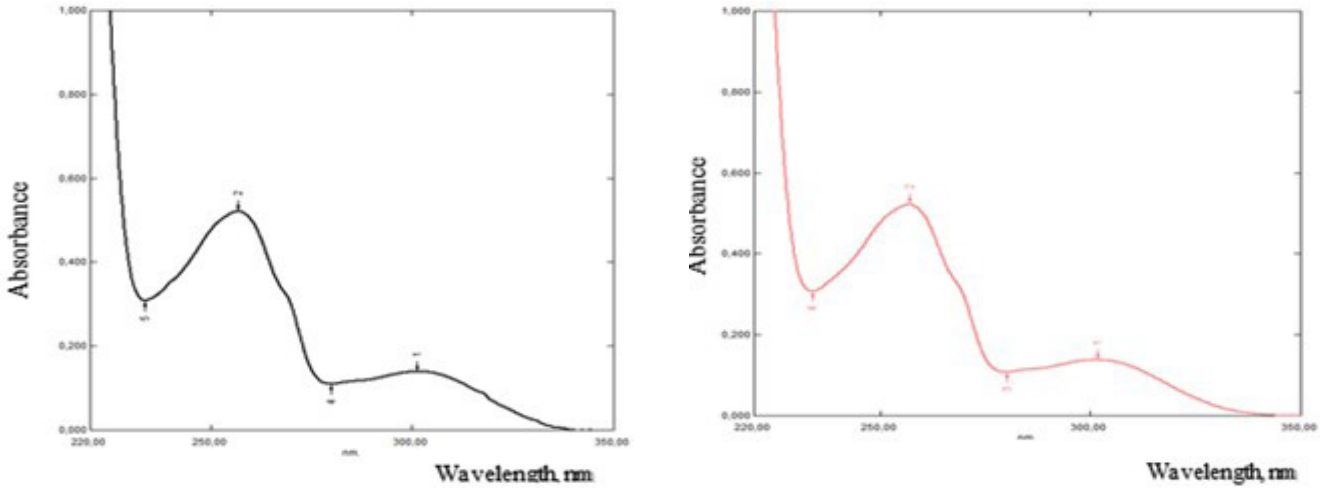

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Fig. 1 Ultraviolet absorption spectra of Dioxoindolinone standard solution (1) and Dioxoindolinone sample (2)

The samples of the substance to be analyzed were subjected to oxidative stress (3% hydrogen peroxide solution), acidic hydrolytic stress (0.1 M hydrochloric acid) and basic hydrolytic stress (0.1 M sodium hydroxide); thermal (60 °C), photolytic (UV light), the substance was investigated under the influence of humidity. The evaluation of the degree of influence of stress factors on Dioxoindolinone was carried out at time intervals: 0 minutes, 3 hours and 24 hours following exposure to the above-mentioned factors (when investigating oxidative, acidic and basic stress); 24 hours and 48 hours (photolytic stress in the UV chamber) or at time intervals: 1 week and 2 weeks (when investigating the influence of humidity and thermal stress). All experiments were conducted in triplicate, and data were expressed as the mean.

Degradation under stress conditions

Oxidative stress

Oxidative degradation can be initiated by three factors: UV light, heat or physical strain in the presence of an oxygen-containing atmosphere, and may occur via two mechanisms: photo-oxidation and thermal oxidation. The selection of an oxidizing agent, its concentration and conditions depend on the drug substance, the most frequently used standard agent is hydrogen peroxide [9].

Under the action of the oxidant (3% hydrogen peroxide solution), complete degradation of Dioxoindolinone was observed, with a change in the absorption spectrum, which made it impossible to determine the absorbance and content (Fig. 2).

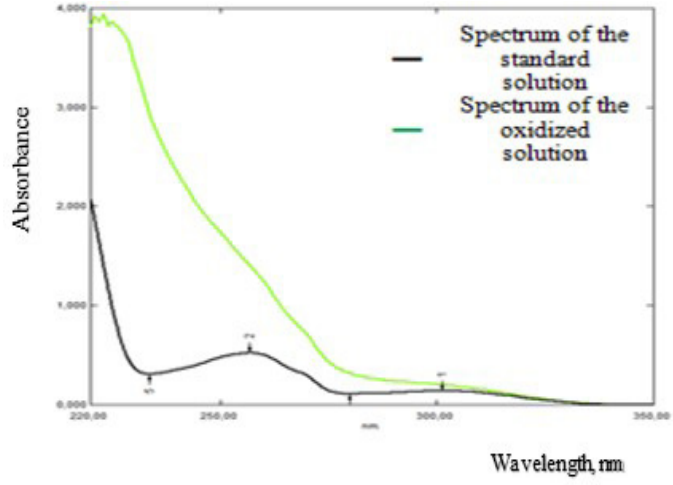

Fig. 2 Modification in the absorbtion spectrum of Dioxoindolinone following oxidative stress

Note: *oxidant – 3% hydrogen peroxide solution,*

Hydrolytic stress

Hydrolysis is one of the most prevalent chemical degradation reactions, which includes the splitting of a chemical compound by reaction with water. The ionizable functional groups present in the molecule are catalyzed during hy-

drolytic investigation in acidic and alkaline environment. Acid and base stress testing involve the forced degradation of drug substances by exposure to acidic and basic environments that produce primary degradants in the desired range. The choice of the type and strength of the acid or base depends on how stable the drug substance is. As suitable hydrolysis agents, hydrochloric acid or sulfuric acid for acid hydrolysis and sodium hydroxide or potassium hydroxide for alkaline hydrolysis are proposed [5, 6, 8, 14].

To highlight the influence of acids on Dioxoindolinone, 0.1 M hydrochloric acid was used, and for creation of the basic medium – 0.1 M sodium hydroxide. Absorption spectra of stressed solutions in acidic and basic media were recorded.

Acidic hydrolytic stress caused insignificant degradation of Dioxoindolinone, by 2.45% at 3 hours and 2.04% at 24 hours (Fig. 3).

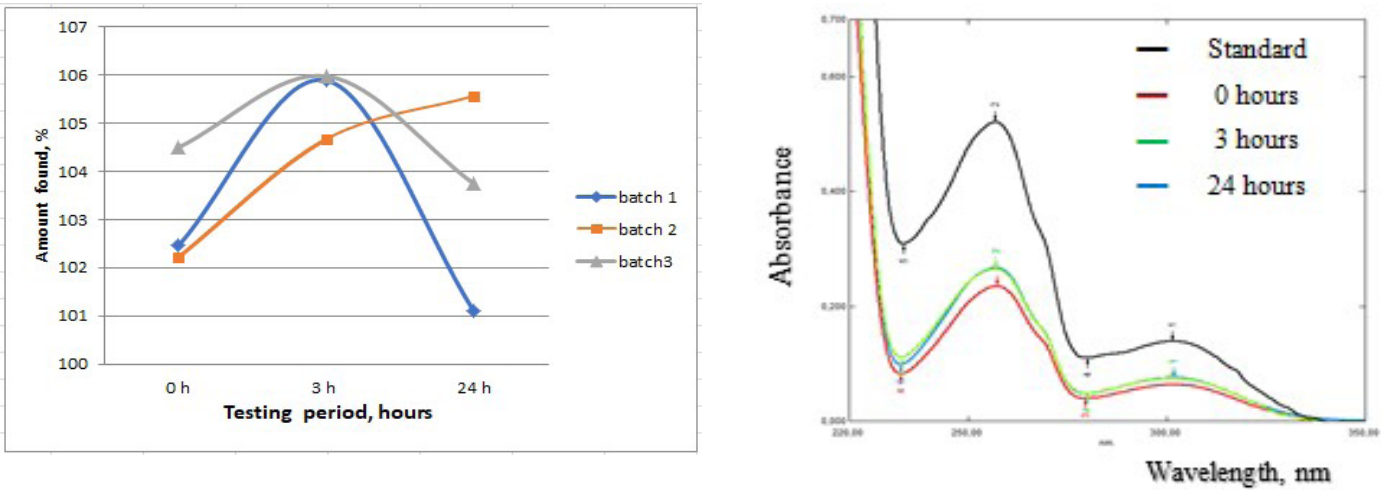

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Fig. 3. Modification of the concentration of Dioxoindolinone following acid hydrolysis (1) and the absorption spectra of Dioxoindolinone under the action of acid hydrolytic stress (2)

Note: *The average results for three batches of substance are shown*

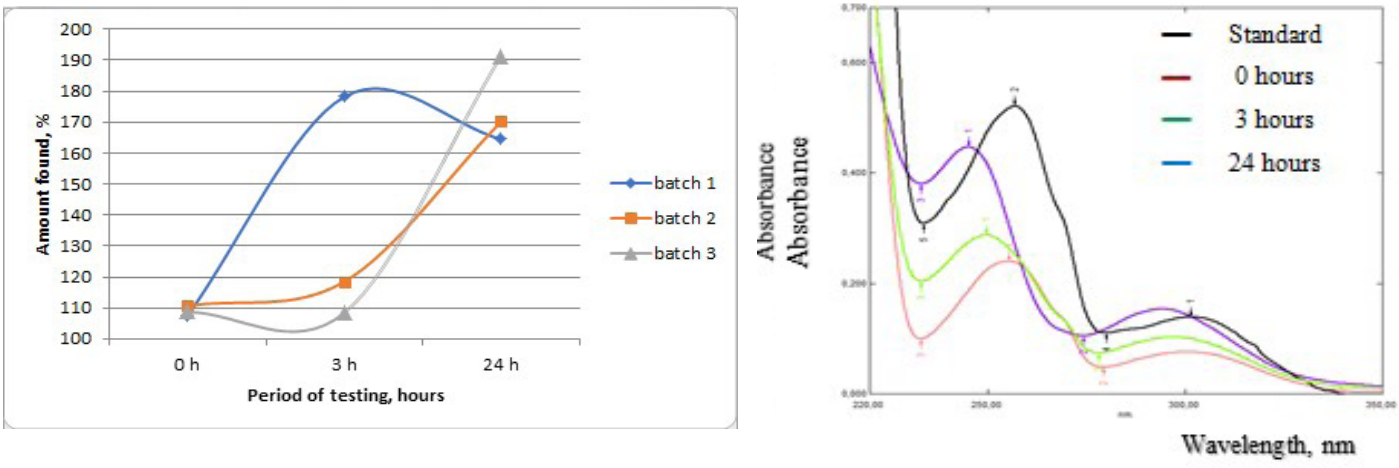

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Fig. 4. Modification of the concentration of Dioxoindolinone following basic hydrolysis (1) and the absorption spectra of Dioxoindolinone under the action of basic hydrolytic stress (2)

Note: *The average results for three batches of substance are shown*

The results obtained from basic hydrolysis indicate degradation processes with the shift of the absorption maximum from 258 nm to 255 nm (0 min), to 250 nm (3 hours) and to 243 nm (24 hours), and the increase in measured concentration due to the formation of degradation products (0 min – 108.99%, 3 hours – 135.04%, 24 hours – 175.33%) (Fig. 4.).

Influence of humidity, photolytic, and thermal stress

Moisture, as an atmospheric factor, creates favorable conditions for oxidation processes, hydrolysis, as well as for microbial growth. At the same time, light and temperature represent activating factors of drug degradation reactions; an increase in temperature leads to an increase in the rate

of the degradation reaction. Therefore, these factors must be studied in the process of analyzing of the substance [8, 9, 14, 15].

Dioxoindolinone exhibited a high degree of stability under humid conditions (in a desiccator above water for 2 weeks). The results obtained indicate that Dioxoindolinone is not hygroscopic and does not undergo degradation, the quantitative content being practically unchanged (0 min – 99.87%, 1 week – 99.51%, 2 weeks – 99.71%) (Fig. 5.).

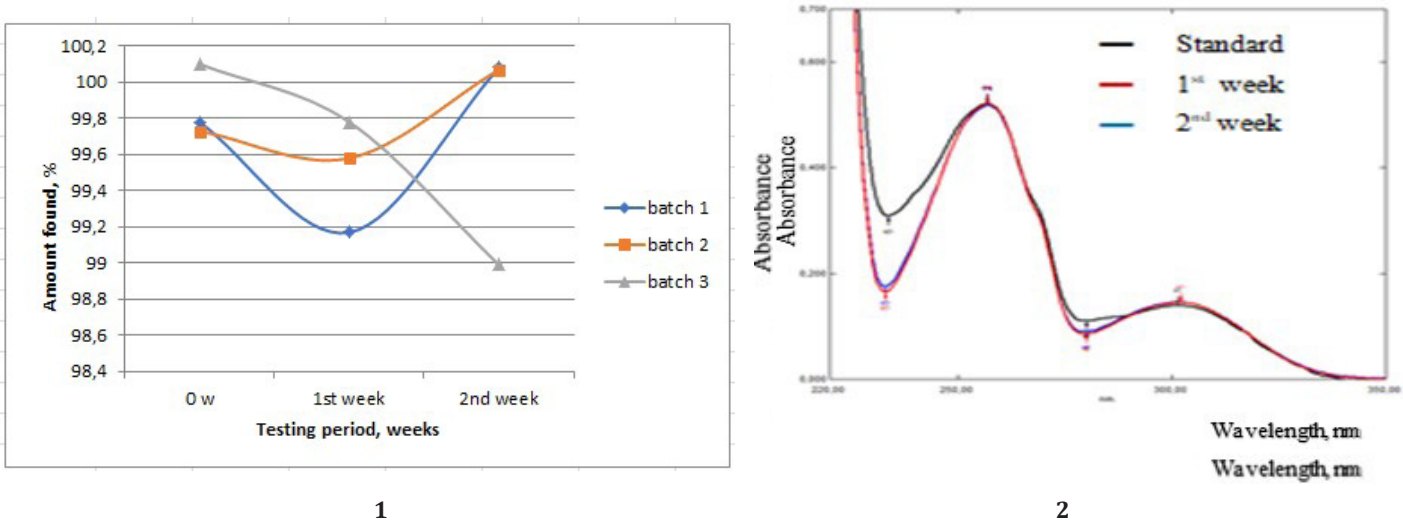

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Fig. 5. Modification of the concentration of Dioxoindolinone following influence of humidity (1) and the absorption spectra of Dioxoindolinone under the influence of humidity (2)/

Note: *The average results for three batches of substance are shown*

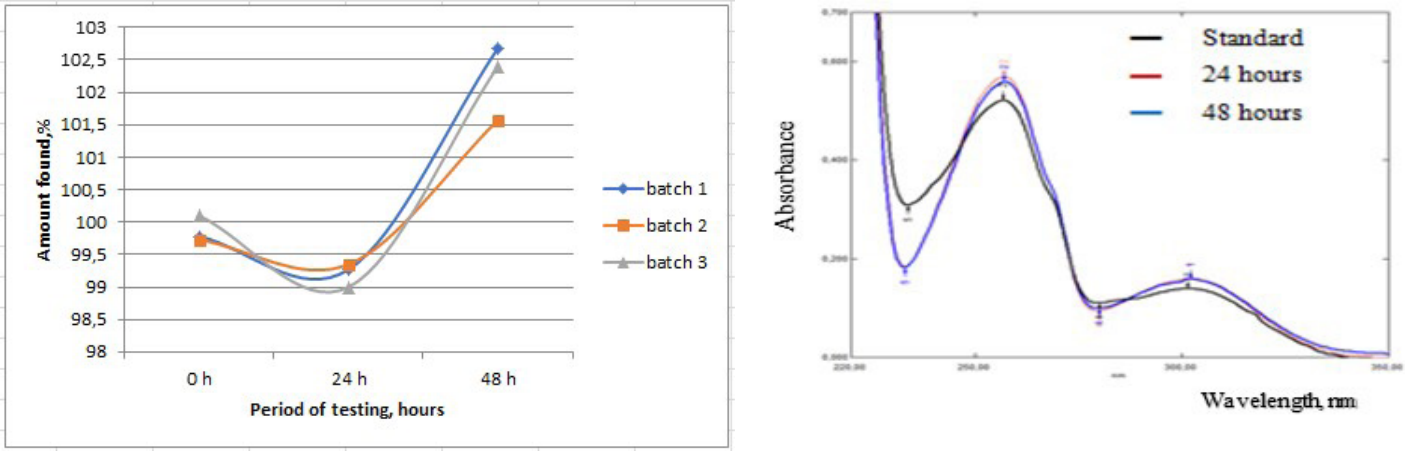

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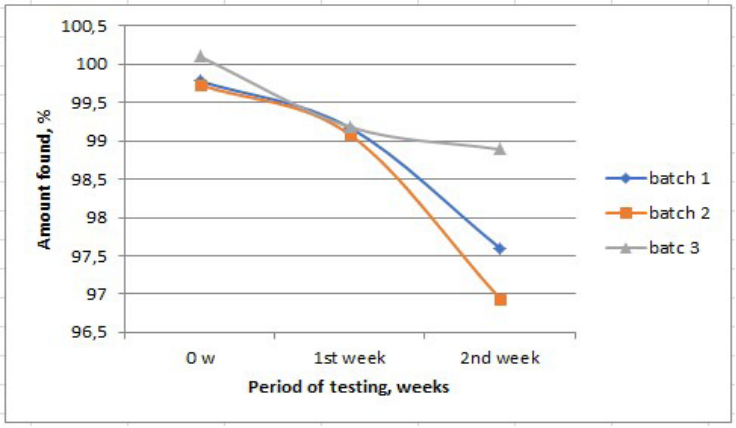
Fig. 6. Modification of the concentration of Dioxoindolinone following photolytic stress (1) and the absorption spectra of Dioxoindolinone under the action of photolytic stress (2)

Note: *The average results for three batches of substance are shown*

Dioxoindolinone was evaluated over 0-48 hours of photolytic stress. The results of the determinations are shown in Figure 6. Upon interaction with ultraviolet radiation, a change in the content of Dioxoindolinone of 2.23% was observed (0 min – 99.87%, 24 hours – 99.21%, 48 hours – 102.1%).

The influence of high temperature on Dioxoindolinone was evaluated. The substance placed in an open container was stored at high temperature (60 ^{0}C) in the thermostat for 2 weeks.

Samples subjected to thermal stress of 60 ^{o}C showed a change of 2.06% (0 min – 99.87%, 1 week – 99.14%, 2 weeks – 97.81%) (Fig. 7.).



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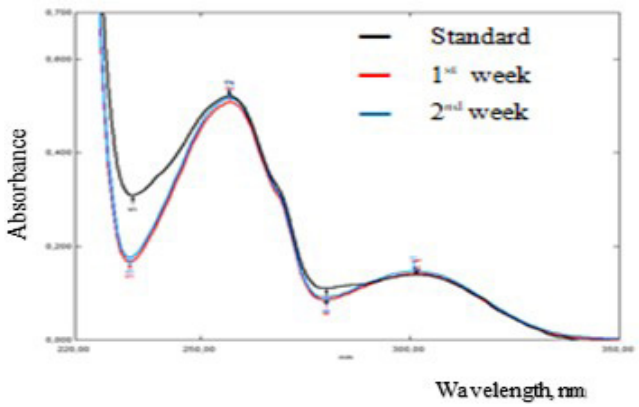

2

Fig. 7. Modification of the concentration of Dioxoindolinone following thermal stress (1) and the absorption spectra of Dioxoindolinone under the action of thermal stress (2)

Note: *The average results for three batches of substance are shown*

The most important changes in the samples subjected to forced degradation occurred under the action of oxidants and in a basic environment. Under the influence of the oxidizing agent the substance degraded, which was demonstrated by the complete change in the absorption spectrum, which made it impossible to determine the content of Dioxoindolinone in the sample. In a basic environment, Dioxoindolinone decomposed, and the degradation products increased the absorbance, causing concentration oscillations. In an acidic environment, the substance was stable, with concentration varying within 2%.

The results of the thermal stability evaluation indicate that Dioxoindolinone is stable under thermal stress, with concentration within 1.57%.

Although light is considered to be a destructive factor for medicinal substances, Dioxoindolinone demonstrated relative stability to UV irradiation, with a concentration increase of 2.9%.

The results of the evaluation of stability to humidity demonstrated that Dioxoindolinone is stable under high humidity, with no significant change in concentration observed.

The main quality parameter denoting the presence or absence of degradation processes in medicinal substances is Assay. In Table 1, the results of sample assay using the UV-Vis spectrophotometric method under various stress factors are presented.

Table 1. Modification of Dioxoindolinone concentrations under the action of stress factors

	Testing frequency	λ_{max}, nm	Batch 1		Batch 2		Batch 3	
			Absorbance	Amount found, %	Absorbance	Amount found, %	Absorbance	Amount found, %
H_2O_2 3 %	-	-	-	-	-	-	-	-
0.1 M HCl	0 hours	251.1	0.299	102.46	0.323	102.22	0.235	102.34
	3 hours	256.6	0.309	105.89	0.298	104.67	0.265	104.50
	24 hours	256.8	0.295	106.09	0.286	105.57	0.268	105.74
0.1 M NaOH	0 hours	256.2	0.314	107.61	0.303	110.7	0.443	108.68
	3 hours	246.3	0.521	178.2	0.321	118.49	0.441	108.43
	24 hours	241.6	0.480	164.49	0.461	170.16	0.781	191.36
t = 60 ˚C	0 hours	257	0.541	99.78	0.531	99.73	0.517	100.10
	1 week	257	0.502	99.17	0.504	99.08	0.503	99.18
	2 weeks	257	0.504	97.6	0.507	96.94	0.516	98.89
UV	0 hours	257	0.541	99.78	0.530	99.73	0.517	100.10
	24 hours	257	0.570	99.27	0.554	99.35	0.570	99.00
	48 hours	257	0.560	102.68	0.546	101.57	0.566	102.04
Moisture	0 hours	257	0.540	99.78	0.531	99.73	0.517	100.10
	1 week	257	0.522	99.17	0.524	99.58	0.524	99.78
	2 weeks	257	0.518	100.08	0.516	100.07	0.516	98.99

Note: λ_{max} *– the wavelength corresponding to maximum absorbance, t - temperature, UV - Ultra-violet,* H_2O_2 *– peroxide of hydrogen; HCl – hydrochloric acid; NaOH – sodium hydroxide;*
The average results for three batches of substance are shown

Conclusions

Forced degradation is an analytical method used to test a drug under more extreme conditions than those encountered in accelerated stability studies.

Under conditions of oxidative, hydrolytic, thermal, acid-base, photolytic stress, the UV-Vis spectrophotometric method demonstrated that the substance is stable under humid conditions humidity and in an acidic environment. Dioxoindolinone was found to degrade under the influence of oxidant, it was unstable in basic environment (a change in concentration was observed). The insignificant influence of UV light and high temperature was demonstrated.

The results obtained will be confirmed by real-time stability studies. Currently, the substance is stored under normal conditions (25°C; 65% RH) for 4 years and 10 months. So far, the drug substance meets all the quality criteria stipulated in the draft specification.

Competing interests

None declared.

Acknowledgements and funding

This work was fulfilled with the financial support from the Project: Development of new pharmaceutical products from local raw materials (No. 080301), *Nicolae Testemițanu* State University of Medicine and Pharmacy.

Ethics approval

No approval was required for this study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Liu W, Zhang Y, Chen J, Li X, Huang Y, Zhao F, Chen F, Qu P and Li Y. Global burden and trends of major mental disorders in individuals under 24 years of age from 1990 to 2021, with projections to 2050: insights from the Global Burden of Disease Study 2021. Front Public Health. 2025;13:1635801. doi: 10.3389/fpubh.2025.1635801.
2. Kumar S, Nair AS, Abdelgawad MA, Mathew B. Exploration of the detailed structure–activity relationships of isatin and their isomers as monoamine oxidase inhibitors. ACS Omega. 2022;7(19):16244-16259. doi: 10.1021/acsomega.2c01470.
3. Macaev F, Stîngaci E, Sucman N, Bilan D, Gorincioi E, inventors. Synthesis procedure monotopic sequential synthesis of β-ethylene acetals of 1-(phenacyl-, acetonyl) isatins. Republic of Moldova Patent Application, Deposit no. a 2025 0008; 2025 Feb 11 (unpublished data).
4. Chiriță C, Marineci CD, Zbârcea CE, Mihai DP, Ștefaneț T, Valica V, Negreș S. Research action antidepressants of a new 2-indolinone derivative. In: Pharmacy: from innovation to good pharmaceutical practice: 18th National Pharmacy Congress of Romania; 2021 Sept 15-17; Oradea, Romania. Oradea; 2021. p.172.
5. Verma A, Singla S, Palia P. Development of forced degradation and stability indicating studies of drugs: a review. Asian J Pharm Res Dev. 2022;10(2):83-89. doi: 10.22270/ajprd.v10i2.1104.
6. Sakaeva IV, Buniatian ND, Kovaleva EL, Sakanian EI, Mit'kina LI, Prokopov IA, Shelekhina ES, Mit'kina IuV. [Basic approaches to drug stability studies: domestic and international experience]. [Regul Res Med Eval] (Moscow). 2013;(3):8-11. Russian [cited 2025 Dec 12]. Available from: https://cyberleninka.ru/article/n/osnovnye-podhody-k-izucheniyu-stabilnosti-lekarstvennyh-sredstv-otechestvennyy-i-mezhdunarodnyy-opyt
7. Enciu M, Chiurciu V, Oltean EG, Nica AG. Degradarea forțată a produsului Romprogestin 1% pentru validarea metodei HPLC și evaluarea stabilității medicamentului [Forced degradation of Romprogestin 1% for HPLC method validation and stability evaluation]. Vet Drug (Timisoara). 2018;12(1):64-71. [cited 2025 Dec 12]. Available from: https://www.veterinarypharmacon.com/docs/1951-2018_VD_12(1)_ART5.RO.pdf
8. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. J Appl Pharm Sci. 2022;2 (3):129-138. [cited 2025 Dec 12]. Available from: https://japsonline.com/admin/php/uploads/409_pdf.pdf
9. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs: a review. J Pharm Anal. 2014;4(3):159-165. doi: 10.1016/j.jpha.2013.09.003.
10. International Council for Harmonization. ICH guideline Q1A(R2): Stability testing of new drug substances and products. Step 5: Note for guidance on stability testing: stability testing of new drug substances and products (CPMP/ICH/2736/99). London: EMA; 2003.
11. International Council for Harmonization. ICH guideline Q1B: Photostability testing of new drug substances and products. 1996. In: Proceedings of the International Conference on Harmonization; 2005.
12. Ștefaneț T, Valica V, Stîngaci E, Macaev F. Elaborarea și validarea metodei spectrofotometrice în ultraviolet și vizibil de dozare a dioxoindolinonei [Development and validation of a UV-Vis spectrophotometric method for Dioxoindolinone dosage]. Rev Farm Mold. 2024;53(Suppl):58-64. Romanian.
13. Ștefaneț T, Macaev F, Stîngaci E, Valica V. Investigation of the stability of Dioxoindolinone under stress conditions. In: The 37th Balkan Medical Week "The perspectives of Balkan medicine in the post COVID-19 era", 2023 June 7-9, Chisinau, Republic of Moldiva: Abstract book. Bucharest; 2023. p. 281.
14. Nicolai E, Vislouh O, Valica V, Parii S, Uncu L. Stability studies of combined ear drops for the treatment of otitis. Mold Med J. 2020;63(3):43-50. doi: 10.5281/zenodo.3958557.
15. Glass BD, Novak C, Brown ME. The thermal and photostability of solid pharmaceuticals. J Therm Anal Calorim. 2004;77:1013-1036. https://doi.org/10.1023/B:JTAN.0000041677.48299.25

https://doi.org/10.52645/MJHS.2026.2.08

UDC: 616-002-092-02:616-001

REVIEW ARTICLE

Proteases involved in distant posttraumatic lesions: a review of literature

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ABSTRACT

Introduction. Inflammation is a state driven by pathogenic stimuli. Trauma is one of the causes of acute onset of the inflammatory pathway. Multiple proteases are capable of inducing distant multiple organ lesions (lungs, brain or spinal cord, heart, kidney, liver and systemic vessel endothelium). The onset of corresponding syndromes will complicate the clinical course of that particular patient. These molecules are potential biomarkers in trauma patients.

Material and methods. There were reviewed the PubMed, Elsevier, ResearchGate, Google Scholar, Cochrane Library, medRxiv databases using the keywords "proteases", "antiproteases" and "trauma". A total of 114 relevant sources were included. An additional74 papers were selected. Overall there 188 literature sources were reviewed.

Results and discussions. There are six classes of proteases: aspartic, glutamic, metalloproteases, cysteine, serine, and threonine proteases of which the glutamic ones are not found in mammals. Multiple processes that involve protein degradation are the fundamental mechanisms through which they mediate tissue and organ destruction after trauma-mediated inflammation. Certain inhibitors of the aforementioned proteases are of importance in these processes – they are vital in the prevention of pathophysiological processes such as fibrosis, although in the case of trauma due to their depletion there is high activity of the proteases system. The release of the protease/antiprotease system is mediated through by leukocytes, thrombocytes, myocytes and endothelium.

Conclusions. In this literature review there was described a high variety of protease and antiproteases. There is an increased complexity for the potential treatment of the distant lesions, thus the necessity for symptomatic treatment is foremost in order to diminish the lesions of the acute phase.

Keywords: protease, protease inhibitors, multiple organ failure.

Cite this article: Croitoru D, Iachimovschi I, Arnaut O. Proteases involved in distant posttraumatic lesions: a systematic review of literature. Mold J Health Sci. 2026;13(2):48-62. https:doi.org/10.52645/MJHS.2026.2.08.

Manuscript received: 20.01.2026

Accepted for publication: 20.05.2026

Published: 15.06.2026

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

There are no broad descriptions of the protease/antiprotease system as a whole in the literature in the context of traumatic distant lesions.

The research hypothesis

There is a high myriad of proteases that are released during the systemic inflammatory response syndrome and respond with an aggressive tissue destruction in multiple tissues and organs.

The novelty added by the manuscript to the already published scientific literature

A systematic approach was chosen in this review article, thus encompassing the high versatility of the targets that need to be inhibited in the context of preventing distant lesions in trauma.

Introduction

Inflammation is a pathophysiological process that has been regarded historically in many cultures and by many researchers. The first descriptions of the inflammation were documented in the ancient Egyptian and Greek cultures. The main signs that allow us to identify inflammation are – *color*, *dolor*, *calor*, *tumor* and *functio laesa*. *Tumor* was added by Hippocrates, then Aulus Celsus added *color*, *dolor* and *calor*. Galen completed this list with the *functio laesa* [1]. The mechanisms involved in the onset of inflammation were studied in detail beginning with the second half of the 19th century. R. Virchow proposed the theory that inflammation is a "nutritional disturbance", in 1870 J. Cohnheim demonstrated that leukocyte diapedesis is present in this process, therebt proposing the vascular theory. Ten years later, C. Weigert proposed another idea – that it is a self-destruction process. In 1890, E. Metchnikoff enacted the theory that inflammation is controlled mainly by phagocytes, in accordance with Cohnheim's view. A significant shift in this field was seen in 1917, when L. Aschoff stated that inflammation is a reaction to the irritants, that may vary in different situations thus complicating the quest to define inflammation even further. Nowadays we differentiate broadly between acute and chronic inflammation [2].

After traumatic events a series of mechanisms that involve the hemostatic, endocrine and neurological systems, including immunocompetent cells act together in order to restore homeostasis [3].

In the conditions of trauma, it is important to take into account both the severity of damage and the host response. Under normal conditions, it is balanced and the inflammation remains local/limited without generalization and systemic effects. In severe trauma or polytrauma, this response leads to systemic inflammatory response syndrome (SIRS) development that occurs because of a disbalance in the pro-inflammatory and anti-inflammatory agents [4]. According to the literature, the appearance of damage-associated molecular patterns (DAMP) and inflammasomes, assembled in the immune cells, platelets and remote organs (the organs situated outside of the primary traumatic lesions), can be observed. As a result, the immune cells activated/attracted via cytokines and/or chemokines, together with increased vascular permeability and the expression of adhesion proteins on the endothelium, facilitates leukocyte diapedesis. They accumulate in injured and intact tissues. Here, these immunocompetent cells undergo degranulation and release reactive oxygen species (ROS) and a series of proteases. Consequently, "distant lesions" and sometimes multiple organ failure (MOF) develop [5]. This evolution represents an important cause of death and remains an unsolved problem in the management of critically injured patients [6].

Returning to the role of the inflammasomes in SIRS, there are a series of commonly recognized lesion sites that involve distant lesions: lungs (acute respiratory distress syndrome, ARDS), brain or spinal cord (disruption of the blood-brain and blood-medullary barriers), heart (acute coronary syndrome), liver (acute liver injury), kidneys (acute kidney injury) and the endothelium of the systemic vessels (disseminated intravascular coagulation) [5]. This concept is in accordance with the data reported in the literature. ARDS is associated with a higher level of plasma inflammatory cytokines [7], which is due to an imbalance in the protease-antiprotease system [8]. The blood-brain barrier in severe trauma had an increased permeability, as a result intracranial hemorrhage and hematomas may occur [9]. Acute coronary syndrome in polytrauma in patients with comorbidities is well recognized [10]. Acute kidney injury is mainly found in association with other injuries in severe trauma, as an indirect consequence [11]and shock associated with major trauma has been proposed to result in inadequate renal perfusion and subsequent AKI in trauma patients. This study aimed to investigate the true incidence and clinical presentation of post-traumatic AKI in hospitalized adult patients and its association with shock at a Level I trauma center. Methods: Detailed data of 78 trauma patients with AKI and 14,504 patients without AKI between January 1, 2009 and December 31, 2014 were retrieved from the Trauma Registry System. Patients with direct renal trauma were excluded from this study. Two-sided Fisher's exact or Pearson's chi-square tests were used to compare categorical data, unpaired Student's t-test was used to analyze normally distributed continuous data, and Mann-Whitney's U test was used to compare non-normally distributed data. Propensity score matching with a 1:1 ratio with logistic regression was used to evaluate the effect of shock on AKI. Results: Patients with AKI presented with significantly older age, higher incidence rates of pre-existing comorbidities, higher odds of associated injures (subdural hematoma, intracerebral hematoma, intra-abdominal injury, and hepatic injury, along with liver injuries [12]. Also, in the early stages of trauma, disseminated intravascular coagulation (DIC) is frequently reported [13-15]. All these „events" complete the pathophysiological portrait of the SIRS complications.

Taking into account the information above, the perspectives in preventing the distant lesions become clear, we propose a potential treatment or to identify the direction of the following researches in this field: usage of ROS and proteases/antiproteases as biomarkers for distant lesions in severe trauma in order to predict the outcomes for trauma patients and assess the severity of trauma.

The goal of this review was to list the elements of the protease/antiprotease system, their effects and to identify the components that can be used for the prediction of outcomes, including the secondary (indirect) lesions or the survival rate.

Material and methods

There were reviewed the PubMed, Elsevier, ResearchGate, Google Scholar, Cochrane Library, medRxiv databases using the keywords "proteases", "antiproteases" and "trauma". There were identified 114 relevant sources.

The inclusion criteria were: relevance to the topic and plausibility of statistical data in the study. Exclusion criteria were: irrelevance to the topic and low-quality statistical

data in the study. There were no inclusion/exclusion criteria pertaining to the year of publication, although sources were preferentially selected from the last 10 years in order to avoid biases outdated findings. Studies on both animal subjects and human patients studies were considered due to the scarcity of information regarding some proteases and antiproteases in the available literature.

The relevant information collected from the databases concerned the classification of proteases or antiproteases, mechanisms of action, pathological potential, clinical significance and prediction capability. Articles were selected that highlighted the potential of these molecules as biomarkers and their positive or negative effects in the context of trauma. The information was carefully studied, revised and critically structured in order to ensure an emphasis in the information structure on the objectives of this literature review.

In order to increase the validity of the information, 74 additional scientific articles were selectively reviewed. A total of 188 sources were included.

Results and discussions

The components of the protease/antiprotease system from the functional point of view can be divided into two groups: the substances that have potential negative effects (typically proteases), that are able to destroy invaders or healthy tissues, and the elements characterized by protective effects (typically antiproteases), that are able to reduce or prevent damage to the tissues.

Proteases. There are 6 main classes of proteases based on their mechanism of catalysis – aspartic, glutamic, metalloproteases, cysteine, serine, and threonine proteases, of which the glutamic class is not found in mammals [16]. Only 4 out of 6 classes are involved in trauma.

Matrix metalloproteases. A major protease class is the matrix metalloproteases, which is composed of 4 main subclasses – gelatinases, collagenases, stromelysins and elastases. Their effects are variable and are highly dependent on the microenvironment in which they act, and the multiple interactions between them and other proteases or antiproteases determine a specific pattern of expression in different conditions (tumors, necrosis or trauma).

Gelatinases

MMP-2 (Matrix metalloproteinase 2/Gelatinase A). It may be found in the granules of polymorphonuclear neutrophils [17, 18]angiogenesis and metastasis. Its remodeling is executed by a family of matrix metalloproteinases (MMPs, macrophages, monocytes, endothelial cells [19, 20], platelets [21, 22], lymphocytes [17] and astrocytes [23].

This protease has many destructive effects: it damages the basal lamina of the blood-brain barrier [17, 23], attacks the tight junctions between endothelial cells [17, 23]. Gelatinase A is able to degrade type I-V collagen, elastin and has lower proteolytic activity against proteoglycans and fibronectin [19], its activity against the type IV collagen and gelatin is notably high [24, 25].

It was highly expressed in trauma patients [26-28]in order to differentiate between a physiological tissue remodelling pattern and that associated with inflammatory tissue destruction. Methods - Analysis of SwissProt protein and EMBL/GenBank nucleotide sequence banks, protein sequence alignment, reverse transcriptase-polymerase chain reaction and nucleotide sequencing were used. Results - MMP-2 (gelatinase A, in spinal cord injury [29, 30], with elevated plasma levels after traumatic brain injury (TBI) [17] and a high level after skeletal muscle trauma in rats [19]. It was shown that it has a proaggregatory effect [31-33] and that it is a good predictor for acute respiratory distress syndrome (ARDS) [34]and involves degradation of the basement membrane. Matrix metalloproteinases (MMPs. The natural inhibitors for this protease are tissue inhibitors of metalloproteinases (TIMP 1-4) [19, 35].

MMP-9 (Matrix metalloproteinase 9/Gelatinase B). Gelatinase B may be found in the granules of the polymorphonuclear neutrophils [17, 18, 36] and lymphocytes [17]. It damages the basal lamina of the blood-brain barrier after spinal cord injury [17, 30, 37, 38], destroys the bone architecture, leading to osteoporosis [39, 40] and acts as a protease of the extracellular matrix and basal membrane (type IV collagen, gelatin, membrane growth factor receptor, tyrosine kinases and vascular endothelium adherence proteins) [32, 36, 38, 41, 42]. It is moderately expressed in trauma patients [26]in order to differentiate between a physiological tissue remodelling pattern and that associated with inflammatory tissue destruction. Methods - Analysis of SwissProt protein and EMBL/GenBank nucleotide sequence banks, protein sequence alignment, reverse transcriptase-polymerase chain reaction and nucleotide sequencing were used. Results - MMP-2 (gelatinase A, with elevated levels after burn trauma [36, 37], without predicting survivability [37], with plasma and cerebrospinal fluid levels higher in TBI before hypothermia induction [43], with increased cerebrospinal fluid (CSF) levels at the early clinical stage of TBI [17], with no difference in serum levels after a TBI [38, 41], with higher levels in case of spinal cord injury (SCI) in samples of post-mortem spinal cords detected with immunohistochemistry in rats [44] and humans [29], with higher serum levels in SCI [30], with lower levels in spinal cord injuries only after the inhibition of MMP-9 [45], with an antiaggregatory platelet effect according to a theoretical review [32] and cleaving the von Willebrand factor [46]but their physiological importance in preventing thrombus formation is unknown. This study investigated if, and which, proteases could cleave VWF in the glomerulus. The content of the glomerular basement membrane (GBM, with lower levels in ARDS along with lower MMP-9/TIMP-1 ratio values in a prospective clinical study [34]and involves degradation of the basement membrane. Matrix metalloproteinases (MMPs. Increased levels in severe sulfur mustard injuries of the eye when the tear meniscus is injured [47]. It is inhibited by TIMP 1-4 [19, 35, 36, 42, 48], melatonin, sulforaphane [30] and rutin [45].

Stromelysins

MMP-3 (Matrix metalloproteinase 3/stromelysin 1). It is found in the polymorphonuclear neutrophils [49] and chondrocytes [50, 51]. It is responsible for the degradation

of the non-collagenous extracellular matrix components and completes the collagen destruction, after it is cleaved by collagenases [49-51], it may induce seizures [52, 53]in which about 30% of patients cannot be treated adequately with anti-epileptic drugs. Brain inflammation and remodeling of the extracellular matrix (ECM the underlying mechanism being endopeptidase activity [49-51]. It is highly expressed in trauma patients [26, 54]in order to differentiate between a physiological tissue remodelling pattern and that associated with inflammatory tissue destruction. Methods - Analysis of SwissProt protein and EMBL/GenBank nucleotide sequence banks, protein sequence alignment, reverse transcriptase-polymerase chain reaction and nucleotide sequencing were used. Results - MMP-2 (gelatinase A and contributes to the lung injury as cited by a series of reviews [34, 49, 55] and has a slightly increased level after acute traumatic injuries in wounds with impaired healing [27]. It is inhibited by TIMP 1-4 [19, 35, 56, 57].

MMP-10 (Matrix metalloproteinase 10/stromelysin 2). It is found in macrophages [30, 58]. It resolves the scar tissue [58, 59], has a thrombolytic effect [60-62], stabilizes the thrombus [63]through a protease-activated receptor-1 (PAR-1, and exerts vascular remodeling effects [64]stromelysin-2 due to its promotion of the collagen lysis [30, 58]. It promotes wound healing [64]stromelysin-2 in mice [58] reported data on smoking behaviors for PLWH by gender. A slight decrease in the plasma level was observed in cases of traumatic brain injury with contusion in the first 72 hours, and a slight increase after 72 hours [65]. It is inhibited by TIMP 1-4 [19, 35, 48, 64]stromelysin-2.

MMP-11 (Matrix metalloproteinase 11/stromelysin 3). It can be found in the endothelium [66] and fibroblasts [67, 68]. It may be activated intracellularly by subtilisin-type serine proteases, due to the fact that it contains a RXK/RR furin-like recognition motif, thus having a different effect [69]. Although unable to hydrolise the extracellular matrix components, it hydrolyses the α_1-proteinase inhibitors [66, 67] and serine protease inhibitors [67], leads to the degradation of connective tissues [70, 71] due to its endopeptidase activity [66]. It is highly expressed in trauma patients [26]in order to differentiate between a physiological tissue remodelling pattern and that associated with inflammatory tissue destruction. Methods - Analysis of SwissProt protein and EMBL/GenBank nucleotide sequence banks, protein sequence alignment, reverse transcriptase-polymerase chain reaction and nucleotide sequencing were used. Results - MMP-2 (gelatinase A. It is inhibited by retinoic acid [67, 68, 72] and TIMP 1-4 [19, 35, 48].

Collagenases

MMP-1 (Matrix metalloproteinase 1/collagenase 1). It can be found in astrocytes [73], [29] and monocytes [29]. It enhances the blood-spinal cord barrier permeability, and induces macrophages and polymorphonuclear neutrophils phagocytosis signaling pathways [29, 73], causes severe cartilage damage [74]sixty rats were\nrandomly selected. Eleven rats were selected as the blank group.\nForty-four rat KOA models were established, and the remaining 5 rats\nwere used for stem cell extraction. The rats were randomly divided into\ntwo groups, and the transplantation group was treated with ADMSCs\ntransplantation. The KOA group was intragastrically administered with\nsaline. The expressions of MMP-13 mRNA and DDR2 in rats were detected by\nRT-qPCR and immunohistochemistry. Correlation analysis was performed in\nMMP-13 mRNA and DDR2 expression levels in the KOA rats. After treatment,\nthe indexes of Lequesne MG knee joints, MMP-13 mRNA and DDR2 in the\ntransplanted rats were significantly lower than those in the KOA group\n(P<0.05 because, at a neutral pH [75], it can destroy type I, II, III, V, and IX collagen [73, 76, 77] and native fibrillary collagen [76-78]. Increased levels have been reported in spinal cord trauma [29, 73], and it participates in wound healing [29, 73]. It is inhibited by TIMP 1-4 [19, 35, 48, 79]which function in extracellular matrix catabolism. Here, phage display was used to identify variants of human TIMP-2 that are selective inhibitors of human MMP-1, a collagenase whose unregulated action is linked to cancer, arthritis, and fibrosis. Using hard randomization of residues 2, 4, 5, and 6 (L1.

MMP-8 (Matrix metalloproteinase 8/Neutrophil collagenase/collagenase 2). It is found in the polymorphonuclear neutrophils [36]. It leads to the degradation of the components of the extracellular matrix [36, 80-82], may induce inflammation [81, 82], contributes to hard and soft tissue repair in mice [82], acts at neutral pH [75] as an endopeptidase [36], destroys type I, II, III, V, and IX collagen as well as native fibrillary collagen [77]. It has higher serum levels in SCI [30], and contributes to ARDS pathogenesis [34]and involves degradation of the basement membrane. Matrix metalloproteinases (MMPs, with increased levels in sepsis [81], elevated expression levels in burn trauma patients [36]. It may be inhibited by TIMP 1-4 [19, 35, 36, 48].

MMP-13 (Matrix metalloproteinase 13/collagenase 3). It can be identified in the chondrocytes [83] and fibroblasts [78]. It destroys the cartilage matrix [78, 84, 85], degrades the extracellular matrix [83, 85], leads to bone degradation [78, 86], acts at neutral pH [75] thus destroying type I, II, III, V, and IX collagen [77] as well as native fibrillary collagen [77, 78] especially type II [74, 83, 84], can bind to the platelet receptor αIIbβ3 and platelet glycoprotein (GP) VI [87]. It induces degenerative processes in cartilage and connective tissue [83], decreases platelet aggregation on collagen fibers in vascular trauma [87]. It is inhibited by MiR-320 [88, 89] and TIMP 1-4 [19, 35, 48].

Elastases

MMP-7 (Matrix metalloproteinase 7/Matrilysin). It can be identified in the macrophages [90]. It damages the extracellular matrix components [27], especially proteoglycans, insoluble elastin, and fibronectin [90], and causes synaptic reorganization in the central nervous system and excitotoxicity [91], by cleaving E-cadherin [91, 92], N-cadherin [90, 91], laminin and fibronectin [91]. Increased levels were observed after acute traumatic injury in wounds with impaired healing that are correlated with the injury severity score (ISS) [27], elevated expression was observed

ARDS [34, 92]and involves degradation of the basement membrane. Matrix metalloproteinases (MMPs, it prevents pulmonary fibrosis when it has low levels of expression [92] but increases the lung injury and fibrosis at high levels of expression [92-95]. It is inhibited by indomethacin, IL-4, IL-10, IFN-γ [96] and TIMP 1-4 [19, 35, 48].

MMP-12 (Matrix metalloproteinase 12/ Macrophage elastase/Human metalloelastase/ME). It can be identified in macrophages [58, 97]. It leads to lung structural destruction with production of mucin in the airways [35], and emphysema [98, 99]macrophage elastase-deficient (MME(- /-, it causes secondary injury processes in spinal cord injuries [30], it has protective effects in corneal injuries [100], [101] by hydrolysing the type III, IV, and V collagen, laminin-1, gelatin, proteoglycans, and elastin [35]. High concentrations that are found in trauma patients lead to extensive vascular damage, reverse repair [97], higher levels in case of SCI in samples of post-mortem spinal cords detected with immunohistochemistry [29, 30]. It can be inhibited by TIMP 1-4 [19, 35, 48].

There are also other proteases involved in trauma and have a varying serum levels following trauma. They were listed below, and grouped based on their catalytic center amino acid residue – serine, cysteine and aspartyl proteases. All of them are identified *in vivo*.

Serine proteases

Cathepsin A. It is found in the endothelium of the pulmonary capillaries [102]. It has hypotensive and immunostimulatory effects [102, 103], it may induce cardiac lesions [104, 105] and plays a major role in the oxidative stress response [105] by exerting a carboxypeptidase activity at acidic pH in the lysosome. It has deamidase and esterase activity at neutral pH [104, 106]Results: The crystal structure of mature and active cathepsin A reveals its mechanism of activation., Conclusion: Removal of a 3.3-kDa peptide (by unidentified proteases. It can metabolize endothelin-1 and angiotensin I in the bloodstream of mice [103, 104] and humans [106]Results: The crystal structure of mature and active cathepsin A reveals its mechanism of activation., Conclusion: Removal of a 3.3-kDa peptide (by unidentified proteases along with bradykinin [104]. It is inhibited by α_2 – Macroglobulin (A2MG) [107] and SAR1 [104, 108].

Cathepsin G. It may be found in the polymorphonuclear neutrophils, monocytes, macrophages and microglia [109]. It activates reactive T cells, increases cytokine and antigen-specific antibody production, increases vascular permeability which may lead to edema, and induces matrix-degrading metalloproteinases, thus leading to microvascular regression [109]. It has antimicrobial effects [110] due to its conversion of prochemerin into chemerin; it activates T cells in mice, augments antigen-specific antibody production, binds to lymphocytes including CD4+, CD8+, NK (natural killer), and B cells via a thrombin-like receptor, and increases the cytotoxicity of NK cells [109]. It activates the coagulation factor VIII *in vitro* [111] and promotes the platelet thrombus formation [112] other sources say that it cleaves this factor, thus deactivating it [46, 113, 114]but their physiological importance in preventing thrombus formation is unknown. This study investigated if, and which, proteases could cleave VWF in the glomerulus. The content of the glomerular basement membrane (GBM. Induces cardiac injury in rats [115] and mice [116]4,5-trisphosphate accumulation, activates ERK, p38 MAPK, and AKT, and decreases contractile function in cardiomyocytes. Because some cathepsin G responses mimic cardiomyocyte activation by thrombin, a role for PARs was considered. Cathepsin G markedly activates phospholipase C and p38 MAPK in cardiomyocytes from PAR-1-/- mice, but it fails to activate phospholipase C, ERK, p38 MAPK, or AKT in PAR-1- or PAR-4-expressing PAR-1-/- fibroblasts (which display robust responses to thrombin. Intensely expressed after TBI [117]. It is inhibited by serpinB1, serpinB6 [118] and secretory leucocyte protease inhibitor (SLPI) [119-121].

Trypsin. It can be found in the polymorphonuclear neutrophils [122, 123]. Has anti-inflammatory, anti-edematous, fibrinolytic, antioxidant and anti-infectious effects [124], also promotes tissue repair [124] by competing with plasmin in binding to the α_1 – antitrypsin and α_2 – macroglobulin, thus leading to an increased α_1 – antitrypsin expression and in turn this leads to an increase in the macrophage phagocytic activity, lower albumin and prealbumin loss, and higher antioxidant levels [124]. Administration of trypsin in trauma reduces the edema and ecchymosis [125], immunoreactive trypsin (IRT) serum levels are positively correlated with the ARDS manifestation in septic patients [126]. It is inhibited by α_1-antitrypsin (A1AT) [127], SLPI [119, 127], urinary trypsin inhibitor (UTI) [128], A2MG [107].

Chymotrypsin and/or chymase. Chymase is secreted by the polymorphonuclear neutrophils [129, 130], and by mast cells [121, 131]. It improves tissue repair and decreases long term proteolytic effects [124] using a mechanism that increases A1AT and A2MG expression in cells, thus increasing their activity for a period of time [124]. In trauma, it improves soft tissue regeneration [124]. It may be inhibited by α_1 – antichymotrypsin (A1ACT) [107], UTI [128], elafin [127], A2MG [107], [130] and SLPI [119, 121].

Urokinase (uPA). It may be found in polymorphonuclear neutrophils, monocytes, endothelium [132, 133]. It activates plasminogen into plasmin [132-134] due to endopeptidase-related mechanism [132-134]. Decreased levels have been reported in ARDS with acute lung injury (ALI) [134]. Increased levels of suPAR (soluble uPA receptor) in sepsis may be a predictor for ARDS [135, 136], but there it has no diagnostic value at the moment [136]. There are controversies regarding the suPAR predictive capability for mortality, some studies report a low predictive value [137], while another study reports a high predictive value [138]. Elevated plasma levels of soluble urokinase plasminogen activator receptor (suPAR) have been associated with acute kidney injury (AKI) in different clinical contexts, without having intel about their level in traumas [139]. It is inhibited by plasminogen activator type 1 inhibitor (PAI1) [132, 134] and plasminogen activator type 2 inhibitor (PAI2) [132].

HNE (Human neutrophil elastase). It is found in the polymorphonuclear neutrophils [140]. It exerts direct injury to cilia, secretory cell hyperplasia, mucin production and increases secretion in the respiratory epithelial cells [141]. In very severe cases, it leads to ARDS [140, 142, 143]. It has an antimicrobial effect [110], and mediates fibrinolysis along with degradation of plasminogen into angiostatin [144], the underlying mechanism being the proteolysis of the elastin fibers in the respiratory tract [141]. Its markedly elevated plasma levels in major trauma patients are inversely correlated with the Horowitz index [145]. It mediates acute pathogenesis in the immature brain of mice [146]. It is highly expressed in after TBI [117]. It is considered the main protease that cleaves the von Willebrand factor (vWF) [46]. It degrades the plasminogen and activates angiostatin K1, K2 and K3, which in turn lead to the shutdown of the fibrinolysis [144]. It is inhibited by elafin [143], serpinB1 [147], UTI [128], A2MG, A1AT [148] and SLPI [119].

Cysteine proteases. This class of proteases is known for its unique property of being globally inhibited by cystatins [127].

Cathepsin B. It may be found in endothelial cells, chondrocytes, synovial cells and ulceration sites [149]. It activates the trypsinogen into trypsin [150]. At an acidic pH it has peptidyl-dipeptidase and carboxypeptidase activity, at neutral-basic pH it has an endopeptidase activity [149]. The plasma levels increase in the first day after a trauma, then they fall to lower concentrations by the 3rd day and remain at the same level for 2 weeks, the increase is correlated with the injury severity, patients with sixfold increase develop multiple organ dysfunction syndrome (MODS) [149] also promotes muscular proteolysis [151]. It represents a potential biomarker, having increased concentrations in acute or subacute TBI in humans [152] and also in rodents [149]. It is inhibited by A2MG [107] and CA-074 [152].

Cathepsin C (Dipeptidyl peptidase I). It may be found in the polymorphonuclear neutrophils, cytotoxic lymphocytes, NK lymphocytes, mastocytes and reactive microglia [153]. It aggravates neuroinflammation [153] due to its protease activity [151] and by promoting the expression of proinflammatory factors such as interleukin-1β (IL-1β) and interleukin-6 (IL-6) [153]. In trauma, it promotes muscular proteolysis [151]. It may be highly expressed in case of neuroinflammation that is determined by the central nervous system injury in mice [154]*Cat C expression and its functional role in the brain under normal conditions or in neuroinflammatory processes remain unclear. Our previous study showed that Cat C promoted the progress of brain demyelination in cuprizone-treated mice. The present study further investigated the Cat C expression and activity in lipopolysaccharide (LPS*. It is inhibited by vigdaliptin [155].

Cathepsin H. It is found in the microglial cells [156]. It promotes inflammation, leads to chronic neuroinflammation and neuronal death [156], due to its exopeptidase and endopeptidase activity at an optimal pH of 6.5-6.8 [156], above a pH of 7.0, it cannot be activated due to its precursor stability [157]. In trauma patients, it promotes muscular proteolysis [151]. It is inhibited by A2MG [107].

Cathepsin V. It may be found in macrophages [158]. It promotes tumor necrosis factor α (TNF-α) and IL-6 expression in macrophages [159] by augmenting the IL-6 and TNF-α expressions via ERK1/2 and STAT1 pathway expression [159]. In trauma patients, it induces tunica media hyperplasia, promotes monocyte adhesion to the arterial walls after vascular lesions [158]. It is inhibited by A2MG [107].

Cathepsin L. It is contained in the endothelium, and microglia [160]. It alters the microvessels integrity in focal cerebral ischemia [160] by degrading the regulatory protein perlecan and collagen IV in the microvessels [160] and contributes to the autophagocytic pathways [161]. In trauma patients it promotes muscular proteolysis [151]. It has increased concentrations after TBI [162]. It is inhibited by recombinant cathepsin L propeptide, P41 of MHC class II molecule, antimicrobial Peptide LL-37, L-kininogen, sialostatin, and cystatins A, B, C, D and F [161].

Aspartyl proteases

Cathepsin D. It can be found in the polymorphonuclear neutrophils [163]. It promotes inflammation [163] by processing various enzymes, antigens, hormones and neuropeptides (ex: C5a), intracellularly at a pH of 3-4 [163]. The plasma concentrations are significantly increased in polytrauma patients within less than one hour after trauma and remain increased for at least 48h after the incident [163]. Promotes muscular proteolysis in trauma patients [151]. It is inhibited by A2MG [107].

Antiproteases and inhibitors

α_1 – Antitrypsin (SerpinA1/A1AT). It is found in the polymorphonuclear neutrophils [164-166]leading to life-threatening multiple organ dysfunction syndrome. Previous work suggested that circulating serum factors during inflammation are critically involved in the suppression of neutrophil cell death although the identity of these antiapoptotic mediators remained elusive. In this study, we identified the acute phase protein α-1 Antitrypsin (AAT but mostly in hepatocytes [166, 167]. It ameliorates the inflammation which may lead to systemic inflammatory response syndrome (SIRS) by granting apoptosis resistance due to the prevention of PKC/Akt pathway inhibition in trauma patients [164]leading to life-threatening multiple organ dysfunction syndrome. Previous work suggested that circulating serum factors during inflammation are critically involved in the suppression of neutrophil cell death although the identity of these antiapoptotic mediators remained elusive. In this study, we identified the acute phase protein α-1 Antitrypsin (AAT. Its expression is increased in a substrate-related concentration pattern – when the substrate concentration elevates there is an increase in the expression of A1AT [124].

α_1 – Antichymotrypsin (SerpinA3). It may be found mostly in hepatocytes, and to a lesser extent in the polymorphonuclear neutrophils [167] and glial cells [168]. It has cytotoxic effects in astrocytes [169] due to the neutralisation of chymotrypsin-like proteases (chymotrypsin, cathepsin G) [167]. It may be expressed in the nervous tissue 3 hours

after a TBI and remain for 1-13 days after the event in reactive glial cells [168].

α_2 – Macroglobulin (A2MD). It can be found in chondrocytes [170] and hepatocytes [134], [170]. It slows cartilage damage in rats and humans by regulating intercellular responses [170], inhibiting almost all proteases including ADAMTS proteins [107, 130, 170]. It may inhibit exogenous proteases, regulate the clotting events along with defensins and bind to numerous cytokines as a carrier or as an inhibitor. It regulates the activity of hepcidin, leptin and neuropeptide Y, inhibits antithrombin III [130]. In trauma patients, it can bind to uPA thus inhibiting it, also it may block the activation of NF-κB pathway in ALI/ARDS [134].

MNEI (SerpinB1/Monocyte Neutrophil Elastase Inhibitor). It may be found in the polymorphonuclear neutrophils and monocytes [107, 171]. Diminishes elastase-related lung injuries [172]elastase has an antimicrobial activity and could participate in neutrophil migration, both events being critically important in host defense, explaining the controversial issue of therapeutic elastase inhibition in the setting of acute lung injury. We assessed the effect of a neutrophil elastase inhibitor, EPI-hNE-4, in single (bleomycin, 1.2 mg/rat intratracheally due to the proteolysis of elastin and chymotrypsin-like proteases [107]. In trauma patients, it can prevent the ALI/ARDS [172]elastase has an antimicrobial activity and could participate in neutrophil migration, both events being critically important in host defense, explaining the controversial issue of therapeutic elastase inhibition in the setting of acute lung injury. We assessed the effect of a neutrophil elastase inhibitor, EPI-hNE-4, in single (bleomycin, 1.2 mg/rat intratracheally.

UTI (Urinary trypsin inhibitor). It is found in human urine, may be administered as an exogenous agent [128]. It prevents apoptosis in the endothelial cells, adipocytes [128] by inhibiting the toll-like receptor 4 (TLR4) and Janus tyrosine kinase (JTK), it decreases the NF-kB, TNF-α and p53 levels in cells thus inhibiting the apoptosis cascades [128]. In traumatic patients, it prevents the manifestation of fat embolism syndrome (FES), reduces edema [128] and has beneficial effects in ARDS [173], [174, 175]. It has protective effects in astrocyte lesions after a TBI [176].

Elafin (Peptidase inhibitor 3/PI3). It may be found in monocytes [171], squamous epithelium and respiratory epithelium [177]. It has antimicrobial, antiviral, anti-inflammatory and immunomodulatory effects [178-180]elafin. It inhibits the HNE [177-180]elafin binding to its active site [181]. In traumatic patients, it is associated with ARDS development [142], further studies indicate that a polymorphism increases its likelihood [178, 180]elafin.

SLPI (Secretory Leucocyte Protease Inhibitor). It may be found in polymorphonuclear neutrophils, monocytes, macrophages [107, 177], mastocytes [121] and mucosal epithelial cells [177]. It inhibits the bacterial growth, has a neuroprotective effect in cerebral ischemia, enhances axonal regeneration in an injured central nervous system (CNS), reduces TNF-α, NF-κB and interleukin-8 (IL-8) expression [119] and has anti-inflammatory effects [177] due to the inhibition of HNE in its active site [181]. In traumatic patients, it reduces neuroinflammation, diminishes the effects of cerebral and medullar ischemia after TBI and spinal cord lesions [119]. It is not involved in ARDS development [142]. It can be cleaved and inactivated by chymase [121, 131].

TIMP-1 (Tissue inhibitor of metalloproteinase-1). It is ocated in fibroblasts [182]. It promotes oligodendrocytes differentiation [183] by receptor mediated signaling pathways [183]. It has extended expression in patients with after-burn scar tissues with elevated levels in the days 3-6 but no effect on survival predictability [37]. It is a promising biomarker for fast assessment of the total body surface area (TBSA) that is affected in burn traumas [36]. May be used for the prediction of the TBI mortality outcome on a 30 days period [38, 41].

TIMP-2 (Tissue inhibitor of metalloproteinase-2). It is located in fibroblasts [182]. It increases the wound healing rate by inhibiting the proteases [184]exudate composition and temperature in wounds to predict healing outcomes and to identify the methods that are employed to measure them. Method: A systematic review based on the outcomes of a search strategy of quantitative primary research published in the English language was conducted. Inclusion criteria limited studies to those involving in vivo and human participants with an existing or intentionally provoked wound, defined as 'a break in the epithelial integrity of the skin', and excluded in vitro and animal studies. Data synthesis and analysis was performed using structured narrative summaries of each included study arranged by concept, pH, exudate composition and temperature. The Evidence Based Literature (EBL. It has increased plasma levels in skeletal traumas [19], along with IGFBP7 may serve as an predictor for acute kidney failure [185].

TIMP-3 (Tissue inhibitor of metalloproteinase-3). It may be obtained *in vitro* from the mesenchymal stem cells. It has a neuroprotective effect in mice [186]. It plays an important role for the onset of cerebral edema after TBI. Higher concentrations lead to an increased risk of ARDS manifestation after TBI. It was proven that high doses of TIMP-3 reduce the neuroinflammation and edema followed by a TBI [187].

TIMP-4 (Tissue inhibitor of metalloproteinase-4). It is found in platelets and is involved in the activation of the pro matrix metalloproteases (pro-MMPs) besides inhibiting them. Low concentrations inhibit the platelet aggregation, high concentrations have no significant effect [188]. Possibly inhibits neovascularization. It has decreased levels in severe sulfur mustard eye injuries (SSMEI). The MMP-9/TIMP-4 ratio is decreased in SSMEI, and increases in cases when the lacrimal meniscus is also injured [47].

Conclusions

Analysis of the available literature identified the components of the protease/antiprotease system, sources/localizations, physiological and pathological effects that are potentially significant in medical practice in the post-traumatic population group and particularly regarding distant body lesions.

Based on the available information, it is not currently possible to specifically predict, prevent or treat the distant lesions due to high versatility of therapeutic targets. It may be possible to prevent the potential negative effects of the proteases and increase the potential positive effects of antiproteases in distant lesions. Moreover, a variety of potential effects of the protease/antiprotease system were identified that could explain pathophysiological processes characteristic of severe trauma.

Competing interest

None declared.

Contribution of authors

DC designed the study and revised the literature, II revised the manuscript critically, OA conceptualized the study, revised the manuscript critically and approved the final version of the manuscript.

Ethics approval

No approval was required for this study.

Acknowledgements and funding

No external funding.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Granger DN, Senchenkova E. Inflammation and the microcirculation. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. 86 p. doi: 10.4199/C00013ED-1V01Y201006ISP008.
2. Parnes O. Inflammation. Lancet. 2008;372(9639):621. doi: 10.1016/S0140-6736(08)61262-3.
3. Lord JM, Midwinter MJ, Chen YF, et al. The systemic immune response to trauma: an overview of pathophysiology and treatment. Lancet. 2014;384(9952):1455-65. doi: 10.1016/S0140-6736(14)60687-5.
4. Osuka A, Ogura H, Ueyama M, et al. Immune response to traumatic injury: harmony and discordance of immune system homeostasis. Acute Med Surg. 2014;1(2):63-69. doi: 10.1002/ams2.17.
5. Bortolotti P, Faure E, Kipnis E. Inflammasomes in tissue damages and immune disorders after trauma. Front Immunol. 2018;9:1900. doi: 10.3389/fimmu.2018.01900.
6. Sauaia A, Moore FA, Moore EE. Postinjury inflammation and organ dysfunction. Crit Care Clin. 2017;33(1):167-191. doi: 10.1016/j.ccc.2016.08.006.
7. Headley AS, Tolley E, Meduri GU. Infections and the inflammatory response in acute respiratory distress syndrome. Chest. 1997;111(5):1306-21. doi: 10.1378/chest.111.5.1306.
8. Acevedo J, Srinivasan M, Ponce M, et al. Protease-antiprotease imbalance in airway secretions in subjects with acute respiratory failure. Eur Respir J. 2012;40(Suppl 56):7-8.
9. McCourt R, Gould B, Kate M, et al. Blood-brain barrier compromise does not predict perihematoma edema growth in intracerebral hemorrhage. Stroke. 2015;46(4):954-60. doi: 10.1161/STROKEAHA.114.007544.
10. Joseph L, Govindarajan R, Jesudoss DS, et al. Major emergency orthopaedic surgery in patients with a concomitant acute coronary event following trauma. Int Orthop. 2019;43(8):1793-1798. doi: 10.1007/s00264-018-4163-9.
11. Lai WH, Rau CS, Wu SC, et al. Post-traumatic acute kidney injury: a cross-sectional study of trauma patients. Scand J Trauma Resusc Emerg Med. 2016;24(1):136. doi: 10.1186/s13049-016-0330-4.
12. Ramaiah SK, Jaeschke H. Role of neutrophils in the pathogenesis of acute inflammatory liver injury. Toxicol Pathol. 2007;35(6):757-66. doi: 10.1080/01926230701584163.
13. Gando S, Otomo Y. Local hemostasis, immunothrombosis, and systemic disseminated intravascular coagulation in trauma and traumatic shock. Crit Care. 2015;19(1):72. doi: 10.1186/s13054-015-0735-x.
14. Gando S. Disseminated intravascular coagulation in trauma patients. Semin Thromb Hemost. 2001;27(6):585-92. doi: 10.1055/s-2001-18864.
15. Lippi G, Cervellin G. Disseminated intravascular coagulation in trauma injuries. Semin Thromb Hemost. 2010;36(4):378-87. doi: 10.1055/s-0030-1254047.
16. López-Otín C, Bond JS. Proteases: multifunctional enzymes in life and disease. J Biol Chem. 2008;283(45):30433-7. doi: 10.1074/jbc.R800035200.
17. Grossetete M, Phelps J, Arko L, et al. Elevation of matrix metalloproteinases 3 and 9 in cerebrospinal fluid and blood in patients with severe traumatic brain injury. Neurosurgery. 2009;65(4):702-8. doi: 10.1227/01.NEU.0000351768.11363.48.
18. Gioia M, Monaco S, Van Den Steen PE, et al. The collagen binding domain of gelatinase A modulates degradation of collagen IV by gelatinase B. J Mol Biol. 2009;386(2):419-34. doi: 10.1016/j.jmb.2008.12.021.
19. Yu TS, Li Z, Zhao R, et al. Time-dependent expression of MMP-2 and TIMP-2 after rats skeletal muscle contusion and their application to determine wound age. J Forensic Sci. 2016;61(2):527-533. doi: 10.1111/1556-4029.13001.
20. Sharma R, Rosenberg A, Bennett ER, et al. A blood-based biomarker panel to risk-stratify mild traumatic brain injury. PLoS One. 2017;12(3):e0173798. doi: 10.1371/journal.pone.0173798.
21. Kuliczkowski W, Radomski M, Gąsior M, et al. MMP-2, MMP-9, and TIMP-4 and response to aspirin in diabetic and nondiabetic patients with stable coronary artery disease: a pilot study. Biomed Res Int. 2017;2017:9352015. doi: 10.1155/2017/9352015.
22. Marturano A, Hendrickx MLV, Falcinelli E, et al. Development of anti-matrix metalloproteinase-2 (MMP-2)

nanobodies as potential therapeutic and diagnostic tools. Nanomedicine. 2020;24:102103. doi: 10.1016/j.nano.2019.102103.
23. Zhang S, Kojic L, Tsang M, et al. Distinct roles for metalloproteinases during traumatic brain injury. Neurochem Int. 2016;96:46-55. doi: 10.1016/j.neuint.2016.02.013.
24. Henriet P, Emonard H. Matrix metalloproteinase-2: Not (just) a "hero" of the past. Biochimie. 2019;166:223-232. doi: 10.1016/j.biochi.2019.07.019.
25. Azhagiya Singam ER, Rajapandian V, Subramanian V. Molecular dynamics simulation study on the interaction of collagen-like peptides with gelatinase-A (MMP-2). Biopolymers. 2014;101(7):779-94. doi: 10.1002/bip.22457.
26. Konttinen YT, Ainola M, Valleala H, et al. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis. 1999;58(11):691-7. doi: 10.1136/ard.58.11.691.
27. Utz ER, Elster EA, Tadaki DK, et al. Metalloproteinase expression is associated with traumatic wound failure. J Surg Res. 2010;159(2):633-9. doi: 10.1016/j.jss.2009.08.021.
28. Wells JE, Rice TK, Nuttall RK, et al. An adverse role for matrix metalloproteinase 12 after spinal cord injury in mice. J Neurosci. 2003;23(31):10107-15. doi: 10.1523/JNEUROSCI.23-31-10107.2003.
29. Buss A, Pech K, Kakulas BA, et al. Matrix metalloproteinases and their inhibitors in human traumatic spinal cord injury. BMC Neurol. 2007;7:17. doi: 10.1186/1471-2377-7-17.
30. Moghaddam A, Heller R, Daniel V, et al. Exploratory study to suggest the possibility of MMP-8 and MMP-9 serum levels as early markers for remission after traumatic spinal cord injury. Spinal Cord. 2017;55(1):8-15. doi: 10.1038/sc.2016.104.
31. Sawicki G, Sanders EJ, Salas E, et al. Localization and translocation of MMP-2 during aggregation of human platelets. Thromb Haemost. 1998 Nov;80(5):836-9.
32. Santos-Martínez MJ, Medina C, Jurasz P, et al. Role of metalloproteinases in platelet function. Thromb Res. 2008;121(4):535-42. doi: 10.1016/j.thromres.2007.06.002.
33. Momi S, Falcinelli E, Giannini S, et al. Loss of matrix metalloproteinase 2 in platelets reduces arterial thrombosis in vivo. J Exp Med. 2009;206(11):2365-79. doi: 10.1084/jem.20090687.
34. Davey A, McAuley DF, O'Kane CM. Matrix metalloproteinases in acute lung injury: mediators of injury and drivers of repair. Eur Respir J. 2011;38(4):959-70. doi: 10.1183/09031936.00032111.
35. Hao W, Li M, Zhang Y, et al. Expressions of MMP-12, TIMP-4, and neutrophil elastase in PBMCs and exhaled breath condensate in patients with COPD and their relationships with disease severity and acute exacerbations. J Immunol Res. 2019;2019:7142438. doi: 10.1155/2019/7142438.
36. Hästbacka J, Fredén F, Hult M, et al. Matrix metalloproteinases -8 and -9 and tissue inhibitor of metalloproteinase-1 in burn patients. A prospective observational study. PLoS One. 2015;10(5):e0125918. doi: 10.1371/journal.pone.0125918.
37. Nagy B, Szélig L, Rendeki S, et al Dynamic changes of matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 after burn injury. J Crit Care. 2015;30(1):162-6. doi: 10.1016/j.jcrc.2014.07.008.
38. Lorente L, Martín MM, López P, et al. Association between serum tissue inhibitor of matrix metalloproteinase-1 levels and mortality in patients with severe brain trauma injury. PLoS One. 2014;9(4):e94370. doi: 10.1371/journal.pone.0094370.
39. Nyman JS, Lynch CC, Perrien DS, et al. Differential effects between the loss of MMP-2 and MMP-9 on structural and tissue-level properties of bone. J Bone Miner Res. 2011;26(6):1252-60. doi: 10.1002/jbmr.326.
40. Collins FL, Williams JO, Bloom AC, et al. CCL3 and MMP-9 are induced by TL1A during death receptor 3 (TNFRSF25)-dependent osteoclast function and systemic bone loss. Bone. 2017;97:94-104. doi: 10.1016/j.bone.2017.01.002.
41. Lorente L, Martín MM, Ramos L, et al. Persistently high circulating tissue inhibitor of matrix metalloproteinase-1 levels in non-survivor brain trauma injury patients. J Crit Care. 2019;51:117-121. doi: 10.1016/j.jcrc.2019.02.014.
42. Wiggins-Dohlvik K, Oakley RP, Han MS, et al. Tissue inhibitor of metalloproteinase-2 inhibits burn-induced derangements and hyperpermeability in microvascular endothelial cells. Am J Surg. 2016;211(1):197-205. doi: 10.1016/j.amjsurg.2015.08.016.
43. Suehiro E, Fujisawa H, Akimura T, et al. Increased matrix metalloproteinase-9 in blood in association with activation of interleukin-6 after traumatic brain injury: influence of hypothermic therapy. J Neurotrauma. 2004;21(12):1706-11. doi: 10.1089/neu.2004.21.1706.
44. Yu F, Kamada H, Niizuma K, et al. Induction of mmp-9 expression and endothelial injury by oxidative stress after spinal cord injury. J Neurotrauma. 2008;25(3):184-95. doi: 10.1089/neu.2007.0438.
45. Zhang P, Ma X. Effect of rutin on spinal cord injury through inhibition of the expression of MIP-2 and activation of MMP-9, and downregulation of Akt phosphorylation. Mol Med Rep. 2015;12(5):7554-60. doi: 10.3892/mmr.2015.4357.
46. Tati R, Kristoffersson AC, Manea Hedström M, et al. Neutrophil protease cleavage of von Willebrand factor in glomeruli - an anti-thrombotic mechanism in the kidney. EBioMedicine. 2017;16:302-311. doi: 10.1016/j.ebiom.2017.01.032.
47. Ghasemi H, Yaraee R, Faghihzadeh S, et al. Tear and serum MMP-9 and serum TIMPs levels in the severe sulfur mustard eye injured exposed patients. Int Im-

munopharmacol. 2019;77:105812. doi: 10.1016/j.intimp.2019.105812.
48. Sato Y, Kishi J, Suzuki K, et al. Sonic extracts from a bacterium related to periapical disease activate gelatinase A and inactivate tissue inhibitor of metalloproteinases TIMP-1 and TIMP-2. Int Endod J. 2009;42(12):1104-11. doi: 10.1111/j.1365-2591.2009.01640.x.
49. Nerusu KC, Warner RL, Bhagavathula N, et al. Matrix metalloproteinase-3 (stromelysin-1) in acute inflammatory tissue injury. Exp Mol Pathol. 2007;83(2):169-76. doi: 10.1016/j.yexmp.2007.04.003.
50. Guo L, Hao R, Tian F, et al. Interferon regulatory factor 5 (IRF5) regulates the expression of matrix metalloproteinase-3 (MMP-3) in human chondrocytes. Int Immunopharmacol. 2018;55:231-236. doi: 10.1016/j.intimp.2017.11.035.
51. Li Y, Zhao M, Xiao W. KLF15 Regulates the expression of MMP-3 in human chondrocytes. J Interferon Cytokine Res. 2018;38(8):356-362. doi: 10.1089/jir.2017.0135.
52. Korotkov A, Broekaart DWM, van Scheppingen J, et al. Increased expression of matrix metalloproteinase 3 can be attenuated by inhibition of microRNA-155 in cultured human astrocytes. J Neuroinflammation. 2018;15(1):211. doi: 10.1186/s12974-018-1245-y.
53. Wang R, Zeng GQ, Liu X, et al. Evaluation of serum matrix metalloproteinase-3 as a biomarker for diagnosis of epilepsy. J Neurol Sci. 2016;367:291-7. doi: 10.1016/j.jns.2016.06.031.
54. Lohmander LS, Roos H, Dahlberg L, et al. Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. J Orthop Res. 1994;12(1):21-8. doi: 10.1002/jor.1100120104.
55. Craig VJ, Zhang L, Hagood JS, et al. Matrix metalloproteinases as therapeutic targets for idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol. 2015;53(5):585-600. doi: 10.1165/rcmb.2015-0020TR.
56. Heuschkel R, MacDonald TT, Monteleone G, et al. Imbalance of stromelysin-1 and timp-1 mrna and protein in the mucosal lesions of children with inflammatory bowel disease. Gut. 2000 Jul;47(1):57-62. doi: 10.1136/gut.47.1.57.
57. Nagase H, Suzuki K, Cawston TE, et al. Involvement of a region near valine-69 of tissue inhibitor of metalloproteinases (TIMP)-1 in the interaction with matrix metalloproteinase 3 (stromelysin 1). Biochem J. 1997;325 (Pt 1)(Pt 1):163-7. doi: 10.1042/bj3250163.
58. McMahan RS, Birkland TP, Smigiel KS, et al. Stromelysin-2 (MMP10) moderates inflammation by controlling macrophage activation. J Immunol. 2016;197(3):899-909. doi: 10.4049/jimmunol.1600502.
59. Rohani MG, McMahan RS, Razumova MV, et al. MMP-10 regulates collagenolytic activity of alternatively activated resident macrophages. J Invest Dermatol. 2015;135(10):2377-2384. doi: 10.1038/jid.2015.167.
60. Orbe J, Barrenetxe J, Rodriguez JA, et al. Matrix metalloproteinase-10 effectively reduces infarct size in experimental stroke by enhancing fibrinolysis via a thrombin-activatable fibrinolysis inhibitor-mediated mechanism. Circulation. 2011;124(25):2909-19. doi: 10.1161/CIRCULATIONAHA.111.047100.
61. Roncal C, Martinez de Lizarrondo S, Salicio A, et al. New thrombolytic strategy providing neuroprotection in experimental ischemic stroke: MMP10 alone or in combination with tissue-type plasminogen activator. Cardiovasc Res. 2017;113(10):1219-1229. doi: 10.1093/cvr/cvx069.
62. Navarro-Oviedo M, Roncal C, Salicio A, et al. MMP10 promotes efficient thrombolysis after ischemic stroke in mice with induced diabetes. Transl Stroke Res. 2019;10(4):389-401. doi: 10.1007/s12975-018-0652-9.
63. Orbe J, Rodríguez JA, Calvayrac O, et al. Matrix metalloproteinase-10 is upregulated by thrombin in endothelial cells and increased in patients with enhanced thrombin generation. Arterioscler Thromb Vasc Biol. 2009;29(12):2109-16. doi: 10.1161/ATVBAHA.109.194589.
64. Batra J, Robinson J, Soares AS, et al. Matrix metalloproteinase-10 (MMP-10) interaction with tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2: binding studies and crystal structure. J Biol Chem. 2012;287(19):15935-46. doi: 10.1074/jbc.M112.341156.
65. Guilfoyle MR, Carpenter KL, Helmy A, et al. Matrix metalloproteinase expression in contusional traumatic brain injury: a paired microdialysis study. J Neurotrauma. 2015;32(20):1553-9. doi: 10.1089/neu.2014.3764.
66. Lijnen HR, Van Hoef B, Vanlinthout I, et al. Accelerated neointima formation after vascular injury in mice with stromelysin-3 (MMP-11) gene inactivation. Arterioscler Thromb Vasc Biol. 1999;19(12):2863-70. doi: 10.1161/01.atv.19.12.2863.
67. Anderson IC, Sugarbaker DJ, Ganju RK, et al. Stromelysin-3 is overexpressed by stromal elements in primary non-small cell lung cancers and regulated by retinoic acid in pulmonary fibroblasts. Cancer Res. 1995;55(18):4120-6.
68. Guérin E, Ludwig MG, Basset P, et al. Stromelysin-3 induction and interstitial collagenase repression by retinoic acid. Therapeutical implication of receptor-selective retinoids dissociating transactivation and AP-1-mediated transrepression. J Biol Chem. 1997;272(17):11088-95. doi: 10.1074/jbc.272.17.11088.
69. Zhang H, Adwanikar H, Werb Z, et al. Matrix metalloproteinases and neurotrauma: evolving roles in injury and reparative processes. Neuroscientist. 2010;16(2):156-70. doi: 10.1177/1073858409355830.
70. Lee J, Lee J, Kim JH. Identification of matrix metalloproteinase 11 as a prognostic biomarker in pancreatic

cancer. Anticancer Res. 2019;39(11):5963-5971. doi: 10.21873/anticanres.13801.
71. Rickaby R, El Khoury LY, Samiric T, et al. Epigenetic status of the human *MMP11* gene promoter is altered in patellar tendinopathy. J Sports Sci Med. 2019;18(1):155-159.
72. von Marschall Z, Riecken EO, Rosewicz S. Stromelysin 3 is overexpressed in human pancreatic carcinoma and regulated by retinoic acid in pancreatic carcinoma cell lines. Gut. 1998;43(5):692-8. doi: 10.1136/gut.43.5.692.
73. Zhou Y, Cui Z, Xia X, et al. Matrix metalloproteinase-1 (MMP-1) expression in rat spinal cord injury model. Cell Mol Neurobiol. 2014;34(8):1151-63. doi: 10.1007/s10571-014-0090-5.
74. Li J, Zhu X, Shao Q, et al. Allogeneic adipose-derived stem cell transplantation on knee osteoarthritis rats and its effect on MMP-13 and DDR2. Exp Ther Med. 2019;18(1):99-104. doi: 10.3892/etm.2019.7554.
75. Inada M, Wang Y, Byrne MH, et al. Critical roles for collagenase-3 (Mmp13) in development of growth plate cartilage and in endochondral ossification. Proc Natl Acad Sci USA. 2004;101(49):17192-7. doi: 10.1073/pnas.0407788101.
76. Ma Y, Iyer R, Bras L, et al. Cross talk between inflammation and extracellular matrix following myocardial infarction. In: Blankesteijn WM, Altara R, editors. Inflammation in heart failure. Amsterdam: Elsevier/Academic Press; 2015. p. 67-79. doi: 10.1016/B978-0-12-800039-7.00004-9.
77. Inanc S, Keles D, Oktay G. An improved collagen zymography approach for evaluating the collagenases MMP-1, MMP-8, and MMP-13. Biotechniques. 2017;63(4):174-180. doi: 10.2144/000114597.
78. Agere SA, Akhtar N, Watson JM, et al. RANTES/CCL5 induces collagen degradation by activating MMP-1 and MMP-13 expression in human rheumatoid arthritis synovial fibroblasts. Front Immunol. 2017;8:1341. doi: 10.3389/fimmu.2017.01341.
79. Bahudhanapati H, Zhang Y, Sidhu SS, et al. Phage display of tissue inhibitor of metalloproteinases-2 (TIMP-2): identification of selective inhibitors of collagenase-1 (metalloproteinase 1 (MMP-1)). J Biol Chem. 2011;286(36):31761-70. doi: 10.1074/jbc.M111.253328.
80. Nwomeh BC, Liang HX, Cohen IK, et al. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res. 1999;81(2):189-95. doi: 10.1006/jsre.1998.5495.
81. Daly MC, Atkinson SJ, Varisco BM, et al. Role of matrix metalloproteinase-8 as a mediator of injury in intestinal ischemia and reperfusion. FASEB J. 2016;30(10):3453-3460. doi: 10.1096/fj.201600242R.
82. Astrom P. Regulatory mechanisms mediating matrix metalloproteinase-8 effects in oral tissue repair and tongue cancer [dissertation]. Oulu (FI): University of Oulu; 2014.
83. Otero M, Plumb DA, Tsuchimochi K, et al. E74-like factor 3 (ELF3) impacts on matrix metalloproteinase 13 (MMP13) transcriptional control in articular chondrocytes under proinflammatory stress. J Biol Chem. 2012;287(5):3559-72. doi: 10.1074/jbc.M111.265744.
84. Yang Q, Ding W, Cao Y, et al. Interferonregulatory-factor-8(IRF-8) regulates the expression of matrix metalloproteinase-13 (MMP-13) in chondrocytes. Cell Stress Chaperones. 2018;23(3):393-398. doi: 10.1007/s12192-017-0849-y.
85. Li H, Wang D, Yuan Y, et al. New insights on the MMP-13 regulatory network in the pathogenesis of early osteoarthritis. Arthritis Res Ther. 2017;19(1):248. doi: 10.1186/s13075-017-1454-2.
86. Li J, Liao EY, Dai RC, et al. Effects of 17 beta-estradiol on the expression of interstitial collagenases-8 and -13 (MMP-8 and MMP-13) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in ovariectomized rat osteoblastic cells. J Mol Histol. 2004;35(8-9):723-31. doi: 10.1007/s10735-004-6206-3.
87. Howes JM, Pugh N, Hamaia SW, et al. MMP-13 binds to platelet receptors αIIbβ3 and GPVI and impairs aggregation and thrombus formation. Res Pract Thromb Haemost. 2018;2(2):370-379. doi: 10.1002/rth2.12088.
88. Zhang HX, Sun C, Yu HC, et al. Targeted inhibition of β-catenin by miR-320 and decreased MMP-13 expression in suppressing chondrocyte collagen degradation. Eur Rev Med Pharmacol Sci. 2018 Sep;22(18):5828-5835. doi: 10.26355/eurrev_201809_15909.
89. Meng F, Zhang Z, Chen W, et al. MicroRNA-320 regulates matrix metalloproteinase-13 expression in chondrogenesis and interleukin-1β-induced chondrocyte responses. Osteoarthritis Cartilage. 2016;24(5):932-41. doi: 10.1016/j.joca.2015.12.012.
90. Halpert I, Sires UI, Roby JD, et al. Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proc Natl Acad Sci USA. 1996;93(18):9748-53. doi: 10.1073/pnas.93.18.9748.
91. Bilousova TV, Rusakov DA, Ethell DW, et al. Matrix metalloproteinase-7 disrupts dendritic spines in hippocampal neurons through NMDA receptor activation. J Neurochem. 2006;97(1):44-56. doi: 10.1111/j.1471-4159.2006.03701.x.
92. Manicone AM, Huizar I, McGuire JK. Matrilysin (Matrix Metalloproteinase-7) regulates anti-inflammatory and antifibrotic pulmonary dendritic cells that express CD103 (alpha(E)beta(7)-integrin). Am J Pathol. 2009;175(6):2319-31. doi: 10.2353/ajpath.2009.090101.
93. Villar J, Cabrera-Benítez NE, Ramos-Nuez A, et al. Early activation of pro-fibrotic WNT5A in sepsis-induced acute lung injury. Crit Care. 2014;18(5):568. doi: 10.1186/s13054-014-0568-z.

94. Raish M, Ahmad A, Ahmad Ansari M, et al. Sinapic acid ameliorates bleomycin-induced lung fibrosis in rats. Biomed Pharmacother. 2018;108:224-231. doi: 10.1016/j.biopha.2018.09.032.
95. Zuo F, Kaminski N, Eugui E, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA. 2002;99(9):6292-7. doi: 10.1073/pnas.092134099.
96. Busiek DF, Baragi V, Nehring LC, et al. Matrilysin expression by human mononuclear phagocytes and its regulation by cytokines and hormones. J Immunol. 1995;154(12):6484-91.
97. Aristorena M, Gallardo-Vara E, Vicen M, et al. MMP-12, secreted by pro-inflammatory macrophages, targets endoglin in human macrophages and endothelial cells. Int J Mol Sci. 2019;20(12):3107. doi: 10.3390/ijms20123107.
98. Hautamaki RD, Kobayashi DK, Senior RM, et al. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science. 1997;277(5334):2002-4. doi: 10.1126/science.277.5334.2002.
99. Churg A, Zhou S, Wright JL. Series "matrix metalloproteinases in lung health and disease": matrix metalloproteinases in COPD. Eur Respir J. 2012;39(1):197-209. doi: 10.1183/09031936.00121611.
100. Wolf M, Maltseva I, Clay SM, et al. Effects of MMP12 on cell motility and inflammation during corneal epithelial repair. Exp Eye Res. 2017;160:11-20. doi: 10.1016/j.exer.2017.04.007.
101. Chan MF, Li J, Bertrand A, et al. Protective effects of matrix metalloproteinase-12 following corneal injury. J Cell Sci. 2013;126(Pt 17):3948-60. doi: 10.1242/jcs.128033.
102. Hyun SW, Liu A, Liu Z, et al. The NEU1-selective sialidase inhibitor, C9-butyl-amide-DANA, blocks sialidase activity and NEU1-mediated bioactivities in human lung in vitro and murine lung in vivo. Glycobiology. 2016;26(8):834-49. doi: 10.1093/glycob/cww060.
103. Pan X, Grigoryeva L, Seyrantepe V, et al. Serine carboxypeptidase SCPEP1 and cathepsin A play complementary roles in regulation of vasoconstriction via inactivation of endothelin-1. PLoS Genet. 2014;10(2):e1004146. doi: 10.1371/journal.pgen.1004146.
104. Petrera A, Gassenhuber J, Ruf S, et al. Cathepsin A inhibition attenuates myocardial infarction-induced heart failure on the functional and proteomic levels. J Transl Med. 2016;14(1):153. doi: 10.1186/s12967-016-0907-8.
105. Petrera A, Kern U, Linz D, et al. Proteomic profiling of cardiomyocyte-specific cathepsin A overexpression links cathepsin A to the oxidative stress response. J Proteome Res. 2016;15(9):3188-95. doi: 10.1021/acs.jproteome.6b00413.
106. Kolli N, Garman SC. Proteolytic activation of human cathepsin A. J Biol Chem. 2014;289(17):11592-11600. doi: 10.1074/jbc.M113.524280.
107. Korkmaz B, Horwitz MS, Jenne DE, et al. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev. 2010;62(4):726-59. doi: 10.1124/pr.110.002733.
108. Tillner J, Lehmann A, Paehler T, et al. Tolerability, safety, and pharmacokinetics of the novel cathepsin A inhibitor SAR164653 in healthy subjects. Clin Pharmacol Drug Dev. 2016;5(1):57-68. doi: 10.1002/cpdd.201.
109. Gao S, Zhu H, Zuo X, et al. Cathepsin G and its role in inflammation and autoimmune diseases. Arch Rheumatol. 2018;33(4):498-504. doi: 10.5606/ArchRheumatol.2018.6595.
110. Campanelli D, Detmers PA, Nathan CF, et al. Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties. J Clin Invest. 1990;85(3):904-15. doi: 10.1172/JCI114518.
111. Gale AJ, Rozenshteyn D. Cathepsin G, a leukocyte protease, activates coagulation factor VIII. Thromb Haemost. 2008;99(1):44-51. doi: 10.1160/TH07-08-0495.
112. Faraday N, Schunke K, Saleem S, et al. Cathepsin G-dependent modulation of platelet thrombus formation in vivo by blood neutrophils. PLoS One. 2013;8(8):e71447. doi: 10.1371/journal.pone.0071447.
113. Lancellotti S, Basso M, De Cristofaro R. Proteolytic processing of von Willebrand factor by adamts13 and leukocyte proteases. Mediterr J Hematol Infect Dis. 2013;5(1):e2013058. doi: 10.4084/MJHID.2013.058.
114. Raife TJ, Cao W, Atkinson BS, et al. Leukocyte proteases cleave von Willebrand factor at or near the ADAMTS13 cleavage site. Blood. 2009;114(8):1666-74. doi: 10.1182/blood-2009-01-195461.
115. Miller SA, Kolpakov MA, Guo X, et al. Intracardiac administration of neutrophil protease cathepsin G activates noncanonical inflammasome pathway and promotes inflammation and pathological remodeling in non-injured heart. J Mol Cell Cardiol. 2019;134:29-39. doi: 10.1016/j.yjmcc.2019.06.016.
116. Sabri A, Alcott SG, Elouardighi H, et al. Neutrophil cathepsin G promotes detachment-induced cardiomyocyte apoptosis via a protease-activated receptor-independent mechanism. J Biol Chem. 2003;278(26):23944-54. doi: 10.1074/jbc.M302718200.
117. Bank U, Reinhold D, Schneemilch C, et al. Selective proteolytic cleavage of IL-2 receptor and IL-6 receptor ligand binding chains by neutrophil-derived serine proteases at foci of inflammation. J Interferon Cytokine Res. 1999;19(11):1277-87. doi: 10.1089/107999099312957.
118. Burgener SS, Leborgne NGF, Snipas SJ, et al. Cathepsin G inhibition by Serpinb1 and Serpinb6 prevents

programmed necrosis in neutrophils and monocytes and reduces GSDMD-driven inflammation. Cell Rep. 2019;27(12):3646-3656.e5. doi: 10.1016/j.celrep.2019.05.065.
119. Hannila SS. Secretory leukocyte protease inhibitor (SLPI): emerging roles in CNS trauma and repair. Neuroscientist. 2015;21(6):630-6. doi: 10.1177/1073858414546000.
120. Renesto P, Balloy V, Kamimura T, et al. Inhibition by recombinant SLPI and half-SLPI (Asn55-Ala107) of elastase and cathepsin G activities: consequence for neutrophil-platelet cooperation. Br J Pharmacol. 1993;108(4):1100-6. doi: 10.1111/j.1476-5381.1993.tb13511.x.
121. Belkowski SM, Masucci J, Mahan A, et al. Cleaved SLPI, a novel biomarker of chymase activity. Biol Chem. 2008;389(9):1219-24. doi: 10.1515/BC.2008.138.
122. Merza M, Hartman H, Rahman M, et al. Neutrophil extracellular traps induce trypsin activation, inflammation, and tissue damage in mice with severe acute pancreatitis. Gastroenterology. 2015;149(7):1920-1931.e8. doi: 10.1053/j.gastro.2015.08.026.
123. Yang ZW, Meng XX, Xu P. Central role of neutrophil in the pathogenesis of severe acute pancreatitis. J Cell Mol Med. 2015;19(11):2513-20. doi: 10.1111/jcmm.12639.
124. Shah D, Mital K. The Role of trypsin:chymotrypsin in tissue repair. Adv Ther. 2018;35(1):31-42. doi: 10.1007/s12325-017-0648-y.
125. Miller RR, De Young DV, Paxinos J. Enzymes for trauma. Postgrad Med J. 1970;46(533):154-6. doi: 10.1136/pgmj.46.533.154.
126. Deby-Dupont G, Haas M, Pincemail J, et al. Immunoreactive trypsin in the adult respiratory distress syndrome. Intensive Care Med. 1984;10(1):7-12. doi: 10.1007/BF00258062.
127. Greene CM, McElvaney NG. Proteases and antiproteases in chronic neutrophilic lung disease - relevance to drug discovery. Br J Pharmacol. 2009;158(4):1048-58. doi: 10.1111/j.1476-5381.2009.00448.x.
128. Xiong L, Sun L, Liu S, et al. The protective roles of urinary trypsin inhibitor in brain injury following fat embolism syndrome in a rat model. Cell Transplant. 2019;28(6):704-712. doi: 10.1177/0963689718814766.
129. Venge P. Kinetic studies of cell migration in a modified Boyden chamber: dependence on cell concentration and effects of the chymotrypsin-like cationic protein of human granulocytes. J Immunol. 1979;122(4):1180-4.
130. Rehman AA, Ahsan H, Khan FH. α-2-Macroglobulin: a physiological guardian. J Cell Physiol. 2013;228(8):1665-75. doi: 10.1002/jcp.24266.
131. Thorpe M, Yu J, Boinapally V, et al. Extended cleavage specificity of the mast cell chymase from the crab-eating macaque (Macaca fascicularis): an interesting animal model for the analysis of the function of the human mast cell chymase. Int Immunol. 2012;24(12):771-82. doi: 10.1093/intimm/dxs081.
132. Mahmood N, Mihalcioiu C, Rabbani SA. Multifaceted role of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR): diagnostic, prognostic, and therapeutic applications. Front Oncol. 2018;8:24. doi: 10.3389/fonc.2018.00024.
133. Sanderson-Smith ML, Zhang Y, Ly D, et al. A key role for the urokinase plasminogen activator (uPA) in invasive Group A streptococcal infection. PLoS Pathog. 2013;9(7):e1003469. doi: 10.1371/journal.ppat.1003469.
134. Komissarov AA, Stankowska D, Krupa A, et al. Novel aspects of urokinase function in the injured lung: role of α2-macroglobulin. Am J Physiol Lung Cell Mol Physiol. 2012;303(12):L1037-45. doi: 10.1152/ajplung.00117.2012.
135. Chen D, Wu X, Yang J, et al. Serum plasminogen activator urokinase receptor predicts elevated risk of acute respiratory distress syndrome in patients with sepsis and is positively associated with disease severity, inflammation and mortality. Exp Ther Med. 2019;18(4):2984-2992. doi: 10.3892/etm.2019.7931.
136. Shan L, Shan F, Li J, et al. Association of circulating suPAR with disease severity and clinical outcomes in patients with ARDS induced by intra-abdominal infections: a prospective observational study. Int J Clin Exp Med. 2016;9(7):12788-12795.
137. Geboers DG, de Beer FM, Tuip-de Boer AM, et al. Plasma suPAR as a prognostic biological marker for ICU mortality in ARDS patients. Intensive Care Med. 2015;41(7):1281-90. doi: 10.1007/s00134-015-3924-9.
138. Jalkanen V, Yang R, Linko R, et al. SuPAR and PAI-1 in critically ill, mechanically ventilated patients. Intensive Care Med. 2013;39(3):489-96. doi: 10.1007/s00134-012-2730-x.
139. Hayek SS, Leaf DE, Samman Tahhan A, et al. Soluble urokinase receptor and acute kidney injury. N Engl J Med. 2020;382(5):416-426. doi: 10.1056/NEJMoa1911481.
140. Bhatia R, Dent C, Topley N, et al. Neutrophil priming for elastase release in adult blunt trauma patients. J Trauma. 2006;60(3):590-6. doi: 10.1097/01.ta.0000205614.51885.ff.
141. Brusselle GG, Van Braeckel E. Sputum neutrophil elastase as a biomarker for disease activity in bronchiectasis. Am J Respir Crit Care Med. 2017;195(10):1289-1291. doi: 10.1164/rccm.201612-2476ED.
142. Wang Z, Chen F, Zhai R, et al. Plasma neutrophil elastase and elafin imbalance is associated with acute respiratory distress syndrome (ARDS) development. PLoS One. 2009;4(2):e4380. doi: 10.1371/journal.pone.0004380.
143. Wang T, Zhu Z, Liu Z, et al. Plasma neutrophil

elastase and elafin as prognostic biomarker for acute respiratory distress syndrome: a multicenter survival and longitudinal prospective observation study. Shock. 2017;48(2):168-174. doi: 10.1097/SHK.0000000000000845.
144. Barrett CD, Moore HB, Banerjee A, et al. Human neutrophil elastase mediates fibrinolysis shutdown through competitive degradation of plasminogen and generation of angiostatin. J Trauma Acute Care Surg. 2017;83(6):1053-1061. doi: 10.1097/TA.0000000000001685.
145. Donnelly SC, MacGregor I, Zamani A, et al. Plasma elastase levels and the development of the adult respiratory distress syndrome. Am J Respir Crit Care Med. 1995;151(5):1428-33. doi: 10.1164/ajrccm.151.5.7735596.
146. Semple BD, Trivedi A, Gimlin K, et al. Neutrophil elastase mediates acute pathogenesis and is a determinant of long-term behavioral recovery after traumatic injury to the immature brain. Neurobiol Dis. 2015;74:263-80. doi: 10.1016/j.nbd.2014.12.003.
147. Uchiyama K, Naito Y, Takagi T, et al. Serpin B1 protects colonic epithelial cell via blockage of neutrophil elastase activity and its expression is enhanced in patients with ulcerative colitis. Am J Physiol Gastrointest Liver Physiol. 2012;302(10):G1163-70. doi: 10.1152/ajpgi.00292.2011.
148. Kunder M, Lakshmaiah V, Moideen Kutty AV. Plasma neutrophil elastase, α_1-antitrypsin, α_2-macroglobulin and neutrophil elastase-α_1-antitrypsin complex levels in patients with dengue fever. Indian J Clin Biochem. 2018;33(2):218-221. doi: 10.1007/s12291-017-0658-1.
149. Hook G, Jacobsen JS, Grabstein K, et al. Cathepsin B is a new drug target for traumatic brain injury therapeutics: evidence for E64d as a promising lead drug candidate. Front Neurol. 2015;6:178. doi: 10.3389/fneur.2015.00178.
150. Talukdar R, Sareen A, Zhu H, et al. Release of cathepsin B in cytosol causes cell death in acute pancreatitis. Gastroenterology. 2016;151(4):747-758.e5. doi: 10.1053/j.gastro.2016.06.042.
151. Farges MC, Balcerzak D, Fisher BD, et al. Increased muscle proteolysis after local trauma mainly reflects macrophage-associated lysosomal proteolysis. Am J Physiol Endocrinol Metab. 2002;282(2):E326-35. doi: 10.1152/ajpendo.00345.2001.
152. Boutté AM, Hook V, Thangavelu B, et al. Penetrating traumatic brain injury triggers dysregulation of cathepsin B protein levels independent of cysteine protease activity in brain and cerebral spinal fluid. J Neurotrauma. 2020;37(13):1574-1586. doi: 10.1089/neu.2019.6537.
153. Liu Q, Zhang Y, Liu S, et al. Cathepsin C promotes microglia M1 polarization and aggravates neuroinflammation via activation of Ca^{2+}-dependent PKC/p38MAPK/NF-κB pathway. J Neuroinflammation. 2019;16(1):10. doi: 10.1186/s12974-019-1398-3.
154. Fan K, Wu X, Fan B, et al. Up-regulation of microglial cathepsin C expression and activity in lipopolysaccharide-induced neuroinflammation. J Neuroinflammation. 2012;9:96. doi: 10.1186/1742-2094-9-96.
155. Laine DI, Busch-Petersen J. Inhibitors of cathepsin C (dipeptidyl peptidase I). Expert Opin Ther Pat. 2010;20(4):497-506. doi: 10.1517/13543771003657172.
156. Fan K, Li D, Zhang Y, et al. The induction of neuronal death by up-regulated microglial cathepsin H in LPS-induced neuroinflammation. J Neuroinflammation. 2015;12:54. doi: 10.1186/s12974-015-0268-x.
157. Kirschke H. Cathepsin H. In: Rawlings ND, Salvesen G, editors. Handbook proteolytic enzymes. 3rd ed. Amsterdam: Elsevier/Academic Press; 2013. p. 1795-1800.
158. Cai J, Zhong H, Wu J, et al. Cathepsin L promotes vascular intimal hyperplasia after arterial injury. Mol Med. 2017;23:92-100. doi: 10.2119/molmed.2016.00222.
159. Leng YP, Ma YS, Li XG, et al. l-Homocysteine-induced cathepsin V mediates the vascular endothelial inflammation in hyperhomocysteinaemia. Br J Pharmacol. 2018;175(8):1157-1172. doi: 10.1111/bph.13920.
160. Gu YH, Kanazawa M, Hung SY, et al. Cathepsin L acutely alters microvessel integrity within the neurovascular unit during focal cerebral ischemia. J Cereb Blood Flow Metab. 2015;35(11):1888-900. doi: 10.1038/jcbfm.2015.170.
161. Dana D, Pathak SK. A Review of small molecule inhibitors and functional probes of human cathepsin L. Molecules. 2020;25(3):698. doi: 10.3390/molecules25030698.
162. Kanbak G, Kartkaya K, Ozcelik E, et al. The neuroprotective effect of acute moderate alcohol consumption on caspase-3 mediated neuroapoptosis in traumatic brain injury: the role of lysosomal cathepsin L and nitric oxide. Gene. 2013;512(2):492-5. doi: 10.1016/j.gene.2012.10.012.
163. Huber-Lang M, Denk S, Fulda S, et al. Cathepsin D is released after severe tissue trauma in vivo and is capable of generating C5a in vitro. Mol Immunol. 2012;50(1-2):60-5. doi: 10.1016/j.molimm.2011.12.005.
164. Sarabhai T, Peter C, Bär AK, et al. Serum α-1 Antitrypsin (AAT) antagonizes intrinsic apoptosis induction in neutrophils from patients with systemic inflammatory response syndrome. PLoS One. 2017;12(5):e0177450. doi: 10.1371/journal.pone.0177450.
165. Chapman KR, Chorostowska-Wynimko J, Koczulla AR, et al. Alpha 1 antitrypsin to treat lung disease in alpha 1 antitrypsin deficiency: recent developments and clinical implications. Int J Chron Obstruct Pulmon Dis. 2018;13:419-432. doi: 10.2147/COPD.S149429.
166. Chiuchiolo MJ, Crystal RG. Gene Therapy for alpha-1 antitrypsin deficiency lung disease. Ann Am Thorac

Soc. 2016;13 Suppl 4:S352-69. doi: 10.1513/AnnalsATS.201506-344KV.
167. Bergman D, Kadner SS, Cruz MR, et al. Synthesis of alpha 1-antichymotrypsin and alpha 1-antitrypsin by human trophoblast. Pediatr Res. 1993;34(3):312-7. doi: 10.1203/00006450-199309000-00015.
168. Hausmann R, Betz P. Course of glial immunoreactivity for vimentin, tenascin and alpha1-antichymotrypsin after traumatic injury to human brain. Int J Legal Med. 2001;114(6):338-42. doi: 10.1007/s004140000199.
169. Park J, Masaki T, Mezaki Y, et al. Alpha-1 antichymotrypsin is involved in astrocyte injury in concert with arginine-vasopressin during the development of acute hepatic encephalopathy. PLoS One. 2017;12(12):e0189346. doi: 10.1371/journal.pone.0189346.
170. Wang S, Wei X, Zhou J, et al. Identification of α2-macroglobulin as a master inhibitor of cartilage-degrading factors that attenuates the progression of posttraumatic osteoarthritis. Arthritis Rheumatol. 2014;66(7):1843-53. doi: 10.1002/art.38576.
171. Jenne DE. Structure of the azurocidin, proteinase 3, and neutrophil elastase genes. Implications for inflammation and vasculitis. Am J Respir Crit Care Med. 1994;150(6 Pt 2):S147-54. doi: 10.1164/ajrccm/150.6_Pt_2.S147.
172. Honoré S, Attalah HL, Azoulay E, et al. Beneficial effect of an inhibitor of leukocyte elastase (EPI-hNE-4) in presence of repeated lung injuries. Shock. 2004;22(2):131-6. doi: 10.1097/01.shk.0000126861.77543.d0.
173. Zhang X, Zhu Z, Jiao W, et al. Ulinastatin treatment for acute respiratory distress syndrome in China: a meta-analysis of randomized controlled trials. BMC Pulm Med. 2019;19(1):196. doi: 10.1186/s12890-019-0968-6.
174. Leng YX, Yang SG, Song YH, et al. Ulinastatin for acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. World J Crit Care Med. 2014;3(1):34-41. doi: 10.5492/wjccm.v3.i1.34.
175. He G, Li Q, Li W, et al. Effect of ulinastatin on interleukins and pulmonary function in bypass patients: a meta-analysis of randomized controlled trials. Herz. 2020;45(4):335-346. doi: 10.1007/s00059-018-4732-0.
176. Zhang S, Yu R, Zhang Y, et al. Cytoprotective effects of urinary trypsin inhibitor on astrocytes injured by sustained compression. Mol Biol Rep. 2014;41(3):1311-6. doi: 10.1007/s11033-013-2976-6.
177. Williams SE, Brown TI, Roghanian A, et al. SLPI and elafin: one glove, many fingers. Clin Sci (Lond). 2006;110(1):21-35. doi: 10.1042/CS20050115.
178. Tejera P, Wang Z, Zhai R, et al. Genetic polymorphisms of peptidase inhibitor 3 (elafin) are associated with acute respiratory distress syndrome. Am J Respir Cell Mol Biol. 2009;41(6):696-704. doi: 10.1165/rcmb.2008-0410OC.
179. Wang Z, Beach D, Su L, et al. A genome-wide expression analysis in blood identifies pre-elafin as a biomarker in ARDS. Am J Respir Cell Mol Biol. 2008;38(6):724-32. doi: 10.1165/rcmb.2007-0354OC.
180. Tejera P, O'Mahony DS, Owen CA, et al. Functional characterization of polymorphisms in the peptidase inhibitor 3 (elafin) gene and validation of their contribution to risk of acute respiratory distress syndrome. Am J Respir Cell Mol Biol. 2014;51(2):262-72. doi: 10.1165/rcmb.2013-0238OC.
181. Moreau T, Baranger K, Dadé S, et al. Multifaceted roles of human elafin and secretory leukocyte proteinase inhibitor (SLPI), two serine protease inhibitors of the chelonianin family. Biochimie. 2008;90(2):284-95. doi: 10.1016/j.biochi.2007.09.007.
182. Babaei S, Bayat M. Pentoxifylline accelerates wound healing process by modulating gene expression of MMP-1, MMP-3, and TIMP-1 in normoglycemic rats. J Invest Surg. 2015;28(4):196-201. doi: 10.3109/08941939.2014.1002642.
183. Nicaise AM, Johnson KM, Willis CM, et al. TIMP-1 promotes oligodendrocyte differentiation through receptor-mediated signaling. Mol Neurobiol. 2019;56(5):3380-3392. doi: 10.1007/s12035-018-1310-7.
184. Power G, Moore Z, O'Connor T. Measurement of pH, exudate composition and temperature in wound healing: a systematic review. J Wound Care. 2017;26(7):381-397. doi: 10.12968/jowc.2017.26.7.381.
185. Liu C, Lu X, Mao Z, et al. The diagnostic accuracy of urinary [TIMP-2]·[IGFBP7] for acute kidney injury in adults: a PRISMA-compliant meta-analysis. Medicine (Baltimore). 2017;96(27):e7484. doi: 10.1097/MD.0000000000007484.
186. Gibb SL, Zhao Y, Potter D, et al. TIMP3 attenuates the loss of neural stem cells, mature neurons and neurocognitive dysfunction in traumatic brain injury. Stem Cells. 2015;33(12):3530-44. doi: 10.1002/stem.2189.
187. Hendrickson CM, Gibb SL, Miyazawa BY, et al. Elevated plasma levels of TIMP-3 are associated with a higher risk of acute respiratory distress syndrome and death following severe isolated traumatic brain injury. Trauma Surg Acute Care Open. 2018;3(1):e000171. doi: 10.1136/tsaco-2018-000171.
188. Radomski A, Jurasz P, Sanders EJ, et al. Identification, regulation and role of tissue inhibitor of metalloproteinases-4 (TIMP-4) in human platelets. Br J Pharmacol. 2002;137(8):1330-8. doi: 10.1038/sj.bjp.0704936.

https://doi.org/10.52645/MJHS.2026.2.09

UDC: [616.12+612.17]:57.034

REVIEW ARTICLE

Circadian rhythms in cardiovascular physiology and disease: extrinsic and intrinsic factors with implications for intensive care

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ABSTRACT

Introduction. Circadian rhythms are endogenous, approximately 24-hour oscillations that coordinate nearly all physiological systems, including cardiovascular function. The suprachiasmatic nucleus serves as the central pacemaker, synchronizing peripheral clocks in the heart, vasculature, and kidneys to generate daily fluctuations in blood pressure, heart rate, endothelial function, coagulation, myocardial metabolism, and autonomic tone. Disruption of circadian organization – through extrinsic factors (shift work, irregular light exposure, altered feeding schedules) or intrinsic factors (aging, inflammation, genetic clock-gene variants) – has been strongly linked to increased cardiovascular morbidity and mortality.

Material and methods. A bibliographic search was conducted in PubMed, Scopus, and Web of Science for English-language publications (2000–2025), focusing on the circadian rhythm, cardiovascular disease, hypertension, chronotherapy, and critical illness. Keywords included "circadian rhythm," "cardiovascular disease," "hypertension," "chronotherapy," and "intensive care." Original research, clinical trials, meta-analyses, and experimental studies were eligible; studies addressing circadian blood pressure variability and its relation to outcomes in critically ill patients were specifically examined. Filters required full-text availability and publication dates from 2000 to 2025. The search yielded 276 full-text articles, of which 79 representative sources were selected for this narrative review.

Results. This review synthesizes current evidence demonstrating that circadian clocks regulate essential cardiovascular processes and that their disruption contributes to disease pathogenesis. Observational data on circadian blood pressure variability are discussed, showing that the attenuation of normal hemodynamic oscillations is associated with a worse prognosis. Particular attention is given to the extrinsic and intrinsic factors that modulate circadian alignment, with implications for the management of patients in intensive care.

Keywords: circadian rhythm, cardiovascular disease, hypertension, chronotherapy, autonomic dysfunction, intensive care.

Cite this article: Camerzan I. Circadian rhythms in cardiovascular physiology and disease: extrinsic and intrinsic factors with implications for intensive care. Mold J Health Sci. 2026;13(2):63-72. https://doi.org/10.52645/MJHS.2026.2.09.

Manuscript received: 03.03.2026

Accepted for publication: 20.05.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Regarding the role of the circadian rhythm in cardiovascular physiology, the prognostic significance of circadian blood pressure variability in critically ill patients remains insufficiently explored. In particular, the relationship between circadian hemodynamic alterations and mortality risk in intensive care settings is not yet fully understood. Furthermore, the integration of circadian parameters with established clinical severity scores to improve prognostic assessment has not been extensively investigated.

The research hypothesis

We hypothesized that the disruption of physiological circadian blood pressure variability is associated with increased mortality in

critically ill patients. Furthermore, we assumed that the incorporation of circadian hemodynamic parameters into established clinical severity scoring systems, such as SOFA and APACHE II, could improve prognostic accuracy and provide additional information regarding patient outcomes.

The novelty added by the manuscript to the already published scientific literature

The present manuscript provides new insights into the prognostic significance of circadian blood pressure variability in critically ill patients. The integration of circadian variability into established prognostic scores may contribute to improved risk stratification and support the development of chronotherapeutic approaches in intensive care medicine.

Introduction

Because the Earth turns on its axis every 24 hours, almost all life on the planet has a mechanism – circadian rhythmicity – to anticipate the daily changes caused by this rotation. The molecular clocks that control circadian rhythms are being revealed as important regulators of physiology and disease. In humans, circadian rhythms have been studied extensively in the cardiovascular system. Many cardiovascular functions, such as endothelial function, thrombus formation, blood pressure, and heart rate, are now known to be regulated by the circadian clock. Additionally, the onset of acute myocardial infarction, stroke, arrhythmias and other adverse cardiovascular events shows circadian rhythmicity [1].

The circadian clock evolved in diverse organisms to integrate external environmental changes with internal physiology. The clock endows the host with temporal precision and robust adaptation to the surrounding environment. When circadian rhythms are perturbed or misaligned–as a result of jet lag, shift work, or other lifestyle factors–adverse health consequences arise, and the risks of diseases such as cancer, cardiovascular diseases, or metabolic disorders increase. Although the negative impact of circadian rhythm disruption is now well established, how to take advantage of biological timing, or correct it for health benefits, remains underappreciated [2].

The circadian clock is an evolutionarily conserved biological system that coordinates physiological and behavioral processes in a 24-hour rhythm, enabling organisms to anticipate and adapt to daily environmental changes, such as the light-dark cycle [3, 4]. At its core, the circadian rhythm is governed by a network of molecular clocks, with the suprachiasmatic nucleus (SCN) in the hypothalamus serving as the central pacemaker that synchronizes peripheral clocks located in virtually all tissues, including the heart, blood vessels, and kidneys [5-7]. The circadian rhythm, orchestrated by the SCN, synchronizes body-wide clocks through neural and hormonal pathways. Light signals, detected by specialized retinal cells, travel via the retinohypothalamic tract to the SCN, initiating a cascade through the paraventricular nucleus (PVN), brainstem, and spinal cord to the pineal gland. The molecular machinery of the circadian clock involves a set of core clock genes, including *CLOCK*, which operate via transcriptional-translational feedback loops (TTFLs) to regulate the expression of clock-controlled genes (CCGs) that influence diverse physiological processes [3, 8]. This intricate system ensures temporal coordination of cardiovascular functions, such as blood pressure, heart rate, and endothelial function, which exhibit robust diurnal variations [9-12]. For instance, blood pressure typically dips during sleep and rises in the early morning, a pattern regulated by the interplay between the SCN and peripheral clocks in the cardiovascular system (CVS) [13-16]. Disruption of these rhythms, as seen in shift workers or individuals with sleep disorders, has been epidemiologically linked to an increased risk of cardiovascular disease (CVD), including hypertension, myocardial infarction (MI), and atherosclerosis [17, 18]. Studies have shown that circadian misalignment can lead to dysregulation of the autonomic nervous system (ANS), impaired glucose metabolism, and increased systemic inflammation, all of which contribute to CVD pathogenesis [19-22]. Evidently, the circadian rhythm is a crucial conduit between the brain and the heart.

The objective of this narrative review is to synthesize the current scientific evidence on the regulation of cardiovascular physiology by circadian rhythms and the consequences of their disruption in disease states. Specifically, we aim to: (1) delineate the molecular and physiological mechanisms linking central and peripheral circadian clocks to key cardiovascular functions (blood pressure, heart rate, endothelial function, coagulation, and autonomic tone); (2) analyze the contributions of extrinsic (e.g., shift work, irregular light exposure, altered feeding schedules) and intrinsic (e.g., aging, inflammation, genetic clock-gene variants) factors to circadian misalignment and its associated cardiovascular morbidity and mortality; and (3) discuss the clinical implications of these disruptions for critically ill patients in intensive care, including the potential value of chronotherapeutic strategies and circadian-aligned environmental interventions.

Material and methods

A comprehensive bibliographic search was initiated to conduct a narrative literature review synthesizing current scientific evidence regarding circadian rhythm disruption and its effects on cardiovascular regulation in critically ill patients. The review followed the general principles of systematic literature analysis to identify, select, and evaluate relevant studies addressing circadian mechanisms and their clinical implications in intensive care settings. The search was performed across electronic databases, including PubMed, Scopus, and Web of Science, and was limited to studies published between 2000 and 2025. The following keywords and their combinations were used: *circadian rhythm*, *circadian disruption*, *blood pressure variability*,

cardiovascular regulation, *critical illness*, *intensive care unit*, *chronobiology*, and *hemodynamic instability*. Additionally, the reference lists of relevant publications were manually screened to identify further studies that met the inclusion criteria. Studies were considered eligible if they addressed circadian rhythm physiology, mechanisms of circadian regulation, circadian disruption in intensive care environments, or the relationship between circadian rhythms and cardiovascular function.

The following types of publications were included:

- clinical studies
- experimental studies
- observational studies
- relevant review articles

Studies were excluded if they:

- were not published in English
- lacked relevance to the topic of circadian rhythm and cardiovascular regulation
- did not include data or discussion related to critically ill patients or cardiovascular physiology.

The identified articles were initially screened based on their titles and abstracts. Publications that appeared relevant were further assessed through full-text evaluation. Studies meeting the eligibility criteria were included in the final analysis. Relevant information from the selected studies was extracted, including:

- study objectives
- study design
- investigated circadian parameters
- cardiovascular outcomes
- major findings related to circadian rhythm disturbances.

The extracted data were organized and analyzed to identify key themes related to circadian regulation and cardiovascular instability in critically ill patients. The findings from the selected studies were qualitatively synthesized to provide an integrated overview of the current understanding of circadian rhythm disruption and its clinical implications in intensive care medicine. As this study represents a review of previously published literature, no original statistical analyses were performed; instead, the results from the included studies were analyzed descriptively. Because this study is based exclusively on previously published scientific literature, ethical approval and informed consent were not required.

The literature search identified 276 records through database searching across PubMed, Scopus, and Web of Science. After screening titles and abstracts, 197 studies were excluded due to a lack of relevance, insufficient data, or non-English language. A total of 82 full-text articles were assessed for eligibility and included in the final review (Fig. 1).

Results

It is undeniable that the circadian orchestration of biological processes is fundamental for both health and disease. During pathological conditions, circadian orchestration is often perturbed, and the disruption of normal rhythms in healthy organisms leads to pathology. Acknowledgment of these principles has led to the concept that perhaps circadian clocks and biology might be targeted for cardiovascular disease treatment [23]. Disruption of normal circadian rhythms, whether through behavioral, environmental, or genetic means, is detrimental in both humans and animal models, leading to an increased risk of pathologies, including cardiovascular disease. Similarly, whole-body circadian rhythmicity is attenuated with age, in association with increased disease risk [24]. Taken together, such observations suggest that the maintenance of normal circadian biology is critical for prevention of disease and maximizing longevity [23]. The master biological clock, known as the central clock, is located in the suprachiasmatic nucleus (SCN) in the hypothalamus [25]. The central clock is essentially involved in adjusting circadian variations in physiological function by regulating the autonomic nervous system, humoral mediators, and other systemic signals. In addition to the central clock in the SCN, each peripheral organ and cell also possesses circadian expression of core clock genes; these are referred to as peripheral clocks. The molecular mechanisms of peripheral clocks are believed to be identical to those of the central clock [26]. The factors that can reset and affect the phase of the internal clock are called zeitgebers (timekeepers). Light is established as the main zeitgeber for the central clock. In contrast, the specific zeitgebers for peripheral clocks are not yet fully elucidated. The discovery of the appropriate zeitgeber for each organ is required to establish novel therapeutic approaches considering time-of-day treatment, namely chronotherapy [27].

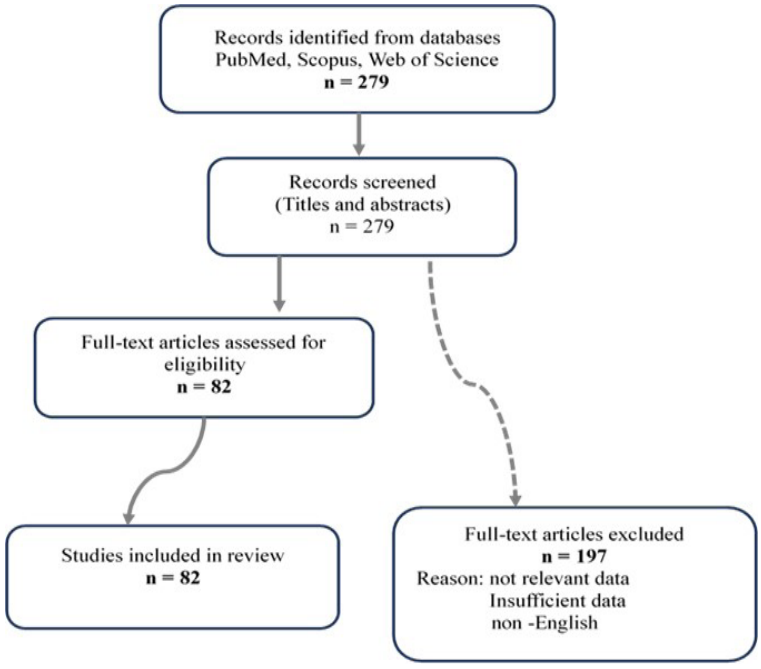

Fig. 1 PRISMA 2020 flow diagram of the literature search and study selection process

The circadian mechanism underlies daily rhythms in cardiovascular physiology, and rhythm disruption is a major risk factor for heart disease and worse outcomes. However, the role of circadian rhythms is generally clinically unappreciated [28]. These rhythms drive daily variations in key parameters, including heart rate [29], blood pressure [30], and cardiac contractility [31]. Circadian rhythm disruption is a major risk factor for cardiovascular disease and is associated with severe health consequences. [32, 33]. Recently, there have been major advances in our understanding of daily rhythmicity and its relevance to the pathogenesis and treatment of cardiac hypertrophy and heart failure [31, 34, 35].

Because circadian rhythms display oscillating patterns, developing treatments that can take advantage of this biological governance holds great therapeutic value. Hence, a crucial therapeutic approach involves mitigating risks during periods of highest vulnerability in the circadian cycle, or reinstating typical circadian phase and amplitude patterns [36].

Circadian rhythms, regulated by the suprachiasmatic nucleus in the brain, profoundly influence cardiovascular health through intricate neurobiological mechanisms. These rhythms regulate gene expression in cardiomyocytes, modulate autonomic nervous system (ANS) activity, and synchronize cardiovascular functions with environmental cues, ultimately impacting heart rate, blood pressure, and susceptibility to cardiac events. The intricate relationship between circadian rhythms and cardiovascular health emphasizes the critical role of brain-heart communication in physiological processes [37].

Peripheral clocks in cardiovascular tissues. Peripheral clocks in cardiovascular tissues play a crucial role in regulating circadian rhythms of cardiovascular function, working in concert with the central clock in the SCN [3, 38, 39]. In the heart, cardiomyocytes exhibit rhythmic expression of core clock genes and CCGs, with up to 10 % of the cardiac transcriptome showing circadian oscillations [40]. These oscillations regulate critical cardiac functions, such as the expression of ion channels like $K_v1.5$, $K_v4.2$, and Scn5a, which are involved in cardiac electrophysiology [41, 42]. The peripheral clock in blood vessels also demonstrates circadian rhythmicity, with smooth muscle BMAL1 participating in blood pressure regulation [13, 43]. Beesley et al. conducted an intriguing study to explore how circadian rhythms affect heart function in mice cardiomyocytes. They found that the circadian clock gene expression of PER2 in cardiomyocytes is cell-autonomous and can be amplified by β-adrenergic signaling, suggesting how circadian rhythms are regulated in cardiomyocytes and their potential implications for cardiac function [44]. Endothelial cells, crucial for vascular homeostasis, exhibit circadian control over coagulation factors like plasminogen activator inhibitor-1 (PAI-1) and thrombomodulin, as well as cell cycle regulators *Ccna1* and *Cdk1* [40, 45, 46]. The interaction between central and peripheral clocks is complex, with the SCN playing a central role in the synchronization of peripheral clocks through neural and humoral signals [47, 48]. However, peripheral clocks can also be affected by local zeitgebers, such as food intake and physical activity, allowing for tissue-specific temporal organization [46, 49, 50]. This intricate network of central and peripheral clocks enables the CVS to anticipate and adapt to daily environmental changes. The SCN affects peripheral clocks via the SNS, while hormones like T_3 and neurotransmitters such as VIP fine-tune cardiac rhythms. Voltage-gated calcium channels (VGCCs) play a crucial role in translating circadian signals into functional cardiac outputs [40, 51]. Disruption of this synchronization, either between the central clock and peripheral clocks or among different peripheral clocks, can lead to cardiovascular dysfunction [52]. For instance, cardiomyocyte-specific deletion of the Bmal1 gene results in a slower heart rate and increased susceptibility to arrhythmias [41, 53, 54]. Moreover, desynchronization between internal clocks and the external environment, as seen in shift work or irregular social schedules, has been associated with an increased risk of cardiovascular diseases [20, 46]. Understanding the molecular mechanisms underlying these peripheral clocks and their interaction with the central clock is crucial for developing novel therapeutic approaches to manage cardiovascular disorders and optimize treatment timing, a concept known as chronotherapy [41, 46, 55, 56].

Mechanism of circadian disruption causing cardiovascular diseases. Studies have shown that major cardiovascular events, such as acute myocardial infarction, sudden cardiac death, and stroke, occur more frequently at certain times of the day – particularly in the early morning – suggesting a dysfunctional circadian regulation. Disruption of circadian rhythms has been associated with resistant hypertension, endothelial dysfunction, and chronic vascular inflammation, all of which contribute to the progression of cardiovascular disease.

Circadian disruption broadly refers to multiple types of circadian clock disturbances, including circadian misalignment [57] and circadian desynchrony [58] or desynchronization [59]. These disturbances can manifest across various biological levels, from cellular and tissue scales to organismal and systemic scales. Circadian misalignment is a mismatch between an individual's internal circadian clock and their external environment or social schedule. Circadian desynchrony or desynchronization both refer to a variance in the cycles of 2 or more rhythms. Both concepts can be quantified by measuring the phase angle differences and comparing the estimated durations of the rhythms [60].

Circadian disruption significantly impacts cardiovascular health through multiple interconnected mechanisms (Table 1). Dysregulation of ANS activity is a key factor, as evidenced by altered heart rate variability and blood pressure patterns in individuals experiencing circadian misalignment [20, 41, 46]. This disruption can lead to prolonged QTc intervals (the time from the start of the Q wave to the end of the T wave on an electrocardiogram (ECG), measuring ventricular depolarization and repolarization) and increased susceptibility to arrhythmias, particularly in shift workers

[38, 46]. Impaired metabolic and hormonal rhythms also play a crucial role in associating circadian disruption with CVD. Cortisol and melatonin, two hormones with strong circadian patterns, are particularly affected. Disrupted cortisol rhythms can lead to increased inflammation and metabolic dysfunction, while altered melatonin secretion due to light exposure at night can impact sleep quality and cardiovascular function [50, 61, 62].

Table 1. Effects of circadian clock gene dysregulation on cardiovascular function

Clock gene manipulation	Cardiovascular effects	References
Bmal1 knockout	Arrhythmic behaviour, loss of circadian rhythms in peripheral tissues, reduced lifespan	Haque et al., 2019
Per2 mutation	Impaired endothelium-dependent relaxation, decreased nitric oxide production	Viswambharan et al., 2007
SCN-specific Bmal1 knockout	Loss of circadian behavioural rhythms while peripheral clocks remain rhythmic	Haque et al., 2019
Astrocyte-specific Bmal1 knockout	Delayed activity onset and slower entrainment to new light–dark cycles	Haque et al., 2019
Ventral forebrain Bmal1 knockout	Altered timing of circadian behavioural patterns	Haque et al., 2019
Per2 knockout in cardiomyocytes	Increased cell death and mitochondrial dysfunction under stress	Bhaskara et al., 2024
VSMC Bmal1 knockout	Aggravated atherosclerotic lesions, increased VSMC migration, ROS levels, and apoptosis	Lin et al., 2022
Postnatal cardiomyocyte Bmal1 knockout	Increased cardiac hypertrophy and fibrosis; pressure overload-induced cardiac remodeling	Liang et al., 2022
Per1 knockout	Circadian rhythm desynchronization and increased salt-sensitive hypertension	Zietara et al., 2022

***Note*:** *VSMC – vascular smooth muscle cells; ROS – reactive oxygen species; SCN – suprachiasmatic nucleus; BMAL1 – Brain and Muscle ARNT-Like 1, a core component of the circadian clock transcription–translation feedback loop; PER1 / PER2 – Period genes (Period Circadian Regulator 1 and 2), essential components of the negative feedback loop regulating circadian rhythms.*

Oxidative stress and inflammation are exacerbated by circadian disruption, contributing significantly to CVD risk. Studies have shown that circadian rhythm disruption impairs tissue homeostasis and exacerbates chronic inflammation [39, 50, 63, 64]. Circadian dysregulation is intricately correlated to various cardio-vascular conditions, highlighting the critical role of the body's internal clock in maintaining cardiovascular health. The onset of cardiovascular disease (CVD) exhibits a diurnal oscillation; for example, acute coronary syndrome or atrial fibrillation often occurs in the early morning (Table 2).

In addition to disease onset, several cardiac functions also show circadian variation, including heart rate (HR) and blood pressure (BP). Recent evidence supports the idea that the diurnal variation of cardiovascular physiology and pathology is tightly related to an intrinsic biological rhythm, named the circadian clock [27].

Table 2. Common onset time of cardiovascular events.

Disease	Onset time
Acute myocardial infarction	Early morning
Cerebral infarction	Morning
Subarachnoid hemorrhage	Daytime
Atrial fibrillation	Morning/night
Ventricular tachycardia/fibrillation	Morning

Diurnal variation and chronotherapy in blood pressure/hypertension. It is well known that there is a 24-hour variation in BP with a distinct peak in the morning. This daily variation in BP is considered representative of both intrinsic and exogenous factors. Intrinsic factors include autonomic nervous system activity and humoral factors such as cortisol, renin, aldosterone, vasoactive intestinal peptide, and ANP [65]. Conversely, exogeneous factors such as physical activity, emotional state, meals, and sleep-wake states also profoundly affect BP variations. In normal subjects, the nocturnal BP decline of less than 10-20%, and these individuals are termed "normal dippers". Persons with a nocturnal decline of less than 10% are classified as "non-dippers". Some subjects can be classified as "extreme dippers" (a decline greater than 20%), while others present as "inverse dippers" or "risers" (a decline less than 0%), indicating that their asleep BP is higher than their awake BP (Table 3) [27].

Table 3. Indices of diurnal blood pressure (BP) decline.

BP Dipping Classification	Nocturnal BP decline ratio
Normal dipper	10—20%
Non-dipper	<10%
Extreme dipper	>20%
Inverse dipper/ riser	<0%

Note: *[(Mean SBP awake – Mean SBP asleep)/Mean SBP awake] × 100.*

Loss of adequate BP decline during the sleep period may be a significant risk factor for cardiovascular organ damage. Hypertensive patients with the non-dipper BP pattern are found to be more susceptible to heart and kidney damage [66]. Patients with a non-dipper BP profile have a higher risk than dipper patients for left ventricular hypertrophy, heart failure, myocardial infarction, stroke, albuminuria, and progression to end-stage renal disease [65]. Non-dip-

per normotensive patients exhibit an equivalent hazard ratio for cardiovascular mortality compared to dipper hypertensive patients [66]. Inverse-dipper or riser patients also demonstrate a significantly higher incidence of stroke compared with those with other patterns of BP variation, suggesting that patients with this BP profile carry the highest overall risk for cardiovascular events [67, 68]. Considering the heterogeneity of BP profiles, it is clinically inappropriate to treat all hypertensive patients with the same regimen. Currently, more than 80% of hypertensive patients take all of their anti-hypertensive medications in the morning. Therefore, it is becoming a physician's responsibility personalize treatment according to a patient's specific diurnal BP profile. Chronotherapy aims to provide treatment with maximum beneficial effects and minimal adverse effects by aligning drug delivery with the intrinsic circadian rhythm of disease processes or symptoms. Chronotherapy with antihypertensive medication can be successfully achieved by adjusting the administration time within a 24-hour cycle [27, 69].

Circadian rhythm in acute coronary syndrome. The onset of acute myocardial infarction (AMI) or pulmonary embolism demonstrates a clear circadian variation with a peak in the early morning [70, 71]. A number of cardiac functions related to the pathogenesis of myocardial infarction are known to exhibit circadian variation. Even the shift to daylight saving time in the spring could influence the onset of AMI; during the first 3 weeks after this seasonal transition, the incidence of AMI significantly increases [72].

The diurnal variation in autonomic nervous system activity could account for the circadian onset of AMI. In the early morning, systolic BP and HR increase, resulting in an elevated myocardial oxygen demand. Conversely, the vascular tone of the coronary arteries rises, and coronary blood flow decreases during morning hours. This mismatch between myocardial oxygen demand and supply in the morning appears to trigger the circadian onset of AMI. Interestingly, of the morning peak in AMI onset is blunted in patients receiving β-blockers, as well as in diabetic patients with autonomic neuropathy, highlighting the central role of autonomic nervous system signaling in driving this diurnal variation [73]. In addition, a state of hypercoagulability in the morning may underlie the circadian onset of AMI. Circulating platelet counts and platelet aggregation pathways fluctuate in a circadian fashion [74]. Because platelets are activated by catecholamines, the rhythmic activation of the autonomic nervous system can induce corresponding oscillations in platelet activity. The coagulation activity also has a circadian variation. The plasma concentration of factor VII, the elevation of which is known as a risk factor for coronary artery disease, exhibits a diurnal oscillation [75]. Other coagulation-related factors, such as fibrinogen, prothrombin, factor VIII, and tissue factor pathway inhibitor also exert circadian activation [76]. The coagulation process can be counterbalanced by activation of the fibrinolytic pathway, which attenuates the coagulation processes and prevents thrombosis. The fibrinolytic function, however, decreases in the morning. Tissue plasminogen activator inhibitor-1 (PAI-1) regulates the activity of tissue plasminogen activator (t-PA), thus strongly influencing fibrinolytic activity. The concentration and activity of PAI-1 were noted to oscillate in a circadian pattern with a peak in the morning, resulting in lower t-PA activity during the morning. Therefore, the efficacy of t-PA therapy to restore the patency of occluded vessels in AMI patients is time-dependent [77]. As such, the circadian variation of PAI-1 activity seems to be strongly related to the morning decrease in fibrinolytic activity [27].

Sudden cardiac arrest is a malfunction of the heart's electrical system, typically caused by ventricular arrhythmias, that can lead to sudden cardiac death (SCD) within minutes. Epidemiological studies have shown that SCD and ventricular arrhythmias are more likely to occur in the morning than in the evening, and laboratory studies indicate that these daily rhythms in adverse cardiovascular events are at least partially under the control of the endogenous circadian timekeeping system. However, the biophysical mechanisms linking molecular circadian clocks to cardiac arrhythmogenesis are not fully understood [78].

The biophysical mechanisms underlying these daily rhythms in adverse cardiovascular events are not fully understood. The master circadian (24-hour) pacemaker in the hypothalamus, the suprachiasmatic nucleus (SCN), influences a variety of cardiovascular phenomena by coordinating daily rhythms in the release of hormones and other circulating molecules. Recently, it has been demonstrated that circadian clocks within heart muscle cells (cardiomyocytes) also regulate rhythms in cardiac electrophysiology [79].

Normal circadian organization is critical for maintaining homeostasis, and a disturbance of this rhythm can induce the progression of organ damage. Therefore, the dysregulation of the circadian rhythm can induce the development of cardiac diseases. Moreover, reversal of the deregulated circadian rhythm or resynchronization of individual tissue clocks may become a promising therapeutic target to prevent disease progression [27].

Discussion

The present review highlights the role of circadian regulation in cardiovascular physiology and its clinical implications in critically ill patients. Our findings support the concept that circadian blood pressure variability represents not only a physiological phenomenon but also a potential marker of systemic integrity and adaptive capacity. At the molecular level, circadian clock genes regulate myocardial metabolism, electrophysiological stability, endothelial function, and vascular tone. Disruption of these mechanisms contributes to autonomic imbalance, oxidative stress, inflammatory activation, and prothrombotic states. These pathophysiological pathways provide a biological explanation for the well-documented morning predominance of acute myocardial infarction, stroke, and malignant arrhythmias. Chronotherapeutic strategies, including time-adjusted antihypertensive administration and structured environmental light management, have demonstrated benefits

in non-ICU populations. However, evidence in critically ill patients remains limited. Prospective, multicenter studies are required to determine whether modulation of circadian patterns can improve survival and reduce cardiovascular complications in this vulnerable group [67, 72].

Several limitations should be acknowledged. The design of the reviewed studies restricts causal inference, and circadian assessment was limited to hemodynamic parameters without biochemical phase markers such as melatonin or cortisol. Additionally, standardized protocols for circadian monitoring in ICU practice are not yet established. Despite these limitations, the results support the hypothesis that preserved circadian variability reflects better physiological regulation and an improved prognosis.

The extrinsic and intrinsic factors discussed above acquire particular relevance in the intensive care environment, where multiple timers are simultaneously disrupted [79].

The study selection process, illustrated in the PRISMA 2020 flow diagram (Figure 1), enhances transparency and methodological rigor in the identification and inclusion of relevant studies. Several limitations should be acknowledged. The design of the reviewed studies restricts causal inference, and circadian assessment was limited to hemodynamic parameters without the inclusion of biochemical phase markers such as melatonin or cortisol. Additionally, standardized protocols for circadian monitoring in ICU practice are not yet established. Furthermore, although a comprehensive literature search was performed using major databases, along with language and full-text availability filters, no formal risk-of-bias assessment was conducted. Therefore, potential selection and publication biases cannot be fully excluded. The heterogeneity of the included studies in terms of design, patient populations, and outcome measures may also limit the generalizability of the findings.

Conclusions

The circadian system plays a vital role in regulating various physiologic processes. The way the body responds to injury is often dependent on the interaction of the injury with the circadian machinery. Circadian rhythms are frequently disrupted in patients in the ICU, and there are a number of factors that likely contribute to this breakdown. Practices leading to circadian rhythm optimization may improve patient outcomes, and the implementation of these practices should be incorporated into ICU care. Circadian rhythm disruption plays a significant role in cardiovascular pathology and is particularly relevant in critically ill patients. Incorporating circadian assessment into clinical evaluation may enhance prognostic accuracy and open new perspectives for individualized therapeutic timing in intensive care medicine.

Competing interests

None declared.

Acknowledgements and funding

No external funding.

Provenance and peer review

Not commissioned, externally peer reviewed.

References

1. Crnko S, Du Pré BC, Sluijter JPG, Van Laake LW. Circadian rhythms and the molecular clock in cardiovascular biology and disease. Nat Rev Cardiol. 2019;16(7):437-447. doi: 10.1038/s41569-019-0167-4.
2. Wei R, Yuan X, Eltzschig HK. Circadian rhythm as a therapeutic target. Nat Rev Drug Discov. 2021;20(4):287-307. doi: 10.1038/s41573-020-00109-w.
3. Bass J, Lazar MA. Circadian time signatures of fitness and disease. Science. 2016;354(6315):994-999. doi: 10.1126/science.aah4965.
4. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017;18(3):164-179. doi: 10.1038/nrg.2016.150.
5. Koronowski KB, Sassone-Corsi P. Communicating clocks shape circadian homeostasis. Science. 2021;371(6530): eabd0951. doi: 10.1126/science.abd0951.
6. Hastings MH, Maywood ES, Brancaccio M. Generation of circadian rhythms in the suprachiasmatic nucleus. Nat Rev Neurosci. 2018;19(8):453-469. doi: 10.1038/s41583-018-0026-z.
7. Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. Annu Rev Neurosci. 2012;35(1):445-462. doi: 10.1146/annurev-neuro-060909-153128.
8. Pilorz V, Helfrich-Forster C, Oster H. The role of the circadian clock system in physiology. Pflugers Arch Eur J Physiol. 2018;470(2):227-239. doi: 10.1007/s00424-017-2103-y.
9. Martino TA, Sole MJ. Molecular time : an often overlooked dimension to cardiovascular disease. Circ Res. 2009;105(11):1047-1061. doi: 10.1161/CIRCRESAHA.109.206201.
10. Monfredi O, Lakatta EG. Complexities in cardiovascular rhythmicity: perspectives on circadian normality, ageing and disease. Cardiovasc Res. 2019;115(11):1576-1595. doi: 10.1093/cvr/cvz112.
11. Atkinson G, Jones H, Ainslie PN. Circadian variation in the circulatory responses to exercise: relevance to the morning peaks in strokes and cardiac events. Eur J Appl Physiol. 2010;108(1):15-29. doi: 10.1007/s00421-009-1243-y.
12. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA. Circadian variation of blood pressure and the vascular response to asynchronous stress. Proc Natl Acad Sci USA. 2007;104(9):3450-3455. doi: 10.1073/pnas.0611680104.
13. Douma LG, Gumz ML. Circadian clock-mediated regulation of blood pressure. Free Radic Biol Med. 2018;119: 108-114. doi: 10.1016/j.freeradbiomed.2017.11.024.

14. Smolensky MH, Hermida RC, Portaluppi F. Circadian mechanisms of 24-hour blood pressure regulation and patterning. Sleep Med Rev. 2017; 33:4-16. doi: 10.1016/j.smrv.2016.02.003.
15. Faraci FM, Scheer FAJL. Hypertension: causes and consequences of circadian rhythms in blood pressure. Circ Res. 2024;134(6):810-832. doi: 10.1161/CIRCRESAHA.124.323515.
16. Hermida RC, Ayala DE, Fernandez JR, Portaluppi F, Fabbian F, Smolensky MH. Circadian rhythms in blood pressure regulation and optimization of hypertension treatment with ACE inhibitor and ARB medications. Am J Hypertens. 2011;24(4):383-391. doi: 10.1038/ajh.2010.217.
17. Wong R, Crane A, Sheth J, Mayrovitz HN. Shift work as a cardiovascular disease risk factor: a narrative review. Cureus. 2023;15(6): e41186. doi: 10.7759/cureus.41186.
18. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, Janszky I, Mrkobrada M, Parraga G, Hackam DG. Shift work and vascular events: systematic review and meta-analysis. BMJ. 2012;345: e4800. doi: 10.1136/bmj.e4800.
19. Chellappa SL, Vujovic N, Williams JS, Scheer FAJL. Impact of circadian disruption on cardiovascular function and disease. Trends Endocrinol Metabol. 2019;30(10):767-779. doi: 10.1016/j.tem.2019.07.008.
20. Young MJ, Heanue S, Kanki M, Moneghetti KJ. Circadian disruption and its impact on the cardiovascular system. Trends Endocrinol Metabol. 2025;36(9):842-854. doi: 10.1016/j.tem.2024.11.010.
21. Morris CJ, Purvis TE, Hu K, Scheer FAJL. Circadian misalignment increases cardiovascular disease risk factors in humans. Proc Natl Acad Sci USA. 2016;113(10): E1402-11. doi: 10.1073/pnas.1516953113.
22. Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci USA. 2009;106(11):4453-4458. doi: 10.1073/pnas.0808180106.
23. Young ME. The cardiac circadian clock: implications for cardiovascular disease and its treatment. JACC Basic Transl Sci. 2023;8(12):1613-1628. doi: 10.1016/j.jacbts.2023.03.024.
24. Hood S, Amir S. The aging clock: circadian rhythms and later life. J Clin Invest. 2017;127(2):437-446. doi: 10.1172/JCI90328.
25. Schibler U. The daily rhythms of genes, cells and organs. Biological clocks and circadian timing in cells. EMBO Rep. 2005;6(Suppl 1): S9-13. doi: 10.1038/sj.embor.7400424.
26. Oishi K, Sakamoto K, Okada T, Nagase T, Ishida N. Antiphase circadian expression between BMAL1 and period homologue mRNA in the suprachiasmatic nucleus and peripheral tissues of rats. Biochem Biophys Res Commun. 1998;253(2):199-203. doi: 10.1006/bbrc.1998.9779.
27. Takeda N, Maemura K. Circadian clock and cardiovascular disease. J Cardiol. 2011;57(3):249-56. doi: 10.1016/j.jjcc.2011.02.006.
28. Alibhai FJ, LaMarre J, Reitz CJ, Tsimakouridze EV, Kroetsch JT, Bolz SS, Shulman A, Steinberg S, Burris TP, Oudit GY, Martino TA. Disrupting the key circadian regulator CLOCK leads to age-dependent cardiovascular disease. J Mol Cell Cardiol. 2017; 105:24-37. doi: 10.1016/j.yjmcc.2017.01.008.
29. Bray MS, Shaw CA, Moore MW, Garcia RA, Zanquetta MM, Durgan DJ, et al. Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression. Am J Physiol Heart Circ Physiol. 2008;294(2):H1036-H1047. doi: 10.1152/ajpheart.01291.2007.
30. Podobed P, Pyle WG, Ackloo S, Alibhai FJ, Tsimakouridze EV, Ratcliffe WF, et al. The day/night proteome in the murine heart. Am J Physiol Regul Integr Comp Physiol. 2014;307(2): R121-R137. doi: 10.1152/ajpregu.00011.2014.
31. Alibhai FJ, Tsimakouridze EV, Reitz CJ, Pyle WG, Martino TA. Consequences of circadian and sleep disturbances for the cardiovascular system. Can J Cardiol. 2015;31(7):860-872. doi: 10.1016/j.cjca.2015.01.015.
32. Ruger M, Scheer FA. Effects of circadian disruption on the cardiometabolic system. Rev Endocr Metab Disord. 2009;10(4):245-260. doi: 10.1007/s11154-009-9122-8.
33. Martino TA, Tata N, Simpson JA, Vanderlaan R, Dawood F, Kabir MG, et al. The primary benefits of angiotensin-converting enzyme inhibition on cardiac remodeling occur during sleep time in murine pressure overload hypertrophy. J Am Coll Cardiol. 2011;57(20): 2020-2028. doi: 10.1016/j.jacc.2010.11.022.
34. Sachan N, Dey A, Rotter D, Grinsfelder DB, Battiprolu PK, Sikder D, et al. Sustained hemodynamic stress disrupts normal circadian rhythms in calcineurin dependent signaling and protein phosphorylation in the heart. Circ Res. 2011; 108(4): 437-445. doi: 10.1161/CIRCRESAHA.110.235309.
35. Pinotti M, Bertolucci C, Portaluppi F, Colognesi I, Frigato E, Foa A, Bernardi F. Daily and circadian rhythms of tissue factor pathway inhibitor and factor VII activity. Arterioscler Thromb Vasc Biol 2005;25(3):646-9. doi: 10.1161/01.ATV.0000153140.13148.e0.
36. Thakur A, Kishore R. Neurobiology of the circadian clock and its role in cardiovascular disease:Mechanisms, biomarkers, and chronotherapy. Neurobiol Sleep Circadian Rhythms. 2025;19:100131. doi: 10.1016/j.nbscr.2025.100131.
37. Meloni M, Setzu D, Del Rio A, Campagna M, Cocco P. QTc interval and electrocardiographic changes by type of shift work. Am J Ind Med. 2013;56(10):1174-1179. doi: 10.1002/ajim.22207.

38. Csoma B, Bikov A. The role of the circadian rhythm in dyslipidaemia and vascular inflammation leading to atherosclerosis. Int J Mol Sci. 2023;24(18):14145. doi: 10.3390/ijms241814145.
39. Lecacheur M, Ammerlaan DJM, Dierickx P. Circadian rhythms in cardiovascular (dys)function: approaches for future therapeutics. NPJ Cardiovasc Health. 2024;1(1):21. doi: 10.1038/s44325-024-00024-8.
40. Richards J, Gumz ML. Mechanism of the circadian clock in physiology. Am J Physiol Regul Integr Comp Physiol. 2013;304(12): R1053-R1064. doi: 10.1152/ajpregu.00066.2013.
41. Diekman CO, Wei N. Circadian rhythms of early afterdepolarizations and ventricular arrhythmias in a cardiomyocyte model. Biophys J. 2021;120(2):319-333. doi: 10.1016/j.bpj.2020.11.2264.
42. Crnko S, Cour M, Van Laake LW, Lecour S. Vasculature on the clock: circadian rhythm and vascular dysfunction. Vasc Pharmacol. 2018;108: 1-7. doi: 10.1016/j.vph.2018.05.003.
43. Xie Z, Su W, Liu S, Zhao G, Esser K, Schroder EA, Lefta M, Stauss HM, Guo Z, Gong MC. Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation. J Clin Invest. 2015;125(1):324-336. doi: 10.1172/JCI76881.
44. Beesley S, Noguchi T, Welsh DK. Cardiomyocyte circadian oscillations are cellautonomous, amplified by β-adrenergic signaling, and synchronized in cardiac ventricle tissue. PLoS One 2016;11(7):e0159618. doi: 10.1371/journal.pone.0159618.
45. Mieda M. The central circadian clock of the suprachiasmatic nucleus as an ensemble of multiple oscillatory neurons. Neurosci Res. 2020;156:24-31. doi: 10.1016/j.neures.2019.08.003.
46. Barclay JL, Tsang AH, Oster H. Interaction of central and peripheral clocks in physiological regulation. Prog Brain Res. 2012;199:163-181. doi: 10.1016/B978-0-444-59427-3.00030-7.
47. Ramkisoensing A, Meijer JH. Synchronization of biological clock neurons by light and peripheral feedback systems promotes circadian rhythms and health. Front Neurol. 2015;6:128. doi: 10.3389/fneur.2015.00128.
48. Hower IM, Harper SA, Buford TW. Circadian rhythms, exercise, and cardiovascular health. J Circadian Rhythms. 2018;16(1):7. doi: 10.5334/jcr.164.
49. Xu W, Jain MK, Zhang L. Molecular link between circadian clocks and cardiac function: a network of core clock, slave clock, and effectors. Curr Opin Pharmacol. 2021;57:28-40. doi: 10.1016/j.coph.2020.10.006.
50. Young ME, Bray MS. Potential role for peripheral circadian clock dyssynchrony in the pathogenesis of cardiovascular dysfunction. Sleep Med. 2007;8(6):656-667. doi: 10.1016/j.sleep.2006.12.010.
51. Lefta M, Campbell KS, Feng HZ, Jin JP, Esser KA. Development of dilated cardiomyopathy in Bmal1-deficient mice. Am J Physiol Heart Circ Physiol. 2012;303(4):H475-H485. doi: 10.1152/ajpheart.00238.2012.
52. Schroder EA, Lefta M, Zhang X, Bartos DC, Feng HZ, Zhao Y, Patwardhan A, Jin JP, Esser KA, Delisle BP. The cardiomyocyte molecular clock, regulation of *Scn5a* and arrhythmia susceptibility. Am J Physiol Cell Physiol. 2013;304(10):C954-C965. doi: 10.1152/ajpcell.00383.2012.
53. Festus ID, Spilberg J, Young ME, Cain S, Khoshnevis S, Smolensky MH, Zaheer F, Descalzi G, Martino TA. Pioneering new frontiers in circadian medicine chronotherapies for cardiovascular health. Trends Endocrinol Metabol. 2024;35(7):607-623. doi: 10.1016/j.tem.2024.02.011.
54. Lee Y, Field JM, Sehgal A. Circadian rhythms, disease and chronotherapy. J Biol Rhythm. 2021;36(6):503-531. doi: 10.1177/07487304211044301.
55. Baron KG, Reid KJ. Circadian misalignment and health. Int Rev Psychiatry. 2014;26(2):139-54. doi: 10.3109/09540261.2014.911149.
56. Lewy AJ, Sack RL. Exogenous melatonin's phase-shifting effects on the endogenous melatonin profile in sighted humans: a brief review and critique of the literature. J Biol Rhythms. 1997;12(6):588-94. doi: 10.1177/074873049701200614.
57. Aschoff J. Circadian rhythms in man. Science. 1965;148(3676):1427-32. doi: 10.1126/science.148.3676.1427.
58. Lin J, Kuang H, Jiang J, Zhou H, Peng L, Yan X, Kuang J. Circadian rhythms in cardiovascular function: implications for cardiac diseases and therapeutic opportunities. Med Sci Monit. 2023;29:e942215. doi: 10.12659/MSM.942215.
59. Myung J, Schmal C, Hong S, Tsukizawa Y, Rose P, Zhang Y, Holtzman MJ, De Schutter E, Herzel H, Bordyugov G, Takumi T. The choroid plexus is an important circadian clock component. Nat Commun. 2018;9(1);1062. doi: 10.1038/s41467-018-03507-2.
60. Ansu Baidoo V, Knutson KL. Associations between circadian disruption and cardiometabolic disease risk: a review. Obesity (Silver Spring). 2023;31(3):615-624. doi: 10.1002/oby.23666.
61. Orozco-Solis R, Aguilar-Arnal L. Circadian regulation of immunity through epigenetic mechanisms. Front Cell Infect Microbiol. 2020;10:96. doi: 10.3389/fcimb.2020.00096.
62. Fanjul-Moles ML, Lopez-Riquelme GO. Relationship between oxidative stress, circadian rhythms, and AMD. Oxid Med Cell Longev. 2016;2016(1):7420637. doi: 10.1155/2016/7420637.
63. Takeda N, Maemura K. Cardiovascular disease, chronopharmacotherapy, and the molecular clock. Adv Drug Deliv Rev. 2010;62:956-66. doi: 10.1016/j.addr.2010.04.011.
64. Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Guerrieri M, Gatteschi C, Zampi I,

Santucci A, Reboldi G. Ambulatory blood pressure: an independent predictor of prognosis in essential hypertension. Hypertension. 1994;24(6):793-801. doi: 10.1161/01.hyp.24.6.793.
65. Ohkubo T, Hozawa A, Yamaguchi J, Kikuya M, Ohmori K, Michimata M, Matsubara M, Hashimoto J, Hoshi H, Araki T, Tsuji I, Satoh H, Hisamichi S, Imai Y. Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study. J Hypertens. 2002;20(11):2183-9. doi: 10.1097/00004872-200211000-00017.
66. Kario K, Pickering TG, Matsuo T, Hoshide S, Schwartz JE, Shimada K. Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. Hypertension. 2001;38(4):852-7. doi: 10.1161/hy1001.092640.
67. Hermida RC, Ayala DE, Calvo C, Portaluppi F, Smolensky MH. Chronotherapy of hypertension: administration-timedependent effects of treatment on the circadian pattern of blood pressure. Adv Drug Deliv Rev. 2007;59(9-10):923-39. doi: 10.1016/j.addr.2006.09.021.
68. Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, de Leeuw PW, Mancia G, Nachev C, Palatini P, Parati G, Tuomilehto J, Webster J. Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. Systolic Hypertension in Europe Trial Investigators. JAMA. 1999;282(6):539-46. doi: 10.1001/jama.282.6.539.
69. Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, et al. Circadian variation in the frequency of onset of acute myocardial infarction. N Engl J Med. 1985;313(21):1315-22. doi: 10.1056/NEJM198511213132103.
70. Janszky I, Ljung R. Shifts to and from daylight saving time and incidence of myocardial infarction. N Engl J Med. 2008;359(18):1966-8. doi: 10.1056/NEJMc0807104.
71. Willich SN, Linderer T, Wegscheider K, Leizorovicz A, Alamercery I, Schroder R. Increased morning incidence of myocardial infarction in the ISAM Study: absence with prior beta-adrenergic blockade. ISAM Study Group. Circulation. 1989;80(4):853-8. doi: 10.1161/01.cir.80.4.853.
72. Manfredini R, Boari B, Smolensky MH, Salmi R, la Cecilia O, Maria Malagoni A, Haus E, Manfredini F. Circadian variation in stroke onset: identical temporal pattern in ischemic and hemorrhagic events. Chronobiol Int. 2005;22(3):417-53. doi: 10.1081/CBI-200062927.
73. Kurnik PB. Circadian variation in the efficacy of tissue-type plasminogen activator. Circulation. 1995;91(5):1341-6. doi: 10.1161/01.cir.91.5.1341.
74. Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH. Circadian variation in the frequency of sudden cardiac death. Circulation. 1987;75(1):131-138. doi: 10.1161/01.cir.75.1.131.
75. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. Am J Cardiol. 1987;60(10):801-806. doi: 10.1016/0002-9149(87)91027-7.
76. Kozak M, Krivan L, Semrad B. Circadian variations in the occurrence of ventricular tachyarrhythmias in patients with implantable cardioverter defibrillators. Pacing Clin Electrophysiol. 2003;26(3):731-735. doi: 10.1046/j.1460-9592.2003.00124.x.
77. Ruwald MH, Moss AJ, Zareba W, Jons C, Ruwald AC, McNitt S, Polonsky B, Kutyifa V. Circadian distribution of ventricular tachyarrhythmias and association with mortality in the MADIT-CRT trial. J Cardiovasc Electrophysiol. 2015;26(3):291-9. doi: 10.1111/jce.12592.
78. Black N, D'Souza A, Wang Y, Piggins H, Dobrzynski H, Morris G, Boyett MR. Circadian rhythm of cardiac electrophysiology, arrhythmogenesis, and the underlying mechanisms. Heart Rhythm. 2019;16(2):298-307. doi: 10.1016/j.hrthm.2018.08.026.
79. Jiménez-Pastor JM, Rodríguez-Cortés F, López-Soto P, López-Coleto L, Cruz MME. Cardiorespiratory and circadian clock markers in intensive care unit patients. Dent Med Probl. 2024;61(5):797-801. doi: 10.17219/dmp/191537.

https://doi.org/10.52645/MJHS.2026.2.10

UDC: 616.853+316.647.8

REVIEW ARTICLE

Epilepsy-specific quality-of-life questionnaires and social stigma scales in adults with epilepsy: a methodological review

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ABSTRACT

Introduction. Adult epilepsy generates a burden that extends beyond seizure counts and includes adverse treatment effects, role restriction, emotional distress, and the social devaluation attached to the diagnosis. The methodological problem is not the absence of patient-reported measures, but the heterogeneity with which disease-specific quality-of-life and stigma instruments are selected, interpreted, and combined in adult studies.

Materials and methods. A structured narrative methodological review was conducted using PubMed/MEDLINE, Scopus, Web of Science, Embase, Cochrane Library, and the institutional repository of the *Nicolae Testemițanu* State University of Medicine and Pharmacy. The synthesis focused on the Quality of Life in Epilepsy Inventory family, especially the 89-, 31-, 31-P, and 10-item forms, the adolescent 48-item comparator, and adult epilepsy stigma measures such as the Epilepsy Stigma Scale (ESS) variants, the Stigma Scale of Epilepsy (SSE), and the Epilepsy Self-Stigma Scale (ESSS). Special attention was given to publications from the Republic of Moldova and Romania because regional evidence is sparse but clinically relevant.

Results. QOLIE-31 emerged as the most defensible adult comparative instrument because it balances breadth, feasibility, and international comparability. QOLIE-31-P was particularly useful for patient-centred and real-world designs, while QOLIE-10 served primarily as a screening instrument and QOLIE-89 retained value for comprehensive psychometric work. The 48-item version was methodologically informative but remained adolescent-oriented rather than a primary adult endpoint. Across the stigma literature, ESS, SSE, and ESSS were clearly not interchangeable because they capture overlapping but distinct constructs, including perceived stigma, felt stigma, and internalized self-stigma.

Conclusions. The working hypothesis was supported across international, regional, and Moldovan sources: the greater the clinical and psychosocial severity of epilepsy, the lower the epilepsy-specific quality of life. Seizure frequency, uncontrolled or drug-resistant epilepsy, polytherapy, adverse medication effects, depression, anxiety, and stigma were the most recurrent determinants of lower scores. For adult studies intended for Moldovan settings and the MJHS submission, QOLIE-31 or QOLIE-31-P, combined with one clearly defined stigma scale and a standardized set of severity variables, offers the strongest methodological balance.

Keywords: epilepsy, quality of life, social stigma, patient-reported outcome measures, questionnaires, narrative review.

Cite this article: Lişinschi-Baranov G, Groppa S, Spinei L, Ojovan V, Chiosa V, Ferdohleb A. Epilepsy-Specific Quality-of-Life Questionnaires and Stigma Scales in adults with epilepsy: a methodological review. Mold J Health Sci. 2026;13(2):73-83. https://doi.org/ https://doi.org/10.52645/MJHS.2026.2.10

Manuscript received: 12.04.2026

Accepted for publication: 22.05.2026

Published: 15.06.2026

Key messages

What is not yet known on this issue addressed in the submitted manuscript

Adult epilepsy research uses multiple disease-specific quality-of-life and stigma instruments, but the Eastern European and

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Moldovan academic community has rarely organized these tools according to construct differences, respondent burden, and study design.

The research hypothesis

Reduced seizure control, higher treatment burden, and stronger perceived or internalized stigma are associated with poorer epilepsy-specific quality-of-life scores across adult studies, regardless of the exact questionnaire version.

The novelty added by the manuscript to the already published scientific literature

This review integrates international, Romanian, and Moldovan literature, converting that evidence into a practical adult questionnaire strategy for future Moldovan epilepsy studies.

Introduction

Epilepsy is among the chronic neurological conditions in which biomedical control and the lived burden do not always move in parallel. Two adults with the same nominal seizure diagnosis can report very different levels of daily restriction because the quality of life in epilepsy is shaped not only by seizure occurrence, but also by medication burden, unpredictability, fear of recurrence, limitations on driving and employment, interpersonal strain, and the social meaning attached to the disorder. For that reason, epilepsy research that reports only seizure outcomes risks underestimating clinically relevant morbidity. Systematic review evidence has repeatedly shown that the health-related quality of life in adults with epilepsy is most strongly eroded by high seizure frequency, depression, anxiety, adverse effects of anti-seizure medication, and broader psychosocial strain [1, 2].

Stigma occupies a special place in this burden architecture because it works simultaneously at social, interpersonal, and intrapsychic levels. Quantitative and qualitative reviews show that adults with epilepsy encounter enacted discrimination, anticipated rejection, concealment behavior, diminished self-worth, and reduced help-seeking; these effects are shaped by illness severity but are not reducible to it. In other words, stigma is not a decorative psychosocial theme added to seizure medicine after the real work is done. It is a mechanism that alters adherence, participation, family functioning, and even the perceived legitimacy of one's own symptoms. Moldovan authors have framed this issue in similar terms, emphasizing that stigmatization and self-stigmatization alter self-perception, reduce self-esteem, affect treatment adherence, and ultimately worsen quality of life [3-5].

The public-health salience of epilepsy stigma has long been recognised internationally. The ILAE/IBE/WHO global campaign "Out of the Shadows" was built on the premise that seizure control alone is not enough if communities continue to mark epilepsy as a condition associated with fear, incompetence, unpredictability, or social inferiority. That insight remains methodologically important because it explains why adult epilepsy research cannot choose instruments as if they all measured a single undifferentiated outcome. A quality-of-life inventory, a perceived-stigma scale, and a self-stigma measure each illuminates a different layer of the patient experience. The more rigorously these constructs are separated, the more interpretable the findings become [6-8].

The best-known disease-specific quality-of-life tools in epilepsy derive from the Quality of Life in Epilepsy Inventory family. QOLIE-89 was developed as the comprehensive parent measure, QOLIE-31 as the more feasible short form for adult comparative work, and QOLIE-10 as a very brief screening instrument. A further 48-item instrument, QOLIE-AD-48, extended the same logic to adolescents rather than adults. Official RAND documentation still presents QOLIE-89 and QOLIE-31 as publicly available survey instruments with scoring manuals, which is valuable for transparency, reproducibility, and local implementation planning. Yet public availability does not solve the deeper methodological problem: each version has a different trade-off between construct richness, respondent burden, and suitability for longitudinal, psychometric, or routine-care use [9-13].

A parallel problem exists in the stigma literature. Across the international adult literature, the abbreviations ESS, SSE, revised ESS, and ESSS are easy to conflate even when they refer to different instruments or to translated variants targeting different stigma domains. Some scales emphasize perceived or felt stigma in everyday social encounters; others move closer to internalized self-stigma or to culturally embedded social discrediting. When investigators refer loosely to "stigma" without naming the construct and instrument precisely, the literature becomes deceptively coherent. In reality, it contains multiple partially overlapping constructs that cannot be pooled naively. This ambiguity matters even more in low-volume national literatures, where a single study can influence local practice disproportionately [14-18].

In the Republic of Moldova, the need for a construct-sensitive approach is especially evident. The adult national clinical protocol formalizes standardized diagnostic and therapeutic pathways and explicitly places quality of care and patient outcomes at the centre of adult epilepsy management. Local scholarly work has also highlighted stigma, adaptation, resocialization, and psychological rehabilitation as clinically meaningful dimensions rather than peripheral

social commentary. Recent Romanian studies add a relevant Eastern European comparator by showing that QOLIE-31-P is operationally feasible in real-life neurology practice and that seizure frequency remains a powerful inverse predictor of epilepsy-specific quality of life over time. These regional contributions justify a dedicated methodological synthesis that moves from general knowledge to a practical questionnaire strategy for future Moldovan adult research [19-24].

The aim of the present review was therefore to examine how epilepsy-specific quality-of-life questionnaires and adult stigma scales are used in adult epilepsy research, to interpret their findings in relation to epilepsy severity and study design, and to integrate international evidence with regional and Moldovan publications. The working hypothesis was that poorer seizure control, greater treatment burden, and stronger perceived or internalized stigma are associated with worse epilepsy-specific quality-of-life scores regardless of the exact questionnaire version. A secondary objective was pragmatic: to identify which instrument combinations appear most suitable for future Moldovan adult studies prepared for the MJHS [20, 21, 25].

Materials and methods

This manuscript was designed as a narrative methodological review rather than a systematic review or meta-analysis. The choice was deliberate. The central question was not simply whether epilepsy severity correlates with quality-of-life or stigma scores, since that pattern is already well documented, but how different questionnaires operationalize adult burden, what methodological tasks they perform best, and how a Moldovan research team should choose among them. Narrative methodological synthesis is appropriate when the aim is to integrate psychometric, conceptual, and practice-oriented literature that spans instrument development, validation, observational studies, follow-up cohorts, regional protocols, and local contextual publications. The interpretive stance was informed by applied policy analysis and by basic psychometric reasoning regarding construct validity, reliability, feasibility, and respondent burden [25, 26].

A structured search framework was used even though the review was not converted into a formal PRISMA flow study. The databases and repositories screened were PubMed/MEDLINE, Scopus, Web of Science, Embase, Cochrane Library, and the institutional repository of the *Nicolae Testemițanu* State University of Medicine and Pharmacy. The search strategy combined the disease term epilepsy with the main instrument families of interest: "QOLIE-89", "QOLIE-31", "QOLIE-31-P", "QOLIE-10", "QOLIE-48" or "QOLIE-AD-48", "Quality of Life in Epilepsy Inventory", "Epilepsy Stigma Scale", "Stigma Scale of Epilepsy", "revised epilepsy stigma scale", and "Epilepsy Self-Stigma Scale". Additional regional searches included Romanian and Moldovan terms linked to quality of life, stigma, adaptation, resocialization, rehabilitation, and adult epilepsy protocols [9, 10, 14, 16, 19].

Studies were considered eligible for the core synthesis when they met at least one of the following criteria: development of an instrument; formal psychometric validation or cross-cultural adaptation; use of a QOLIE or epilepsy-specific stigma scale in adult clinical cohorts; explicit analysis of associations between questionnaire scores and seizure burden, drug-resistant epilepsy, medication profile, psychiatric comorbidity, or stigma; or contextual regional relevance for Moldovan adult epilepsy research. Systematic reviews and meta-syntheses were used to stabilize the broader interpretive frame. Pediatric studies were excluded from the core adult synthesis, except where QOLIE-AD-48 was needed as a methodological comparator clarifying why the 48-item form should not be treated as a default adult endpoint. Non-public local files were not treated as citable evidence [1, 3, 4, 12].

Data extraction was thematic rather than numeric. Each source was read for one or more of the following analytical dimensions: target population; stigma construct or quality-of-life construct; respondent burden; study design; severity indicators used in analysis; psychometric properties; regional transferability; and practical usefulness for future Moldovan adult studies. Severity was operationalized broadly, because the adult epilepsy literature rarely relies on a single measure. Recurrent indicators included seizure frequency, uncontrolled or drug-resistant epilepsy, number of anti-seizure medications, medication adverse effects, psychiatric symptoms, epileptiform activity, employment restriction, and social participation. This broad severity lens was retained because it better reflects how the quality-of-life and stigma instruments function in real practice [1, 21, 27, 28].

Special effort was made to integrate Moldovan sources constructively rather than ceremonially. Local articles and protocol documents were not inserted merely to "nationalize" an international review. Instead, they were treated as a regional evidence layer showing how social stigma, adaptation, resocialization, and psychological rehabilitation are currently conceptualized within Moldovan clinical and academic discourse. Recent Moldovan conference and review contributions from 2024 and 2025 were therefore included as contextual sources, while stronger empirical weight was assigned to instrument-development studies, psychometric validations, multicentre cohorts, and the Romanian adult QOLIE-31-P studies that provide a more directly comparable regional measurement context [5, 20-24, 29, 30].

Because the review synthesized published and publicly accessible literature and did not involve human participants, ethics approval and informed consent were not required. The methodological purpose was practical: to convert a heterogeneous body of adult epilepsy literature into a coherent decision framework for questionnaire choice in future Moldovan studies and in an MJHS-compatible manuscript format [19, 26].

Results

Why quality of life and stigma should be synthesized together. The screened literature converged on a stable starting point: in adult epilepsy, quality of life is not an optional soft endpoint added after seizure control, but an integrated expression of how neurological, psychiatric, social, and

cultural burdens accumulate in daily life. The systematic review literature shows that quality-of-life scores are consistently damaged by uncontrolled seizures, psychiatric symptoms, adverse treatment effects, and social restriction. The stigma literature adds that some of these burdens are amplified by how the disorder is interpreted by others and by the patient. Thus, an adult with frequent seizures is not only managing a biological risk state, but also a social identity under threat. This is precisely why the quality-of-life and stigma instruments should be treated as complementary rather than competing measures [1-4].

Family context further strengthens this conclusion. Studies of adult patients and their relatives show that the epilepsy-related burden is distributed across households through emotional strain, supervision demands, role negotiation, worry, and uncertainty about future functioning. Family-centered qualitative work similarly indicates that epilepsy alters the routines and self-understandings of adult family units, not only those of the individual with seizures. These observations matter methodologically because some quality-of-life domains, such as social function, energy, or emotional well-being, are partly mediated through family ecology, while stigma can affect concealment, disclosure, and care-seeking at the household level. A narrowly clinical instrument strategy risks losing these downstream pathways [31, 32].

Historical and contemporary stigma scholarship makes the same point from another direction. Public perceptions of epilepsy have long been coloured by fear, misconceptions about competence and contagion, and the tendency to define the person through the illness. Classic work on epilepsy as a damaged self-concept anticipated the more recent distinction between public stigma and self-stigma, while contemporary analyses show that felt stigma, enacted discrimination, and internalized inferiority can coexist without being reducible to one another. This is why the label "stigma" is methodologically too broad unless it is tied to a named instrument and an explicit construct [8, 33-35].

Table 1. Epilepsy-specific quality-of-life instruments relevant to adult methodological synthesis

Instrument	Adult applicability	Best methodological use	Main advantage	Main caution
QOLIE-89 [9, 36, 37]	Yes	Deep psychometric profiling and comprehensive baseline characterization	Broad domain coverage with rich subscales	High respondent burden; less suitable for routine clinics
QOLIE-31 [10, 13, 38]	Yes	Default adult comparative and multicenter observational work	Best balance between breadth and feasibility	Less granular than the parent inventory
QOLIE-31-P [20, 21, 39, 40]	Yes	Patient-centred, real-world, longitudinal, and regional implementation studies	Adds patient weighting and preserves feasibility	Requires careful handling of local translation and scoring workflows
QOLIE-10 [11]	Yes	Rapid clinical screening and service triage	Very brief and easy to administer	Too limited for richer methodological characterization
QOLIE-AD-48 [12]	No, adolescent comparator	Contextual comparison of developmental measurement logic	Shows how severity can be captured in a broader developmental frame	Not a primary adult endpoint

Note: *QOLIE = Quality of Life in Epilepsy Inventory. Key supporting references are embedded in the first column. The 48-item instrument in the QOLIE family is predominantly QOLIE-AD-48 and is retained here only as a methodological comparator, not as a default adult outcome measure.*

QOLIE family: same lineage, different methodological roles

QOLIE-89 remains the psychometric anchor of the family. Its large domain structure allows investigators to trace the many ways epilepsy affects adult life, including cognitive complaints, emotional well-being, social isolation, seizure worry, medication effects, work and driving function, and health perceptions. This breadth makes QOLIE-89 particularly useful in studies whose purpose is scale development, responsiveness testing, comprehensive baseline phenotyping, or validation of derived measures. The trade-off is obvious and repeatedly acknowledged in practice: administration burden increases, completion is slower, and the instrument becomes less attractive for busy clinics or repeated-measure designs. In the logic of questionnaire architecture, QOLIE-89 is the instrument chosen when the objective is depth [9, 13, 36, 37].

QOLIE-31 is the most stable adult workhorse because it captures the major disease-specific domains without reproducing the full burden of the parent scale. Its development history, cross-cultural translations, repeated validation work, and use in global comparative studies make it the strongest candidate when investigators need international comparability and clinically interpretable breadth. This is particularly important for narrative synthesis, where one wants an instrument that has traveled across settings and languages without collapsing into a generic quality-of-life measure. The global comparison by Saadi et al. is especially useful in this regard, because it showed that QOLIE-31 has been used across a broad range of countries and that mean scores vary meaningfully across resource settings [10, 38, 41, 42].

QOLIE-31-P is best understood not as a trivial variant, but as a patient-weighted adult form that is especially attractive for real-world and patient-centred research. It retains a manageable length while foregrounding patient priorities more explicitly than the standard short form. This makes it valuable in longitudinal outpatient care, in refractory epilepsy services, and in studies exploring the lived salience of symptom clusters rather than merely their presence. Lithuanian psychometric work and Chinese validation studies confirm its adaptability, while Romanian studies demonstrate that it can be implemented in real Eastern European clinical prac-

tice without collapsing under logistical demands. In pragmatic research terms, QOLIE-31-P is the scale most compatible with adult follow-up studies that want both feasibility and interpretive richness [20, 21, 39, 40].

The formal role of QOLIE-10 is different. It is an efficient brief screen, useful in outpatient environments where clinicians need a fast signal of impaired epilepsy-specific well-being, but it is not a replacement for more nuanced adult measurement when the research question centres on mediation, domain profiles, or cross-cultural instrument behaviour. QOLIE-10 is at its best when the study question is operational and triage-oriented. It is at its weakest when investigators later try to extract domain-level interpretations that the instrument was never designed to provide. The adolescent QOLIE-AD-48 reinforces the same methodological lesson from the opposite direction: more items do not automatically make an instrument suitable for adults if the developmental target is wrong [11-13].

Taken together, the QOLIE family behaves less like a simple length ladder and more like a set of related instruments optimized for different analytic environments. The literature therefore supports a principle that is surprisingly often ignored in applied studies: the right instrument is not the longest or shortest one, but the one whose measurement logic matches the study's clinical setting, respondent burden tolerance, and inferential ambitions [21, 25, 38].

The adult stigma scale landscape is conceptually fragmented

The stigma literature is more fragmented than the QOLIE literature because the field is still negotiating what exactly should count as the measured object. Some instruments are closer to perceived stigma in everyday life, others to felt stigma or enacted discrimination, and the more recent self-stigma scales move inward to internalized shame, self-devaluation, and identity threat. This fragmentation is not a defect; it reflects real differences in the phenomenon. The problem arises when authors present these scales as though they were measuring the same thing with different wording. The current synthesis found that adult epilepsy stigma research is much easier to interpret when the construct is named first and the instrument second [8, 33, 35].

The Stigma Scale of Epilepsy (SSE) became one of the most visible perceived-stigma instruments after its validation in Brazil and its uptake in several clinical cohorts. The revised Epilepsy Stigma Scale associated with incident-population work is frequently used in adult epidemiological analyses and demonstrates how stigma can be treated as a variable alongside seizure burden and sociodemographic status. More recent work has expanded the field through translated adult ESS variants, including the Japanese version, and through the Epilepsy Self-Stigma Scale, which explicitly targets internalized self-stigma. The German ESSS-G further underscores that self-stigma is increasingly being treated as a distinct psychometric target rather than a hidden subset of public stigma [14-17, 43].

The Kilifi Stigma Scale for Epilepsy, although developed in Kenya and not a core Moldovan adult instrument, remains methodologically useful because it demonstrates how cultural adaptation can change not only language but also the experiential salience of stigma items. That is highly relevant for low- and middle-resource settings and for Eastern European contexts where social meanings, disclosure norms, and family expectations may differ from those embedded in original English-language instruments. In practice, this means a local team should not assume that a translated scale automatically preserves the same construct structure. Adaptation must test, not presume, equivalence [18, 25].

Table 2. Main adult stigma instruments encountered in the epilepsy literature

Instrument	Dominant construct	Typical adult use	Methodological strength	Main caution
Revised Epilepsy Stigma Scale / ESS variants [15, 43]	Felt or enacted stigma	Cross-sectional cohorts and epidemiological association studies	Works well alongside clinical severity variables	The abbreviation ESS is not fully stable across the literature
Stigma Scale of Epilepsy (SSE) [14, 44, 45]	Perceived epilepsy-related stigma	Clinical adult cohorts and quality-of-life studies	Often pairs naturally with QOLIE-31-type measures	Should not be equated with self-stigma instruments
Translated adult Epilepsy Stigma Scale forms [15]	Perceived/felt stigma	Cross-cultural adult validation work	Useful when local language equivalence is important	Translation quality must be reported explicitly
Epilepsy Self-Stigma Scale (ESSS) [16, 17]	Internalized self-stigma	Psychological and intercultural studies	Captures identity-level burden invisible to public-stigma scales	Not interchangeable with public or perceived stigma measures
Kilifi Stigma Scale for Epilepsy [18]	Perceived culturally embedded stigma	Cross-cultural comparator	Illustrates adaptation principles in non-Western settings	Not a default adult Moldovan instrument

Note: *ESS = Epilepsy Stigma Scale or revised Epilepsy Stigma Scale variants; SSE = Stigma Scale of Epilepsy; ESSS = Epilepsy Self-Stigma Scale. Key supporting references are embedded in the first column. Instrument naming in the stigma literature is inconsistent; therefore, the abbreviation and full-scale name should be expanded in the Methods and again at first use in the Results.*

Severity variables repeatedly shape both quality-of-life and stigma scores. Across the reviewed adult studies, epilepsy severity was almost never represented by one variable alone. Instead, researchers used overlapping markers of disease burden: seizure frequency, drug-resistant or uncontrolled epilepsy, polytherapy, adverse medication effects, depression, anxiety, insomnia, epileptiform activity, and social restriction. This multidimensional pattern is methodologically coherent. A patient can have infrequent seizures but a major medication burden and persistent stigma, or monthly seizures with relatively preserved emotional resilience. Quality-of-life and stigma instruments become valuable precisely because they capture the cumulative effect of these non-identical but related burdens [1, 27, 28].

The German multicentre data illustrate this well. Lower QOLIE-31 scores were associated with higher seizure fre-

quency, depressive symptoms, adverse medication effects, seizure worry, and epilepsy stigma. The Spanish refractory epilepsy cohort using QOLIE-31-P reached a similar conclusion from a service environment in which drug resistance, depression, anxiety, and sleep problems converged. These studies are methodologically important because they show that the association between worse quality of life and worse clinical status does not disappear when psychosocial variables are included; instead, clinical and psychosocial severity reinforce one another [27, 28].

The two Romanian studies make the regional signal even clearer. In the 2022 Brașov cohort, seizure frequency was negatively correlated with almost all QOLIE-31-P domains, demonstrating that a disease-specific adult measure can detect clinically meaningful variation in a real Eastern European neurology setting. The 2023 follow-up study refined the pattern: patients with epileptiform activity, polytherapy, uncontrolled seizures, and at least one seizure per month had lower QOLIE-31-P total scores at baseline and follow-up, while seizure frequency remained the strongest inverse predictor in regression analysis. This gives the regional literature something especially valuable for Moldovan planning: not only cross-sectional relevance, but proof that repeated adult measurement is feasible and informative [20, 21].

Stigma studies align with the same severity narrative. In Serbia, stigma contributed to lower health-related quality of life beyond seizure frequency and number of anti-seizure medicines. In China and Turkey, greater stigma was associated with poorer quality-of-life outcomes in adults with epilepsy. Meta-analytic evidence published in 2025 confirmed that stigmas remain common and clinically relevant, although instrument heterogeneity still limits pooled interpretation. The implication is straightforward: stigma should not be appended as an optional add-on when the real hypothesis concerns disease burden. It should be measured as part of the burden model itself [44-47].

Table 3. Selected regional and international studies informing adult epilepsy questionnaire choice

Setting and key source	Design and sample	Instrument(s)	Severity variables linked to scores	Methodological implication
Romania (Cioriceanu et al. [20])	Cross-sectional adult cohort, n=91	QOLIE-31-P	Higher seizure frequency; selected sociodemographic and clinical factors	Demonstrates regional feasibility of patient-weighted adult QOL assessment
Romania (Cioriceanu et al. [21])	Follow-up adult cohort, n=35	QOLIE-31-P	Epileptiform activity, polytherapy, uncontrolled seizures, and at least 1 seizure/month	Supports longitudinal sensitivity in real-life practice
Germany (Siebenbrodt et al. [28])	Multicentre cross-sectional study, n=476 complete QOLIE-31 datasets	QOLIE-31 plus revised Epilepsy Stigma Scale	High seizure frequency, adverse ASM effects, depressive symptoms, seizure worry, and stigma	Illustrates integrated clinical and psychosocial modelling
Spain, tertiary refractory epilepsy clinic (González-Martínez et al. [27])	Cross-sectional cohort, n=84	QOLIE-31-P	Drug-resistant epilepsy setting, depression, anxiety, and insomnia	Supports QOLIE-31-P use in high-burden tertiary cohorts
Republic of Moldova (Doțen et al. [22])	Single-case psychological rehabilitation study, n=1	Psychological rehabilitation outcome framework	Drug-resistant epilepsy, cognitive complaints, anxiety-depressive symptoms, and social isolation	Shows local relevance of rehabilitation-sensitive outcomes; evidentiary weight remains case-based

Note: *QOL = quality of life; ASM = anti-seizure medication. The table highlights anchor studies most relevant to instrument selection for adult Moldovan epilepsy research and is not intended as an exhaustive evidence inventory.*

Moldovan literature adds clinically useful context

The Moldovan evidence base is not yet dominated by large psychometric studies, but it contributes a clinically meaningful social and organizational context. The adult national clinical protocol PCN-290 is particularly important because it formalizes epilepsy care pathways using international evidence sources and explicitly frames optimization of diagnostic quality, treatment effectiveness, and patient quality of life as protocol goals. In other words, the national framework already recognises that adult epilepsy management cannot be reduced to seizure classification alone. For methodological planning, the protocol legitimizes the inclusion of quality-of-life and psychosocial endpoints in Moldovan adult studies and supports standardized reporting of clinical severity variables [19].

The 2016 Moldovan article by *Groppa et al.* places stigma and self-stigma near the center of epilepsy-related suffering. Its conceptual emphasis is highly compatible with the modern adult literature: social discrediting alters self-perception, lowers self-esteem, reduces adherence, complicates social adjustment, and worsens prognosis. Even though the paper is not a psychometric validation study, it performs an important translational role by showing that the stigma-quality-of-life relationship is already articulated in local clinical language. This matters because instrument selection is easier to justify when the construct already has local intellectual legitimacy [5].

The 2022 Moldovan papers on adaptation and resocialization deepen this contextual layer. The adaptation article frames epilepsy as a condition that creates obstacles in family life, couple relations, and wider social functioning, while also underscoring the rehabilitative value of the National Centre of Epileptology and the importance of lifestyle recommendations. The resocialization paper moves further to-

ward reintegration logic by focusing on professional participation, matrimonial status, family impact, and the place of psychotherapeutic conversations in helping patients return to ordinary social roles. These are not redundant observations. Together they suggest that Moldovan adult epilepsy research would benefit from measuring not only symptoms and seizure variables, but also how those clinical variables are translated into everyday participation [23, 24].

The 2024 Moldovan case study on psychological rehabilitation is especially valuable because it gives local evidence that psychosocial intervention can change patient-relevant domains even when seizure reduction is small. In a 39-year-old patient with drug-resistant epilepsy, ten individual counselling sessions were associated with slight improvement in memory, attention, and reaction speed, a reduction of anxiety-depressive symptoms from moderate to mild, lower hostility, greater desire for socialization, increased interest in employment, and improved treatment compliance and trust in specialists. Methodologically, this case study points toward an important future use-case for combined QOL and stigma measurement in Moldova: evaluation of rehabilitation and psychosocial care, not only pharmacological control [22].

Recent Moldovan contributions from 2025, including a narrative synthesis focused on epilepsy and social stigmatization and a conference contribution on the stigmatization phenomenon in epilepsy, suggest that local scholarship is beginning to consolidate around stigma as a core quality-of-life determinant. Even when such sources are more contextual than definitive, they still matter because they indicate what questions are becoming visible within the local research culture. For a future Moldovan adult study, that visibility is strategically important: it creates room for an instrument choice that is psychometrically serious and socially relevant at the same time [29, 30, 50].

Table 4. Recommended instrument combinations for future Moldovan adult epilepsy studies and principal supporting references

Research goal	Preferred quality-of-life tool	Preferred stigma tool	Minimal clinical covariates to report
Cross-sectional adult outpatient survey [10, 14, 15, 28, 38]	QOLIE-31	SSE or clearly defined ESS variant	Seizure frequency, seizure type, number of anti-seizure medications, adverse effects, depression/anxiety screen
Drug-resistant or tertiary-centre cohort [21, 27]	QOLIE-31-P	ESSS if internalized burden is central; SSE if perceived stigma is central	Drug resistance, seizure frequency, ASM polytherapy, adverse effects, insomnia, and psychiatric symptoms
Psychological rehabilitation or psychoeducation study [16, 22]	QOLIE-31-P	ESSS or SSE according to intervention target	Baseline stigma level, social participation, compliance, emotional symptoms, and seizure change over time
Translation and validation study [10, 15, 16, 18, 39-42]	QOLIE-31 or QOLIE-31-P	One single stigma instrument only	Reliability indices, construct validity, known-group validity, and completion burden
Rapid clinical screening in routine care [11]	QOLIE-10	Brief stigma screen or none if visit time is limited	Seizure frequency, medication burden, and brief mood assessment

Note: *QOLIE-31-P = patient-weighted Quality of Life in Epilepsy Inventory-31; SSE = Stigma Scale of Epilepsy; ESS = Epilepsy Stigma Scale or revised Epilepsy Stigma Scale variants; ESSS = Epilepsy Self-Stigma Scale; ASM = anti-seizure medication. Recommendations are synthesis-based and should be adapted to study design, translation status, and available sample size.*

Implementation issues: translation, access, and study design

A recurrent weakness in the adult literature is the dominance of cross-sectional designs. Cross-sectional studies are indispensable for mapping associations and comparing instruments, but they cannot determine whether poor quality of life precedes stigma, follows it, or co-evolves with it through recurrent seizure burden. The literature is therefore stronger on psychometric description and correlational patterning than on temporal causality. This limitation is not a reason to dismiss existing evidence; rather, it clarifies what a future Moldovan agenda should add, namely longitudinal designs, repeated measures, and more explicit modelling of clinical severity together with psychosocial mediators [1, 21, 46].

Translation and implementation deserve equal methodological attention. RAND continues to host QOLIE-89 and QOLIE-31 as public documents with scoring manuals, which lowers barriers to transparent use. At the same time, the existence of COA catalogues and licensing resources reminds investigators that instrument governance, translation procedures, and version control should be verified before local deployment, especially for short forms, patient-weighted variants, or non-English implementations. In practice, a responsible Moldovan study should document the translation pathway, forward-backward translation, cognitive debriefing, pilot burden, and basic psychometric performance rather than treating the questionnaire as a neutral import [13, 25, 48, 49]. From an implementation perspective, the literature supports a severity core set for adult Moldovan studies: seizure frequency, seizure type or focality, drug-resistant epilepsy status, number of anti-seizure medications, adverse medication effects, depression and anxiety screening, sleep problems where relevant, work status, and a clearly defined stigma measure. These variables recur often enough across international and regional studies to form a credible minimum standard, yet they remain realistic for service-based research. When this clinical core is paired with QOLIE-31 or QOLIE-31-P, the resulting design becomes both locally feasible and internationally interpretable [20, 21, 27, 28].

Discussion

This narrative methodological review supports the working hypothesis in a remarkably consistent way. Across

foundational instrument studies, psychometric validations, regional adult cohorts, and Moldovan contextual publications, lower clinical control and higher psychosocial burden are repeatedly linked to worse epilepsy-specific quality of life. What varies is not the direction of the relationship, but how investigators choose to observe it. Some focus on seizure frequency and medication profile; others foreground depression, anxiety, or stigma. The strongest adult studies, however, are not those that choose one domain and ignore the rest, but those that model quality of life as the meeting point of neurological severity and psychosocial mediation [1, 27, 28, 46].

As detailed in the Results, QOLIE-31 remains the preferred default for adult comparative research, while QOLIE-31-P better suits longitudinal or patient-centred designs, and QOLIE-10 serves screening rather than primary outcome purposes. This hierarchy follows directly from instrument design and observed use patterns, not from an ideological preference [10, 11, 20, 21, 38].

The stigma findings are equally clear but conceptually more delicate. As shown in the Results, SSE-type instruments target perceived stigma in social encounters, while ESSS-type measures capture internalized self-stigma; these constructs are overlapping but not interchangeable. The practical implication is direct: future Moldovan methods sections must name the full scale, define the target construct, and explain why that specific form of stigma was selected. That discipline would eliminate a substantial amount of interpretive noise from the local literature [14-17].

The regional and Moldovan evidence layers add more than local colour. The Romanian studies show that a disease-specific adult quality-of-life instrument can be used successfully in an Eastern European clinical environment and that repeated measurement is realistic. The Moldovan sources, in turn, show that local clinicians and researchers already view epilepsy through a psychosocial lens that includes stigma, adaptation, resocialization, and rehabilitation. This means the local research ecosystem is ready for a more formal patient-reported outcomes agenda. The necessary next step is not to discover that quality of life matters, because that is already known, but to operationalize it with methodologically coherent tools and clinically standardized covariates [5, 20-24].

One especially promising direction for Moldova is the evaluation of psychosocial and rehabilitation interventions. The 2024 local case study showed improvements in emotional stability, self-confidence, compliance, and social openness despite only slight and non-significant seizure change. This pattern mirrors a broader insight from the international literature: quality-of-life gains need not wait passively for perfect seizure control. Some may be obtained by reducing emotional distress, isolation, and self-stigma, or by improving the person's capacity to participate in work, family, and social life. For that reason, studies of psychoeducation, counselling, or multidisciplinary rehabilitation should not rely solely on crude symptom counts when selecting outcomes [22, 31, 32].

A practical Moldovan research pathway can therefore be outlined. A first phase could validate or culturally adapt one adult quality-of-life instrument, preferably QOLIE-31 or QOLIE-31-P, together with one stigma scale chosen for construct fit. A second phase could apply those instruments in a multicentre cross-sectional study reporting a minimum severity core set. A third phase could move toward follow-up design in refractory epilepsy services or psychosocial rehabilitation programs. Such sequencing would be more productive than attempting a single over-ambitious project with too many scales and too few patients. The goal is disciplined accumulation of comparable data, not decorative measurement abundance [19, 21, 25, 49].

Equally important is the way results are reported. A future Moldovan adult study should avoid the common pattern of presenting only one total quality-of-life score with minimal clinical annotation. Domain scores should be reported when sample size permits, because seizure worry, social function, emotional well-being, medication effects, and energy or cognitive complaints do not necessarily move together. The Methods section should state exactly which score version was used, how missing items were handled, whether the instrument was self-completed or interviewer-assisted, how long administration required, and whether respondents found any items culturally unclear. Such details may appear mundane, but they determine whether a study can actually be replicated or meaningfully compared with international cohorts. They are also the details most likely to matter when a questionnaire is transferred into a new linguistic and clinical environment [13, 20, 25, 40].

The literature also suggests that future Moldovan work should resist false dichotomies between neurological and psychosocial outcomes. Stigma is not merely a social afterthought, and seizure control is not the sole legitimate endpoint. A strong adult design can report both without methodological confusion: clinical variables establish the burden profile, while QOL and stigma instruments show how that burden is lived. This is particularly relevant for patients with drug-resistant epilepsy, recurrent hospital use, work exclusion, or family strain, where relatively small clinical improvements may still generate meaningful patient-reported benefit. If Moldovan studies adopt this dual-outcome logic, they will be better positioned to evaluate pharmacological management, psychosocial rehabilitation, and service organization within the same evidence framework [1, 22, 31, 32].

The review also exposes several limitations. As a narrative methodological review rather than a systematic review, this synthesis is inherently subject to selection bias in source identification and to the interpretive judgements of the authors; no pooled effect sizes can be calculated, and the breadth-versus-depth trade-off cannot be fully eliminated. A PRISMA-compliant systematic review or meta-analysis would provide a more rigorous evidential foundation but would also restrict the scope in ways incompatible with the primarily methodological aim of this work. Within the evidence base itself: first, much of the adult literature re-

mains cross-sectional, which constrains causal inference. Second, the stigma field continues to suffer from inconsistent terminology and construct overlap. Third, local Moldovan evidence is still stronger on conceptual and contextual relevance than on large-scale psychometric validation. Fourth, not all recent Moldovan 2025 items were available as complete indexed full texts at the time of synthesis, so they were used mainly as contextual rather than core empirical evidence. Finally, inclusion of QOLIE-AD-48 was deliberately limited because it is developmentally informative but not a primary adult outcome instrument. None of these limitations invalidate this synthesis, but they do define the agenda ahead [3, 29, 30, 46].

The principal strength of this review is that it integrates global psychometric literature, contemporary adult cohort data, regional Romanian measurement experience, and Moldovan clinical-social scholarship within a single methodological frame. Instead of asking only whether epilepsy worsens quality of life, it asks which questionnaire strategy best captures that worsening in adult patients and how stigma should be measured without conceptual shortcuts. That is the level at which future Moldovan research can move from general awareness to genuinely comparable evidence [5, 20-22, 38].

Conclusions

This methodological review shows that adult epilepsy burden is best assessed when epilepsy-specific quality of life and stigma are treated as complementary patient-reported outcomes rather than as interchangeable measures. For future adult studies in the Republic of Moldova, the most practical strategy is to pair QOLIE-31 or QOLIE-31-P with one stigma scale selected according to construct and to report a standardized clinical-severity core set. The added value of this synthesis is a locally adaptable measurement framework that can generate patient-reported outcome data that are clinically meaningful and internationally comparable.

Competing interests

None declared.

Authors' contributions

Conception and design of the work: AF, SG, LS, VO, VC, GL-B. Literature search and data collection: GL-B, AF. Drafting the article: AF, GL-B. Critical revision for important intellectual content: SG, LS, AF. Neurology and clinical epilepsy expertise: SG, VC. Methodology and public health expertise: LS, VO. The authors critically reviewed the work and approved the final version of the manuscript.

Acknowledgements and funding

This study is part of the doctoral thesis of Gabriela Lişinschi-Baranov, carried out within the Laboratory of Brain Health and Bioethics, Centre for Brain Health, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Republic of Moldova.

Provenance and peer review

Not commissioned, externally peer reviewed.

Abbreviations:

ASM – anti-seizure medication; ESS – Epilepsy Stigma Scale; ESSS – Epilepsy Self-Stigma Scale; ESSS-G – German version of the Epilepsy Self-Stigma Scale; IBE – International Bureau for Epilepsy; ILAE – International League Against Epilepsy; MJHS – Moldovan Journal of Health Sciences; QOLIE – Quality of Life in Epilepsy Inventory; QOLIE-10 – Quality of Life in Epilepsy Inventory-10; QOLIE-31 – Quality of Life in Epilepsy Inventory-31; QOLIE-31-P – patient-weighted Quality of Life in Epilepsy Inventory-31; QOLIE-89 – Quality of Life in Epilepsy Inventory-89; QOLIE-AD-48 – Quality of Life in Epilepsy Inventory for Adolescents-48; SSE – Stigma Scale of Epilepsy; WHO – World Health Organization.

References

1. Taylor RS, Sander JW, Taylor RJ, Baker GA. Predictors of health-related quality of life and costs in adults with epilepsy: a systematic review. Epilepsia. 2011;52(12):2168-2180. doi: 10.1111/j.1528-1167.2011.03213.x.
2. Jacoby A. Stigma, epilepsy, and quality of life. Epilepsy Behav. 2002;3(6 Suppl 2):10-20. doi: 10.1016/S1525-5050(02)00545-0.
3. Baker D, Eccles FJR, Caswell HL. Correlates of stigma in adults with epilepsy: a systematic review of quantitative studies. Epilepsy Behav. 2018;83:67-80. doi: 10.1016/j.yebeh.2018.02.016.
4. Shi Y, Liu S, Wang J, Li C, Zhang J. Stigma experienced by patients with epilepsy: a systematic review and meta-synthesis of qualitative studies. Epilepsy Behav. 2021;118:107926. doi: 10.1016/j.yebeh.2021.107926.
5. Groppa S, Glavan A, Glavan D. Stigmatul social şi impactul asupra calității vieții în epilepsie [Social stigma and impact on quality of life in epilepsy]. Bull Acad Sci Mold. Med Sci. 2016;(3):154-160. Romanian.
6. Reynolds EH. ILAE/IBE/WHO global campaign "Out of the Shadows": global and regional developments. Epilepsia. 2001;42(8):1094-1100. doi: 10.1046/j.1528-1157.2001.0420081094.x.
7. Reis R, Meinardi H. ILAE/WHO "Out of the Shadows campaign" stigma: does the flag identify the cargo? Epilepsy Behav. 2002;3(6 Suppl 2):33-37. doi: 10.1016/S1525-5050(02)00546-2.
8. Morrell MJ. Stigma and epilepsy. Epilepsy Behav. 2002;3(6 Suppl 2):21-25. doi: 10.1016/S1525-5050(02)00547-4.
9. Devinsky O, Vickrey BG, Cramer J, Perrine K, Hermann B, Meador K, et al. Development of the quality of life in epilepsy inventory. Epilepsia. 1995;36(11):1089-1104. doi: 10.1111/j.1528-1157.1995.tb00467.x.
10. Cramer JA, Perrine K, Devinsky O, Bryant-Comstock L, Meador K, Hermann B. Development and cross-cultural translations of a 31-item quality of life in epilepsy inventory. Epilepsia. 1998;39(1):81-88. doi: 10.1111/j.1528-1157.1998.tb01278.x.

11. Cramer JA, Perrine K, Devinsky O, Meador K. A brief questionnaire to screen for quality of life in epilepsy: the QOLIE-10. Epilepsia. 1996;37(6):577-582. doi: 10.1111/j.1528-1157.1996.tb00612.x.
12. Cramer JA, Westbrook LE, Devinsky O, Perrine K, Glassman MB, Camfield C. Development of the Quality of Life in Epilepsy Inventory for Adolescents: the QOLIE-AD-48. Epilepsia. 1999;40(8):1114-1121. doi: 10.1111/j.1528-1157.1999.tb00828.x.
13. RAND Health. Quality of Life in Epilepsy Inventory (QOLIE-89 and QOLIE-31) [Internet]. Santa Monica (CA): RAND Corporation; ©2026 [cited 2026 Apr 8]. Available from: https://www.rand.org/health/surveys/qolie.html.
14. Fernandes PT, Salgado PCB, Noronha ALA, Sander JW, Li LM. Stigma Scale of Epilepsy: validation process. Arq Neuropsiquiatr. 2007;65(Suppl 1):35-42. doi: 10.1590/S0004-282X2007001000006.
15. Ogawa M, Fujikawa M, Tasaki K, Jin K, Kakisaka Y, Nakasato N. Development and validation of the Japanese version of the Epilepsy Stigma Scale in adults with epilepsy. Epilepsy Behav. 2022;134:108832. doi: 10.1016/j.yebeh.2022.108832.
16. Kuramochi I, Iwayama T, Horikawa N, Shimotsu S, Watanabe S, Yamanouchi H, et al. Development and validation of the Epilepsy Self-Stigma Scale. Epilepsia Open. 2021;6(4):748-756. doi: 10.1002/epi4.12547.
17. Kuramochi I, Iwayama T, Brandt C, Yoshimasu H, Bien CG, Hagemann A. Assessment of self-stigma in epilepsy: validation of the German version Epilepsy Self-Stigma Scale (ESSS-G). Epilepsia Open. 2023;8(3):946-958. doi: 10.1002/epi4.12765.
18. Mbuba CK, Abubakar A, Odermatt P, Newton CR, Carter JA. Development and validation of the Kilifi Stigma Scale for Epilepsy in Kenya. Epilepsy Behav. 2012;24(1):81-85. doi: 10.1016/j.yebeh.2012.02.019.
19. Ministerul Sănătății al Republicii Moldova [Ministry of Health of the Republic of Moldova]; Groppa S, Chiosa V, Cobîleanschi Oleg, et al. Epilepsia la adult: protocol clinic national: PCN-290 [Adult epilepsy: national clinical protocol: NCP-290]. Chișinău: The Ministry; 2017 [cited 2026 Apr 8]. Romanian. Available from: https://repository.usmf.md/handle/20.500.12710/10670.
20. Cioriceanu IH, Constantin DA, Marceanu LG, Anastasiu CV, Șerbănică AN, Rogozea L. Impact of clinical and socio-demographic factors on the quality of life in Romanian people with epilepsy. Healthcare (Basel). 2022;10(10):1909. doi: 10.3390/healthcare10101909.
21. Cioriceanu IH, Constantin DA, Bobescu E, Marceanu LG, Rogozea L. Influence of clinical factors on the quality of life in Romanian people with epilepsy: a follow-up study in real-life clinical practice. J Pers Med. 2023;13(5):752. doi: 10.3390/jpm13050752.
22. Doțen N, Vataman A, Aftene D, Dragan D, Chiosa V. Reabilitarea psihologică în epilepsie: studiu de caz = Psychological rehabilitation in epilepsy: a case study. Mold J Health Sci. 2024;11(3 Suppl 2):431 [cited 2026 Apr 8]. Romanian, English. Available from: https://repository.usmf.md/handle/20.500.12710/29451.
23. Ciobanu M, Cobîleanschi O, Bugneac I. Adaptarea pacienților cu epilepsie [Adaptation of patients with epilepsy]. Bull Acad Sci Mold. Med Sci. 2022;2(73):120-125. doi: 10.52692/1857-0011.2022.2-73.20. Romanian.
24. Cobîleanschi O, Bugneac I. Resocializarea pacienților cu epilepsie [Resocialization of patients with epilepsy]. Bull Acad Sci Mold. Med Sci. 2022;2(73):167-172. doi: 10.52692/1857-0011.2022.2-73.28. Romanian.
25. Nunnally JC, Bernstein IH. Psychometric theory. 3rd ed. New York: McGraw-Hill; 1994.
26. Ritchie J, Spencer E. Qualitative data analysis for applied policy research. In: Bryman A, Burgess RG, editors. Analyzing qualitative data. London: Routledge; 1994. p. 172-194.
27. González-Martínez A, Planchuelo-Gómez Á, Vieira Campos A, Martínez-Dubarbie F, Vivancos J, de Toledo-Heras M. Perceived quality of life (QOLIE-31-P), depression (NDDI-E), anxiety (GAD-7), and insomnia in patients with epilepsy attended at a refractory epilepsy unit in real-life clinical practice. Neurol Sci. 2022;43(3):1955-1964. doi: 10.1007/s10072-021-05595-3.
28. Siebenbrodt K, Willems LM, von Podewils F, Mross PM, Strüber M, Langenbruch L, et al. Determinants of quality of life in adults with epilepsy: a multicentre, cross-sectional study from Germany. Neurol Res Pract. 2023;5(1):41. doi: 10.1186/s42466-023-00265-5.
29. Lișinschi-Baranov G, Ferdohleb A, Chiosa V. Epilepsia și stigmatizarea socială: efecte asupra calității vieții = Epilepsy and social stigmatization: effects on quality of life. Mold J Health Sci. 2025;12(2 Suppl 1):7. Romanian, English.
30. Lișinschi-Baranov G, Ferdohleb A. The phenomenon of stigmatization of patients with epilepsy. In: Condraticova L., editor. Yesterday's cultural heritage – contribution to the development of tomorrow's sustainable society: international scientific conference, 11th ed., 2025 Feb 11-12; Iași-Chișinău-Lviv. Chișinău; 2025. p. 292-293.
31. Mahrer-Imhof R, Jaggi S, Bonomo A, Hediger H, Eggenschwiler P, Krämer G, et al. Quality of life in adult patients with epilepsy and their family members. Seizure. 2013;22(2):128-135. doi: 10.1016/j.seizure.2012.11.012.
32. Saada F, Wang ZS, Bautista RED. In focus: the everyday lives of families of adult individuals with epilepsy. Epilepsy Behav. 2015;50:10-13. doi: 10.1016/j.yebeh.2015.05.041.
33. Ryan R, Kempner K, Emlen AC. The stigma of epilepsy as a self-concept. Epilepsia. 1980;21(4):433-444. doi: 10.1111/j.1528-1157.1980.tb04091.x.

34. McLin WM, de Boer HM. Public perceptions about epilepsy. Epilepsia. 1995;36(10):957-959. doi: 10.1111/j.1528-1157.1995.tb00952.x.
35. Lee SA, Yoo HJ, Lee BI. Factors contributing to the stigma of epilepsy. Seizure. 2005;14(3):157-163. doi: 10.1016/j.seizure.2005.01.001.
36. Kim S, Hays RD, Birbeck GL, Vickrey BG. Responsiveness of the quality of life in epilepsy inventory (QOLIE-89) in an antiepileptic drug trial. Qual Life Res. 2003;12(2):147-155. doi: 10.1023/A:1022209105926.
37. Ebrahimi A, Barekatain M, Bornamanesh A, Najafi MR, Salehzadeh M, Maracy MR. Psychometric properties and validation of Persian version of quality of life in epilepsy inventory (QOLIE-89). J Res Med Sci. 2013;18(11):990-994.
38. Saadi A, Patenaude B, Mateen FJ. Quality of life in epilepsy-31 inventory (QOLIE-31) scores: a global comparison. Epilepsy Behav. 2016;65:13-17. doi: 10.1016/j.yebeh.2016.09.032.
39. Hu Y, Guo Y, Wang YQ, Du Q, Ding MP. Reliability and validity of a Chinese version of the Quality of Life in Epilepsy Inventory (QOLIE-31-P). Zhejiang Da Xue Xue Bao Yi Xue Ban. 2009;38(6):605-610. doi: 10.3785/j.issn.1008-9292.2009.06.009.
40. Puteikis K, Mameniškienė R. Psychometric properties of the Lithuanian version of the patient-weighted inventory on quality of life in epilepsy. Epilepsy Behav. 2022;130:108648. doi: 10.1016/j.yebeh.2022.108648.
41. May TW, Pfafflin M, Cramer JA. Psychometric properties of the German translation of the QOLIE-31. Epilepsy Behav. 2001;2(2):106-114. doi: 10.1006/ebeh.2001.0170.
42. Picot MC, Crespel A, Daurès JP, Baldy-Moulinier M, El Hasnaoui A. Psychometric validation of the French version of the quality of life in epilepsy inventory (QOLIE-31): comparison with a generic health-related quality of life questionnaire. Epileptic Disord. 2004;6(4):275-285.
43. Taylor J, Baker GA, Jacoby A. Levels of epilepsy stigma in an incident population and associated factors. Epilepsy Behav. 2011;21(3):255-260. doi: 10.1016/j.yebeh.2011.04.002.
44. Lalatović S, Milovanović M, Krstić N. Stigma and its association with health-related quality of life in adults with epilepsy. Epilepsy Behav. 2022;135:108874. doi: 10.1016/j.yebeh.2022.108874.
45. Hu C, Zhao Y, Xiao Z. Effects of stigma on the quality of life in patients with epilepsy. Acta Epileptol. 2024;6(1):10. doi: 10.1186/s42494-024-00154-7.
46. Leite e Silva MH, Lima JVS, Lopes IPS, da Silva ACV, Lucchi IM, Oliveira GP, et al. Stigmas in epilepsy: systematic review and meta-analysis. J Epilepsy Res. 2025;15(1):23-32. doi: 10.14581/jer.25003.
47. Mollaoğlu M, Yılmaz M. The impact of stigma on quality of life in patients with epilepsy. Soc Neurosci. 2025;20(2):109-119. doi: 10.1080/17470919.2025.2536572.
48. Mapi Research Trust. COA questionnaires licensing [Internet]. Lyon: Mapi Research Trust; ©2026 [cited 2026 Apr 8]. Available from: https://www.mapi-trust.org/services/questionnaire-licensing.
49. Bryant-Comstock L, Cramer JA, Devinsky O, Hermann B, Meador K, Perrine K. Quality of Life in Epilepsy Inventory-31 (QOLIE-31) [Internet]. ePROVIDE platform. Lyon: Mapi Research Trust; ©2026 [cited 2026 Apr 8]. Available from: https://eprovide.mapi-trust.org/instruments/quality-of-life-in-epilepsy-inventory-31.

https://doi.org/10.52645/MJHS.2026.2.11

UDC: 616-006.441

REVIEW ARTICLE

Follicular lymphoma and its transformation to diffuse large B-cell lymphoma - a brief introduction to disease biology

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ABSTRACT

Introduction. Follicular lymphoma (FL) is a slow-growing B-cell lymphoma with a generally favorable prognosis. Nevertheless, its clinical course is heterogeneous, with a significant subset of patients experiencing early progression or histological transformation into diffuse large B-cell lymphoma (DLBCL), both considered to be high-risk events associated with treatment resistance and markedly inferior outcomes. Importantly, clinical risk factors have limited value in predicting these complications. This review outlines the key biologic features of FL, discussing how the novel molecular biology approaches can explain the clinical heterogeneity and high-risk disease evolution of FL.

Materials and methods. A focused literature review was conducted using the PubMed/MEDLINE database to identify studies on follicular lymphoma and its histological transformation to diffuse large B-cell lymphoma. Priority was given to original research or review articles investigating genetic, epigenetic, transcriptional, or microenvironmental determinants of FL.

Results. Evidence from early cytogenetic and DNA sequencing studies established BCL2 deregulation as an initiating lesion in FL, with further genetic alterations in epigenetic regulators like *KMT2D, EZH2, CREBBP/EP300* occurring early on and persisting throughout the disease course. Studies of transformed FL samples indicate that aggressive evolution is associated with acquisition of additional genetic lesions, such as those affecting the cell cycle regulators *CDKN2A/2B* and *TP53*. More recently, integrated genomic, transcriptomic and spatial resolved techniques have demonstrated substantial transcriptional heterogeneity within individual genetic subclones, suggesting that the genotype alone does not determine the phenotype of the malignant cells and supporting a pathogenetic model in which clinical trajectories reflect the combined effects of genomic evolution, transcriptional cell state, and tumor-microenvironment crosstalk. Important findings, including greater infiltration with $LAG3^{+}CD8^{+}$ T cells in cases of histological transformation to DLBCL and upregulation of transcriptional programs that promote stromal expansion and B-cell receptor signaling in cases of early FL relapse, indicate that integrated profiling represents a promising avenue for identifying the biomarkers and treatment targets that are specific to high-risk disease.

Conclusions. Continued research concentrated on multiomic profiling of both malignant and non-malignant tumor compartments is essential in order to reveal the mechanisms of FL heterogeneity and translate these data into practical biomarkers and therapeutic strategies.

Keywords: follicular lymphoma, diffuse large B-cell lymphoma, molecular biology, multiomics.

Cite this article: Negara I, Arnaut O, Buruiana S. Follicular lymphoma and its transformation to diffuse large B-cell lymphoma - a brief introduction to disease biology. Mold J Health Sci. 2026;13(2):84-94. https://doi.org/10.52645/MJHS.2026.2.11.

Manuscript received: 28.01.2026

Accepted for publication: 20.05.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Follicular lymphoma is a malignancy characterized by substantial heterogeneity in terms of clinical trajectories. Early progression and histological transformation to diffuse large B-cell lymphoma,

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both events characterized by poor prognosis and incompletely understood biology, cannot be reliably predicted, limiting the possibility of personalized upfront treatment decisions.

The research hypothesis

Recent studies have demonstrated that the clinical heterogeneity inherent to follicular lymphoma arises from the combined effects of multiple biological determinants that are currently being resolved using novel molecular biology approaches.

The novelty added by the manuscript to the already published scientific literature

Herein, a clinician-oriented synthesis is provided of how these techniques are shaping the current pathogenetic models of de novo and transformed follicular lymphoma, and how they may contribute to the identification of practical biomarkers and therapeutic vulnerabilities.

Introduction

Follicular lymphoma (FL) represents the most prevalent indolent B-cell lymphoma, comprising up to 25% of all non-Hodgkin lymphoma cases [1]. A combination of anti-CD20 agents with chemotherapy in the frontline setting results in favorable initial treatment responses in the majority of patients [2-6]0 to 54.5, leading to overall survival rates rivaling normal life expectancy [7]. Importantly, a significant number of patients follow clinical trajectories that are distinct from the otherwise prolonged and indolent course. In particular, approximately 20% experience early disease progression (commonly referred to as progression of disease within 24 months; POD24), which is associated with markedly inferior clinical outcomes [8-11]588 patients with stage 2 to 4 FL received first-line rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP. A sizeable minority also undergoes histological transformation into an aggressive lymphoma, most commonly diffuse large B-cell lymphoma (DLBCL), a complication likewise linked to rapid progression and inferior survival [12-14]some patients experience early disease progression, including progression of disease within 24 months (POD24.

Technological breakthroughs in the fields of genomics, transcriptomics, and epigenomics have advanced the understanding of FL from being a single entity primarily driven by the t(14;18) translocation to a highly heterogeneous group of tumors with diverse molecular features [15]epigenetic, microenvironmental, and clinical features. It is the most prevalent indolent non-Hodgkin lymphoma, characterized by a relapsing course and risk of transformation to aggressive diffuse large B-cell lymphoma. Recent advances in high-throughput sequencing, spatial transcriptomics, and imaging technologies uncovered genetic, epigenetic, and immunogenetic features underpinning FL, offering insights into its biology and potential therapeutic vulnerabilities. Although FL is primarily driven by the hallmark t(14;18. These advances have also provided a rationale for the introduction of multiple novel therapeutic agents which target the various pathways related to the underlying disease pathogenesis, including epigenetic regulators [16, 17], the B-cell receptor (BCR) pathway [18, 19]randomized study that assessed the efficacy and safety of ZO versus obinutuzumab in patients with relapsed/refractory (R/R, E3 ubiquitin ligase complexes [20, 21] and the tumor microenvironment (TME) - via T-cell engagers such as bispecific antibodies [22-25]a novel CD3 × CD20 bispecific antibody, in the third-line and later setting of follicular lymphoma.\nMETHODS: EPCORE NHL-1 is a multicohort, single-arm, phase 1-2 trial conducted at 88 sites across 15 countries. Here, we report the primary analysis of patients with relapsed or refractory follicular lymphoma in the phase 2 part of the trial, which included the pivotal (dose expansion, chimeric antigen receptor (CAR) T cells [26-28], or via promoting tumor cell phagocytosis [29].

In view of the expanding range of novel treatment options, it is essential to reliably identify the high-risk patients who are unlikely to benefit from conventional chemoimmunotherapy, such as those predisposed to early progression or transformation. Although a number of clinical predictors were shown to be associated with these poor outcomes, including advanced stage, elevated LDH, poor performance status, presence of B symptoms, extranodal site involvement, and high overall Follicular Lymphoma International Prognostic Index (FLIPI) score [9, 30-33]such as progression of disease within 24 months (POD24, none of these risk factors have sufficient pre-treatment predictive capacity. Moreover, the biologic events that shape a particular disease trajectory in FL, and, therefore, have the potential to serve as candidate biomarkers for poor clinical outcomes, are still incompletely understood. Although genomic studies have provided a foundation for the mechanisms of FL lymphomagenesis, it has become increasingly evident that the disease cannot be fully explained at the DNA level alone, highlighting the need to investigate additional layers of tumor biology. These challenges are currently being addressed through the use of novel techniques such as single-cell profiling and multiomics, which allow for a more comprehensive interrogation of both the malignant and non-malignant tumor compartments. In this review, a brief clinician-oriented

overview is provided of how these and other techniques are reshaping our understanding of de novo and transformed FL, presents a rationale for how this knowledge may eventually translate into clinically meaningful risk assessment and therapy development.

Materials and methods

The PubMed/MEDLINE database was searched using combinations of terms including "follicular lymphoma", "transformation", "diffuse large B-cell lymphoma", "POD24", "early progression", "tumor microenvironment", "single-cell", "spatial", "integrated profiling" and "multiomics." Reference lists of publications were manually searched to identify additional relevant studies. Titles and abstracts were screened for relevance, followed by full-text review of potentially eligible articles. This review considered peer-reviewed original research, reviews and conference abstracts addressing FL and its histological transformation to DLBCL. Non–peer-reviewed sources and isolated case reports were excluded.

Results

The role of genomic sequencing in follicular lymphoma BCL2 dysregulation. Before the advent of next-generation sequencing (NGS) technologies, the knowledge of specific events leading to FL development was largely limited to a single genetic lesion (Fig. 1). Early cytogenetic studies from the mid-1980s revealed that 85-90% of FL patients carry a hallmark reciprocal translocation involving the *B-cell lymphoma 2* (*BCL2)* oncogene on chromosome 18 and the immunoglobulin heavy chain (*IgH*) gene locus on chromosome 14 [34-36]hybrid bcl-2/immunoglobulin heavy chain transcripts are produced that consist of the 5′ half of the bcl-2 mRNA fused to a "decapitated" immunoglobulin heavy chain mRNA. Nucleotide sequence analyses confirmed that the hybrid transcripts continue to encode a normal bcl-2 protein. Our results suggest that t(14;18. As a consequence, the *BCL2* gene is placed under the control of strong *IgH* gene enhancer elements, resulting in constitutive *BCL2* overexpression [37]. The *BCL2* gene product then acts as its role as an anti-apoptotic protein, providing a selective survival advantage to the FL precursors carrying the t(14;18) translocation [38] and allowing them to accumulate secondary genetic lesions without undergoing apoptosis [39]the structure in which B cells undergo somatic hypermutation (SHM).

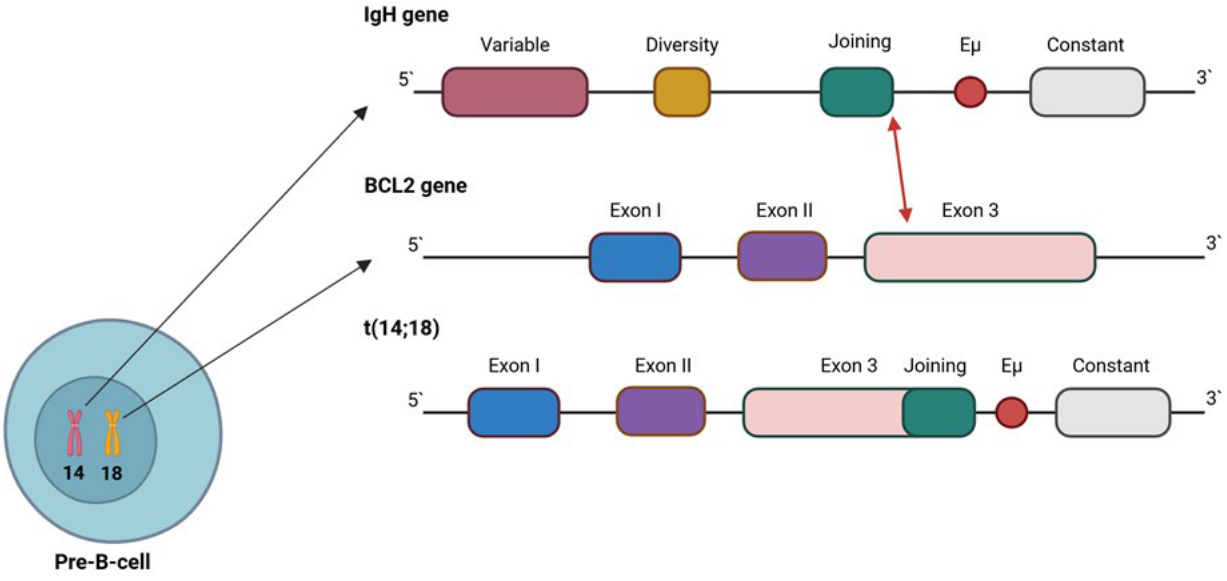

Fig. 1 A simplified schematic depiction of the hallmark t(14;18) rearrangement.

Note: *The reciprocal translocation, arising during the physiological V(D)J recombination process in developing B cells, juxtaposes BCL2 (chromosome 18, typically involving the region adjacent to exon 3) to the IgH (chromosome 14) Joining (J) region (red arrow), resulting in constitutive BCL2 expression driven by IgH regulatory sequences (such as Eμ) and promoting apoptosis resistance in B cells carrying the rearrangement.*

More recently, a role for somatic mutations in the *BCL2* gene has also been demonstrated. Correia *et al.* used Sanger sequencing to characterize *BCL2* mutations in FL and reported that such mutations, identified in 12% of patients, represent an independent risk factor for transformation and death from lymphoma [40]Follicular lymphoma (FL. However, the reliance on conventional Sanger sequencing likely underestimated the true prevalence and spectrum of *BCL2* alterations. Because Sanger sequencing produces only a single continuous read per DNA fragment, it is not sensitive enough to reliably detect mutations present below a variant allele frequency (VAF) of 15-20% [41]Sanger sequencing, single-strand conformation polymorphism (SSCP. This limitation is particularly important in the context of heterogeneous tumor samples, where a high background of DNA coming from normal cells within the tumor could mask mutations of the malignant clone. In contrast, NGS allows detection of less common variants through redundant sequencing of the same genomic regions. In line with the above, in a subsequent study by the same group

employing NGS, *BCL2* mutations were found in 52% of newly diagnosed FL [42]we examined 11 AICDA mutational targets, including BCL2, BCL6, PAX5, PIM1, RHOH, SOCS, and MYC, in 199 newly diagnosed grade 1 and 2 FLs. BCL2 mutations with VAF ≥20% occurred in 52% of cases. Among 97 FL patients who did not initially receive rituximab-containing therapy, nonsynonymous BCL2 mutations at VAF ≥20% were associated with increased transformation risk (HR 3.01, 95% CI 1.04–8.78, p = 0.043, in stark contrast to the 12% prevalence reported using Sanger sequencing [40]Follicular lymphoma (FL. Beyond sensitivity, Sanger sequencing approaches are also inherently targeted, focusing on preselected genes or regions, and therefore may miss pathogenic alterations outside the interrogated targets. Importantly, the observation that the t(14;18) translocation can be detected in a fraction of B cells in the majority of healthy individuals [43]chromosomal translocations are considered to be an early oncogenic hit. We investigated whether the lymphoma-associated t(14;18 further supports the notion that BCL2 deregulation alone is insufficient for malignant transformation, and that additional oncogenic events are required for FL development.

Epigenetic modifiers. Early NGS studies were foundational in defining the recurrent mutational landscape of FL. In a seminal study, Morin et al. conducted whole genome sequencing (WGS) of FL and DLBCL tumor samples, identifying recurrent mutations in EZH2, a gene encoding a H3K27 histone methyltransferase [44]. In a subsequent study by the same group, the authors discovered frequent inactivating mutations in the *KMT2D* (*MLL2*) gene, encoding for another histone methyltransferase [45], in 89% of FL patients [46]. In parallel, Pasqualucci et al. reported frequent mutations in the functionally related histone/protein lysine acetyltransferases *CREBBP* and *EP300*, detected in 32.6% and 8.7% of FL samples, respectively [47]. Building on these observations, Okosun et al. conducted WGS or whole exome sequencing (WES) of multiple samples that were collected from 10 patients at different time points during the course of their disease [48]. This sequential approach enabled the construction of phylogenetic trees, reflecting the clonal evolution of FL for each patient. The analysis demonstrated a branching evolution pattern in each of the trees, with the "trunk" representing the putative driver lesions that are shared between the initially identified and the subsequent clones in the FL tumors, thus supporting the existence of a common precursor and divergent subclones. In all patients, the precursor populations demonstrated an enrichment for mutations in the above-mentioned epigenetic modifiers, including *KMT2D, CREBBP, EP300*, and *EZH2*, and these findings were validated in an additional cohort of over 100 FL biopsies, where concurrent mutations in at least 2 epigenetic modifiers were found in more than 70% of cases. Interestingly, a small number of patients showed distinct mutations between diagnostic and subsequent biopsies that occurred within the same genes, a finding that was recently also observed in DLBCL [49], suggesting that certain driver lesions may be indispensable for lymphomagenesis and determine the commitment of the tumor towards a particular genetic phenotype.

Genetic determinants of FL transformation to DLBCL. The above data established epigenetic dysregulation as a central driving mechanism in the pathogenesis of de novo FL, with oncogenic effects that include immune evasion, cell cycle dysregulation, and alteration of multiple signaling pathways, including BCR, JAK-STAT, and NF-κB [15]. Importantly, analysis of sequential patient biopsies in the study of Okosun et al. reported that other genetic events, particularly abnormalities affecting cell cycle regulation and apoptosis (e.g., *MDM2*, *MYC*), as well as NF-κB signaling (*REL*, *MYD88*, *TNFAIP3*), appeared by the time of histological transformation of FL into DLBCL, and were not detectable in the initial FL biopsies [48]. Consistent with this, subsequent studies have demonstrated an association between transformation and increased mutational burden, in addition to recurrent genetic lesions affecting immune escape (B2M), confinement of B cells to the germinal center (*GNA13, S1PR2, P2RY8*), and cell cycle regulation and apoptosis (C*DKN2A, CDKN2B, TP53, MYC*) [50-53]. Notably, such abnormalities, in particular those affecting CDKN2A/2B, TP53 and MYC have also been implicated in transformation of chronic lymphocytic leukemia into DLBCL [54-56], and represent defining features of several prognostically inferior genetic subtypes of de novo DLBCL [53, 57-59]. Collectively, these findings support the notion that acquisition of specific genetic events contributes to the transformation of FL (Fig. 2)

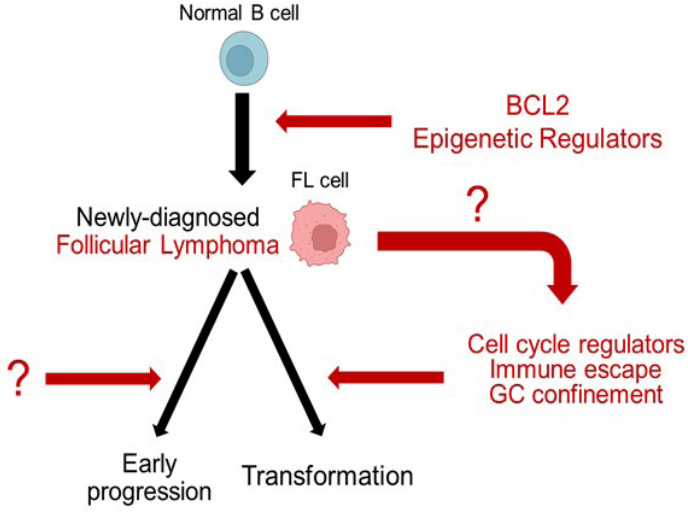

Fig. 2 Conceptual model of follicular lymphoma pathogenesis.

Note: *Early molecular events, including BCL2 deregulation and recurrent alterations in epigenetic regulators (upper right), contribute to malignant transformation of normal B cells into follicular lymphoma. Following diagnosis, FL may follow distinct evolutionary and clinical courses, including high-risk outcomes such as early progression and histological transformation to aggressive lymphoma. Transformation is accompanied by gain of additional genetic lesions, such as those affecting cell cycle regulators, immune escape, and germinal center confinement (lower right). Black arrows indicate established evolutionary relationships; red arrows indicate the mechanisms driving these relationships. Question marks denote biologic events that are incompletely understood: the determinants of early progression (lower left) and the events leading to acquisition of transformation-enabling genetic lesions (middle right).*

and raise the possibility that similar mechanisms play an important role in the development of more aggressive phenotypes across a spectrum of lymphoid malignancies [60].

The above findings indicate that transformation of FL into DLBCL is driven by an expansion of a tumor subclone with the propensity to acquire transformation-enabling genetic lesions [52, 61], a tendency that may arise as a functional consequence of pre-existing genetic abnormalities or other biologic events. In this context, it appears to be of more value to identify how the mutations at initial diagnosis influence the risk of FL transformation, rather than those acquired later during the course of disease progression, given that such an approach could eventually help guide up-front clinical decision-making. In a recent study that analyzed WGS data from 423 FL and de novo DLBCL patients, two genetically distinct FL subgroups were resolved using a machine learning classifier trained to discriminate between DLBCL and FL based on mutational profiles [62]. The first group, which included 53% of untransformed FL from the training cohort, was termed the constrained FL (cFL). Patients within this group were less likely to undergo transformation, and were distinguished by the presence of missense mutations in the lysine acetyltransferase domain of *CREBBP*, in addition to mutations in several genes involved in the *mTORC1* signaling pathway (*RRAGC*, *ATP6AP1*, and *ATP6V1B2*). The remaining 47% of untransformed FL were classified as DLBCL-like FL (*dFL*), a subgroup presenting with increased rates of aberrant somatic hypermutation (as demonstrated by a higher prevalence of mutations within the transcription start sites of commonly affected genes, such as *BCL6*, *BCL7A*, *RHOH*, and *ZFP36L1*), nonsense *CREBBP* mutations, and higher risk of transformation to *DLBCL*. These findings indicate that certain early genetic lesions may predispose FL to a commitment toward histological transformation, and thus serve as potential predictors for this clinical outcome. Whether these early genetic events contribute to transformation directly, or determine a state in which additional transformation-inducing genetic events accumulate, is currently unclear.

Genetic risk stratification models. Data from the aforementioned studies have laid the foundation for developing pretreatment prognostic scoring systems that incorporate somatic mutations for FL risk stratification. The most well-known of these models is the m7-FLIPI, a molecular prognostic tool created using a targeted NGS panel of 74 genes applied to pretreatment biopsies from 151 FL patients [63]. The score integrates several clinical parameters with the mutation status of 7 genes: *EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP*, and *CARD11*. In the initial trials, it was able to stratify patients into low- and high-risk subgroups based on failure-free survival [63], as well as identify those at highest risk for POD24 [9]. However, multiple follow-up studies attempting to validate the m7-FLIPI score have yielded inconsistent results in external patient cohorts [33, 64-67], suggesting that specific genetic lesions may be associated with distinct functional consequences in different clinical and biologic contexts. Given these challenges, no mutation-based prognostic score is currently adopted in clinical practice. As expanded upon below, the extensive inter- and intraclonal heterogeneity inherent to FL likely diminishes the ability of simplified models that are based on individual genes to consistently identify high-risk patients across different settings. At earlier time points, such as at diagnosis, the smaller, potentially prognostically relevant subclones may, at best, only be detectable using ultra-sensitive technologies [52, 61]. Moreover, none of the existing risk-stratification models were specifically trained for the most pertinent predictor of inferior survival, POD24 [68]. Finally, the risk of adverse clinical outcomes, such as transformation, may be associated with exposure to specific treatment regimens [69], further complicating our understanding of the predictive value of genetic lesions in FL. Collectively, these findings indicate that conventional, bulk DNA-level profiling alone is not sufficient to identify the core determinants underlying the clinical trajectories seen in FL.

The role of single-cell sequencing and integrated multiomic approaches in follicular lymphoma

Single-cell profiling and tumor microenvironment as a driver of clinical behavior. Bulk DNA sequencing is readily accessible, and widely used across cancer studies for diagnosis and biomarker identification. However, every tumor cell can exhibit a unique genomic, epigenomic, and transcriptional profile. Modifications at the RNA level appear particularly important, given that genomic and epigenomic changes are ultimately reflected at the RNA level. With the development of the 10x Genomics Chromium systems, single-cell RNA sequencing has recently become more accessible to the scientific community, allowing for analysis of gene expression profiles from thousands of individual cells per run [70]splicing variants, mutations/indels in addition to differential gene expression, thus providing a more complete genetic picture than DNA sequencing. This most widely used technology in genomics tool box has evolved from classic bulk RNA sequencing (RNAseq, exposing subclones and cell states that would otherwise be invisible in bulk sequencing.

Multiple studies employing single-cell RNA sequencing have reaffirmed that FL tumors consist of several coexisting subclones, either at different anatomical sites, or within the same lesion. In a recent study, Haebe *et al.* used single-cell RNA, B- and T-cell receptor sequencing, as well as flow cytometry to profile synchronously acquired tumors from different sites in 10 patients with FL [71]FL can exhibit site-to-site genetic and phenotypic divergence as well as differential Tfh abundance and tumor-Tfh cross talk.In FL, biopsy of a single anatomical site may not capture the full scope of a patient's disease., Tumor heterogeneity complicates biomarker development and fosters drug resistance in solid malignancies. In lymphoma, our knowledge of site-to-site heterogeneity and its clinical implications is still limited. Here, we profiled 2 nodal, synchronously acquired tumor samples from 10 patients with follicular lymphoma (FL. Unexpectedly, the authors found that in many patients the disease evolved independently at different sites, exhibiting site-to-site divergence in BCR evolution, gene expression and surface protein profiles. Supporting these observations, bulk WES of samples obtained from multiple disease sites

within the same patients revealed spatially discordant genetic abnormalities, including alterations in several m7-FLIPI-related genes, such as *EZH2* and *EP300* [72]. These findings appear particularly relevant given that most FL patients manifest disseminated tumor involvement. They also support the notion that a diagnostic biopsy from a single anatomical site may be insufficient, and further add to the limited predictive ability of the approaches relying just on bulk genome sequencing and clinical data. Moving forward, the potential requirement for multiple biopsies may be circumvented by genomic profiling of circulating tumor DNA [73] yet the identification of poor-risk groups remains challenging. In addition, the biology underlying these differences is incompletely understood. We hypothesized that characterization of mutational heterogeneity and genomic evolution using circulating tumor DNA (ctDNA, although the utility of such an approach in FL remains to be validated.

In a study integrating single-cell RNA and bulk exome sequencing, Andor *et al.* also identified the presence of multiple subclones within individual FL tumors, each associated with transcriptional profiles that reflected their distinct genetic backgrounds [74]characterized by differential pathway activities.In CD4+ Tregs, known immune checkpoint genes are coexpressed with transcription factors and immune regulators, including CEBPA and B2M., Follicular lymphoma (FL. Notably, substantial transcriptional heterogeneity was also observed within individual genetic subclones, suggesting that phenotypic diversity is not solely determined by the genotype. Consistent with this, malignant B cells in FL were shown to cycle between different transcriptional states largely independently of their mutational profile, a process likely driven by extrinsic signals from the non-malignant components of the lymphoma microenvironment [75]the second most frequent lymphoma in adults, often presents as a disseminated disease at diagnosis. Despite a generally slow progression and a median overall survival of more than 15 years with current chemo-immunotherapies, FL patients often suffer from multiple relapses. Yet, the biological mechanisms promoting FL dissemination, progression and relapse are still poorly understood. FL, like most B-cell lymphomas, originates from germinal centers (GC.

The structure of lymph nodes affected by FL retains features of normal lymphoid tissue, but the architecture is disorganized, with complete or partial effacement by the neoplastic follicles [76]primarily, within lymph nodes (LNs. It is enriched for T cells, including mostly $CD4^+$ subsets, such as T follicular helper cells, T regulatory cells, T follicular regulatory cells, and others, which were shown to contribute to tumor growth and treatment resistance via pro-survival signals (such as CD40L or IL-4), or by suppressing the normal anti-tumor immune response. Other cell types include the various macrophages and dendritic cells, which are frequently polarized into a tumor-supportive phenotype by the malignant cells, as well as a variety of stromal cells, which are able to promote tumor growth by altering the microenvironment or by interacting directly with the tumor cells and secreting tumor-promoting cytokines. Dave *et al.* were among the first to demonstrate the role of the FL TME in determining the patient clinical trajectory [77]. Using microarray-based gene-expression profiling, the authors resolved two survival-associated gene expression signatures, each reflecting the biologic characteristics of the non-malignant cells within the analyzed tumors. The first, termed immune-response 1, was associated with a favorable prognosis and enriched for expression of genes encoding T-cell and macrophage markers, whereas the second, termed immune-response 2, was associated with a poor prognosis and enriched only for genes preferentially expressed in macrophages and dendritic cells, thus suggesting a role for the TME-derived T cells in preventing tumor growth. These early bulk gene-expression data provided the first evidence that the FL microenvironment is a prognostically meaningful structure that could shape the disease trajectory. Single-cell technologies have since expanded this concept substantially. Han *et al.* used single-cell RNA sequencing to subdivide FL into four major subtypes based on the phenotype and relative abundance of the various T cell populations [78]and associations with characteristics of tumor-infiltrating T-cell subsets., Follicular lymphoma (FL. Importantly, the subtype characterized by T cell depletion was associated with poor survival, reinforcing the role of T cells in suppressing tumor growth and suggesting that immune evasion by the tumor cells is an important contributor to poor prognosis.

Integrated multiomic approaches in early relapse FL and transformation to DLBCL. A multiomic approach refers to the integrated analysis of two or more biologic data layers (e.g., genomics, transcriptomics, epigenomics, proteomics) to reconstruct a more complete view of tumor biology. In FL, where clonal heterogeneity and microenvironmental interactions appear to significantly influence disease behavior, multiomic profiling is particularly valuable for capturing the complexity that may otherwise be missed by single-modality methods. Using WES, bulk and single-cell RNA sequencing, and iterative bleaching extends multiplexity (IBEX) imaging (an immunofluorescence technique that allows visualization of more than 65 proteins in the same tissue section) [79]lacking a spatial context, and traditional immunofluorescence, capturing only two to six molecular features, cannot resolve these issues. Imaging technologies have been developed to address these problems, but each possesses limitations that constrain widespread use. Here we report a method that overcomes major impediments to highly multiplex tissue imaging. \"Iterative bleaching extends multiplexity\" (IBEX, Radtke *et al.* constructed a molecular and cellular atlas of lymph nodes affected by FL [80]. The authors demonstrated that malignant B cells in high-risk patients undergoing early relapse exhibited increased expression of genes related to BCR signaling and TME remodeling, unlike the malignant B cells derived from all other FL patients. The unique imaging technique enabled spatial resolution of the non-malignant cell populations within individual tumors, revealing that early-relapse cases were characterized by an expansion of specific stromal cell communities and desmoplasia. Notably, the tumor B cells were frequently found to be in close physi-

cal proximity to certain TME components (such as dendritic cells), a state that may facilitate sustained BCR signaling.

Additional insights were obtained from another integrative single-cell study by Sarkozy et al., who applied single-cell genome and transcriptome sequencing to investigate the co-evolution of the malignant B cells and the surrounding microenvironment during transformation of FL into DLBCL [61]. Transcriptomic profiling showed differential expression of MYC target genes, in addition to activation of oxidative phosphorylation and mTORC1 pathways during transformation. By integrating WGS and single-cell RNA sequencing data across sequential time points, the authors further identified an association between genomic evolution (i.e., emergence of additional genetic lesions) and acquisition of a transformation-related transcriptional phenotype. However, significant transcriptional diversity in the absence of detectable genomic evolution was also observed, reinforcing the notion that genotype alone does not fully determine the tumor cell state. Analysis of the non-B cell fraction revealed marked remodeling of the TME, with decreases in naive/memory-like and follicular helper-like T cells, accompanied by expansion of the exhausted/regulatory-like and effector T-cell clusters, findings that were further supported by immunofluorescence evidence of increased CD8+ T-cell exhaustion marker expression during transformation. Spatial profiling additionally showed that the exhausted T cells were more abundant in close proximity to the malignant B cells after transformation, suggesting that the malignant B cells engaging with the surrounding T cells may contribute to T-cell dysfunction, and, potentially, to transformation-associated transcriptional reprogramming in the tumor B cells. Importantly, expression of the exhaustion marker LAG3 on CD8+ T cells in two independent pre-treatment FL cohorts was significantly associated with shorter time to transformation, indicating that specific TME features could represent candidate biomarkers for histological transformation. Collectively, these studies support the notion that both transformation and early relapse are accompanied by a shift in the composition and spatial organization of the TME, thus indicating that the tumor-microenvironment crosstalk is a core component of the biologic process that drives the development of more aggressive disease phenotypes (Fig. 3).

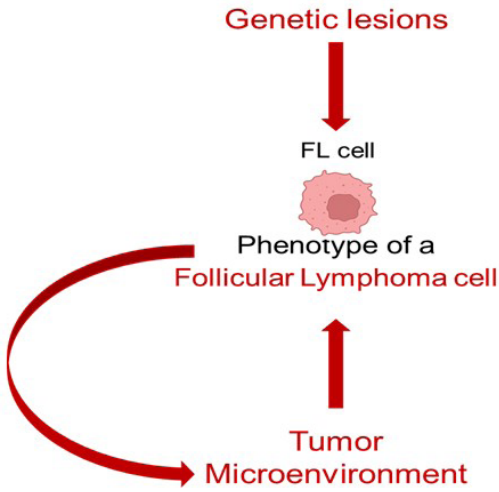

Fig. 3 Conceptual model for the determinants of a follicular lymphoma cell phenotype.

Note: *The phenotype of a malignant follicular lymphoma cell is shaped by both intrinsic genetic lesions (top arrow) and extrinsic signals from the tumor microenvironment (bottom arrow). The curved arrow denotes bidirectional crosstalk between tumor cells and surrounding non-malignant components, emphasizing the dynamic and reciprocal process determining the tumor cell phenotype.*

Discussion

Over the last few decades, the application of omics technologies has transformed the understanding of follicular lymphoma from a disease centered on BCL2 deregulation to a complex and heterogeneous entity shaped by multiple molecular mechanisms. Genomic sequencing has established epigenetic dysregulation as a core component of FL pathogenesis, with recurrent mutations in chromatin modifiers like *KMT2D*, *CREBBP*, *EP300* and *EZH2* occurring early and being maintained throughout disease evolution. In contrast, other genetic lesions, including those affecting cell cycle regulators, are more often acquired later on and are associated with more aggressive disease phenotypes such as transformation into DLBCL.

More recently, integrative approaches that combine genomics, transcriptomics, and spatial profiling have further shown that the mechanisms driving FL are multifaceted and cannot be explained by the genotype alone, instead reflecting the interplay between genetic lesions, transcriptional programs, and dynamic crosstalk with the lymphoma microenvironment. Collectively, the data presented in this review support a model of FL pathogenesis in which malignant cells undergo genetically driven phenotypic changes and engage in dynamic, bidirectional interactions with surrounding immune cells, thereby remodeling the immune microenvironment into a tumor-supporting framework that promotes further phenotypic changes in the malignant cells.

Despite these advances, FL continues to pose significant clinical challenges, with markedly inferior outcomes in patients with histological transformation or early progression. Recent discoveries, including the identification of $LAG3^{+}CD8^{+}$ T-cell infiltration as a candidate biomarker of histological transformation and the emergence of potential therapeutic vulnerabilities in early relapse, such as fibrosis-associated TME remodeling that may be targetable with antifibrotic agents and BCR pathway inhibitors, support the notion that multiomic profiling may continue to reveal clinically actionable biomarkers of high-risk disease and inform risk-adapted therapeutic strategies.

Conclusions

Follicular lymphoma is a biologically complex disease in which clinical outcomes are determined not only by the genetic background of the malignant cells, but also by their transcriptional states and interactions with the surrounding microenvironment. The data reviewed here indicate that continued research focused on multiomic profiling is essential to identify the distinct biologic determinants that drive

the various clinical trajectories in FL, thereby improving our ability to predict poor outcomes and facilitating therapeutic approaches that are focused on timely prevention of transformation and early progression.

Competing interests

None declared.

Authors' contributions

IN, OA and SB participated in conceptualization of the manuscript. IN conducted the literature review and drafted the manuscript. OA and SB critically revised the manuscript. All authors approved the final version of the manuscript.

Ethics approval

Not needed for this study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of Generative AI and AI-assisted technologies in the writing process

The GPT-5.2 model was used during copy-editing of the finished manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of this publication.

References

1. Cerhan JR. Epidemiology of follicular lymphoma. Hematol Oncol Clin North Am. 2020 Aug;34(4):631-46. doi: 10.1016/j.hoc.2020.02.001.
2. Marcus R, Davies A, Ando K, Klapper W, Opat S, Owen C, et al. Obinutuzumab for the first-line treatment of follicular lymphoma. N Engl J Med. 2017 Oct 5;377(14):1331-44. doi: 10.1056/NEJMoa1614598.
3. Bachy E, Seymour JF, Feugier P, Offner F, López-Guillermo A, Belada D, et al. Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: long-term results of the PRIMA study. J Clin Oncol. 2019 Nov;37(31):2815-24. doi: 10.1200/JCO.19.01073.
4. Townsend W, Hiddemann W, Buske C, Cartron G, Cunningham D, Dyer MJS, et al. Obinutuzumab versus rituximab immunochemotherapy in previously untreated iNHL: final results from the GALLIUM study. HemaSphere. 2023 Jul;7(7):e919. doi: 10.1097/HS9.0000000000000919.
5. Kahl BS, Jegede OA, Peterson C, Swinnen LJ, Habermann TM, Schuster SJ, et al. Long-term follow-up of the RESORT study (E4402): a randomized phase III comparison of two different rituximab dosing strategies for low-tumor burden follicular lymphoma. J Clin Oncol. 2024 Mar 1;42(7):774-8. doi: 10.1200/JCO.23.01912.
6. Schulz H, Bohlius J, Skoetz N, Trelle S, Kober T, Reiser M, et al. Chemotherapy plus rituximab versus chemotherapy alone for B-cell non-Hodgkin's lymphoma. Cochrane Database Syst Rev. 2007 Oct 17;2007(4):CD003805. doi: 10.1002/14651858.CD003805.pub2.
7. Tan D, Horning SJ, Hoppe RT, Levy R, Rosenberg SA, Sigal BM, et al. Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. Blood. 2013 Aug 8;122(6):981-7. doi: 10.1182/blood-2013-03-491514.
8. Casulo C, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: an analysis from the national lymphocare study. J Clin Oncol. 2015 Aug 10;33(23):2516-22. doi: 10.1200/JCO.2014.59.7534.
9. Jurinovic V, Kridel R, Staiger AM, Szczepanowski M, Horn H, Dreyling MH, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. Blood. 2016 Aug 25;128(8):1112-20. doi: 10.1182/blood-2016-05-717355.
10. Maurer MJ, Bachy E, Ghesquières H, Ansell SM, Nowakowski GS, Thompson CA, et al. Early event status informs subsequent outcome in newly diagnosed follicular lymphoma. Am J Hematol. 2016 Nov;91(11):1096-101. doi: 10.1002/ajh.24492.
11. Casulo C, Dixon JG, Le-Rademacher J, Hoster E, Hochster HS, Hiddemann W, et al. Validation of POD24 as a robust early clinical end point of poor survival in FL from 5225 patients on 13 clinical trials. Blood. 2022 Mar 17;139(11):1684-93. doi: 10.1182/blood.2020010263.
12. Hori Y, Hosoi H, Hiroi T, Wan K, Murata S, Morimoto M, et al. Utilizing clinical transformation criteria for prognostic stratification in follicular lymphoma prior to initial immunochemotherapy. Hematol Rep. 2024 Oct 4;16(4):612-23. doi: 10.3390/hematolrep16040060.
13. Mozessohn L, Cheung MC, Crump M, Buckstein R, Berinstein N, Imrie K, et al. Chemoimmunotherapy resistant follicular lymphoma: predictors of resistance, association with transformation and prognosis. Leuk Lymphoma. 2014 Nov;55(11):2502-7. doi: 10.3109/10428194.2014.885513.
14. Sarkozy C, Trneny M, Xerri L, Wickham N, Feugier P, Leppa S, et al. Risk factors and outcomes for patients with follicular lymphoma who had histologic transformation after response to first-line immunochemotherapy in the PRIMA trial. J Clin Oncol. 2016 Aug 1;34(22):2575-82. doi: 10.1200/JCO.2015.65.7163.
15. Araujo-Ayala F, Béguelin W. Biology as vulnerability in follicular lymphoma: genetics, epigenetics, and immunogenetics. Blood. 2025 Oct 9;146(15):1759-69. doi: 10.1182/blood.2024026020.
16. Morschhauser F, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S, et al. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. Lancet Oncol. 2020 Nov;21(11):1433-42. doi: 10.1016/S1470-2045(20)30441-1.
17. Shi Y, Gui L, Cheng Y, Wang H, Cao J, Li Y, et al. A phase II multicenter study of abexinostat, an oral histone

deacetylase inhibitor, in patients with relapsed/refractory follicular lymphoma. J Clin Oncol. 2024 Jun;42(16_suppl):7059-7059. doi: 10.1200/JCO.2024.42.16_suppl.7059.
18. Zinzani PL, Mayer J, Flowers CR, Bijou F, De Oliveira AC, Song Y, et al. ROSEWOOD: a phase II randomized study of zanubrutinib plus obinutuzumab versus obinutuzumab monotherapy in patients with relapsed or refractory follicular lymphoma. J Clin Oncol. 2023 Nov 20;41(33):5107-17. doi: 10.1200/JCO.23.00775.
19. Shah NN, Zinzani PL, Wang ML, Nasta SD, Lech-Maranda E, Ogawa Y, et al. Pirtobrutinib, a highly selective, non-Ccvalent (reversible) BTK inhibitor in relapsed/refractory follicular lymphoma: results from the phase 1/2 BRUIN study. Blood. 2023 Nov 2;142(Suppl 1):3026. https://doi.org/10.1182/blood-2023-181309
20. Leonard JP, Trneny M, Izutsu K, Fowler NH, Hong X, Zhu J, et al. AUGMENT: a phase III study of lenalidomide plus rituximab versus placebo plus rituximab in relapsed or refractory indolent lymphoma. J Clin Oncol. 2019 May 10;37(14):1188-99. doi: 10.1200/JCO.19.00010.
21. Morschhauser F, Fowler NH, Feugier P, Bouabdallah R, Tilly H, Palomba ML, et al. Rituximab plus lenalidomide in advanced untreated follicular lymphoma. N Engl J Med. 2018 Sep 6;379(10):934-47. doi: 10.1056/NEJMoa1805104.
22. Linton KM, Vitolo U, Jurczak W, Lugtenburg PJ, Gyan E, Sureda A, et al. Epcoritamab monotherapy in patients with relapsed or refractory follicular lymphoma (EPCORE NHL-1): a phase 2 cohort of a single-arm, multicentre study. Lancet Haematol. 2024 Aug;11(8):e593-605. doi: 10.1016/S2352-3026(24)00166-2.
23. Budde LE, Sehn LH, Matasar M, Schuster SJ, Assouline S, Giri P, et al. Safety and efficacy of mosunetuzumab, a bispecific antibody, in patients with relapsed or refractory follicular lymphoma: a single-arm, multicentre, phase 2 study. Lancet Oncol. 2022 Aug;23(8):1055-65. doi: 10.1016/S1470-2045(22)00335-7.
24. Hutchings M, Morschhauser F, Iacoboni G, Carlo-Stella C, Offner FC, Sureda A, et al. Glofitamab, a novel, bivalent CD20-targeting T-cell-engaging bispecific antibody, induces durable complete remissions in relapsed or refractory B-cell lymphoma: a phase I trial. J Clin Oncol. 2021 Jun 20;39(18):1959-70. doi: 10.1200/JCO.20.03175.
25. Bannerji R, Arnason JE, Advani RH, Brown JR, Allan JN, Ansell SM, et al. Odronextamab, a human CD20×CD3 bispecific antibody in patients with CD20-positive B-cell malignancies (ELM-1): results from the relapsed or refractory non-Hodgkin lymphoma cohort in a single-arm, multicentre, phase 1 trial. Lancet Haematol. 2022 May;9(5):e327-39. doi: 10.1016/S2352-3026(22)00072-2.
26. Neelapu SS, Chavez JC, Sehgal AR, Epperla N, Ulrickson M, Bachy E, et al. Three-year follow-up analysis of axicabtagene ciloleucel in relapsed/refractory indolent non-Hodgkin lymphoma (ZUMA-5). Blood. 2024 Feb 8;143(6):496-506. doi: 10.1182/blood.2023021243.
27. Fowler NH, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, et al. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. Nat Med. 2022 Feb;28(2):325-32. doi: 10.1038/s41591-021-01622-0.
28. Morschhauser F, Dahiya S, Palomba ML, Martin Garcia-Sancho A, Reguera Ortega JL, Kuruvilla J, et al. Lisocabtagene maraleucel in follicular lymphoma: the phase 2 TRANSCEND FL study. Nat Med. 2024 Aug;30(8):2199-207. doi: 10.1038/s41591-024-02986-9.
29. Mehta A, Popplewell L, Collins GP, Smith SM, Flinn IW, Bartlett NL, et al. Magrolimab plus rituximab in relapsed/refractory indolent non-Hodgkin lymphoma: 3-year follow-up of a phase 1/2 trial. Blood Adv. 2024 Sep 3;8(22):5855-63. doi: 10.1182/bloodadvances.2024013277.
30. Link BK, Maurer MJ, Nowakowski GS, Ansell SM, Macon WR, Syrbu SI, et al. Rates and outcomes of follicular lymphoma transformation in the immunochemotherapy era: a report from the University of Iowa/MayoClinic Specialized Program of Research Excellence Molecular Epidemiology Resource. J Clin Oncol. 2013 Sep 10;31(26):3272-8. doi: 10.1200/JCO.2012.48.3990.
31. Wagner-Johnston ND, Link BK, Byrtek M, Dawson KL, Hainsworth J, Flowers CR, et al. Outcomes of transformed follicular lymphoma in the modern era: a report from the National LymphoCare Study (NLCS). Blood. 2015 Aug 13;126(7):851-7. doi: 10.1182/blood-2015-01-621375.
32. Marcus R, Imrie K, Solal-Celigny P, Catalano JV, Dmoszynska A, Raposo JC, et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. J Clin Oncol. 2008 Oct 1;26(28):4579-86. doi: 10.1200/JCO.2007.13.5376.
33. Jurinovic V, Passerini V, Oestergaard MZ, Knapp A, Mundt K, Araf S, et al. Evaluation of the m7-FLIPI in patients with follicular lymphoma treated within the Gallium trial: *EZH2* mutation status may be a predictive marker for differential efficacy of chemotherapy. Blood. 2019 Nov 13;134(Suppl 1):122. https://doi.org/10.1182/blood-2019-130208
34. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for *bcl*-2 and a hybrid *bcl*-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell. 1986 Oct 10;47(1):19-28. doi: 10.1016/0092-8674(86)90362-4.
35. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 1985 Jun 21;228(4706):1440-3. doi: 10.1126/science.3874430.
36. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A. Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. N Engl J Med. 1982 Nov 11;307(20):1231-6. doi: 10.1056/NEJM198211113072002.

37. Graninger WB, Seto M, Boutain B, Goldman P, Korsmeyer SJ. Expression of Bcl-2 and Bcl-2-Ig fusion transcripts in normal and neoplastic cells. J Clin Invest. 1987 Nov 1;80(5):1512-5. doi: 10.1172/JCI113235.
38. Vogler M, Braun Y, Smith VM, Westhoff MA, Pereira RS, Pieper NM, et al. The BCL2 family: from apoptosis mechanisms to new advances in targeted therapy. Signal Transduct Target Ther. 2025 Mar 21;10(1):91. doi: 10.1038/s41392-025-02176-0.
39. Pasqualucci L, Bhagat G, Jankovic M, Compagno M, Smith P, Muramatsu M, et al. AID is required for germinal center-derived lymphomagenesis. Nat Genet. 2008 Jan;40(1):108-12. doi: 10.1038/ng.2007.35.
40. Correia C, Schneider PA, Dai H, Dogan A, Maurer MJ, Church AK, et al. BCL2 mutations are associated with increased risk of transformation and shortened survival in follicular lymphoma. Blood. 2015 Jan 22;125(4):658-67. doi: 10.1182/blood-2014-04-571786.
41. Rohlin A, Wernersson J, Engwall Y, Wiklund L, Björk J, Nordling M. Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques. Hum Mutat. 2009;30(6):1012-20. doi: 10.1002/humu.20980.
42. Correia C, Maurer MJ, McDonough SJ, Schneider PA, Ross PE, Novak AJ, et al. Relationship between BCL2 mutations and follicular lymphoma outcome in the chemoimmunotherapy era. Blood Cancer J. 2023 May 17;13(1):81. doi: 10.1038/s41408-023-00847-1.
43. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJB, et al. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood. 1995 May 1;85(9):2528-36.
44. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutation of EZH2 (Y641) in follicular and diffuse large B-cell lymphomas of germinal center origin. Nat Genet. 2010 Feb;42(2):181-5. doi: 10.1038/ng.518.
45. Shilatifard A. Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Curr Opin Cell Biol. 2008 Jun 1;20(3):341-8. doi: 10.1016/j.ceb.2008.03.019.
46. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011 Aug;476(7360):298-303. doi: 10.1038/nature10351.
47. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011 Mar 10;471(7337):189-95. doi: 10.1038/nature09730.
48. Okosun J, Bödör C, Wang J, Araf S, Yang CY, Pan C, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. Nat Genet. 2014 Feb;46(2):176-81. doi: 10.1038/ng.2856.
49. Hilton LK, Ngu HS, Collinge B, Dreval K, Ben-Neriah S, Rushton CK, et al. Relapse timing is associated with distinct evolutionary dynamics in diffuse large B-cell lymphoma. J Clin Oncol. 2023 Sep 1;41(25):4164-77. doi: 10.1200/JCO.23.00570.
50. Pasqualucci L, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, et al. Genetics of follicular lymphoma transformation. Cell Rep. 2014 Jan 16;6(1):130-40. doi: 10.1016/j.celrep.2013.12.027.
51. Bouska A, Zhang W, Gong Q, Iqbal J, Scuto A, Vose J, et al. Combined copy number and mutation analysis identifies oncogenic pathways associated with transformation of follicular lymphoma. Leukemia. 2017 Jan;31(1):83-91. doi: 10.1038/leu.2016.175.
52. Kridel R, Chan FC, Mottok A, Boyle M, Farinha P, Tan K, et al. Histological transformation and progression in follicular lymphoma: a clonal evolution study. PLoS Med. 2016 Dec 13;13(12):e1002197. doi: 10.1371/journal.pmed.1002197.
53. Wright GW, Huang DW, Phelan JD, Coulibaly ZA, Roulland S, Young RM, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. Cancer Cell. 2020 Apr;37(4):551-568.e14. doi: 10.1016/j.ccell.2020.03.015.
54. Chakraborty S, Martines C, Porro F, Fortunati I, Bonato A, Marija Dimishkovska, et al. B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation. Blood. 2021 Sep 23;138(12):1053-66. doi: 10.1182/blood.2020008276.
55. Parry EM, Leshchiner I, Guièze R, Johnson C, Tausch E, Parikh SA, et al. Evolutionary history of transformation from chronic lymphocytic leukemia to Richter syndrome. Nat Med. 2023 Jan;29(1):158-69. doi: 10.1038/s41591-022-02113-6.
56. Martines C, Chakraborty S, Felician G, Hofmann K, Blasutig S, Negara I, et al. Richter syndrome-associated genetic lesions in TP53 and the cell cycle inhibitors CDKN2A and CDKN2B induce transformation of a CD5+ B cell subset characterized by a restricted IGHV repertoire, autoreactivity and BCR dependence. Blood. 2024 Nov 5;144(Suppl 1):759. https://doi.org/10.1182/blood-2024-207095
57. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med. 2018 May;24(5):679-90. doi: 10.1038/s41591-018-0016-8.
58. Chapuy B, Wood T, Stewart C, Dunford A, Wienand K, Khan SJ, et al. DLBclass: a probabilistic molecular classifier to guide clinical investigation and practice in diffuse large B-cell lymphoma. Blood. 2025 May 1;145(18):2041-2055. doi: 10.1182/blood.2024025652.
59. Stokes ME, Wenzl K, Huang CC, Ortiz M, Hsu CC, Maurer MJ, et al. Transcriptomic classification of diffuse large B-cell lymphoma identifies a high-risk activated B-cell-like subpopulation with targetable MYC dysregulation. Nat Commun. 2024 Aug 8;15(1):6790. doi: 10.1038/s41467-024-50830-y.

60. Negara I, Tomuleasa C, Buruiana S, Efremov DG. Molecular subtypes and the role of TP53 in diffuse large B-cell lymphoma and Richter syndrome. Cancers (Basel). 2024 Jan;16(12):2170. doi: 10.3390/cancers16122170.
61. Sarkozy C, Wu S, Takata K, Aoki T, Neriah SB, Milne K, et al. Integrated single cell analysis reveals co-evolution of malignant B cells and tumor micro-environment in transformed follicular lymphoma. Cancer Cell. 2024 Jun 10;42(6):1003-1017.e6. doi: 10.1016/j.ccell.2024.05.011.
62. Dreval K, Hilton LK, Cruz M, Shaalan H, Ben-Neriah S, Boyle M, et al. Genetic subdivisions of follicular lymphoma defined by distinct coding and noncoding mutation patterns. Blood. 2023 Aug 10;142(6):561-73. doi: 10.1182/blood.2022018719.
63. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015 Sep;16(9):1111-22. doi: 10.1016/S1470-2045(15)00169-2.
64. Russler-Germain DA, Krysiak K, Ramirez C, Mosior M, Watkins MP, Gomez F, et al. Mutations associated with progression in follicular lymphoma predict inferior outcomes at diagnosis: Alliance A151303. Blood Adv. 2023 Sep 26;7(18):5524-39. doi: 10.1182/bloodadvances.2023010779.
65. Wu W, Bruscaggin A, Valera A, Flospergher E, Bonfiglio F, Spina V, et al. Evaluation of the different stratification models for POD24 prediction in patients with follicular lymphoma. Blood. 2020 Nov 5;136(Suppl 1):24-5. https://doi.org/10.1182/blood-2020-142000
66. Lockmer S, Ren W, Brodtkorb M, Østenstad B, Wahlin BE, Pan-Hammarström Q, et al. M7-FLIPI is not prognostic in follicular lymphoma patients with first-line rituximab chemo-free therapy. Br J Haematol. 2020;188(2):259-67. doi: 10.1111/bjh.16159.
67. Rodríguez-Sevilla JJ, Fernández-Rodríguez C, Bento L, Diez-Feijóo R, Pinzón S, Gibert J, et al. Evaluation of 4 prognostic indices in follicular lymphoma treated in first line with immunochemotherapy. Blood Adv. 2023 Apr 25;7(8):1606-14. doi: 10.1182/bloodadvances.2022007949.
68. Wright GW, Ramsower CA, Cerhan JR, Novak AJ, Link BK, Maurer MJ, et al. Prediction of early disease progression at 24 months (POD24) using pre-treatment biopsies from patients with follicular lymphoma (FL) treated with immunochemotherapy (IC). Blood. 2022 Nov 15;140(Supp 1):6426-7. https://doi.org/10.1182/blood-2022-160351
69. Freeman CL, Kridel R, Moccia AA, Savage KJ, Villa DR, Scott DW, et al. Early progression after bendamustine-rituximab is associated with high risk of transformation in advanced stage follicular lymphoma. Blood. 2019 Aug 29;134(9):761-4. doi: 10.1182/blood.2019000258.
70. Li X, Wang CY. From bulk, single-cell to spatial RNA sequencing. Int J Oral Sci. 2021 Nov 15;13(1):36. doi: 10.1038/s41368-021-00146-0.
71. Haebe S, Shree T, Sathe A, Day G, Czerwinski DK, Grimes SM, et al. Single-cell analysis can define distinct evolution of tumor sites in follicular lymphoma. Blood. 2021 May 27;137(21):2869-80. doi: 10.1182/blood.2020009855.
72. Araf S, Wang J, Korfi K, Pangault C, Kotsiou E, Rio-Machin A, et al. Genomic profiling reveals spatial intra-tumor heterogeneity in follicular lymphoma. Leukemia. 2018;32(5):1261-5. doi: 10.1038/s41375-018-0043-y.
73. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AFM, Esfahani MS, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2016 Nov 9;8(364):364ra155. doi: 10.1126/scitranslmed.aai8545.
74. Andor N, Simonds EF, Czerwinski DK, Chen J, Grimes SM, Wood-Bouwens C, et al. Single-cell RNA-Seq of follicular lymphoma reveals malignant B-cell types and coexpression of T-cell immune checkpoints. Blood. 2019 Mar 7;133(10):1119-29. doi: 10.1182/blood-2018-08-862292.
75. Attaf N, Cervera-Marzal I, Gil L, Dong C, Navarro JM, Spinelli L, et al. Single-cell RNA sequencing identifies a pseudo-immune differentiation axis as the main source of functional heterogeneity in follicular lymphoma B-cells. Blood. 2019 Nov 13;134(Suppl 1):548. https://doi.org/10.1182/blood-2019-125718
76. Radtke AJ, Roschewski M. The follicular lymphoma tumor microenvironment at single-cell and spatial resolution. Blood. 2024 Mar 21;143(12):1069-79. doi: 10.1182/blood.2023020999.
77. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004 Nov 18;351(21):2159-69. doi: 10.1056/NEJMoa041869.
78. Han G, Deng Q, Marques-Piubelli ML, Dai E, Dang M, Ma MCJ, et al. Follicular lymphoma microenvironment characteristics associated with tumor cell mutations and MHC class II expression. Blood Cancer Discov. 2022 Sep 6;3(5):428-43. doi: 10.1158/2643-3230.BCD-21-0075.
79. Radtke AJ, Kandov E, Lowekamp B, Speranza E, Chu CJ, Gola A, et al. IBEX: A versatile multiplex optical imaging approach for deep phenotyping and spatial analysis of cells in complex tissues. Proc Natl Acad Sci USA. 2020 Dec 29;117(52):33455-65. doi: 10.1073/pnas.2018488117.
80. Radtke AJ, Postovalova E, Varlamova A, Bagaev A, Sorokina M, Kudryashova O, et al. Multi-omic profiling of follicular lymphoma reveals changes in tissue architecture and enhanced stromal remodeling in high-risk patients. Cancer Cell. 2024 Mar 11;42(3):444-463.e10. doi: 10.1016/j.ccell.2024.02.001.

https://doi.org/10.52645/MJHS.2026.2.12

UDC: 616.995.42

REVIEW ARTICLE

Tick-borne mix infection diagnosis, challenges, and current practices

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ABSTRACT

Introduction. Tick-borne infections (TBIs) are increasingly recognized as a public health concern in North America and Europe, with Lyme disease being the most notable. The Centers for Disease Control and Prevention (CDC) acknowledges that official statistics likely underestimate the true incidence of TBIs due to diagnostic challenges and underreporting. Co-infections, where multiple pathogens are transmitted through a single tick bite or multiple bites, complicate diagnosis and treatment, leading to more severe symptoms and longer illness durations. Studies indicate a significant percentage of Lyme disease patients also have co-infections, with babesiosis being a common co-infection.

Materials and methods. A comprehensive narrative literature review was conducted using PubMed and Scopus, resulting in 52 manuscripts. Additional reports from the CDC and European Centre for Disease Prevention and Control (ECDC), as well as relevant academic books, were included to meet the study's objectives. The Elicit platform was utilized to enhance reference identification and information synthesis.

Results. The paper provides an overview of tick-borne co-infections, emphasizing the diagnostic challenges posed by overlapping and nonspecific symptoms. It discusses various diseases, including Lyme disease, babesiosis, anaplasmosis, ehrlichiosis, Rocky Mountain spotted fever, and tick-borne encephalitis, detailing their causative organisms, vectors, clinical features, and common co-infections. The review critically examines diagnostic methods such as serological tests, molecular tests, and blood smears, highlighting issues like the „window period" false negatives/positives, and differentiating active from past infections. It also explores emerging technologies and biomarkers, including multiplex assays and next-generation sequencing, which enhance detection capabilities but face challenges in data analysis and standardization.

Conclusions. Accurate diagnosis is crucial to manage these infections effectively, particularly in vulnerable populations. The rise in co-infections and inadequate testing presents a significant public health challenge, necessitating improved surveillance and diagnostic approaches.

Keywords: tick-borne infections, coinfections, TBIs, diagnosis, challenges.

Cite this article: Sofronie O, Balan G. Tick-borne mix infection diagnosis, challenges, and current practices. Mold J Health Sci. 2026;13(2):95-103. https://doi.org/10.52645/MJHS.2026.2.12.

Manuscript received: 8.09.2025

Accepted for publication: 18.02.2026

Published: 15.06.2026

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

The prevalence of tick-borne co-infections in humans, both globally and specifically in Moldova, is largely unknown or poorly understood. The true number of tick-borne disease cases may be much higher than reported, due to underreporting and lack of testing, as many patients are not tested, especially if symptoms appear late or if they do not recall a previous tick bite.

The research hypothesis

Patients with tick-borne co-infections are at higher risk of delayed

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or inaccurate diagnoses and more severe clinical outcomes compared to patients with single tick-borne infections, due to overlapping symptoms and limitations of current diagnostic methods.

The novelty added by the manuscript to the already published scientific literature

It consolidates disparate information on diagnostic challenges, current practices, and emerging technologies in the field of tick-borne co-infections into a single structured document; it also explicitly highlights what is not yet known, potentially guiding future research directions.

Introduction

Tick-borne infections are a growing public health issue in North America and Europe, caused by various pathogens (bacteria, viruses, protozoa) transmitted through tick bites [1]. The Centers for Disease Control and Prevention (CDC) reports an increasing trend in these infections, particularly Lyme disease, but admits that official statistics likely underestimate the true incidence due to diagnostic challenges and underreporting [2].

Managing tick-borne infections (TBIs) is complicated by co-infections, where a single tick bite can transmit multiple pathogens or multiple ticks can infect a person [3]. Epidemiological data show that 4% to 45% of Lyme disease patients also have co-infections, which often lead to more severe symptoms and longer illness duration [4]. A recent study found that 42% of babesiosis patients were co-infected with another tick-borne disease, with Lyme disease being the most common co-infection in 41% of those cases [5].

Accurate and timely diagnosis of tick-borne coinfections is essential for effective patient management, reducing morbidity, and preventing severe, potentially life-threatening complications, particularly in vulnerable groups such as older adults or immunocompromised individuals [4]. The presence of multiple pathogens significantly complicates the clinical picture, often resulting in overlapping symptoms that may mask underlying infections. Failure to identify all coinfecting agents may result in inadequate or inappropriate treatment, contributing to persistent symptoms and adverse patient outcomes [6].

The rising prevalence of tick-borne co-infections, along with inadequate testing, poses a significant public health challenge. Many patients with co-infections, which worsen disease severity, remain undiagnosed, leading to an underestimation of the true impact of tick-borne infections in clinical practice and surveillance data. This situation calls for a more comprehensive approach to surveillance and diagnosis that accounts for multiple pathogens, ensuring better allocation of public health resources and improved patient care [7,8].

Prominent bacterial agents include *Borrelia burgdorferi*, the causative agent of Lyme disease, and other *Borrelia* species such as *Borrelia mayonii* and *Borrelia miyamotoi*, which cause Lyme-like illness and relapsing fever, respectively. *Anaplasma phagocytophilum* is responsible for anaplasmosis, while various *Ehrlichia* species cause ehrlichiosis. *Rickettsia rickettsii* is the pathogen responsible for Rocky Mountain Spotted Fever (RMSF), while *Rickettsia conorii* is the causative agent of Boutonneuse fever. Other recognized bacterial infections include Southern Tick-Associated Rash Illness (STARI) and tularemia. Among protozoan pathogens, *Babesia* species, particularly *Babesia microti*, are significant causes of babesiosis [1]. Important viral agents include the Tick-borne encephalitis virus (TBEV), Powassan virus, and Crimean-Congo hemorrhagic fever virus [9].

Environmental factors like temperature and humidity affect tick populations and their spread, while invasive plants create favorable conditions for ticks, hosts and pathogens [2]. Co-infections in ticks vary by region, with specific combinations such as *Borrelia burgdorferi-Babesia microti* and *Borrelia burgdorferi-Anaplasma phagocytophilum* being more common in the U.S. and others like *Babesia-Anaplasma phagocytophilum* and *Rickettsia-Anaplasma phagocytophilum* in Europe [3].

Materials and methods

This review was designed as a structured narrative synthesis of current knowledge about tick-borne co-infections, their clinical significance, and the diagnostic challenges they present. To ensure reproducibility and comprehensive coverage, a systematic approach was applied to the identification, selection, and analysis of the relevant literature. A literature search was conducted in PubMed and Scopus between January and June 2025, using a combination of keywords and MeSH terms such as „tick-borne infections," „co-infection," „mixed infection," „Lyme disease," „babesiosis," „anaplasmosis," „ehrlichiosis," „tick-borne encephalitis," „diagnosis," „multiplex assays," and „next-generation sequencing". Boolean operators were used to refine the queries, and the reference lists of relevant articles and reviews were also reviewed to identify additional sources.

The initial search yielded 52 manuscripts. After removing duplicates, titles and abstracts were screened, and potentially relevant studies were assessed in full text. To complement the database search, official reports from the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC), relevant academic textbooks, and additional records retrieved through the artificial intelligence-based Elicit platform were included. The selection was guided by predefined el-

igibility criteria: peer-reviewed articles and authoritative reports published in English between 2000 and 2025 that addressed the epidemiology, clinical aspects, or diagnostic approaches of tick-borne co-infections in humans were included, while single case reports without broader implications, studies conducted exclusively in animals, and records without accessible full text were excluded. Screening and selection of studies were performed independently by two reviewers, and discrepancies were resolved through discussion to ensure consistency.

From each eligible publication, data were extracted on the type of infection studied, the geographical setting, the diagnostic methods used, and the main clinical or epidemiological findings. The information was thematically synthesized into categories that reflected the study objectives: epidemiology and burden of co-infections, clinical characteristics and outcomes, current diagnostic practices, and advances in emerging technologies such as multiplex testing and next-generation sequencing. Quantitative results, such as prevalence ranges of co-infections or sensitivity and specificity values of diagnostic methods, were reported descriptively, as provided in the original studies.

Overall, the described methodology combines a systematic literature search, clear inclusion/exclusion criteria, independent dual screening, and descriptive synthesis of quantitative results to produce a reproducible and comprehensive narrative summary of the literature on tick-borne co-infections in humans and their diagnoses.

Results

Tick-borne co-infections present a complex clinical picture due to nonspecific and overlapping symptoms, making diagnosis challenging. Patients often experience common symptoms like fever, fatigue, joint and muscle pain, and headaches, which can resemble other viral illnesses or autoimmune disorders, leading to misdiagnosis or delays in treatment [10]. The erythema migrans rash, indicative of early Lyme disease, is not always present, occurring in only 70–80% of cases. Its absence can complicate the initial diagnosis of tick-borne diseases, especially when co-infections are present [9].

With Lyme-Babesia co-infection, patients often present with a greater number and severity of symptoms than those with Lyme disease alone. These may include increased fatigue, headaches, profuse sweating, chills, loss of appetite, emotional lability, nausea, conjunctivitis, and splenomegaly [11].

Co-infection with *Babesia* is also associated with a significantly longer duration of illness in patients with Lyme disease. One study observed that 50% of co-infected patients remained symptomatic for three months or more, compared with only 4% of patients with Lyme disease alone [11]. Rash is not a common symptom of babesiosis, and its presence may suggest co-infection with Lyme disease [12].

Symptoms of anaplasmosis typically include headache, fever, chills, malaise, and muscle aches, with some infections being asymptomatic [13]. The presence of multiple pathogens can lead to overlapping or intensified symptoms, greatly confusing physicians and significantly delaying appropriate treatment [14]. The clinical spectrum of babesiosis alone can range from asymptomatic to severe multiple organ failure, with severity often dependent on the immunocompetence of the host [12].

Polish studies documented lower co-infection rates, with *Borrelia* species co-infections with *A. phagocytophilum* and *Babesia* spp. each occurring at 4.2%. However, in patients with tick-borne encephalitis (TBE), 27% were co-infected with *Borrelia* species, 10.9% with *A. phagocytophilum*, and 0.9% with *Babesia* spp., with triple co-infections (TBE-*Borrelia*-*Anaplasma*) occurring in 2.7% of patients [15].

These co-infections can complicate diagnosis and treatment, as they may exacerbate symptoms or mimic other tick-borne diseases [16]. Co-infections between *Borrelia burgdorferi* s.l. and TBEV are particularly notable, especially in patients presenting with high fever, erythema migrans, or neurological symptoms [17].

An overview of common tick-borne pathogens, detailing the diseases they cause and their main tick vectors, is provided in Table 1. It also highlights the typical clinical features associated with each infection and mentions their common co-infection partners, illustrating the diverse and interconnected nature of tick-borne diseases.

Table 1. Common tick-borne pathogens, associated diseases, and primary vectors

Disease name	Causative organism	Primary tick vector(s)	Key clinical features	Common co-infection organism
Lyme Disease	*Borrelia burgdorferi, B. mayonii, B. miyamotoi*	*Ixodes scapularis, I. pacificus, I. ricinus, I. persulcatus*	Erythema migrans rash (70-80%), fever, chills, fatigue, headache, joint pain, neurological issues	*Babesia microti, Anaplasma phagocytophilum*
Babesiosis	*Babesia microti, B. duncani, B. venatorum, B. divergens*	*Ixodes scapularis, I. pacificus, I. ricinus*	Fever, chills, sweats, malaise, fatigue, headache, splenomegaly, anemia, thrombocytopenia (rash uncommon, suggests co-infection)	*Borrelia burgdorferi, Anaplasma phagocytophilum*
Anaplasmosis	*Anaplasma phagocytophilum*	*Ixodes scapularis, I. pacificus*	Fever, chills, malaise, headache, myalgia, cytopenia, liver enzyme abnormalities (rash uncommon, may occur with Borrelia co-infection)	*Borrelia burgdorferi, Babesia* spp.
Ehrlichiosis	*Ehrlichia chaffeensis, E. ewingii*	*Amblyomma americanum, Dermacentor variabilis*	Fever, headache, myalgia, fatigue, cytopenia, liver enzyme abnormalities (rash less common)	*Borrelia burgdorferi, Anaplasma phagocytophilum*

Rocky Mountain Spotted Fever (RMSF)	*Rickettsia rickettsii*	*Dermacentor variabilis, Rhipicephalus sanguineus*	Fever, headache, rash (maculopapular or petechial, often on extremities), myalgia, nausea, vomiting	Other *Rickettsia* spp. (cross-reactivity in tests)
Tick-borne Encephalitis	Tick-borne Encephalitis Virus (TBEV)	*Ixodes ricinus, I. persulcatus, I. ovatus*	Neurological phase: higher fever, severe headache, stiff neck (meningeal signs), confusion or altered mental status, sensitivity to light, dizziness, lack of coordination, tremors, seizures, weakness or paralysis (especially of the limbs or facial nerves)	*Borrelia burgdorferi, Anaplasma phagocytophilum, Babesia* spp.
Powassan Encephalitis	Powassan virus	*Ixodes scapularis, I. cookei*	Fever, headache, vomiting, weakness, confusion, seizures, memory loss, encephalitis	None specified

Limitations of current standard diagnostic methods. Recent studies have highlighted the challenges in diagnosing tick-borne mixed infections. Early diagnosis can be difficult without laboratory confirmation [18]. However, researchers have developed predictive models using clinical and laboratory parameters to differentiate between mixed infections and mono-infections with excellent accuracy [19]. These models incorporate factors such as fever, intoxication syndrome score, and various blood count parameters. To address the limitations of current diagnostic methods, a multiplex, array-based assay called TBD-Serochip has been developed, capable of discriminating antibody responses to eight major tick-borne pathogens [20]. This platform allows for accurate identification of specific immunodominant epitopes, enhancing diagnostic accuracy. Despite these advancements, challenges remain in the molecular and serologic diagnosis of tick-borne co-infections, primarily due to limitations in sensitivity, specificity, and the capacity to include multiple agents in a single assay [6].

Serological tests (ELISA, Western Blot, indirect immunofluorescence assay - IFA). Serological tests primarily detect the host antibody response, which typically takes several weeks to develop. This „window period" often results in false-negative results during the crucial early stages of infection, when treatment is most effective [21]. In contrast, antibodies can persist for years after infection, making it difficult to differentiate between an acute, active infection and a previous exposure. Confirmation of a recent infection usually requires demonstration of a fourfold increase in antibody titers between acute and convalescent samples [22].

Intrathecal antibody production is the gold standard for diagnosing Lyme neuroborreliosis in Europe, particularly with *B. garinii* linked to neurological cases. However, interpreting results is challenging due to the absence of a definitive gold standard, varying case definitions, different assays, and limited comparisons among labs. The sensitivity of detecting intrathecal antibody production in acute cases is approximately 50% [23]. Cross-reactivity with antibodies from other infections is a significant problem, particularly in rickettsial diseases, where serological tests may fail to differentiate between the highly lethal RMSF and similar less severe infections [24]. Many laboratory-developed tests for TBIs are not approved by regulatory bodies, and diagnostic methods are not standardized across clinical laboratories, leading to inconsistencies [25].

Molecular tests (polymerase chain reaction - PCR). Although PCR offers greater specificity and directly indicates active infection by detecting pathogen DNA/RNA, its sensitivity may be limited in early or late infection due to low pathogen burden or transient presence in the bloodstream [6]. In some infections, PCR results may remain positive for months or even years after treatment, complicating the assessment of active infection versus residual genetic material [26].

PCR for detecting *Rickettsia* is a powerful tool but has limitations, such as the risk of false negatives in early infections or low bacterial loads. Sensitivity can vary based on the timing of sample collection and the specific *Rickettsia* species, and PCR assays may struggle to differentiate between closely related species [27,28].

PCR methods present significant advancements in diagnosing Lyme disease, particularly through digital PCR, which enhances sensitivity by detecting *Borrelia burgdorferi* DNA even at low levels. This technique effectively addresses challenges related to low spirochete counts by utilizing larger sample volumes and pre-examination processing, with platelet-rich plasma being particularly useful [29]. However, standardization is essential due to variability in results among laboratories. While PCR is highly specific and sensitive, its effectiveness is influenced by several factors, highlighting the need for standardized protocols to ensure consistent results [30].

Molecular methods are effective for detecting tick-borne encephalitis virus RNA in ticks and clinical samples. Nested RT-PCR targeting the NS5 gene and real-time PCR targeting the E gene have been developed for epidemiological surveillance and strain identification [31,32]. However, the diagnostic utility of PCR in clinical samples is limited, as positive results are typically only obtained early in the disease, making serological diagnosis more reliable for patient care [33].

Blood smear examination (for babesiosis, anaplasmosis). Direct microscopic examination of peripheral blood smears can diagnose babesiosis and anaplasmosis, but it is a laborious method that requires a highly skilled specialist. In early infection, the parasite load may be low, requiring examination of multiple smears to increase the sensitivity of detection. Morphological similarities can lead to misinterpretations, such as confusing ring forms of *Babesia* with *Plasmodium falciparum* [12].

Overview of standard methods for individual infections. The standard diagnostic approach for Lyme disease involves

a two-tiered serological testing method, typically starting with an ELISA followed by a confirmatory Western blot. Modified tests like multi-antigen ELISA or C6 ELISA are also used [34]. However, serological tests can be misleading, as they may be negative early in the infection and can remain positive for years, complicating the distinction between active and past infections [35].

Babesiosis diagnosis is primarily made by identifying the *Babesia* organism in a Giemsa- or Wright-stained blood smear, looking for ring shapes and tetrads (Maltese crosses). Due to potentially low parasite loads in early infection, multiple smears are recommended for better detection. PCR testing provides higher sensitivity than smears, while serology, especially indirect immunofluorescent antibody testing, can confirm the diagnosis but requires a fourfold increase in titers to indicate a recent infection [9].

Anaplasmosis diagnosis involves various methods such as culture, histopathology, PCR, and serology. A key diagnostic indicator is the presence of morulae, which are characteristic intracytoplasmic aggregates found in neutrophils, detectable in 20–80% of symptomatic patients, particularly during the first week of infection. Confirmation of the diagnosis often relies on serological tests or blood smear examination [36].

For ehrlichiosis, similar to anaplasmosis, the diagnosis is frequently confirmed by serologies or blood smears. While serology can confirm past infection, PCR is generally more useful for detecting active infection. Although PCR tests are available for *Ehrlichia*, their widespread accessibility and speed for real-time clinical decisions remain limitations [37].

Diagnosis of tick-borne encephalitis virus (TBEV) primarily relies on serological methods, as viral RNA is seldom detectable during neurological symptoms. TBEV-specific IgM ELISA tests in serum and cerebrospinal fluid (CSF) demonstrate high sensitivity and specificity (94-100%) for diagnosing tick-borne encephalitis in humans. However, IgG ELISAs may lack specificity due to potential cross-reactions with other flaviviruses and require confirmatory virus neutralization. The presence of intrathecal TBEV IgG synthesis can support the diagnosis in chronic cases, with about 55% of TBE cases showing this response. In immunocompromised patients, detecting TBEV RNA in CSF might be necessary for accurate diagnosis [38–40].

The diagnosis of Rocky Mountain spotted fever, like other tick-borne rickettsial diseases, is usually based on a combination of clinical symptoms and epidemiologic evidence. Serological tests, particularly the indirect immunofluorescence test, are considered the gold standard for rickettsial infections. PCR testing can confirm an active infection, but a negative PCR result does not definitively rule out RMSF [41].

Emerging diagnostic technologies and new biomarkers. Recent advancements in multiplex testing platforms have improved the diagnosis of tick-borne infections. New array-based assays can differentiate antibody responses to eight major pathogens, while real-time multiplex PCR assays enable quick and cost-effective screening for *Borrelia burgdorferi, Anaplasma phagocytophilum*, and *Babesia microti*. Additionally, a customizable multiplex protein microarray enhances sensitivity and specificity by detecting multiple antibodies simultaneously. A multiplex qPCR method has also been developed to efficiently detect *Ehrlichia* spp., *Rickettsia* spp., and *Borrelia* spp. in one reaction. These innovations significantly enhance the molecular diagnosis of tick-borne diseases, facilitating earlier intervention and better patient outcomes [20,42–44].

Beyond direct pathogen detection, research is actively exploring novel host-response biomarkers that could improve the diagnosis and monitoring of tick-borne infections and co-infections. Standard clinical markers, such as cytopenias and liver function test abnormalities, are already recognized as typical laboratory findings in tick-borne diseases and can aid in diagnosis [37]. Studies have investigated various immune biomarkers, including CD57+ and CD19+ lymphocyte counts, CD3%, CD4%, CD4+ Helper T cell count, CD4+/CD8+ ratio, white cell count, and total IgG. Notably, a significant percentage of patients with clinically diagnosed tick-borne infections exhibited low CD57+ counts. Changes in iron studies, specifically transferrin and transferrin saturation percentages, have also shown statistically significant alterations in TBI patients before and after antibiotic treatment, suggesting their potential as diagnostic or prognostic markers [45,46]. For tick-borne encephalitis, specific immunoglobulins, free light chains, metalloproteinases, and cytokines show promise as biomarkers [47].

The latest evolution in sequencing technologies, particularly next-generation sequencing (NGS), has greatly enhanced the detection and characterization of tick-borne pathogens. Techniques such as the TBDCapSeq assay, which utilizes hybridization capture probes, have shown superior sensitivity compared to traditional PCR methods, allowing for the identification of a broader range of pathogens, including previously unknown infections and co-infections [48,49]. Additionally, a 16S rRNA gene PCR followed by NGS has demonstrated effectiveness in detecting tick-borne bacteria in whole blood [50]. Nanopore adaptive sampling (NAS) further improves biosurveillance by enabling real-time enrichment of targeted sequences, facilitating the simultaneous detection of multiple pathogens. These advancements underscore the complexity of microbial communities associated with ticks and emphasize the need to study the entire pathobiome. Despite these promising developments, challenges in data analysis, particularly in understanding complex microbial interactions, remain a significant hurdle [51,52].

A summary comparison is provided in Table 2 on various diagnostic methods used for tick-borne co-infections, including traditional approaches such as blood smears and serology, alongside emerging technologies such as multiplex assays and sequencing. It outlines the strengths, limitations, and optimal use cases for each method, highlighting the continuing advances in the detection of these complex infections [25].

Table 2. Comparison of diagnostic methods for tick-borne coinfections

Diagnostic Method	Primary Pathogens Detected	Key Strengths	Key Limitations	Optimal Use Case
Blood Smear	*Babesia*, *Anaplasma*	Direct visualization of parasites, can assess parasitemia	Requires skilled microscopist, time-consuming, low sensitivity in early infection (low parasite burden), misinterpretation risk (e.g., *Plasmodium*)	Acute babesiosis/ anaplasmosis, initial suspicion
Serology (ELISA/ Western Blot/IFA)	*Borrelia*, *Babesia*, *Anaplasma*, *Ehrlichia*, *Rickettsia,* TBEV	Relatively accessible, can confirm exposure	Low sensitivity in early infection (window period), cannot distinguish active vs. past infection (single positive), variable antibody responses, cross-reactivity, low completion rates for two-step tests	Later stage infection, confirmation of exposure, epidemiological studies
PCR (Molecular Assays)	*Borrelia*, *Babesia*, *Anaplasma*, *Ehrlichia*, *Rickettsia*, *B. miyamotoi*	Direct detection of pathogen DNA/RNA (active infection), high specificity, can detect multiple strains	Sensitivity limited by transient/ low pathogen burden, can remain positive post-treatment, not widely accessible/fast enough for all pathogens (e.g., *Rickettsia*), expensive	Early acute infection, immunocompromised patients, confirmation of active infection
Multiplex Assays (e.g., Protein Microarrays, Chemiluminescent Arrays)	Multiple pathogens (e.g., *Borrelia*, *Babesia*, *Bartonella*, *Anaplasma*, *Ehrlichia*)	Broad-spectrum detection in single run, enhanced diagnostic efficiency, reduced turnaround time, improved sensitivity/specificity for multiple agents, reduced cross-reactivity (specific peptides)	Regulatory hurdles, complex validation for multiple analytes and interactions, may still rely on antibody detection (window period)	Comprehensive screening for co-infections, differential diagnosis of overlapping symptoms
Next-Generation Sequencing (NGS)	Broad range of known and potentially novel pathogens	High-throughput, can detect novel/ unsequenced pathogens, large number of probes, reduces lab equipment needs	High cost, complex data analysis, not yet standard for routine clinical use, still may face challenges with very low pathogen loads	Research, complex/ unresolved cases, pathogen discovery
Novel Biomarker Panels (e.g., Immune markers, Metabolomics, Peptidoglycan fragments)	Host response to infection, PTLDS	Potential for earlier detection, differentiation of active vs. past infection, objective measures for post-treatment syndromes, insight into host-pathogen interaction	Still largely research-based, lack of clinical validation and standardization, complex interpretation, not yet widely available clinically	Research, future diagnostics for early disease and PTLDS, monitoring treatment response

Note: *ELISA - Enzyme-linked immunosorbent assay; IFA - Indirect immunofluorescence assay; PCR - polymerase chain reaction; TBEV - Tick-borne encephalitis virus; PTLDS - Post-Treatment Lyme Disease Syndrome.*

Discussion

The complexities of co-infections in tick-borne diseases highlight significant limitations in current diagnostic tools, which often fail to detect a wide range of pathogens due to the variable presence of microbial DNA/RNA or antigens. This results in missed diagnoses and inadequate treatments, as patients are rarely tested for all potential tick-borne agents. Additionally, the differing transmission rates of pathogens complicate diagnostic approaches. These deficiencies lead to increased healthcare costs and prolonged patient suffering, while also hindering public health efforts and research. There is an urgent need for a shift toward integrated, multi-pathogen testing strategies to improve patient outcomes and control the spread of these diseases [53].

The increasing prevalence and complexity of tick-borne co-infections impose a substantial economic and public health burden. Lyme disease alone is estimated to cost the U.S. healthcare system between $712 million and $1.3 billion annually in direct medical costs, averaging nearly $3,000 per patient in follow-up visits and testing. Patients with Lyme disease and Post-Treatment Lyme Disease Syndrome (PTLDS) incur significantly higher healthcare costs, with one study finding an additional $3,798 in costs compared to those without post-treatment symptoms [54,55]. The challenges in diagnosis and treatment, particularly for co-infections, amplify patient suffering and contribute to these rising healthcare costs.

Tick-borne encephalitis (TBE) presents a significant and increasing burden in many European countries, including Slovenia and Sweden. Studies have used disability-adjusted life years (DALYs) to quantify the burden, with Slovenia reporting 3,450 DALYs (167.8 per 100,000 population) in 2011. Permanent sequalae contribute most to the total burden, emphasizing the importance of vaccination as a preventive strategy [56,57]. In Sweden, a 17-year study revealed that TBE patients had significantly more hospitalizations, specialist outpatient visits, and sick leave days compared to the general population, with differences increasing over time [58]. The true burden of TBE may be underestimated, as shown in Italy's Veneto region, where only 80.8% of cases were reported through mandatory notifications [59].

The Altai region of Russia faces a heightened risk of tick-borne diseases, with spotted fever group rickettsiosis being the most significant, contrary to the national prevalence of Lyme borreliosis. The growing threat is compounded by suboptimal diagnostics, limited treatment options for emerging pathogens, and a lack of vaccines. Mixed infections and poorly studied pathogens further complicate the

landscape of tick-borne diseases, highlighting the need for improved prevention and management strategies [54,60].

Conclusions

Addressing the challenge of tick-borne co-infections requires a comprehensive strategy that includes increased investment in research into advanced diagnostic tools and biomarkers. Optimizing regulatory processes is essential to accelerate the clinical availability of these innovations. Raising public and professional awareness of the complex epidemiology and clinical manifestations of these diseases is also crucial. Promoting a more proactive diagnostic approach enables the early detection of tick-borne pathogens, improving treatment strategies and reducing long-term suffering. This multifaceted effort aims to mitigate the significant public health and economic impact associated with tick-borne co-infections.

Competing interests

None declared.

Contribution of authors

OS designed the study, collected, and analyzed the data. GB critically revised the manuscript and analyzed the data. Both authors reviewed the work critically and approved the final version of the manuscript.

Acknowledgements and funding

The authors declare no external funding.

Ethics approval

No approval was required for this study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Nathavitharana RR, Mitty JA. Diseases from North America: focus on tick-borne infections. Clin Med (Lond). 2015 Feb;15(1):74-7. doi: 10.7861/clinmedicine.14-6-74.
2. National Invasive Species Council, Invasive Species Advisory Committee. The interface between invasive species and the increased incidence of tick-borne diseases, and the implications for federal land managers, adopted May 2, 2019 [Internet]. Washington: NISC; 2019 [cited 2025 Jun 24]. Available from: https://www.doi.gov/sites/doi.gov/files/uploads/tick-borne_disease_white_paper.pdf
3. Rocha SC, Velásquez CV, Aquib A, Al-Nazal A, Parveen N. Transmission cycle of tick-borne infections and co-infections, animal models and diseases. Pathogens. 2022 Nov 8;11(11):1309. doi: 10.3390/pathogens11111309.
4. Feng J, Lin T, Mihalca AD, Niu Q, Oosthuizen MC. Coinfections of Lyme disease and other tick-borne diseases. Front Microbiol. 2023 Feb 9;14:1140545. doi: 10.3389/fmicb.2023.1140545.
5. Penn State Health News [Internet]. 2024 [cited 2025 Jun 24]. Rates of a tick-borne parasitic disease are on the rise. Available from: https://pennstatehealthnews.org/2024/10/rates-of-a-tick-borne-parasitic-disease-are-on-the-rise/
6. Sanchez-Vicente S, Tokarz R. Tick-borne co-infections: challenges in molecular and serologic diagnoses. Pathogens. 2023 Nov;12(11):1371. doi: 10.3390/pathogens12111371.
7. Institute of Medicine (US); Committee on Lyme Disease and Other Tick-Borne Diseases. Surveillance, spectrum, and burden of tick-borne disease, and at-risk populations. In: Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes: Workshop report [Internet]. Washington: National Academies Press; 2011 [cited 2025 Jun 28]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK57009/
8. Brooks C, McNeely CL, Maxwell SP, Thomas KC. Assessing tick-borne disease risk and surveillance: toward a multi-modal approach to diagnostic positioning and prediction. Microorganisms. 2022 Apr;10(4):832. doi: 10.3390/microorganisms10040832.
9. Madison-Antenucci S, Kramer LD, Gebhardt LL, Kauffman E. Emerging tick-borne diseases. Clin Microbiol Rev. 2020 Jan 2;33(2):e00083-18. doi: 10.1128/CMR.00083-18.
10. Choi E, Pyzocha NJ, Maurer DM. Tick-borne illnesses. Curr Sports Med Rep. 2016 Apr;15(2):98. doi: 10.1249/JSR.0000000000000238.
11. Cameron D. What are Lyme disease co-infections? 2020 Oct 13 [cited 2025 Jun 24]. In: Daniel Cameron MD [Internet]. New York: Daniel Cameron & Associates; 2020. Available from: https://danielcameronmd.com/lyme-disease-co-infections/
12. Zimmer AJ, Simonsen KA. Babesiosis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Jun 24]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK430715/
13. Centers for Disease Control and Prevention (CDC). Anaplasmosis. Clinical signs and symptoms of anaplasmosis. 2024 May 15 [cited 2025 Jun 24]. Available from: https://www.cdc.gov/anaplasmosis/hcp/clinical-signs/index.html
14. Ely I, Barer J. Tick-borne illness diagnosis enters new era with multiplex testing. 2025 Apr 9 [cited 2025 Jun 24]. In: Technology Networks [Internet]. Sudbury: Technology Networks; ©2025. Available from: http://www.technologynetworks.com/diagnostics/blog/tick-borne-illness-diagnosis-enters-new-era-with-multiplex-testing-398341
15. Moniuszko A, Dunaj J, Święcicka I, Zambrowski G, Chmielewska-Badora J, Żukiewicz-Sobczak W, et al. Co-infections with Borrelia species, Anaplasma phagocytophilum and Babesia spp. in patients with tick-borne encephalitis. Eur J Clin Microbiol Infect Dis. 2014 Oct 1;33(10):1835-41. https://doi.

org/10.1007/s10096-014-2134-7.
16. Stinco G, Bergamo S. Impact of co-infections in Lyme disease. Open Dermatol J. 2016;10(Suppl 1):55-61. doi: 10.2174/1874372201610010055.
17. Boyer PH, Lenormand C, Jaulhac B, Talagrand-Reboul E. Human co-infections between Borrelia burgdorferi s.l. and other ixodes-borne microorganisms: a systematic review. Pathogens. 2022 Mar;11(3):282. doi: 10.3390/pathogens11030282.
18. Ilyinskikh EN, Bondarenko EI, Voronkova OV, Karpova MR, Filatova EN, Reshetova AV, et al. Epidemiological and clinical characteristics of cases and detection of pathogens' markers of tick-borne infections in the Tomsk region. Epidemiol Infect Dis. 2024;29(4):260-73. https://doi.org/10.17816/EID634387.
19. Ilyinskikh E, Filatova E, Bulankov Y, Nekrasov V, Reshetova A, Portnyagina E, et al. [Predictive value of complete blood count for early diagnosis of mixed infection of the non-erythema migrans form of Lyme borreliosis and tick-borne encephalitis]. J Infectol. 2022;14(4):69-76. Russian. doi: 10.22625/2072-6732-2022-14-4-69-76
20. Tokarz R, Mishra N, Tagliafierro T, Sameroff S, Caciula A, Chauhan L, et al. A multiplex serologic platform for diagnosis of tick-borne diseases. Sci Rep. 2018 Feb 16;8(1):3158. doi: 10.1038/s41598-018-21349-2.
21. Moore A, Nelson C, Molins C, Mead P, Schriefer M. Current guidelines, common clinical pitfalls, and future directions for laboratory diagnosis of Lyme disease, United States. Emerg Infect Dis. 2016 Jul;22(7):1169-77. doi: 10.3201/eid2207.151694.
22. European Centre for Disease Prevention and Control. Factsheet about tick-borne encephalitis (TBE) [Internet]. Solna: ECDC; 2024- [cited 2025 Jun 24]. Available from: https://www.ecdc.europa.eu/en/tick-borne-encephalitis/facts/factsheet
23. Marques AR. Laboratory diagnosis of Lyme disease: advances and challenges. Infect Dis Clin North Am. 2015 Jun;29(2):295-307. doi: 10.1016/j.idc.2015.02.005.
24. Liao H, Hollingsworth BD, Cassidy CA, Zychowski D, Ursery L, Giandomenico DA, et al. Completion of paired serological testing algorithms for spotted fever rickettsiosis and ehrlichiosis, North Carolina: 2017-2020. Clin Infect Dis. 2025 Apr 3:ciaf176. https://doi.org/10.1093/cid/ciaf176.
25. Department of Health and Human Services, U.S; Office of the Assistant Secretary for Health (OASH). Diagnostics subcommittee report to the Tick-Borne Working Group [Internet]. Washington: OASH; 2025- [cited 2025 Jun 25]. Available from: https://health.gov/advisory-committees/tick-borne-disease-working-group/tbdwg-reports/diagnostics-2022
26. Dessau RB, van Dam AP, Fingerle V, Gray J, Hovius JW, Hunfeld KP, et al. To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis. Clin Microbiol Infect. 2018 Feb 1;24(2):118-24. doi: 10.1016/j.cmi.2017.08.025.
27. Chung IH, Robinson LK, Stewart-Juba JJ, Dasch GA, Kato CY. Analytically sensitive Rickettsia species detection for laboratory diagnosis. Am J Trop Med Hyg. 2022 May;106(5):1352-7. doi: 10.4269/ajtmh.21-0757.
28. Stewart AG, Stewart AGA. An update on the laboratory diagnosis of Rickettsia spp. infection. Pathogens. 2021 Oct 13;10(10):1319. doi: 10.3390/pathogens10101319.
29. Das S, Hammond-McKibben D, Guralski D, Lobo S, Fiedler PN. Development of a sensitive molecular diagnostic assay for detecting Borrelia burgdorferi DNA from the blood of Lyme disease patients by digital PCR. PLoS One. 2020 Nov 30;15(11):e0235372. doi: 10.1371/journal.pone.0235372.
30. Grąźlewska W, Ferra B, Holec-Gąsior L. Przydatność diagnostyczna reakcji PCR w rozpoznawaniu Boreliozy [Diagnostic usefulness of PCR in the recognition of Lyme disease]. Adv Microbiol. 2021;59(4):367-77. Polish. doi: 10.21307/PM-2020.59.4.28.
31. Makówka A, Gut W, Rogalska J, Michalik J, Wodecka B, Rymaszewska A, et al. [Detection of TBEV RNA in ticks as a tool for valuation of endemic area wide and sensitivity of TBE surveillance]. Przegl Epidemiol. 2009;63(3):375-8. Polish.
32. Pedersen BN, Jenkins A, Paulsen KM, Basset C, Andreassen ÅK. Development of a real-time PCR method for the detection of European and Siberian subtypes of tick-borne encephalitis virus. Microbiol Res. 2023 Dec;14(4):1545-58. https://doi.org/10.3390/microbiolres14040106.
33. Saksida A, Duh D, Lotrič-Furlan S, Strle F, Petrovec M, Avšič-Županc T. The importance of tick-borne encephalitis virus RNA detection for early differential diagnosis of tick-borne encephalitis. J Clin Virol. 2005 Aug 1;33(4):331-5. doi: 10.1016/j.jcv.2004.07.014.
34. Hara Y, Chin CY, Mohamed R, Puthucheary SD, Nathan S. Multiple-antigen ELISA for melioidosis: a novel approach to the improved serodiagnosis of melioidosis. BMC Infect Dis. 2013 Dec;13(1):165. doi: 10.1186/1471-2334-13-165.
35. Skar GL, Blum MA, Simonsen KA. Lyme disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Jun 26]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK431066/
36. Guzman N, Yarrarapu SNS, Beidas SO. Anaplasma Phagocytophilum. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Jun 26]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK513341/
37. Snowden J, Simonsen KA. Ehrlichiosis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Jun 26]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK441966/
38. Reusken C, Boonstra M, Rugebregt S, Scherbeijn S, Chandler F, Avšič-Županc T, et al. An evaluation of serological methods to diagnose tick-borne encephalitis

from serum and cerebrospinal fluid. J Clin Virol. 2019 Nov 1;120:78-83. doi: 10.1016/j.jcv.2019.09.009.
39. Steininger P, Ensser A, Knöll A, Korn K. Results of tick-borne encephalitis virus (TBEV) diagnostics in an endemic area in Southern Germany, 2007 to 2022. Viruses. 2023 Dec;15(12):2357. doi: 10.3390/v15122357.
40. Dobler G. Chapter 10: Diagnosis. In: Dobler G, Erber W, Bröker M, Schmitt HJ, editors. The TBE Book. 6th ed. Singapore: Global Health Press; 2023. doi: 10.33442/26613980_10-6
41. Chapman AS, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, et al. Diagnosis and management of tick-borne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis - United States: a practical guide for physicians and other health-care and public health professionals. MMWR Recomm Rep. 2006 Mar 31;55(RR-4):1-27.
42. Hojgaard A, Lukacik G, Piesman J. Detection of Borrelia burgdorferi, Anaplasma phagocytophilum and Babesia microti, with two different multiplex PCR assays. Ticks Tick Borne Dis. 2014 Apr 1;5(3):349-51. doi: 10.1016/j.ttbdis.2013.12.001.
43. Krishnamurthy HK, Jayaraman V, Krishna K, Wang T, Bei K, Changalath C, et al. A customizable multiplex protein microarray for antibody testing and its application for tick-borne and other infectious diseases. Sci Rep. 2025 Jan 20;15(1):2527. doi: 10.1038/s41598-024-84467-0.
44. Cardenas-Cadena SA, Castañeda-Lopez ME, Mollinedo-Montaño FE, Vazquez-Reyes S, Lara-Arias J, Marino-Martinez IA, et al. Tick-borne pathogens screening using a multiplex real-time polymerase chain reaction-based method. Acta Parasitol. 2023;68(3):705-10. doi: 10.1007/s11686-023-00702-0.
45. Xi D, Garg K, Lambert JS, Rajput-Ray M, Madigan A, Avramovic G, et al. Scrutinizing clinical biomarkers in a large cohort of patients with Lyme disease and other tick-borne infections. Microorganisms. 2024 Feb;12(2):380. doi: 10.3390/microorganisms12020380.
46. Garg K, Thoma A, Avramovic G, Gilbert L, Shawky M, Ray MR, et al. Biomarker-based analysis of pain in patients with tick-borne infections before and after antibiotic treatment. Antibiotics (Basel). 2024 Jul 25;13(8):693. doi: 10.3390/antibiotics13080693.
47. Gudowska-Sawczuk M, Mroczko B. Selected biomarkers of tick-borne encephalitis: a review. Int J Mol Sci. 2021 Sep 30;22(19):10615. doi: 10.3390/ijms221910615.
48. Madugundu AK, Muthusamy B, Sreenivasamurthy SK, Bhavani C, Sharma J, Kumar B, et al. A next-generation sequencing-based molecular approach to characterize a tick vector in Lyme disease. OMICS. 2018 Aug 1;22(8):565-74. doi: 10.1089/omi.2018.0089.
49. Sanchez-Vicente S, Jain K, Tagliafierro T, Gokden A, Kapoor V, Guo C, et al. Capture sequencing enables sensitive detection of tick-borne agents in human blood. Front Microbiol. 2022 Mar 7;13:837621. doi: 10.3389/fmicb.2022.837621.
50. Rodino KG, Wolf MJ, Sheldon S, Kingry LC, Petersen JM, Patel R, et al. Detection of tick-borne bacteria from whole blood using 16S ribosomal RNA gene PCR followed by next-generation sequencing. J Clin Microbiol. 2021 Apr 20;59(5):e03129-20. doi: 10.1128/JCM.03129-20.
51. Kipp EJ, Lindsey LL, Khoo B, Faulk C, Oliver JD, Larsen PA. Metagenomic surveillance for bacterial tick-borne pathogens using nanopore adaptive sampling. Sci Rep. 2023 Jul 7;13(1):10991. doi: 10.1038/s41598-023-37134-9.
52. Cabezas-Cruz A, Vayssier-Taussat M, Greub G. Tick-borne pathogen detection: what's new? Microbes Infect. 2018 Aug 1;20(7):441-4. doi: 10.1016/j.micinf.2017.12.015.
53. Department of Health and Human Services, U. S.; Office of the Assistant Secretary for Health. Tick-borne disease Working Group: 2018 report to Congress [Internet]. Washington: OASH; 2018 [cited 2025 Jun 27]. Available from: https://www.hhs.gov/sites/default/files/tbdwg-report-to-congress-2018.pdf
54. Paules CI, Marston HD, Bloom ME, Fauci AS. Tick-borne diseases – confronting a growing threat. N Engl J Med. 2018 Aug 23;379(8):701-3. doi: 10.1056/NEJMp1807870.
55. Adrion ER, Aucott J, Lemke KW, Weiner JP. Health care costs, utilization and patterns of care following Lyme disease. PLoS One. 2015 Feb 4;10(2):e0116767. doi: 10.1371/journal.pone.0116767.
56. Šmit R, Postma MJ. The burden of tick-borne encephalitis in disability-adjusted life years (DALYs) for Slovenia. PLoS One. 2015 Dec 16;10(12):e0144988. doi: 10.1371/journal.pone.0144988.
57. Šmit R. Reviewing estimates of the burden in disability-adjusted life years (DALYs) of tick-borne encephalitis in Slovenia. Expert Rev Pharmacoecon Outcomes Res. 2019 Jun;19(3):299-303. doi: 10.1080/14737167.2019.1573677.
58. Slunge D, Boman A, Studahl M. Burden of tick-borne encephalitis, Sweden. Emerg Infect Dis. 2022 Feb;28(2):314-22. doi: 10.3201/eid2802.204324.
59. Cocchio S, Bertoncello C, Napoletano G, Claus M, Furlan P, Fonzo M, et al. Do we know the true burden of tick-borne encephalitis? A cross-sectional study. Neuroepidemiology. 2019 Sep 19;54(3):227-34. https://doi.org/10.1159/000503236.
60. Dedkov VG, Simonova EG, Beshlebova OV, Safonova MV, Stukolova OA, Verigina EV, et al. The burden of tick-borne diseases in the Altai region of Russia. Ticks Tick Borne Dis. 2017 Aug 1;8(5):787-94. doi: 10.1016/j.ttbdis.2017.06.004.a

https://doi.org/10.52645/MJHS.2026.2.13

UDC: 616.151-022-053.2:614.21

REVIEW ARTICLE

Healthcare-associated bloodstream infections in children: epidemiology, risk factors and prevention strategies

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ABSTRACT

Introduction. Health care-associated bloodstream infections represent a major public health concern, significantly impacting morbidity, mortality, and the overall cost of pediatric medical care.

Materials and methods. A literature review was conducted based on systematic searches in PubMed, SCOPUS, and Web of Science, following PRISMA guidelines.

Results. The incidence of healthcare-associated bloodstream infections ranges from 2 to 25 cases per 1,000 central venous catheter days, with higher rates reported in pediatric and neonatal intensive care units, where patients are frequently exposed to risk factors such as central venous catheter use, mechanical ventilation, and immunosuppression. Pediatric patients with health care-associated bloodstream infections experience significantly longer hospital stays compared to those without infection (25 vs. 7 days, $P < 0.0001$). In pediatric intensive care units, the average length of hospital stay due to these infections varies between 11.40 and 21.10 days, while in neonatal intensive care units, it ranges from 4 to 27.80 days. Mortality associated with these infections among children varies between 15% and 50%, depending on the severity of infection and underlying comorbidities. Additionally, health care-associated bloodstream infections lead to increased use of medical resources and generate substantial additional costs for the healthcare system-costs that are, in fact, largely preventable.

Conclusions. Evidence-based strategies, such as strict hand hygiene and standardized protocols for medical device use, can significantly reduce the incidence of these infections.

Keywords: bloodstream infections, pediatric patients, mortality, length of hospital stay, healthcare-associated infections, costs, central line catheterization.

Cite this article: Marga I. Healthcare-associated bloodstream infections in children: epidemiology, risk factors and prevention strategies. Mold J Health Sci. 2026;13(2):104-115. https://doi.org/10.52645/MJHS.2026.2.13.

Manuscript received: 5.01.2026

Accepted for publication: 18.02.2026

Published: 15.06.2026

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Despite substantial progress in infection control, there is still limited and heterogeneous evidence regarding the true burden, epidemiological patterns, and modifiable risk factors of healthcare-associated bloodstream infections (HA-BSIs) in pediatric populations, particularly across different healthcare settings. Data on age-specific vulnerabilities, device-related risks, and the effectiveness of bundled prevention strategies in children remain inconsistent and underreported, especially in low- and middle-income countries.

The research hypothesis

I hypothesize that inadequate management of invasive medical devices, younger patient age, and the presence of underlying comorbidities are independent risk factors for healthcare-associated

bloodstream infections in children, and that the application of pediatric-specific, evidence-based infection prevention measures leads to a significant reduction in infection incidence.

The novelty added by manuscript to the already published scientific literature

This manuscript provides an integrated and up-to-date synthesis of epidemiological data, risk factors, and prevention strategies specific to healthcare-associated bloodstream infections in children. It highlights age-adapted and device-focused prevention approaches, emphasizing pediatric-specific vulnerabilities that are insufficiently addressed in existing literature. Additionally, the study contributes a structured framework for translating evidence-based prevention strategies into routine pediatric clinical practice, with relevance across diverse healthcare settings.

Introduction

Healthcare-associated bloodstream infections (HA-BSIs) are among the most severe complications of modern medical care, particularly in pediatric patients. They significantly contribute to increased morbidity, mortality, prolonged hospitalization, and higher treatment costs [1-3]. Bloodstream infections, including central line-associated bloodstream infections (CLABSIs), represent the most frequent type of healthcare-associated infections (HAIs) in neonates admitted to neonatal intensive care units (NICUs) [4, 5], with considerable variation depending on region and the development level of the healthcare system [6]. According to ECDC data, 6.4% of patients admitted to intensive care units (ICUs) develop BSIs if hospitalization exceeds two days [7].

A review of the literature estimated between 575,000 and 677,000 episodes of BSIs annually in North America (536,000–628,000 in the U.S. and 40,000–49,000 in Canada), associated with 79,000–94,000 deaths (72,000–85,000 in the U.S. and 7,000–9,000 in Canada), and more than 1.2 million BSI episodes and 157,000 deaths per year occurring in Europe. BSIs have a major global impact on morbidity and mortality and are ranked among the top seven causes of death worldwide [8]. In the United States, the annual mortality rate associated with BSIs is estimated at 23.5–27.5 deaths per 100,000 population – surpassing the number of deaths caused by any other infectious disease, including influenza and pneumonia combined (16.2 deaths per 100,000) [8]. In Europe, BSIs are the second leading cause of disability and premature death and are also associated with the highest number of preventable deaths among HAIs [9]. The costs associated with BSIs vary widely, with estimates ranging from $960 million to $18.2 billion annually [10].

These infections are often caused by the use of central and peripheral venous catheters, which allow direct access for pathogenic microorganisms into the bloodstream [11, 12]. According to data from the literature, approximately 36.7% of BSI cases are attributed to vascular catheter use, of which 28.4% are associated with central venous catheters and 8.3% with peripheral ones. Additionally, 32.4% of cases occur secondary to other preexisting infections [13]. The incidence of catheter-associated infections varies considerably, ranging from 1.6 to 44.6 CLABSIs per 1,000 catheter-days in adult and pediatric ICUs, and from 2.6 to 60.0 CLABSIs per 1,000 catheter-days in NICUs [14].

This study aims to provide a systematic review of the literature regarding the incidence, causative pathogens, risk factors, and effective strategies to reduce healthcare-associated bloodstream infections in children, in order to improve prevention and management practices in pediatric healthcare settings.

Materials and methods

To conduct this literature review study, an advanced search was performed in the electronic databases PubMed, SCOPUS, and Web of Science using the following keywords: *"bloodstream infections," "antimicrobial resistance," "pediatric patients," "healthcare-associated infections," "mortality," "length of hospital stay,"* and *"costs"*, in combination with the Boolean operators *"AND," "OR,"* and *"NOT."* The search yielded a total of 957 publications – 549 from PubMed, 284 from SCOPUS, and 124 from Web of Science. Of these, 23.72% (n = 227) were duplicates.

The remaining articles were screened following the PRISMA guidelines [15]. In the initial screening phase, 730 publications were reviewed, of which 75.2% (n = 549) did not meet the study objective. A total of 181 scientific papers were assessed for eligibility based on the following inclusion criteria:

- Articles published between 2010 and 2024.
- Language of publication: English and/or Romanian.
- Open-access publications.
- Types of articles: literature reviews, research articles, and meta-analyses.

The following were excluded from the review: articles published in Russian, French, Polish, Chinese, Turkish, Spanish, and Italian; publications dated between 1980 and 2009; editorial letters and article corrections; and restricted-access papers (Figure 1).

A total of 67 publications were selected for the final study. The analysis of the national epidemiological situation was carried out based on data collected from Statistical Form No. 2, "Report on selected infectious and parasitic diseases registered in the Republic of Moldova" for the year 2023, as provided by the National Agency for Public Health.

Results

The literature review highlighted the main epidemiological characteristics of the epidemic process caused by healthcare-associated bloodstream infections in pediatric patients. Epidemiological data were extracted from 67 scientific articles that met the inclusion criteria established for this study. In the Republic of Moldova, HA-BSIs and, more

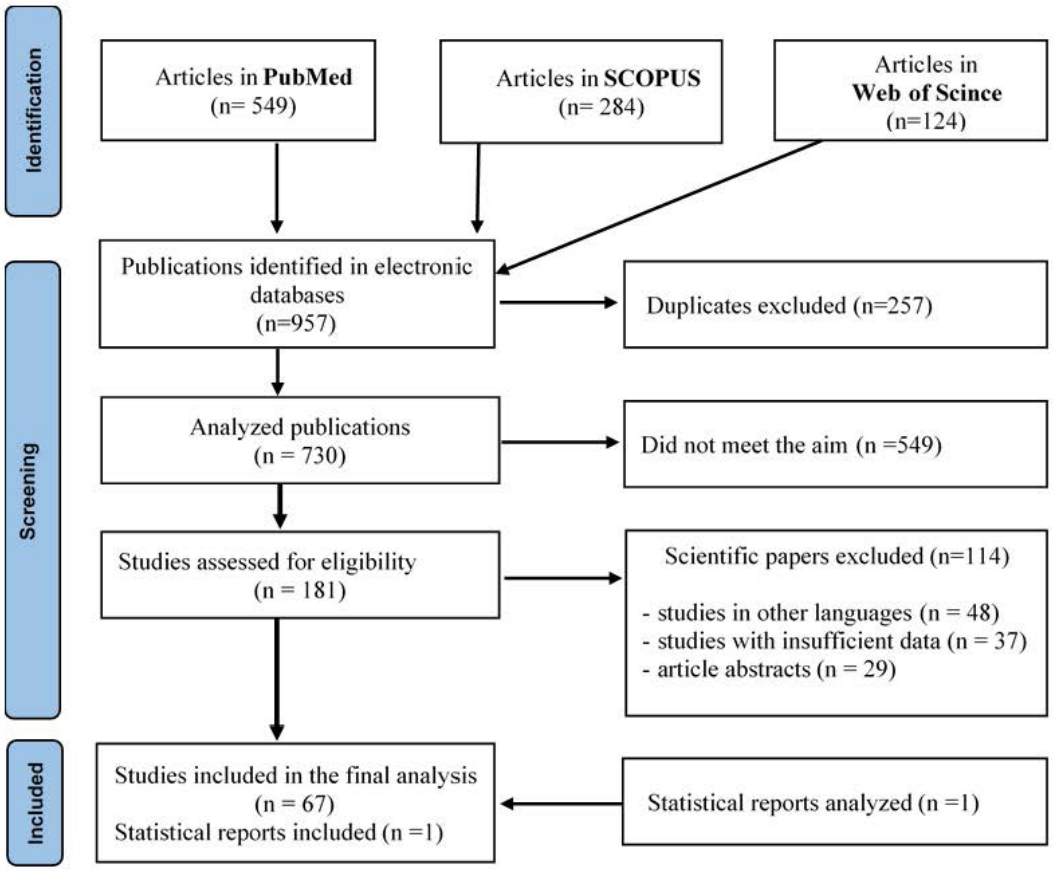

Fig. 1 Publication selection criteria according to the PRISMA 2020 guidelines

broadly, healthcare-associated infections in children remain an underexplored topic. Therefore, we aimed to identify the key epidemiological features, risk factors, and strategies for the prevention and control of HA-BSIs in the pediatric population, based on internationally published scientific evidence.

In 2012, the International Nosocomial Infection Control Consortium (INICC) reported device-associated infection rates in 16 PICUs in low- and middle-income countries (LMICs), revealing significantly higher values compared to high-income countries. According to INICC data, the incidence of ventilator-associated pneumonia (VAP) was 6 infections per 1,000 device-days, CLABSIs had a rate of 8.1 infections per 1,000 device-days, and catheter-associated urinary tract infections (CAUTIs) were reported at 4.1 infections per 1,000 device-days [12]. In contrast, PICUs in the United States reported significantly lower rates: 0.7 for VAP, 1.0 for CLABSI, and 3.5 for CAUTI. These marked differences reflect the challenges that LMIC healthcare systems face in terms of infection prevention and control. Furthermore, the actual burden of these infections in LMICs is likely underestimated, considering that 75% of the PICUs included in the INICC report were located in private hospitals, where practices and resources may differ significantly from those in the public sector [13].

Another study conducted in a developing country found that 56.5% of HAIs occurred in children under 1 year of age, and the incidence density of BSIs in children was 18.1 per 1,000 central venous catheter (CVC) days. Of all identified HAIs, 60.49% were BSIs, and most of them (38 out of 49) were associated with the use of venous catheters. Moreover, bloodstream infections were significantly more common in children with CVCs than in those without (20% vs. 4.7%, $p < 0.05$) [5].

According to a study conducted in Brazil as part of the SCOPE project, out of a total of 342 bloodstream infection episodes, 50.2% occurred in children under one year of age. Nearly half of the patients (45.3%) were admitted to pediatric or neonatal intensive care units, while 21.0% were treated in general pediatric units. Additionally, 66.4% of patients had central venous catheters, 23.1% had peripheral venous catheters, and 2.6% had arterial catheters, highlighting the high prevalence of device-associated infections [16].

Similar results were reported in a study by the European Centre for Disease Prevention and Control (ECDC), which identified 770 cases of HAIs, of which 45% were bloodstream infections. Although most BSIs were reported in infants under 12 months of age, the percentages remained significant across other age groups as well [17].

In another study analyzing children admitted to PICUs, bloodstream infections accounted for 45.4% of all healthcare-associated infections (HAIs), followed by respiratory tract infections (RTIs) at 27.8% and urinary tract infections (UTIs) at 15.8%. Among the 244 BSI cases analyzed, 79.9% were associated with central venous catheter (CVC) use, most of which were diagnosed in patients who had the cath-

eter in place for more than 48 hours [18]. High BSI rates were also reported in a study conducted in Morocco, where these infections represented 44% of HAI cases, followed by nosocomial pneumonia (30%) and surgical site infections and UTIs (each at 7%) [19].

An important aspect regarding the incidence of BSIs is the significant disparity between high-income countries (HICs) and low- and middle-income countries (LMICs). A study conducted in PICUs in LMICs highlighted a significantly higher incidence of HAIs, with CLABSI, VAP, and CAUTI rates all markedly higher compared to HICs: 12.2 vs. 7.0 per 1,000 central line-days; 9.0 vs. 5.4 per 1,000 ventilator-days; and 5.9 vs. 3.7 per 1,000 urinary catheter-days, respectively [20].

Research conducted by Saeghi et al. identified 404 episodes of bacteremia in 272 pediatric patients, of which 82.4% were healthcare-associated, with an incidence rate of 11.1 per 1,000 hospitalizations. Community-onset bacteremia accounted for 17.6% of cases. Almost two-thirds of the patients (85.6%) were under 5 years of age, with 34.4% being infants aged 1 month to 1 year. Underlying comorbidities were identified in 90.6% of patients, the most common being prematurity (26.2%), hematologic malignancies (22.3%), intestinal pathologies (17.6%), and metabolic/genetic syndromes (11.6%). A central venous catheter was present in 45.0% of the BSI cases, and in 7.7% of cases, the source of infection was unidentified [21].

BSIs also predominated in a study conducted at Besat Hospital in western Iran, being the most common infection form in both investigated age groups: 37.38% in children aged 0–4 years and 34.75% in those aged 5–14 years. *Escherichia coli* was more frequently isolated in girls (25.84% in the 0–4 age group and 24.53% in the 5–14 age group), while *Staphylococcus aureus* was more common in boys (33.6% in the 0–4 age group and 29.55% in the 5–14 age group) [22].

Despite the significant variation in BSI pathogens among children across different countries, age groups, and clinical settings [23], Gram-positive microorganisms continue to play a dominant role in the onset of healthcare-associated BSIs. One recent study reported that Gram-positive bacteria were responsible for 57.0% of infections, Gram-negative organisms for 40.2%, and *Candida* species for 2.8%. The most frequently isolated microorganisms were *Staphylococcus aureus* (26.0%), *Escherichia coli* (13.0%), coagulase-negative staphylococci (8.3%), and *Enterococcus* spp. Among *S. aureus* strains, 26.0% were oxacillin-resistant; however, susceptibility to ceftaroline was nearly universal at 99.8%. Among Gram-negative bacteria, *Enterobacteriaceae* and *Pseudomonas aeruginosa* together accounted for over 85% of isolates, all of which were susceptible to ceftazidime-avibactam [24].

Consistent with the above, another study reported that Gram-positive organisms accounted for 56% of all identified isolates, while Gram-negative organisms accounted for 44%. The most common Gram-positive bacteria were *Staphylococcus aureus* (78%), followed by coagulase-negative staphylococci (CoNS) (12%), *Enterococcus faecalis* (6%), and *Streptococcus pneumoniae* (4%). In the European HAI surveillance network, the study by Garcia and data collected from PICUs in France also confirmed the predominance of coagulase-negative staphylococci as the primary etiological agents of bacteremia [25, 26].

Even higher rates of Gram-positive microorganisms were observed in a U.S.-based study, where they accounted for 70.6% of pathogens isolated in healthcare-associated BSIs in children, compared to 29.4% for Gram-negative bacteria. The most frequently identified pathogen was coagulase-negative *Staphylococcus* (CoNS), isolated in 47.1% of cases, followed by *Escherichia coli* (8.3%), *Staphylococcus aureus* (7.0%), *Streptococcus pneumoniae* (5.9%), *Klebsiella* spp. (5.6%), and *Enterococcus* spp. (4.4%). A study analyzing isolation trends between 2015 and 2018 reported a significant decrease in the frequency of CoNS and *Serratia marcescens* ($p < 0.05$), alongside a significant increase in the prevalence of *E. coli*, *S. aureus*, *S. pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium* ($p < 0.05$) [24].

Nevertheless, it is important to note that over the past decade, there has been a significant increase in the number of Gram-negative microorganisms isolated from children with healthcare-associated infections, a trend increasingly reported in international studies [20, 22, 27-30]. One study confirmed the predominance of Gram-negative bacteria in the etiology of HA-BSIs, highlighting a pattern increasingly emphasized in the literature: 60% of infections were caused by Gram-negative pathogens, compared to 33% by Gram-positive bacteria and 7% by fungal pathogens. *Klebsiella pneumoniae* (17%), *Staphylococcus aureus* (14%), and *Escherichia coli* (9.11%) were the most frequently detected pathogens. The crude mortality rate among children with BSIs was 20% (176/864); multivariate analysis identified HIV infection, fungal and Gram-negative sepsis, and the presence of HAIs as significant predictors of mortality [31].

Evidence of the growing dominance of Gram-negative bacteria in the etiological landscape of pediatric HA-BSIs was also reported in a study where, out of 573 isolated strains, 55% were Gram-negative bacteria, 32% Gram-positive bacteria, and 13% fungi. The most frequently isolated species included *Enterobacteriaceae* (30.9%), *Pseudomonas aeruginosa* (19.2%), and *Staphylococcus aureus* (11.0%) [18]. Similar findings on the spectrum of microorganisms isolated from children with HA-BSIs were reported in the United Kingdom [32], Italy [33], Brazil [16], and the United States, particularly among neonates with very low birth weight (<1500 g) [34].

Despite this global trend, some studies from LMICs found that fungi were the predominant etiological agents of pediatric BSIs. For instance, *Candida* spp. was the most commonly isolated pathogen (41%) among children with BSIs, followed by coagulase-negative *Staphylococcus* (17%) [5]. In line with these findings, fungemia was the most common in studies from Turkey [27] and Vietnam [28].

Alarmingly, infections caused by multidrug-resistant (MDR) Gram-negative bacteria have expanded significantly

in recent years, becoming a major global public health concern [24, 29]. This trend is further compounded by the high rate of antimicrobial resistance (AMR), which was significantly higher in hospital-acquired infections (70%) compared to community-acquired infections (25%; p < 0.0001). Factors associated with AMR included nosocomial infection, age under one year, HIV infection, and sepsis caused by Gram-negative bacteria [31].

The link between Gram-negative bacilli-caused BSIs and AMR was underscored by a recent U.S. study analyzing 721 *E. coli* isolates (including 393 associated with BSIs), which reported high rates of non-susceptibility to commonly used empirical antibiotics such as: ampicillin (66.8%) and gentamicin (16.8%), and the presence of extended-spectrum beta-lactamases (ESBLs) in 20% of isolates [30]. In another study conducted in Italy and Brazil, 44% of isolates were MDR; among 175 *Enterobacteriaceae* isolates, 45% were ESBL-positive. Carbapenem resistance was observed in 2% of *Enterobacteriaceae*, 42% of *P. aeruginosa*, and 60% of *Acinetobacter baumannii*. Among Gram-positive bacteria, 56% of *S. aureus* strains were methicillin-resistant; however, no vancomycin-resistant *Enterococcus* spp. were detected. Additionally, of 76 coagulase-negative Staphylococcus (CoNS) isolates, 47 (62%) were classified as multidrug-resistant [18].

According to another study conducted in LMICs, *Klebsiella* spp. was the most common pathogen isolated from neonates with MDR neonatal sepsis [35]. In contrast, *Escherichia coli* and methicillin-sensitive *Staphylococcus aureus* were the most frequently isolated pathogens in children with BSIs in a multicenter US. study [36].

Antimicrobial susceptibility data were also reported in a study from a pediatric hospital in Srinagar. Gram-positive bacteria remained fully susceptible to vancomycin, linezolid, daptomycin, teicoplanin, and tigecycline, with no resistance observed. However, a high rate of methicillin resistance was noted, affecting 72% of *S. aureus* isolates and 50% of CoNS species. Among Gram-negative bacteria, colistin was the only antibiotic uniformly effective across all isolates. In contrast, high levels of resistance were observed for ampicillin, ceftriaxone, ciprofloxacin, and piperacillin-tazobactam, highlighting the growing challenges in treating infections caused by these organisms [37].

The analysis of pathogen distribution by age revealed marked differences across pediatric age groups. Bloodstream infections caused by Gram-positive bacteria predominated in neonates, accounting for 69.0% of all isolates. The most common pathogens in this group were coagulase-negative *Staphylococcus* (48.0%), *Escherichia coli* (11.2%), and *Klebsiella pneumoniae* (8.3%). CoNS was present in all age groups but showed a decreasing frequency with increasing age, while *Staphylococcus aureus* showed an upward trend. *Streptococcus pneumoniae* and Viridans group streptococci were more frequently isolated in children aged 3–5 years (14.3% and 3.7%, respectively), whereas beta-hemolytic streptococci were more prevalent in neonates (4.2%). *Enterococcus faecium* and *E. faecalis* had the highest frequency in neonates (6.1% each), with a progressive decline in older age groups. Among Gram-negative bacteria, *E. coli* and *K. pneumoniae* isolates showed a significant decrease between the 0–28 days and 13 months–2 years age groups, followed by a notable increase in children over 9 years. *Salmonella* spp. infections peaked in the 13 months–2 years group (3.1%) [24].

Regarding the significant risk factors for acquiring healthcare-associated infections, including BSIs, the following were identified in the reviewed studies: recent hospitalization (8/19, 42.1% vs. 17/132, 12.9%; p < 0.001), presence of comorbidities (17/19, 89.5% vs. 72/132, 54.5%; p < 0.004) [38], admission to the PICU (OR 2.0), malnutrition (OR 1.6), HIV infection (OR 1.7), "fatal" McCabe score (OR 2.0), comorbid conditions (OR 1.6), use of implanted medical devices (OR 1.9), blood transfusions (OR 2.5), and transfer from another healthcare facility (OR 1.4) [39].

Specifically, multivariate analysis showed that BSIs caused by extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) were independently associated with the following risk factors: neonatal period (OR = 11.4), sickle cell anemia (OR = 3.1), malnutrition (OR = 2.0), and mechanical ventilation (OR = 3.5) [40]. Additionally, neutropenia was consistently identified as a significant risk factor for infections with multidrug-resistant (MDR) Gram-negative bacteria in several studies [41-43]. The use of tunneled central venous catheters such as Broviac and Hickman was also associated with a higher risk of CLABSI compared to fully implantable devices such as Port-a-Cath [44]. A study from Qatar reported BSI densities of 3.98 per 1,000 catheter-days for Port-a-Cath and 5.13 for Hickman catheters [45].

The insertion site of the central venous catheter also plays a key role in the development of BSIs. A study conducted in adults demonstrated a significantly higher risk of bacteremia with femoral catheters (24.5%; 100 out of 407) compared to those placed in the internal jugular vein (10.4%; 36 out of 346), with a relative risk of 2.36. Moreover, the average time to infection onset was shorter for femoral catheters (20.11 ± 6.91 days) compared to internal jugular catheters (25.97 ± 6.56 days) [46].

Among children with hemato-oncological conditions, significant risk factors for the development of BSIs included corticosteroid treatment (50.3% vs. 68.4%; p = 0.02), antibiotic therapy (62.7% vs. 77.2%; p = 0.05), chemotherapy (67.3% vs. 84.2%; p = 0.01) in the 30 days preceding BSI onset, neutropenia (21.6% vs. 54.4%; p < 0.001), and mucositis (12.4% vs. 29.8%; p < 0.001) [47]. On the other hand, in children with solid tumors, surgical interventions (7.2% vs. 0%; p = 0.03), bed immobilization (41.8% vs. 15.8%; p < 0.001), and mechanical ventilation (12.4% vs. 1.8%; p = 0.01) in the 72 hours preceding the onset of bacteremia were identified as significant risk factors [48].

Bloodstream infections represent a severe and frequent complication among children who develop HA-BSIs, with mortality rates increasing significantly in immunocompromised patients [49]. A multicenter study conducted in New York, which included 4,500 children, found that the probability of death was nearly six times higher in patients with BSIs compared to those without (95% CI: 3.02–16.00;

p < 0.05) [50]. Clinical outcomes were unfavorable, with a crude mortality rate of 41.3% and a mean PICU length of stay of 15 days [51]. Recent studies have reported that mortality rates associated with BSIs range from 21 to 32 deaths per 100,000 population [51, 52], while the one-month post-diagnosis mortality is estimated at 17%–28% for HA-BSIs and 10%–19% for community-acquired BSIs [53, 54]. Higher mortality rates of 40%–50% were observed in studies involving pediatric patients with BSIs admitted to intensive care units [55]. For instance, 45.4% of pediatric patients with BSIs caused by *Enterobacteriaceae* died, with significantly higher mortality in infections caused by ESBL-positive strains (54.8%) compared to ESBL-negative strains (15.4%) (p < 0.001). Univariate analysis showed that fatal outcomes were more frequent in neonates and younger children, while multivariate analysis identified ESBL production as an independent risk factor for mortality (OR = 2.9; 95% CI: 1.8–7.3; p = 0.001) [40]. The average length of stay (LOS) for patients with BSIs caused by ESBL-positive strains was 22.5 days (95% CI: 18.5–26 days), compared to 12.6 days (95% CI: 9.5–15.8 days) for those with ESBL-negative strains (p < 0.0001) [40]. Children with BSIs and respiratory tract infections (RTIs) had 4.0- and 2.9-fold higher mortality risk, respectively, compared to children with other types of HAIs [18]. A retrospective cohort study in the USA showed that patients with BSIs had a significantly higher crude mortality rate than those without (5% vs. 0.34%; p < 0.001) [56].

In pediatric oncology patients, early (7-day) and late (30-day) mortality rates were 3.8% and 13.8%, respectively. These rates were similar between patients with solid tumors (ST) and those with hematologic malignancies (HM). However, PICU care was significantly more frequent in children with ST than in those with HM (23.5% vs. 10.5%; p = 0.05), while the duration of hospitalization after a BSI episode was significantly longer in children with HM compared to those with ST (median 19 vs. 13 days; p = 0.02) [47]. Unit-stratified outcomes showed a greater impact of HA-BSIs in pediatric intensive care units (PICUs), with a mean attributable LOS of 16.4 days, compared to neonatal ICUs (NICUs), where the mean was 11.4 days. In PICUs, the attributable LOS ranged from 11.4 to 21.1 days, while in NICUs, it varied from 4 to 27.8 days [2]. This trend was also reflected in cumulative mortality rates, which were higher in PICUs (0.13) compared to NICUs (0.08). Furthermore, both the average LOS and mortality rate were significantly higher in patients who developed HAIs, with an LOS of 25 days versus 7 days in patients without HAIs (p < 0.0001) and a mortality rate of 50% versus 27.8% (p < 0.005) [57].

The relationship between total cost and independent variables in PICU patients showed that the median cost was highest for children under two months of age, at €6,903.30. Additionally, there was a significant difference between the median cost of patients with bloodstream infections (€37,356.68) and those with pneumonia (€34,912.96). Costs increased proportionally with the number of HAIs diagnosed; the cost difference between patients with one infection and those with three or four reached €38,202.57. Multivariate analysis revealed that patients with bloodstream infections (p < 0.001) incurred significantly higher costs compared to those without HAIs. The average direct healthcare cost for patients with HA-BSIs ranged between $1,642.16 and $160,804 (in 2019). Moreover, direct costs were estimated at $371,887, which included 2,275 additional hospital days, 2,365 days of antimicrobial therapy, and 3,575 additional laboratory investigations [39].

In Greece, the average LOS and cost attributable to CLABSI in the pediatric and neonatal population were 21 days and €13,727, respectively (Table 1). The high incidence of CLABSI reported in the literature (4.41 infections per 1,000 central line-days) further emphasizes the need for preventive measures. This incidence varies by ICU type, reaching 6.02 in NICUs, 6.09 in PICUs, and 2.78 in Hematology-Oncology units [58].

Table 1. Length of hospital stay and costs of bloodstream infections in children in Greece (58)

Variable	CLABSI (n = 94)	Non - CLABSI (n = 94)	Difference	95% IC
LOS, Overall days[1]	57.1	36.6	21	7.3-34.8
By unit				
PICU	52.1	33.2	19.1	5.7-32.6
NICU	75	47.8	27.8	8.7-46.9
Hematology-Oncology Unit	69.8	44.5	24.8	6.8-42.9
Bone marrow transplantation unit	34	21.7	12.4	4.1-20.8
Cost, Overall €	31.302	17.788	13.727	5.758-21.677
By unit				
PICU	29.282	16.951	13.159	4.642-21.677
NICU	36.582	20.788	16.275	6.194-26.357
Hematology-Oncology Unit	38.503	21.881	16.442	5.646-27.238
Bone marrow transplantation unit	21.166	12.028	9.272	3.661-14.883

Note: *CI, Confidence interval; LOS, Length of hospital stay after study enrollment.*

[1]LOS and cost are adjusted for age, gender, hospital, hospitalization unit, LOS prior to study enrollment, CVC outcomes after study enrollment and for propensity score. Propensity score variables include catheter placement, presence of stomy the last 48 h prior to study enrollment, presence of neutropenia and transfusion of blood products the last 14 days prior to study enrollment, LOS in ICU and length of catheter stay prior to study enrollment. €- Euro, the monetary unit and official currency of the European Union.

Although the incidence of this group of infections is high among pediatric patients, existing evidence in the scientific literature shows that bloodstream infections (BSIs) can be prevented. For example, in one study, during the control period, the rate of central line-associated bloodstream infections (CLABSI) was 7.4. After implementing preventive measures, during the intervention period, this rate significantly decreased to 4.78. In the follow-up period, the downward trend was maintained, reaching 2 [59]. A similar reduction in the incidence of bloodstream infections was observed in another study conducted in a pediatric intensive care unit. During the reference period, the infection rate was 88 per 1000 admissions, and after prevention measures were implemented, it decreased to 41 per 1000 admissions. Most of these infections were associated with the use of central venous catheters (CVCs), and the catheter-related infection rate decreased from 25.2 per 1000 CVC-days to 9.3 per 1000 CVC-days ($p < 0.05$). These results emphasize the importance of applying effective protocols to reduce CLABSI and improve patient outcomes [60].

A remarkable example of success was recorded in the neonatal services at Nationwide Children's Hospital, where the CLABSI rate was reduced from 6.0 to 1.43 per 1000 catheter-days in less than two years and then maintained at 0.68 for over five years [61]. Another study conducted in China showed a significant decrease in CLABSI incidence during the COVID-19 pandemic compared to the pre-pandemic period, from 9.4 to 2.2 per 1000 catheter days ($P < 0.001$) [62]. This downward trend was correlated with several factors, the main ones being improved infection prevention and control measures, with the implementation of stricter hygiene protocols and the use of personal protective equipment; reduced hospital admissions and invasive procedures; closer monitoring of patients with central venous catheters; and limiting access of visitors and auxiliary staff [62].

According to data from the Republic of Moldova, in 2023, 163 cases of healthcare-associated infections (HAIs) were reported in children aged 0 to 17 years. Among these, the following were recorded: surgical site infections – 32 cases (19.6%), infections following therapeutic injections, infusions, or transfusions – 21 cases (12.9%), nosocomial pneumonia due to mechanical ventilation – 106 cases (65%), and urinary tract infections following medical interventions – 4 cases (2.5%). These represented approximately 7.5% of the total reported infections (Figure 2).

Data regarding the structure of morbidity due to healthcare-associated infections in newborns in the Republic of Moldova differ from those identified in children aged 0–17 years (Figure 3). Additionally, the number of HAI cases was higher in newborns, emphasizing the biological fragility of this group and the increased exposure to infectious risks from the very first days of life.

Until now, no comprehensive study has been conducted addressing the issue of healthcare-associated infections (HAIs) in children in the Republic of Moldova [6]. However, it is noteworthy that a component of this topic was investigated within the framework of the "First National Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in Hospitals of the Republic of Moldova," which highlighted that septicemia accounts for 1.2% of all HAIs identified among both adults and children [63].

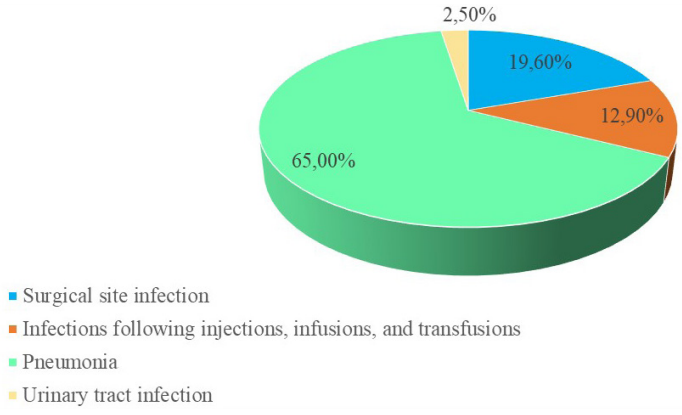

Fig. 2 Structure of morbidity due to healthcare-associated infections in children aged 0–17 years, in the Republic of Moldova, 2023.

Note: *Data are presented as percentages of the total number of reported healthcare-associated infection cases in children aged 0–17 years (n = 163). The figure was developed based on data collected through Statistical Form No. 2, "Report on Certain Infectious and Parasitic Diseases Registered in the Republic of Moldova" for the year 2023, as officially reported by the National Agency for Public Health. Percentages may not sum to 100% due to rounding.*

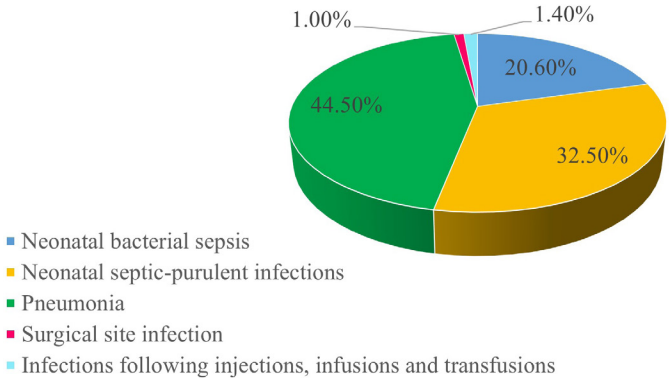

Fig. 3 Structure of morbidity due to healthcare-associated infections in newborns, Republic of Moldova, 2023

Note: *Data are presented as percentages of the total number of reported healthcare-associated infection cases in newborns (n = 209). The figure was developed based on data collected through Statistical Form No. 2, "Report on Certain Infectious and Parasitic Diseases Registered in the Republic of Moldova" for the year 2023, as officially reported by the National Agency for Public Health. Percentages may not sum to 100% due to rounding.*

Discussion

Healthcare-associated bloodstream infections represent a major concern in pediatric care globally, significantly impacting morbidity, mortality, and healthcare system costs. Despite sustained efforts in prevention and control, incidence rates remain high, underscoring the need for the development and implementation of more effective strategies

tailored to the pediatric population. Published data on pediatric HA-BSI vary considerably depending on the country, the profile of healthcare facilities, and the year of publication [12, 18, 20].

Epidemiological studies report a high incidence of HA-BSI ranging between 2 and 25 cases per 1,000 central venous catheter days, with higher rates observed in pediatric and neonatal intensive care units [2, 12, 16-18]. Although bloodstream infections represent one of the predominant forms of healthcare-associated infections, the greatest burden is borne by newborns and children under one year of age, accounting for over 50% of all healthcare-associated infections identified in these vulnerable groups. Additionally, children with solid tumors and hematologic-oncologic conditions exhibit increased susceptibility to HA-BSI due to severe immunosuppression induced by chemotherapy, radiotherapy, and immunosuppressive treatments. In these patients, the risk of infection is further amplified by the frequent and prolonged use of central venous catheters [47].

Another critical aspect of the impact of HA-BSI is the prolongation of hospital stays. Studies show that pediatric patients who develop bloodstream infections require longer hospitalizations, averaging approximately 25 days, compared to only 7 days among children without these infections, directly leading to increased treatment costs and prolonged exposure to nosocomial pathogens [2]. Furthermore, each episode of bloodstream infection can add between 10 and 21 additional days to the length of stay, increasing the risk of complications and the need for additional medical resources [2]. It has also been observed that bloodstream infections caused by ESBL-positive strains are associated with a significantly longer average hospital stay compared to those caused by non-ESBL strains [18, 34, 40].

The economic impact of healthcare-associated bloodstream infections is considerable, generating both direct and indirect costs that place significant pressure on healthcare systems. Hospital expenses are significantly higher in patients who develop bloodstream infections compared to those who do not, varying according to the number, severity, and complexity of infections. Total costs can range from 1,642.16 USD to 160,804 USD per patient [39, 58].

Direct costs are mainly related to prolonged hospital stays, the use of broad-spectrum antibiotics, the need for intensive supportive therapies, as well as the necessity for additional laboratory investigations. Beyond direct costs, there is also a significant economic impact on patients' families, reflected in economic losses caused by parents' absence from work during the child's hospitalization [64-66]. Mortality associated with these infections ranges between 15% and 50%, depending on case severity, patient comorbidities, and the type of microorganisms involved. Severe cases, especially in intensive care units and among children with hematologic-oncologic conditions, have higher mortality, with antibiotic-resistant and fungal strains posing additional risk factors [47].

Among the main pathogens involved in healthcare-associated bloodstream infections are Gram-positive bacteria, especially *Staphylococcus* spp., including coagulase-negative staphylococci, followed by Gram-negative bacteria such as *Klebsiella* spp., *Acinetobacter* spp., *and Escherichia coli*, which in the last decade have played an increasingly significant role in the etiology of HA-BSI [16, 24-26, 30]. Furthermore, there is a notable spread of fungal infections, particularly those caused by *Candida* spp., which continue to increase and can reach incidences of 20–30 cases per 1,000 catheter-days, with mortality rates ranging from 12% to 41%. Fungal infections are often associated with prolonged use of central venous catheters and antimicrobial treatments, which can disrupt the normal microbial flora and favor fungal proliferation. Increased risk of *Candida* infections is also associated with factors such as immunosuppression, parenteral nutrition, diabetes, and prolonged hospitalization [5, 31, 32].

Early diagnosis and prompt initiation of treatment are essential in managing HA-BSI, especially since empirical treatment is often started before blood culture results are available due to the urgent need to control infection [24]. However, a significant challenge in treatment is the growing presence of resistant microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), and vancomycin-resistant enterococci, which further complicate therapies [24, 33, 34]. Additionally, a major difficulty in managing HA-BSI is diagnosis, as in approximately 30.9% of cases, the source of infection cannot be identified, either due to inconclusive clinical evaluation (20.8%) or lack of complete medical data (10.1%) [21]. In this context, the rising antimicrobial resistance – especially to beta-lactams and carbapenems – necessitates adopting more precise and personalized therapeutic strategies to limit complications and improve prognosis in pediatric patients [33-35].

Among the main risk factors involved in the development of HA-BSI identified in the analyzed studies are young age, admission to intensive care units, recent hospitalization, use of invasive medical devices, interhospital transfer, malnutrition, presence of comorbidities, HIV infection, and blood transfusion [21,38-40]. Specific factors such as the neonatal period, sickle cell anemia, mechanical ventilation, and neutropenia have been independently associated with an increased risk of acquiring bloodstream infections caused by extended-spectrum beta-lactamase-producing bacteria [18, 27, 40].

Studies show that strict implementation of prevention and control measures can significantly reduce the incidence of nosocomial infections, thereby helping to decrease the costs associated with patient care [61, 67]. For example, it has been estimated that reducing central line-associated bloodstream infections in neonatal units can generate savings of approximately 348,000 USD per year by decreasing hospitalization duration by 84 days [67]. Additionally, it has been found that femoral catheter insertion represents a

higher risk for acquiring HA-BSI and requires more careful management. The use of femoral catheters should be limited to strictly necessary situations, and patients must be closely monitored to prevent complications [46].

Conclusions

Healthcare-associated bloodstream infections remain a major public health issue among pediatric patients, with a significant impact on morbidity, mortality, length of hospital stay, and costs. Despite the high prevalence of these infections, successful examples from various studies suggest that bloodstream infections can be prevented. Thus, the implementation of effective strategies – such as strict adherence to hygiene measures, judicious use of invasive medical devices, rational antibiotic use, and continuous case monitoring – can significantly contribute to reducing the incidence of healthcare-associated bloodstream infections. In this context, epidemiological surveillance and the adoption of evidence-based protocols are crucial for improving the quality of medical care and reducing the impact of these infections on the pediatric population.

Competing interests

None declared.

Acknowledgements and funding

The study had no external funding.

Provenance and peer review

Not commissioned, externally peer review.

Bibliography

1. Zingg W, Holmes A, Dettenkofer M, Goetting T, Secci F, Clack L, et al. Hospital organisation, management, and structure for prevention of health-care-associated infection: a systematic review and expert consensus. Lancet Infect Dis. 2015;15(2):212-24. doi: 10.1016/S1473-3099(14)70854-0.
2. Karagiannidou S, Triantafyllou C, Zaoutis TE, Papaevangelou V, Maniadakis N, Kourlaba G. Length of stay, cost, and mortality of healthcare-acquired bloodstream infections in children and neonates: a systematic review and meta-analysis. Infect Control Hosp Epidemiol. 2020;41(3):342-54. doi: 10.1017/ice.2019.353.
3. World Health Organization. Report on the burden of endemic health care-associated infection worldwide clean care is safer care [Internet]. Geneva: WHO; 2011 [cited 2025 Jun 6]. Available from: https://www.who.int/publications/i/item/report-on-the-burden-of-endemic-health-care-associated-infection-worldwide.
4. Jansen SJ, van der Hoeven A, van den Akker T, Veenhof M, von Asmuth EGJ, Veldkamp KE, et al. A longitudinal analysis of nosocomial bloodstream infections among preterm neonates. Eur J Clin Microbiol Infect Dis. 2022;41(11):1327-36. doi: 10.1007/s10096-022-04502-8.
5. Becerra MR, Tantaleán JA, Suárez VJ, Alvarado MC, Candela JL, Urcia FC. Epidemiologic surveillance of nosocomial infections in a Pediatric Intensive Care Unit of a developing country. BMC Pediatr. 2010;10:66. https://doi.org/10.1186/1471-2431-10-66.
6. Marga I, Paraschiv A. Infecţiile asociate asistenţei medicale la copii [Medical-associated infections in children]. Public Health Econ Manag Med (Chisinau). 2024 Oct;(4):41-9. Romanian. https://doi.org/10.52556/2587-3873.2024.4(101).07.
7. European Centre for Disease Prevention and Control. Healthcare-associated infections acquired in intensive care units. In: ECDC. Annual Epidemiological Report for 2020. Stockholm: ECDC; 2024.
8. Goto M, Al-Hasan M. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect. 2013 Jun;19(6):501-9. doi: 10.1111/1469-0691.12195.
9. Cassini A, Plachouras D, Eckmanns T, Abu Sin M, Blank HP, Ducomble T, et al. Burden of six healthcare-associated infections on European population health: estimating incidence-based disability-adjusted life years through a population prevalence-based modelling study. PLoS Med. 2016 Oct 1;13(10):e1002150. doi: 10.1371/journal.pmed.1002150.
10. Umscheid CA, Mitchell MD, Doshi JA, Agarwal R, Williams K, Brennan PJ. Estimating the proportion of healthcare-associated infections that are reasonably preventable and the related mortality and costs. Infect Control Hosp Epidemiol. 2011 Feb;32(2):101-14. doi: 10.1086/657912.
11. Campbell AJ, Blyth CC, Hewison CJ, Chen YP, Gough L, Goff Z, et al. Lessons learned from a hospital-wide review of blood stream infections for paediatric central line-associated blood stream infection prevention. J Paediatr Child Health. 2019 Jun 1;55(6):690-4. doi: 10.1111/jpc.14276.
12. Singhi S, Nallaswamy K. Catheter related blood stream infection in Indian PICUs: several unanswered issues! Indian J Crit Care Med. 2013;17(3):127-8. doi: 10.4103/0972-5229.117035.
13. European Centre for Disease Prevention and Control; Suetens C, Kärki T, Plachouras D. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2022–2023 [Internet]. Stockholm: ECDC; 2024 [cited 2025 Apr 15]. Available from: www.ecdc.europa.eu
14. Rosenthal VD, Myatra SN, Divatia JV, Biswas S, Shrivastava A, Al-Ruzzieh MA, et al. The impact of COVID-19 on health care–associated infections in intensive care units in low- and middle-income countries: International Nosocomial Infection Control Consortium (INICC) findings. Int J Infect Dis. 2022 May 1;118:83-88. doi: 10.1016/j.ijid.2022.02.041.
15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 state-

ment: An updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi: 10.1136/bmj.n71.
16. Pereira CAP, Marra AR, Camargo LFA, Pignatari ACC, Sukiennik T, Behar PRP, et al. Nosocomial bloodstream infections in Brazilian pediatric patients: microbiology, epidemiology, and clinical features. PLoS One. 2013 Jul 4;8(7):e68144. doi: 10.1371/journal.pone.0068144.
17. Zingg W, Hopkins S, Gayet-Ageron A, Holmes A, Sharland M, Suetens C, et al. Healthcare-associated infections in neonates and children in the first European point prevalence survey. Lancet Infect Dis. 2017 Apr;17(4):381-389. doi: 10.1016/S1473-3099(16)30517-5.
18. Folgori L, Bernaschi P, Piga S, Carletti M, Cunha FP, Lara PHR, et al. Healthcare-associated infections in pediatric and neonatal intensive care units: Impact of underlying risk factors and antimicrobial resistance on 30-day case-fatality in Italy and Brazil. Infect Control Hosp Epidemiol. 2016 Nov 1;37(11):1302-9. doi: 10.1017/ice.2016.185.
19. Younous S, Nadifiyine D, Yassine A, Mouaffak Y, Sahraoui HE, Sihami A, et al. High rates of nosocomial infections and antimicrobial resistance in a Moroccan PICU: a cause for alarm. IJID Reg. 2024;13:100423. doi: 10.1016/j.ijregi.2024.100423.
20. Rosenthal VD, Jarvis WR, Jamulitrat S, Silva CP, Ramachandran B, Dueñas L, et al. Socioeconomic impact on device-associated infections in pediatric intensive care units of 16 limited-resource countries: International Nosocomial Infection Consortium findings. Pediatr Crit Care Med. 2012;13(4):399-406. doi: 10.1097/PCC.0b013e318238b260.
21. Al Saeghi N, Al Thuhli M, Al Hamrashi H, Al Shibli N, Al Wahaibi A, Al Adawi B, et al. Bloodstream infection in children managed at a tertiary hospital in Oman. Sultan Qaboos Univ Med J. 2024;24(4):501-506. doi: 10.18295/squmj.7.2024.044.
22. Khazaei S, Adabi M, Bashirian S, Shojaeian M, Bathaei SJ, Karami M. Epidemiologic profile of nosocomial infections among paediatric patients in a referral hospital in Hamadan, west of Iran. New Microbes New Infect. 2020 Nov 1;38. doi: 10.1016/j.nmni.2020.100823.
23. Wang C, Hao W, Yu R, Wang X, Zhang J, Wang B. Analysis of pathogen distribution and its antimicrobial resistance in bloodstream infections in hospitalized children in East China, 2015–2018. Trop Pediatr. 2021;67(1):fmaa077. doi: 10.1093/tropej/fmaa077.
24. Sader HS, Castanheira M, Streit JM, Carvalhaes CG, Mendes RE. Frequency and antimicrobial susceptibility of bacteria causing bloodstream infections in pediatric patients from United States (US) medical centers (2014–2018): therapeutic options for multidrug-resistant bacteria. Diagn Microbiol Infect Dis. 2020 Oct 1;98(2):115108. doi: 10.1016/j.diagmicrobio.2020.115108.
25. Paut O, Orliaguet G. Réanimation pédiatrique. Cham: Springer; 2013.
26. Jordan Garcia I, Esteban Torné E, Bustinza Arriortua A, De Carlos Vicente JC, García Soler P, Concha Torre JA, et al. Trends in nosocomial infections and multidrug-resistant microorganisms in Spanish pediatric intensive care units. Enferm Infecc Microbiol Clin. 2016 May 1;34(5):286-92. doi: 10.1016/j.eimc.2015.07.010.
27. Kepenekli E, Soysal A, Yalindag-Ozturk N, Ozgur O, Ozcan I, Devrim I, et al. Healthcare-associated infections in pediatric intensive care units in Turkey: a national point-prevalence survey. Jpn J Infect Dis. 2015 Sep 19;68(5):381-6. doi: 10.7883/yoken.JJID.2014.385.
28. Le NK, Hf W, Vu PD, Khu DTK, Le HT, Hoang BTN, et al. High prevalence of hospital-acquired infections caused by gram-negative carbapenem resistant strains in Vietnamese pediatric ICUs. Medicine (United States). 2016 Jul 1;95(27):e4099. doi: 10.1097/MD.0000000000004099.
29. Castanheira M, Deshpande LM, Mendes RE, Canton R, Sader HS, Jones RN. Variations in the occurrence of resistance phenotypes and carbapenemase genes among enterobacteriaceae isolates in 20 years of the SENTRY antimicrobial surveillance program. Open Forum Infect Dis. 2019 Mar 15;6(Suppl 1):S23-33. doi: 10.1093/ofid/ofy347.
30. Flannery DD, Akinboyo IC, Mukhopadhyay S, Tribble AC, Song L, Chen F, et al. Antibiotic susceptibility of Escherichia coli among infants admitted to neonatal intensive care units across the US from 2009 to 2017. JAMA Pediatr. 2021;175(2):168-75. doi: 10.1001/jamapediatrics.2020.4719.
31. Dramowski A, Cotton MF, Rabie H, Whitelaw A. Trends in paediatric bloodstream infections at a South African referral hospital. BMC Pediatr. 2015 Apr 2;15(1):33. doi: 10.1186/s12887-015-0354-3.
32. Lipworth S, Vihta KD, Davies T, Wright S, Tabirao M, Chau K, et al. Molecular epidemiology and antimicrobial resistance phenotype of paediatric bloodstream infections caused by Gram-negative bacteria. Commun Med. 2022;2:101. doi: 10.1038/s43856-022-00161-0.
33. Venturini E, Montagnani C, Benni A, Becciani S, Biermann KP, De Masi S, et al. Central-line associated bloodstream infections in a tertiary care children's university hospital: a prospective study. BMC Infect Dis. 2016 Dec 1;16(1):725. doi: 10.1186/s12879-016-2061-6.
34. Stoll BJ, Puopolo KM, Hansen NI, Sánchez PJ, Bell EF, Carlo WA, et al. Early-onset neonatal sepsis 2015 to 2017, the rise of *Escherichia coli*, and the need for novel prevention strategies. JAMA Pediatr. 2021;175(2):212. doi: 10.1001/jamapediatrics.2020.6111.
35. Sands K, Carvalho MJ, Portal E, Thomson K, Dyer C, Akpulu C, et al. Characterization of antimicrobial-re-

sistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. Nat Microbiol. 2021 Apr 1;6(4):512-23. doi: 10.1038/s41564-021-00870-7.
36. Spaulding AB, Watson D, Dreyfus J, Heaton P, Grapentine S, Bendel-Stenzel E, et al. Epidemiology of bloodstream infections in hospitalized children in the United States, 2009–2016. Clin Infect Dis. 2019 Aug 30;69(6):995-1002. doi: 10.1093/cid/ciy1030.
37. Hussain MS, Basher H, Lone MS, Wani K. Bloodstream infections in pediatric population in a tertiary care hospital. Int J Contemp Med Res. 2019 Feb;6(2). doi:10.21276/ijcmr.2019.6.2.27
38. Olivier C, Kunneke H, O'Connell N, von Delft E, Wates M, Dramowski A. Healthcare-associated infections in paediatric and neonatal wards: a point prevalence survey at four South African hospitals. S Afr Med J. 2018 May 1;108(5):418-22. doi: 10.7196/SAMJ.2018.v108i5.12862.
39. Dramowski A, Whitelaw A, Cotton MF. Burden, spectrum, and impact of healthcare-associated infection at a South African children's hospital. J Hosp Infect. 2016 Dec 1;94(4):364-72. doi: 10.1016/j.jhin.2016.08.022.
40. Ndir A, Diop A, Faye PM, Cissé MF, Ndoye B, Astagneau P. Epidemiology and burden of bloodstream infections caused by extended-spectrum beta-lactamase producing enterobacteriaceae in a pediatric hospital in Senegal. PLoS One. 2016 Feb 1;11(2):e0143729. doi: 10.1371/journal.pone.0143729.
41. Kelly MS, Conway M, Wirth KE, Potter-Bynoe G, Billett AL, Sandora TJ. Microbiology and risk factors for central line-associated bloodstream infections among pediatric oncology outpatients: a single institution experience of 41 cases. J Pediatr Hematol Oncol. 2013;35(2):e71-6. doi: 10.1097/MPH.0b013e3182820edd.
42. Gaur AH, Bundy DG, Werner EJ, Hord JD, Miller MR, Tang L, et al. A prospective, holistic, multicenter approach to tracking and understanding bloodstream infections in pediatric hematology-oncology patients. Infect Control Hosp Epidemiol. 2017 Jun 1;38(6):690-6. doi: 10.1017/ice.2017.57.
43. Murshed IAS, Zhao L, Zhang W, Yin Y, Li Y, Peng Y, et al. Bloodstream infections in pediatric hematology/oncology patients: a single-center study in Wuhan. Front Cell Infect Microbiol. 2024;14:1480952. doi: 10.3389/fcimb.2024.1480952.
44. Wagner M, Bonhoeffer J, Erb TO, Glanzmann R, Häcker FM, Paulussen M, et al. Prospective study on central venous line associated bloodstream infections. Arch Dis Child. 2011 Sep;96(9):827-31. doi: 10.1136/adc.2010.208595.
45. Alsaad T, Qaisuddin M, AlSaad D, Chandra P, AlAbd O, Nasser A, et al. Central line-associated bloodstream infection in pediatric oncology patients in Qatar: a prospective study. J Appl Hematol. 2017;8(2):49-53. doi: 10.4103/joah.joah_13_17.
46. Hussain M, Nadeem M, Khan A, Rehman K, Mirza A, Rehman M, et al. Bacterial spectrum and antimicrobial pattern of blood stream infections associated with non-tunneled double lumen catheter in hemodialysis. Pak Armed Forces Med J. 2021;71(4):1161-66. https://doi.org/10.51253/pafmj.v71i4.4438.
47. Skogberg K, Lyytikäinen O, Ollgren J, Nuorti JP, Ruutu P. Population-based burden of bloodstream infections in Finland. Clin Microbiol Infect. 2012;18(6):E170-6. doi: 10.1111/j.1469-0691.2012.03845.x.
48. Garrido MM, Garrido RQ, Cunha TN, Ehrlich S, Martins IS. Comparison of epidemiological, clinical and microbiological characteristics of bloodstream infection in children with solid tumours and haematological malignancies. Epidemiol Infect. 2019 Nov 8;147:e298. doi: 10.1017/S0950268819001845.
49. Ammann RA, Laws HJ, Schrey D, Ehlert K, Moser O, Dilloo D, et al. Bloodstream infection in paediatric cancer centres - leukaemia and relapsed malignancies are independent risk factors. Eur J Pediatr. 2015 May 1;174(5):675-86. doi: 10.1007/s00431-015-2525-5.
50. Thurman CB, Abbott M, Liu J, Larson E. Risk for health care–associated bloodstream infections in pediatric oncology patients with various malignancies. J Pediatr Oncol Nurs. 2017 May 1;34(3):196-202. doi: 10.1177/1043454216680596.
51. Ramsunder P, Appel I, Dramowski A. Epidemiology of healthcare-associated bloodstream infections in the paediatric intensive care unit 2021 [Internet]. Stellenbosch: Stellenbosch University; 2021 [cited 2025 May 26]. Available from: https://scholar.sun.ac.za/server/api/core/bitstreams/aebd45cd-563a-423f-b97e-c347061b4c29/content
52. Mehl A, Åsvold BO, Kümmel A, Lydersen S, Paulsen J, Haugan I, et al. Trends in antimicrobial resistance and empiric antibiotic therapy of bloodstream infections at a general hospital in Mid-Norway: a prospective observational study. BMC Infect Dis. 2017 Feb 2;17(1):116. doi: 10.1186/s12879-017-2210-6.
53. Abubakar U, Amir O, Rodríguez-Baño J. Healthcare-associated infections in Africa: a systematic review and meta-analysis of point prevalence studies. J Pharm Policy Pract. 2022;15(1):99. doi: 10.1186/s40545-022-00500-5.
54. Holmbom M, Giske CG, Fredrikson M, Balkhed ÅÖ, Claesson C, Nilsson LE, et al. 14-year survey in a Swedish county reveals a pronounced increase in bloodstream infections (BSI). Comorbidity - an independent risk factor for both BSI and mortality. PLoS One. 2016 Nov 1;11(11):e0166527. doi: 10.1371/journal.pone.0166527.
55. Prowle JR, Echeverri JE, Ligabo EV, Sherry N, Taori GC, Crozier TM, et al. Acquired bloodstream infection in the intensive care unit: incidence and attributable

mortality. Crit Care. 2011 Mar 21;15(2):R100. doi: 10.1186/cc10114.
56. Jin L, Zhao C, Li H, Wang R, Wang Q, Wang H. Clinical profile, prognostic factors, and outcome prediction in hospitalized patients with bloodstream infection: results from a 10-year prospective multicenter study. Front Med (Lausanne). 2021 May 20;8:629671. doi: 10.3389/fmed.2021.629671.
57. Ekubu Otim M, Nawaz Khan H, Grima S, Naylor NR, Yamashita K, Iwami M, et al. Code-sharing in cost-of-illness calculations: an application to antibiotic-resistant bloodstream infections cost-of-illness for antibiotic resistance. Front Public Health. 2020 Nov 27;8:562427. doi: 10.3389/fpubh.2020.562427.
58. Karagiannidou S, Zaoutis T, Maniadakis N, Papaevangelou V, Kourlaba G. Attributable length of stay and cost for pediatric and neonatal central line-associated bloodstream infections in Greece. J Infect Public Health. 2019 May 1;12(3):372-9. doi: 10.1016/j.jiph.2018.12.004.
59. Wassef MA, Ghaith DM, Hussien MM, El-Shazly MA, Yousef RHA. Bundle care approach to reduce device-associated infections in post-living-donor-liver transplantation in a tertiary care hospital, Egypt. BMC Infect Dis. 2024 Dec 1;24(1):674. doi: 10.1186/s12879-024-09525-4.
60. Ng YY, Abdel-Latif MEA, Gan CS, Siham A, Zainol H, Lum LCS. Impact of infection control training for interns on PICU-acquired bloodstream infections in a middle-income country. Singapore Med J. 2015 Sep 1;56(9):506-12. doi: 10.11622/smedj.2015135.
61. Shepherd EG, Kelly TJ, Vinsel JA, Cunningham DJ, Keels E, Beauseau W, et al. Significant reduction of central-line associated bloodstream infections in a network of diverse neonatal nurseries. J Pediatr. 2015 Jul 1;167(1):41-46.e3. doi: 10.1016/j.jpeds.2015.03.046.
62. Huang H, Wu K, Chen H, Wang J, Chen L, Lai Z, et al. The impact of the COVID-19 pandemic on nosocomial infections: a retrospective analysis in a tertiary maternal and child healthcare hospital. Front Public Health. 2023;11:1132323. doi: 10.3389/fpubh.2023.1132323.
63. Busuioc E, Caterinciuc N. Primul studiu național de prevalență de moment a infecțiilor asociate asistenței medicale și a consumului antimicrobienelor în spitalele din Republica Moldova [First national point prevalence survey of healthcare-associated infections and antimicrobial use in acute care hospitals in the Republic of Moldova]. One Health Risk Manag. 2022;3(1):21-8. Romanian. doi: 10.38045/ohrm.2021.4.05.
64. Friedman C. The costs of healthcare-associated infections. In: IFIC Basic Concepts of Infection Control. 3rd edition. Portadown (UK): IFIC; 2016 [cited 2025 May 29]. Available from: https://www.theific.org/wp-content/uploads/2016/04/CostsCh29.pdf
65. Leoncio JM, de Almeida VF, Ferrari RAP, Capobiango JD, Kerbauy G, Tacla MTGM. Impact of healthcare-associated infections on the hospitalization costs of children. Rev Esc Enferm USP. 2019;53:e03486. doi: 10.1590/S1980-220X2018016303486.
66. De Angelis G, Murthy A, Beyersmann J, Harbarth S. Estimating the impact of healthcare-associated infections on length of stay and costs. Clin Microbiol Infect. 2010 Dec 1;16(12):1729-35. doi: 10.1111/j.1469-0691.2010.03332.x.
67. Ceballos K, Waterman K, Hulett T, Makic MBF. Nurse-driven quality improvement interventions to reduce hospital-acquired infection in the NICU. Adv Neonatal Care. 2013 Jun;13(3):154-63. doi: 10.1097/ANC.0b013e318285fe70.

https://doi.org/10.52645/MJHS.2026.2.14

UDC: 618.33-005.98-02:616.972-053.1

CASE STUDY

Non-immune fetal hydrops and intestinal obstruction: rare manifestations of congenital syphilis

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ABSTRACT

Introduction. Fetal hydrops is defined as the pathological accumulation of extracellular fluid in at least two fetal anatomical compartments, including skin edema (> 5 mm thickness), pericardial effusion, pleural effusion, and ascites. Non-immune fetal hydrops (NIHF) accounts for over 90% of all fetal hydrops cases and has a heterogeneous etiology. Congenital infections contribute to approximately 6–7% of NIHF cases and are associated with a severe neonatal prognosis.

Case presentation. A preterm newborn was delivered from a pregnancy complicated by untreated maternal primary syphilis. The fetus had been diagnosed antenatally with NIHF, heart failure, and massive ascites. Postnatally, the infant required early ascitic drainage and subsequently underwent surgery for congenital intestinal obstruction in the context of ileal stenosis. Neonatal serological testing revealed a positive rapid plasma reagin (RPR) and a reactive Treponema pallidum Hemagglutination Assay (TPHA). Management of congenital syphilis was carried out according to the standardized national clinical protocol. The collected data were compared with those reported in the existing literature to assess clinical significance.

Results. The neonate showed a favorable clinical evolution following multidisciplinary management, including intensive care support, anti-infective therapy, and surgical correction of the intestinal obstruction. Progressive improvement allowed successful postoperative recovery and discharge in satisfactory condition.

Conclusions. Early identification of the infectious etiology of fetal hydrops is essential for the implementation of appropriate management and the improvement of neonatal outcomes. Close collaboration between maternal–fetal medicine, neonatology, and pediatric surgery is crucial in managing such complex cases.

Keywords: congenital syphilis; non-immune hydrops fetalis; intestinal obstruction.

Cite this article: Neagu A, Crivceanscaia L. Non-immune fetal hydrops and intestinal obstruction: rare manifestations of congenital syphilis. Mold J Health Sci. 2026;13(2):116-120. https://doi.org/10.52645/MJHS.2026.2.14.

Manuscript received: 31.03.2026

Accepted for publication: 18.05.2026

Published: 15.06.2026

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Although congenital syphilis is a preventable condition, it continues to be reported with increasing incidence worldwide. Cases of non-immune fetal hydrops secondary to congenital syphilis are rarely described in the literature and are frequently associated with high perinatal mortality. The present case report contributes to the existing literature by providing a detailed description of a favorable outcome in an extremely severe clinical context.

The research hypothesis

Early antenatal identification of progression toward non-immune fetal hydrops (NIHF) is independently associated with improved perinatal survival in pregnancies complicated by congenital infections. Furthermore, systematic and continuous monitoring of

pregnant women with sexually transmitted infections is associated with earlier detection of fetal and maternal complications, including Ballantyne syndrome, thereby contributing to improved clinical outcomes.

The novelty added by the manuscript to the already published scientific literature

The presented case demonstrates that survival is possible even in extreme forms of congenital syphilis complicated by fetal hydrops and associated surgical pathology, provided that rigorous and individualized multidisciplinary management is implemented.

Introduction

Following the introduction of anti-D immunoglobulin prophylaxis in 1968, the incidence of immune fetal hydrops decreased significantly [1]. Currently, non-immune etiologies account for approximately 95% of all cases of fetal hydrops [1, 2].

Historically, NIHF was considered a lethal condition. However, understanding NIHF as a clinical sign of a wide spectrum of underlying disorders, together with advances in prenatal diagnosis, neonatal intensive care, and pediatric surgery, has changed this perception [3, 4]. Cardiovascular malformations represent the most frequent etiology of NIHF (21.4%), followed by idiopathic causes (18.2%), chromosomal abnormalities (12.5%), hematologic disorders (10.1%), and congenital lymphatic dysplasia (7.5%). Infections account for 6.8% of NIHF cases, with the most common being parvovirus B19, cytomegalovirus, adenovirus, and enterovirus infections [1].

Maternal infection with *Treponema pallidum* can affect the fetus at all stages of the disease, regardless of the gestational trimester. During the primary and secondary stages of syphilis, the likelihood of vertical transmission from an untreated pregnant woman to her fetus approaches 100% [1]. The global prevalence of congenital syphilis is increasing, rising from 203 cases per 100,000 live births in 2015 to 295 cases per 100,000 in 2021 [5]. Clinical manifestations of fetal infection with *Treponema pallidum* include hepatosplenomegaly, thymic involution, placentomegaly, increased peak systolic velocity in the middle cerebral artery (a marker of fetal anemia), ascites, low birth weight, fetal hydrops, preterm birth, and perinatal death [6, 7]. Proinflammatory cytokines and an imbalance of angiogenic factors (PlGF – placental growth factor, VEGF – vascular endothelial growth factor) induce endothelial injury, contributing to placental dysfunction characteristic of Ballantyne syndrome ("mirror syndrome") [8].

Case presentation

According to the anamnestic and serological data, maternal syphilitic infection was acquired during the current pregnancy, most likely in the second or third trimester of gestation, and was serologically detected in the active stage (primary syphilis). The absence of specific etiological treatment favored transplacental transmission of the infection and the development of a severe form of congenital syphilis.

We report the case of a preterm neonate born at 31 weeks of gestation, with a birth weight of 2254 g, body length of 42 cm, head circumference of 32 cm, and Apgar scores of 3 and 5 at 1 and 5 minutes, respectively. The pregnancy was complicated by untreated maternal primary syphilis, intrauterine infection, and polyhydramnios.

At birth, the neonate was in an extremely critical condition, presenting with generalized edema, severe respiratory distress, marked abdominal distension, and hemorrhagic cutaneous lesions. The abdominal circumference measured at the level of the umbilical stump was 36 cm. Endotracheal intubation was performed in the first minute of life, and the patient was placed on mechanical ventilation. Ultrasonography revealed hepatomegaly, a large amount of free intraperitoneal fluid, and intestinal loops compressed toward the posterior abdominal wall. Subsequent computed tomography confirmed the ultrasound findings, demonstrating hepatomegaly (right lobe 7.0 cm, left lobe 2.6 cm) extending into the splenic space with a mass effect displacing the spleen medially and caudally; the splenic size remained within normal limits. Abdominal radiography showed pronounced distension with an absence of intraluminal intestinal gas. Chest imaging revealed severe type I respiratory distress syndrome, right-sided polysegmental pneumonia, alveolar pulmonary edema, and marked cardiomegaly. Transthoracic echocardiography demonstrated nondilated cardiac chambers with preserved left ventricular systolic function. A patent foramen ovale (PFO) with a left-to-right shunt at the interatrial septum measuring 3 mm was identified, along with false chordae tendineae in the left ventricular cavity. Mild tricuspid regurgitation (grade I) and mild pulmonary valve regurgitation (grade I) were present, associated with mild pulmonary hypertension, estimated echocardiographically by a systolic pulmonary artery pressure of approximately 39 mmHg.

At 2 hours of life, a right-sided drainage microlaparotomy was performed, with the evacuation of clear serous ascitic fluid. The abdominal circumference decreased by 2 cm. Over time, ventilatory support parameters were reduced, while corrective measures addressed anemia (Hb 59 g/L, Ht 17%), thrombocytopenia (platelets 22 × 10^9/L), hypoproteinemia (total protein 39.26 g/L), and hypoglycemia (glucose 1.1 mmol/L). Neonatal serology confirmed active treponemal infection, and microbiological investigations excluded other bacterial infectious etiologies. During the course of the disease, the neonate developed syphilitic infectious hepatitis (ALT 278 U/L, AST 333 U/L) associated with severe cholestasis, evidenced by a total bilirubin level of 235.62 μmol/L with a predominance of direct bilirubin (192.12 μmol/L, approximately 82% of the total), consist-

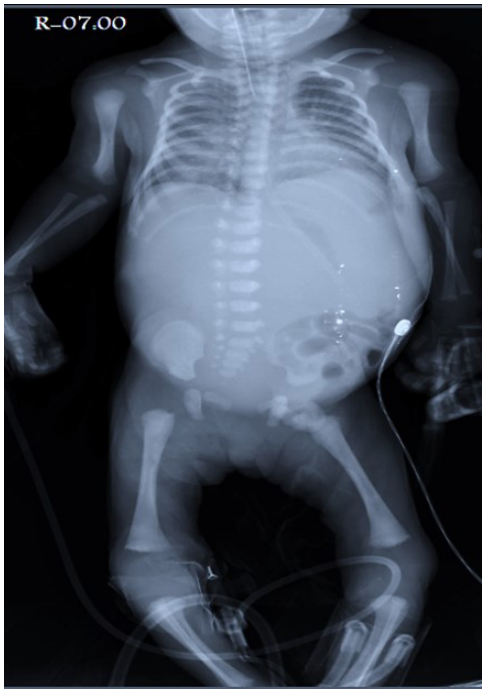

Fig. 1 Radiograph of the long bones showing metaphyseal changes suggestive of congenital syphilitic osteochondritis (metaphyseal irregularity and areas of osteolysis).

ent with syphilitic hepatocellular injury. Acute renal impairment was also documented (urea 28.46 mmol/L, creatinine 166.38 µmol/L). Radiography of the long bones demonstrated metaphyseal syphilitic osteochondritis (Fig. 1).

By the 8th day of life, the general condition showed slight improvement, and regression of respiratory failure allowed de-escalation of ventilatory therapy to noninvasive support.

On the 12th day of life, given the presence of blood streaks in the gastric aspirate, progressive abdominal distension, the absence of meconium passage since birth, and a lack of stool following a cleansing enema, an upright abdominal radiographic study with contrast at predefined intervals was performed (Fig. 2). This revealed retention of contrast within the small intestine, suggestive of meconium ileus, which was subsequently confirmed by computed tomography. The neonate underwent segmental small bowel resection with end-to-end enteroenterostomy. The postoperative diagnosis was: "Congenital distal intestinal obstruction. Ileal stenosis. Diffuse serofibrinous peritonitis. Fetal hepatitis."

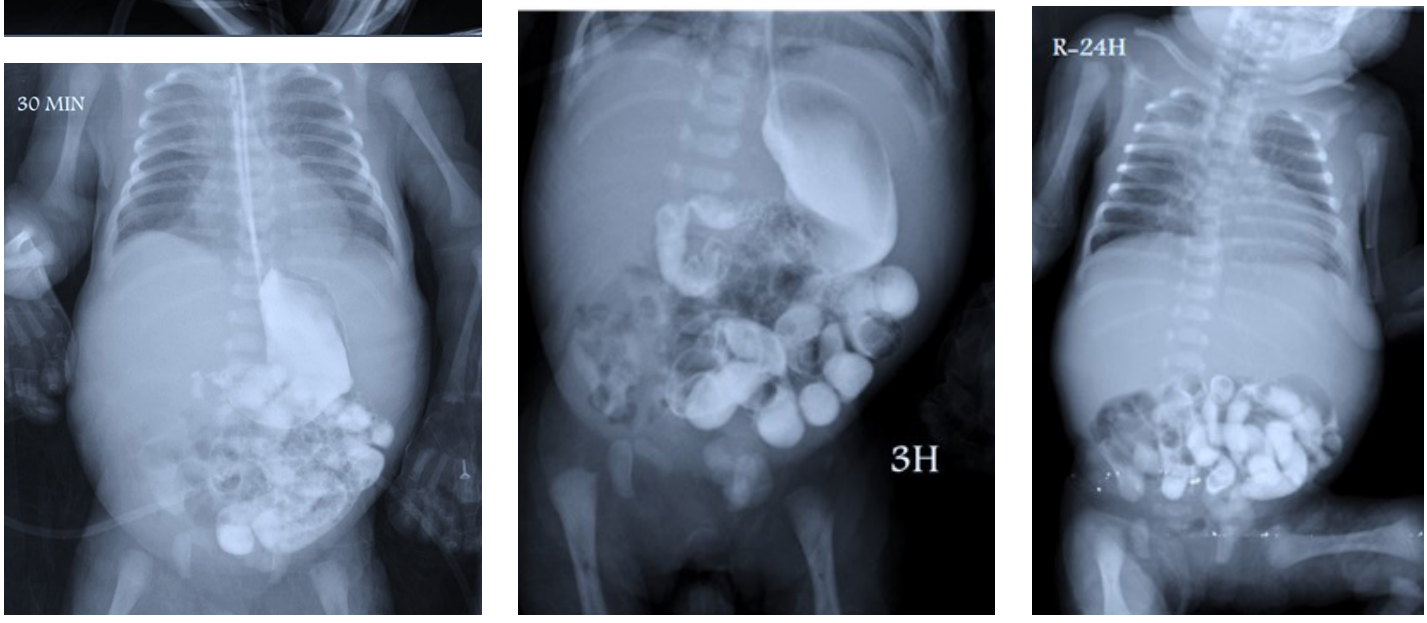

Fig. 2 Serial upright abdominal radiographs

A - 30 minutes, B - 3 hours, and C - 24 hours following administration of an iodinated contrast medium (Ultravist), demonstrating a lack of distal progression and retention of contrast within the small intestine.

The postoperative course was complicated by anemia, thrombocytopenia, pulmonary hemorrhage, toxic hepatitis, retinopathy of prematurity stage I–II, and neonatal jaundice. The neonate was weaned from ventilatory support 4 days postoperatively and passed stool on the 7th postoperative day. Histopathological examination confirmed the postoperative diagnosis, revealing connective tissue with micro- and macrofocal hemorrhages, intravascular erythrocyte thrombi, and focal and diffuse lymphocytic infiltration. The infant was completely weaned from oxygen support at 46 days of life and discharged in a satisfactory condition at 73 days of life.

Antepartum, maternal manifestations of NIHF included moderate peripheral edema, hypoalbuminemia (albumin 30.80 g/L), and hemodilution (urea 2.11 mmol/L, creatinine 39.80 µmol/L), which persisted postpartum. Histopathological examination of the placenta revealed discirculatory-anemic placentopathy associated with amnionitis.

Discussion

Although syphilitic infection is a preventable and treatable condition through prenatal screening and appropriate antimicrobial therapy, it continues to represent a public health problem, particularly in regions with limited access to healthcare services. The persistence of congenital syphilis cases primarily reflects deficiencies in the early detection of maternal infection and delayed or absent initiation of etiological treatment. The lack of adequate treatment during pregnancy is associated with increased perinatal mortality, severe neonatal morbidity, and long-term sequelae.

The present case demonstrates that untreated congenital syphilis may progress with severe systemic manifestations, including multiorgan involvement and rare surgical complications, requiring complex multidisciplinary interventions to ensure survival. Preterm neonates exhibit immature intestinal motility and a vascular system predisposed to injury, which may facilitate the extension of inflammatory lesions and increase susceptibility to severe intestinal obstruction in the context of treponemal inflammation [9]. The presence of non-immune fetal hydrops indicates an advanced stage of congenital infection and reflects the systemic impact of fetal inflammation and associated placental dysfunction. The pathogenic mechanisms involved include fetal anemia secondary to hemolysis, high-output cardiac failure, hepatic impairment with hypoalbuminemia, and placental dysfunction caused by treponemal vasculitis. These mechanisms cumulatively contribute to fluid accumulation in the fetal compartments and the development of hydropic syndrome.

Antenatal diagnosis of intestinal stenosis or obstruction remains challenging and is primarily based on indirect imaging findings. Fetal ultrasonography may reveal dilated bowel loops, increased echogenicity, and reduced or absent peristalsis, frequently associated with polyhydramnios and intrauterine growth restriction. Fetal magnetic resonance imaging (MRI) can provide additional information regarding meconium distribution and the probable level of obstruction. However, precise confirmation of the affected segment and the degree of stenosis is achieved in most cases postnatally, sometimes only during the surgical intervention [10].

In the present case, the antenatal diagnosis of fetal hydrops allowed anticipation of a guarded neonatal prognosis and facilitated the early initiation of intensive supportive measures immediately after birth. Prompt intervention through early ascitic drainage was essential to reduce intra-abdominal pressure, improve respiratory mechanics, and achieve hemodynamic stabilization. The combination of invasive respiratory support, hematological corrections, and anti-infective therapy played a decisive role in the initial stabilization of the neonate.

A particular feature of this case is the association of fetal hydrops with distal congenital intestinal obstruction. The literature describes only isolated cases of intestinal obstruction or necrotizing enterocolitis associated with congenital syphilis, and the causal relationship remains insufficiently elucidated [7, 11]. In the absence of specific histopathological evidence of treponemal obliterative vasculitis at the intestinal level, a direct causal link cannot be established. Nevertheless, prenatal systemic inflammation, mesenteric ischemia, and subclinical vascular injury may represent plausible contributory mechanisms.

The postoperative course was marked by complications commonly encountered in severe congenital syphilis, necessitating close monitoring and continuous therapeutic adjustments. The progressive recovery and survival of the neonate underscore the importance of an integrated multidisciplinary approach involving neonatologists, pediatric surgeons, infectious disease specialists, and intensive care physicians.

Another relevant aspect of this case is the presence of maternal manifestations associated with fetal hydrops, consistent with Ballantyne syndrome ("mirror syndrome"). Maternal edema, hemodilution, and hypoproteinemia suggest the involvement of placental dysfunction and angiogenic imbalance in the pathogenesis of this syndrome. Early recognition of maternal signs may serve as an additional indicator for assessing the severity of fetal involvement.

Prevention of congenital syphilis remains essential, as once fetal hydrops develops, the neonatal prognosis is often unfavorable, and postnatal interventions become complex and resource-intensive. Systematic screening of pregnant women, repeat testing in subsequent trimesters for high-risk populations, and the prompt treatment of maternal infection are key measures for reducing the incidence of these severe forms.

In line with the existing literature, this report highlights the need for increased clinical vigilance regarding atypical and rare manifestations of congenital syphilis. The limitations of this report include its single-center nature and the inability to establish a definitive causal relationship between treponemal infection and intestinal malformation, underscoring the need for further studies to clarify these rare associations.

Conclusions

This case demonstrates that even severe congenital syphilis complicated by non-immune fetal hydrops may result in a favorable outcome when an early diagnosis and timely, coordinated multidisciplinary management are ensured. The report underscores the essential role of systematic prenatal screening for maternal treponemal infection and the importance of integrated perinatal care in improving survival and reducing severe neonatal morbidity.

Competing interests

None declared.

Acknowledgements and funding

No external funding.

Authors' contributions

AN contributed to the acquisition, analysis, and interpretation of the primary data, and LC participated in the study design and drafted the manuscript. All authors critically re-

viewed the work and approved the final version of the manuscript.

Ethics approval

Not needed for this study.

Informed consent for publication

Obtained.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Dysart K, Moldenhauer J. Nonimmune hydrops fetalis. In: Eichenwald E, Hansen A, Martin C, Stark A, editors. Cloherty and Stark's Manual of neonatal care. 9th ed. Philadelphia: Wolters Kluwer; 2023. p. 52-64.
2. Crivceanscaia L, Pintea I. Sifilis congenital [Congenital syphilis]. In: Crivceanscaia L, et al. Protocoale clinice standardizate pentru secțiile neonatale [Standardized clinical protocols for neonatal units]. Chisinau; 2022. p. 288-295. Romanian.
3. Pegoraro V, Urbinati D, Visser G, Renzo G, Zipursky A, Stotler B, et al. Hemolytic disease of the fetus and newborn due to Rh(D) incompatibility: a preventable disease that still produces significant morbidity and mortality in children. PLoS One. 2020;15(7):e0235807. doi: 10.1371/journal.pone.0235807.
4. Swearingen C, Colvin Z, Leuthner S. Nonimmune hydrops fetalis. Clin Perinatol. 2020;47(1):105-121. doi: 10.1016/j.clp.2019.10.001.
5. Duan B, Zhou Y, Wang X, Wang H, Li Y, Zhou X, et al. Congenital syphilis: adverse pregnancy outcomes and neonatal disorders. Infection. 2025;53(6):2303-2319. doi: 10.1007/s15010-025-02591-z.
6. Rac M, Revell P, Eppes C. Syphilis during pregnancy: a preventable threat to maternal-fetal health. Am J Obstet Gynecol. 2017;216(4):352-363. doi: 10.1016/j.ajog.2016.11.1052.
7. Serra G, Carta M, Pace MR, Sala E, Piro E, Salerno S, Schierz IA, Vassallo A, Giuffrè M, Corsello G. Congenital syphilis in a preterm newborn with gastrointestinal disorders and postnatal growth restriction. Ital J Pediatr. 2022;48(1):205. doi: 10.1186/s13052-022-01404-5.
8. Chandra S, Chowdhury S. Systemic effect of different physiological parameter associated with mirror syndrome. J Obstet Gynaecol Res. 2025;51(9):e70062. doi: 10.1111/jog.70062.
9. Lee HS, Lee JI, Jeon J. Congenital syphilis unusually presenting with prematurity-related severe neonatal morbidities including meconium obstruction: a case report and review of the literature. Medicine (Baltimore). 2020;99(40):e22321. doi: 10.1097/MD.0000000000022321.
10. Rubio EI, Blask AR, Badillo AT, Bulas DI. Prenatal magnetic resonance and ultrasonographic findings in small-bowel obstruction: imaging clues and postnatal outcomes. Pediatr Radiol. 2017;47(4):411-421. doi: 10.1007/s00247-016-3770-0.
11. Çelik M, Bülbül A, Uslu S. Congenital syphilis presenting with prenatal bowel hyperechogenicity and necrotizing. Med Bull Şişli Etfal Hospital. 2020;54(1):113-116. doi: 10.14744/SEMB.2018.22605.

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All persons who have made substantial contributions to the work (technical editing, writing assistance, general administrative support, financial and material support) **but do not meet all four criteria for authorship** are listed in *Acknowledgments* section and have given us their written permission to be named.

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MJHS Moldovan Journal of Health Sciences
REVISTA DE ȘTIINȚE ALE SĂNĂTĂȚII DIN MOLDOVA

[Revised January, 2025]

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ABSTRACT AND KEYWORDS	> Structured abstract > Keywords > Clinical trial registration information
MAIN TEXT AND REFERENCES	> Introduction > Materials and methods > Results > Discussion > Conclusions > References
TABLES AND FIGURES	> Tables are inserted immediately after the first paragraph in which they are cited > Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figures should be submitted also as separate files.

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The Introduction section should: provide a context or background for the study that would allow readers outside the field to understand the purpose and significance of the study; define the problem addressed and explain why it is important; include a brief review of recent literature in the field; mention any relevant controversies or disagreements existing in the field; formulate research hypothesis and present the main and secondary assessed outcomes; conclude with the research' propose and a short comment whether the purpose has been achieved. The Introduction should not contain either results or conclusions.

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Authors must present results logically using text, tables, and figures, giving the main or most important findings first. Results should be explained (not justified or compared in this section) and include fundamental statements related to hypothesis behind the study.

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This section should conclude laconically entire study, and highlight the added-value brought on the studied issue. The conclusions should not provide new information or double (repeat) those presented in the *Results* section.

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It may be cited only articles orabstracts that have been published and are available through public servers. Personal communications, manuscripts in preparation, and other unpublished data should not be included in the reference list, but may be mentioned in parentheses in the text as "unpublished data" or "unpublished observations", indicating the involved researchers. It is of manuscript authors' responsibility to obtain the permission to refer to unpublished data.

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Examples of references

Journal article

Belîi A, Cobâleţchi S, Casian V, Belîi N, Severin G, Chesov I, Bubulici E. Les aspects pharmacoéconomiques dans la gestion de la douleur périopératoire [Pharmaco-economic aspects of perioperative pain management]. Ann Fr Anesth Reanim. 2012;31(1):60-6. French. doi: 10.1016/j.annfar.2011.09.008.

Book

Razin MP, Minaev SV, Turabov IA. Detskaia khirurgiia [Pediatric surgery]. 2nd ed. Moscow: Geotar-Media; 2020. 696 p. Russian.

Chapter in a book

Steiber AL, Chazot C, Kopple JD. Vitamin and trace element needs in chronic kidney disease. In: Burrowes J, Kovesdy C, Byham-Gray L, editors. Nutrition in kidney disease. 3rd ed. Cham: Humana Press; 2020. p. 607-623.

Conference paper

Ojovan V. Medical rehabilitation of children with type 1 diabetes: medical bioethical and psychosocial aspects. In: MedEspera: 9th International Medical Congress for Students and Young Doctors, 12-14 May 2022, Chisinau, Republic of Moldova: Abstract book. Chișinău; 2022. p. 77.

Website reference

World Health Organization (WHO). Therapeutics for Ebola virus disease [Internet]. Geneva: WHO; 2022 [cited 2022 Sep 5]. Available from: https://www.who.int/publications/i/item/9789240055742

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Table 1. Intra-anesthetic and immediately post-extubation adverse events

	Experimental Cohort (n=100)	Control Cohort (n=100)	p
Dysrhythmia	6.0%	30%	0.49
Hemodynamic instability	7.0%	1.0%	0.034
Prolonged awakening*	11.0%	4.0%	0.19
PONV post-intubation	8.0%	27.0%	0.007
Strong pain on awakening	17.0%	19.0%	1.0

Note: *Unusually slow awaking, after that cerebral concentration of the anesthetic reach the under hypnotic level.
Used statistical analysis: Fisher's exact test.

Figures (photographs or radiographs, drawings, graphs, bar charts, flow charts, and pathways) should be submitted in a suitable format for print publication. Figures should be either professionally drawn and photographed, or submitted as photographic-quality digital prints. Figures' quality should assure the visibility of details. The following file formats are accepted: ".jpeg", ".tiff", ".eps" (preferred format for diagrams), ".ppt", ".pptx" (figures should be of the size of a single slide), with a resolution of at least 300 dpi.
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4. agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements and funding

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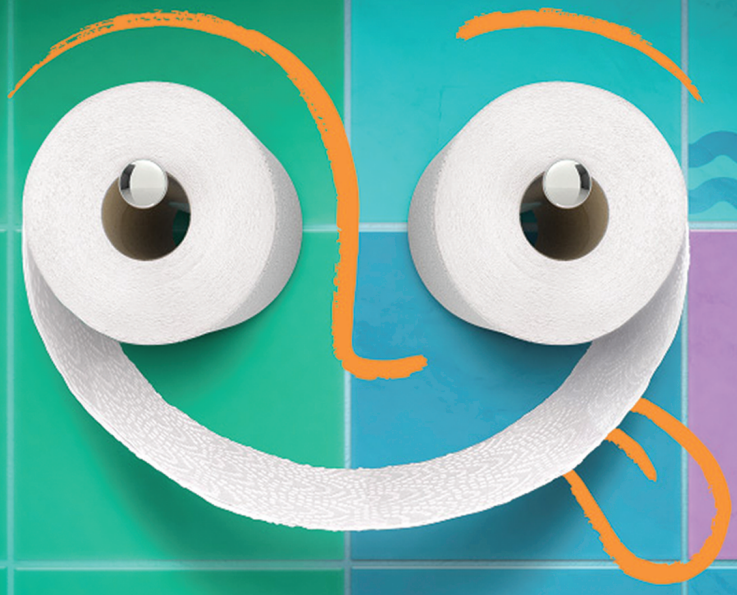

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Activitatea medicală și academică este susținută de **DR. VIOREL ISTRATE**, Conferențiar Universitar, Doctor în științe medicale, medic endoscopist, categorie superioară endoscopie și chirurgie, expert în domeniul endoscopiei digestive și lider de opinie profesional. Prin experiența clinică acumulată, implicarea în formarea medicală continuă și promovarea practicilor bazate pe dovezi științifice, Dr. Istrate contribuie la dezvoltarea și consolidarea standardelor moderne de endoscopie, având permanent ca prioritate siguranța și beneficiul pacientului.

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