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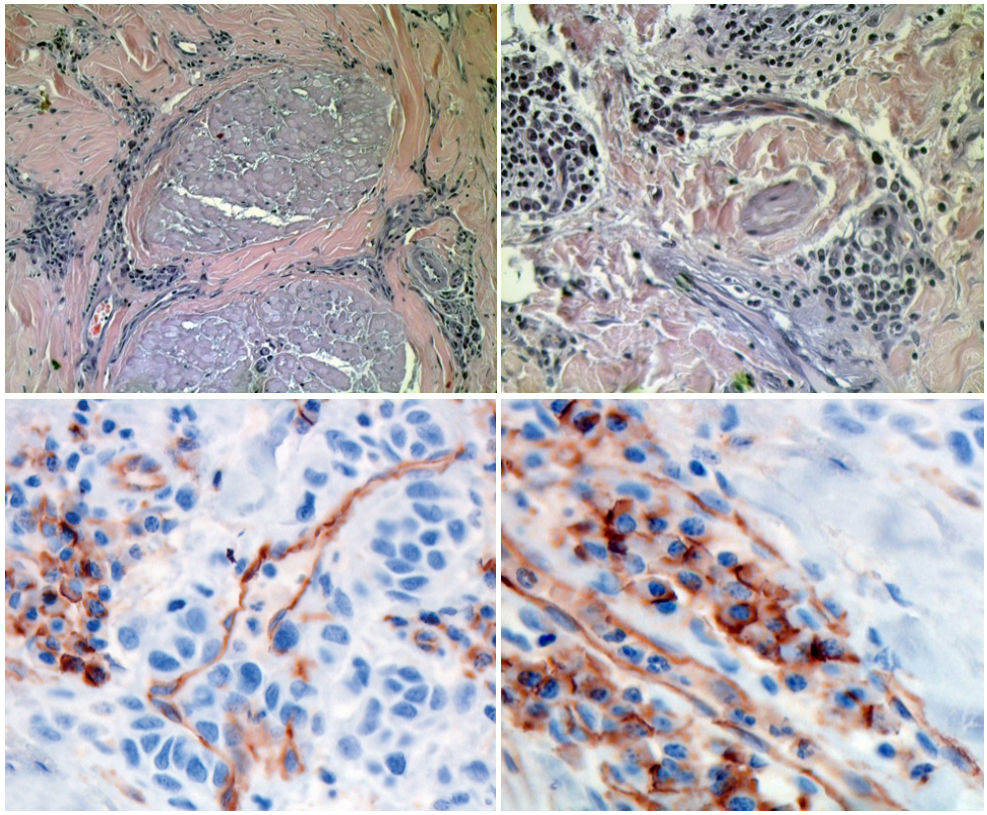
CONTENT HIGHLIGHTS:

Alexandrina Cenușa, Ecaterina Foca, Ecaterina Carpenco, Dumitru Brinza, Valeriu David, Lilian Șaptefrați, Veaceslav Fulga
Invasive potential of cutaneous malignant melanoma



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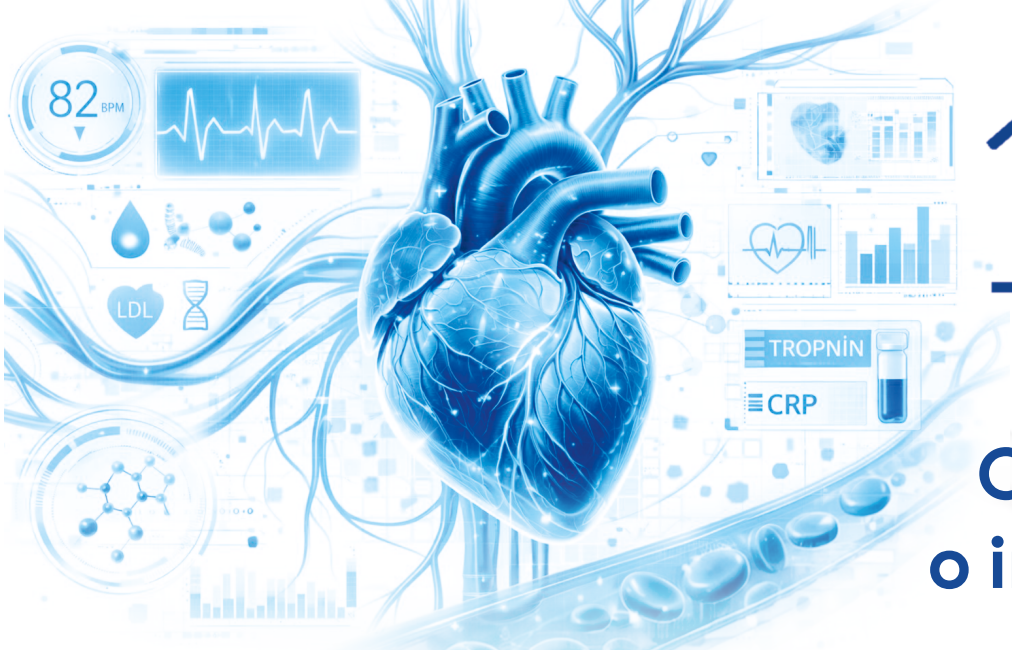

15 ani

synevo
A MEDICOVER COMPANY

Cu grijă pentru o inimă sănătoasă

Laboratorul medical Synevo Moldova, parte a Grupului european MEDICOVER, în anul 2026 marchează 15 ani de activitate în RM. 15 Ani de progres în domeniul diagnosticului de laborator – ani în care excelența profesională, rigoarea și calitatea au rămas constantele care ne definesc. Misiunea noastră este: oferim diagnostic de laborator precis și de încredere, care stă la baza deciziilor medicale corecte și a grijii autentice pentru pacienți.

Synevo oferă o serie de teste pentru diagnosticarea precoce a riscului cardiovascular. Diagnosticul modern de laborator permite identificarea modificărilor aterogene cu mult înainte de apariția simptomelor și elaborarea unei strategii personalizate de prevenție.

În practica de laborator, un rol din ce în ce mai important îl are profilul lipidic extins, care include:

- Colesterol total
- LDL
- HDL
- Trigliceride
- VLDL
- Lipide totale

Această abordare permite o stratificare mai precisă a riscului comparativ cu evaluarea exclusivă a LDL. Lipoproteinele cu densitate foarte mică (VLDL) sunt sintetizate la nivel hepatic și reprezintă principalii transportori ai trigliceridelor endogene. În procesul de lipoliză, acestea se transformă în elemente reziduale cu potențial aterogen pronunțat.

Importanța clinică a VLDL: reflectă intensitatea producției hepatice de trigliceride, corelează cu insulinorezistența și sindromul metabolic, sunt asociate cu steatoza hepatică non-alcoolică, explică „riscul rezidual" la pacienții cu LDL în limite normale aflați sub terapie cu statine. Creșterea trigliceridelor (>1,7 mmol/L) și a VLDL necesită o evaluare aprofundată a statusului metabolic al pacientului, chiar și în prezența unor valori țintă ale LDL. În practica clinică, acest aspect este deosebit de important la pacienții cu obezitate, diabet zaharat tip 2 și hipertensiune arterială.

Astfel, includerea VLDL în interpretarea standard a lipidogramei extinde posibilitățile diagnostice și crește acuratețea evaluării prognostice.

Profilul lipidic se recomandă pentru:

- Persoanele 45+, ca monitorizare regulată a riscului cardiovascular
- Persoane 30-50 ani, care muncesc mult, cu stres, alimentație neregulată
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- Persoanele su sindrom metabolic, obezitate
- Monitorizarea tratamentului hipolipemiant
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- Persoanele cu boli autoimune, cu boala renală cronică

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LETTER TO THE EDITOR

Leadership in living organ transplantation: beyond deceased donor shortages in Turkey and South Korea

Yasin Uzuntarla

Organ Transplant Coordination Center, Gülhane Training and Research Hospital, Ankara, Turkey

Dear Editor,

Turkey and South Korea rank among the countries with the highest rates of living donor organ transplantation, particularly kidney and liver transplants, worldwide [1]. This phenomenon is often attributed solely to the scarcity of deceased donor organs. However, current data and the literature suggest that this pattern arises from a more complex, multi-layered, and structural set of factors [2]. In this letter, I aim to discuss the common dynamics underlying this leadership.

According to The Transplantation Society, the rate of deceased donors in Turkey and South Korea is approximately 7–8 per million population, which is markedly lower than that in many Western European countries [1]. Nevertheless, both countries have developed robust living donor transplant systems and have achieved substantially high transplant volumes.

Firstly, strong family ties and collectivist cultural frameworks facilitate societal acceptance of living donation in both countries. Norms of intra-family altruism, moral responsibility, and caregiving encourage a perspective that prioritizes family welfare over individual autonomy. This socio-cultural context frames the decision to become a living donor not merely as an individual choice but also as a moral duty [3-5].

Secondly, the legal and institutional infrastructure in both countries supports living donor transplantation. In Turkey, Law No. 2238 and the subsequently developed national coordination system facilitate living donor programs; in South Korea, living donor transplantation legislation enables relatively fast and accessible donation processes [6, 7]. In contrast, deceased donor systems remain limited due to challenges in brain death diagnosis, intensive care infrastructure, and reporting chains.

Thirdly, high surgical and academic expertise constitutes a crucial common factor. Both countries host high-volume transplant centers, experienced surgical teams, and advanced techniques, particularly in living donor liver transplantation. This ensures acceptable risk profiles for both patients and donors and encourages clinicians to more frequently recommend living donation [8, 9].

Fourth, and often overlooked, is the differential perception of ethics compared to Western countries. While Western bioethics centers on individual autonomy, relational autonomy and family-centered decision-making predominate in Turkey and South Korea. Consequently, the ethical boundaries of living donation may be interpreted more broadly [10, 11].

In conclusion, the leadership of Turkey and South Korea in living organ transplantation cannot be explained solely by deceased donor shortages. This phenomenon emerges at the intersection of cultural norms, legal frameworks, healthcare system organization, academic surgical capacity, and ethical paradigms. Therefore, when evaluating "success" in international comparisons, the ethical, societal, and systemic costs of high living donor rates should also be considered.

I hope this discussion contributes to the ongoing international academic dialogue on transplantation policies and ethical frameworks.

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Corresponding author: Yasin Uzuntarla, MD, PhD, associate professor
Organ Transplant Coordination Center
Gülhane Training and Research Hospital, Ankara, Turkey
e-mail: yasinuzuntarla@gmail.com

Author's ORCID ID
Yasin Uzuntarla – https://orcid.org/0000-0002-5021-3763

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RESEARCH ARTICLE

Invasive potential of cutaneous malignant melanoma

Alexandrina Cenușa[1], Ecaterina Foca[1], Ecaterina Carpenco[1], Dumitru Brinza[2], Valeriu David[1,3], Lilian Șaptefrați[1,3], Veaceslav Fulga[1*]

[1]Department of Histology, Cytology and Embryology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[2]Department of Pathology, Institute of Oncology, Chișinău, Republic of Moldova

[3]Laboratory of Morphology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

ABSTRACT

Introduction. Cutaneous malignant melanoma is the most aggressive skin cancer, with a high mortality rate despite advances in therapy. This study aimed to evaluate the relationship between lymphovascular and perineural invasion and key clinicopathological parameters in superficial spreading melanoma, in order to assess their potential prognostic significance.

Materials and methods. A retrospective analysis was conducted on 47 cases of superficial spreading melanoma obtained from the Oncology Institute in Chisinau. All cases were histologically confirmed and reviewed for tumor thickness, Clark level, ulceration, mitotic activity, microsatellitosis, pigmentation, and lymph node involvement. Lymphovascular and perineural invasion were assessed using hematoxylin–eosin staining and, where available, immunohistochemistry. Correlations between invasion patterns and clinicopathologic features were analyzed using Pearson correlation coefficients, with statistical significance set at $p < 0.05$.

Results. Lymphovascular invasion was positive and significantly correlated with tumor thickness ($r = 0.54$, $p< 0.001$), Clark level ($r = 0.46$, $p < 0.001$), microsatellitosis ($r = 0.50$, $p < 0.001$), tumor stage ($r = 0.33$, $p = 0.01$), and lymph node involvement ($r = 0.29$, $p = 0.02$). A negative correlation was observed with pigmentation ($r = –0.26$, $p = 0.04$). Perineural invasion was less frequent, but correlated positively with lymphovascular invasion ($r = 0.28$, $p = 0.03$) and showed a trend toward association with amelanotic tumors ($r = –0.24$, $p = 0.05$). No significant relationships were found with ulceration or mitotic activity.

Conclusions. Lymphovascular invasion represents a significant indicator of aggressive biological behavior in superficial spreading melanoma, closely associated with established prognostic factors. Perineural invasion occurs less frequently, but may further reflect invasive potential, particularly in amelanotic variants. Routine histopathologic assessment of both invasion patterns is recommended to improve prognostic evaluation.

Keywords: cutaneous melanoma, lymphovascular invasion, perineural invasion, tumor aggressiveness, prognostic factors.

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*__Corresponding author__: **Veaceslav Fulga**, MD, PhD, associate professor
Department of Histology, Cytology and Embryology
Nicolae Testemițanu State University of Medicine and Pharmacy
165, Stefan cel Mare si Sfant blvd., Chișinău, Republic of Moldova, MD-2004
email: veaceslav.fulga@usmf.md

Authors' ORCID IDs
Alexandrina Cenusa – https://orcid.org/0009-0007-9135-5569
Ecaterina Foca – https://orcid.org/0000-0001-7629-4875
Ecaterina Carpenco – https://orcid.org/0000-0003-1464-3149
Dumitru Brinza – https://orcid.org/0000-0002-3133-1502
Valeriu David – https://orcid.org/0000-0001-9799-7369
Lilian Șaptefrați – https://orcid.org/0000-0003-2779-718X
Veaceslav Fulga – https://orcid.org/0000-0002-7589-7188

Key messages

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

The prognostic significance of lymphovascular invasion (LVI) and perineural invasion (PNI) in superficial spreading melanoma remains insufficiently defined, with inconsistent findings across studies. The relationship between these invasive patterns and key histopathologic indicators, particularly pigmentation status, is also not well established.

The research hypothesis

Lymphovascular and perineural invasion correlate with the tumor's clinicopathological parameters and may serve as meaningful prognostic markers in superficial spreading melanoma.

The novelty added by manuscript to the already published scientific literature

This study identifies a significant link between both invasion patterns and multiple markers of melanoma aggressiveness, including a noteworthy inverse association with pigmentation, suggesting enhanced invasive potential in amelanotic tumors. The manuscript also highlights a positive correlation between LVI and PNI, supporting shared invasive pathways and reinforcing the need for their combined assessment in routine histopathology.

Introduction

Cutaneous melanoma, with a continuously rising incidence worldwide, represents one of the most aggressive forms of skin cancer. Although it accounts for only about 4% of all cutaneous malignancies, melanoma is responsible for approximately 80% of skin cancer-related deaths, reflecting its high metastatic potential and resistance to treatment [1]. The global burden of melanoma has increased significantly over recent decades, with current estimates suggesting over 331,722 new cases annually worldwide [1]. In the United States, the American Cancer Society projects that in 2025, nearly 105,000 new cases of invasive melanoma and over 107,000 cases of melanoma *in situ* will be diagnosed, resulting in approximately 8,400 deaths [2]. Among white populations, the overall lifetime risk of developing invasive melanoma is estimated at 1 in 29 for males and 1 in 40 for females. Despite significant advancements in understanding melanoma biology and the development of immunotherapeutic and targeted treatment strategies, a substantial proportion of patients continue to succumb to the disease due to therapeutic resistance and metastatic spread [3]. Hence, identification of histopathologic parameters associated with aggressive behavior remains a crucial component of melanoma research and patient management [4]. Among the histopathologic features that may influence tumor aggressiveness, lymphovascular invasion (LVI) and perineural invasion (PNI) have attracted increasing attention. While the prognostic role of LVI remains controversial, several studies have associated it with tumor-positive sentinel node metastasis and poorer survival, particularly in tumors showing regression [5]. Perineural invasion, although rare, is increasingly recognized as a potential route of local spread and recurrence, especially in desmoplastic melanoma, which displays neurotropic characteristics [6].

Materials and methods

A total of 47 tumor specimens (from 24 women and 23 men with a mean age of 65.6 ± 12.2 years, median age 68, range 31-88 years) with confirmed diagnoses of superficial spreading melanoma were selected from the archival database (2020-2024) of the Oncology Institute, Chisinau, Republic of Moldova, along with corresponding morphology reports. Each case was reviewed by two pathologists for tumor thickness, Clark level, ulceration, mitotic activity, microsatellitosis, lymphocytic infiltration, pigmentation status, and lymph node involvement. The presence of lymphovascular and perineural invasion was assessed using routine hematoxylin and eosin (HE) staining (ST Infinity H&E Staining System, 3801698, Leica), as well as immunohistochemically (Leica autostainer XL ST5010) following the S100 (BSB5918/ clone 4C4.9/ RTU/ 45 minutes room temperature/ BioSB) and CD31 (PA0414/ clone JC70A/ RTU/ 25 minutes room temperature/ Leica) protocols (Envision FLEX Target retrieval solution high pH/ DM828/ 20 minutes/ Agilent Dako; Mouse/Rabbit PolyDetector Plus DAB HRP Brown, BSB 0261, 60 minutes/ BioSB) (Figure 1, 2). Statistical correlations were calculated using Pearson or Spearman coefficients, depending on the variable type, with significance level set at $p \leq 0.05$.

Results

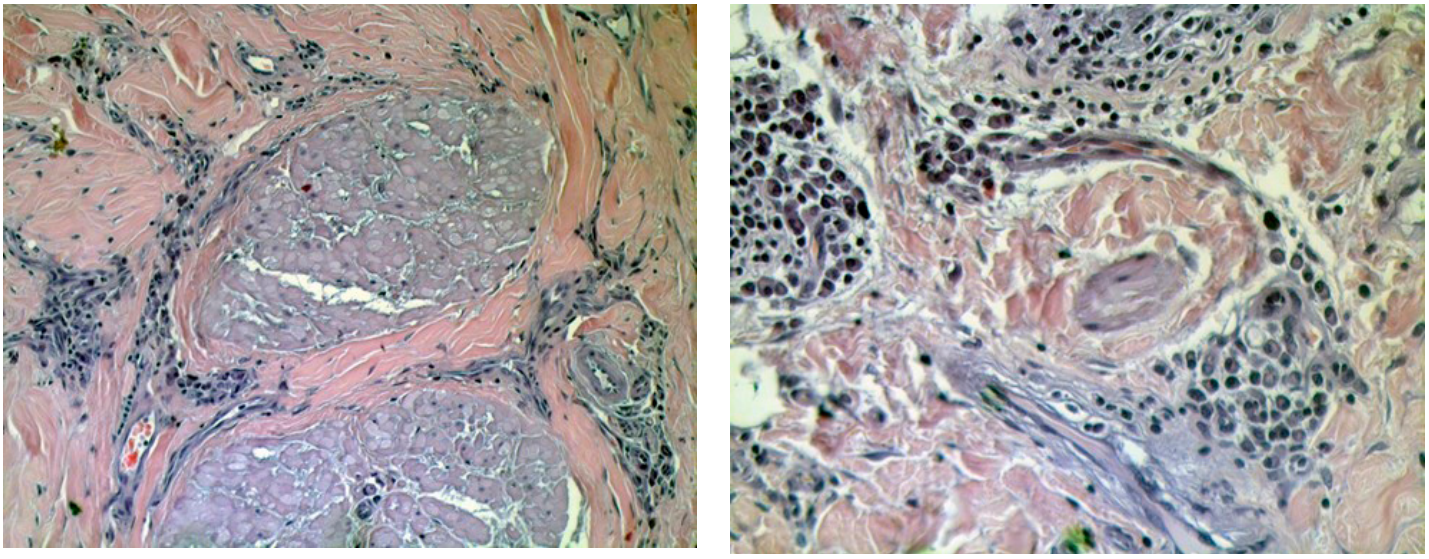
Lymphovascular invasion (LVI) was significantly correlated with several indicators of tumor aggressiveness (Table 1).

Table 1. Correlation coefficients of lymphovascular and perineural invasion with clinicopathologic variables

Variable	Lymphovascular invasion		Perineural invasion	
	r	p	r	p
Age	0.01	0.48	0.20	0.09
Gender	0.22	0.07	–0.14	0.17
Tumor thickness	0.54	0.001*	–0.03	0.41
Clark level	0.46	0.001*	0.11	0.22
Ulceration	0.14	0.17	0.08	0.30
Mitotic activity	–0.09	0.27	–0.13	0.19
Lymphocytic invasion	–0.21	0.07	0.04	0.38
Microsatellitosis	0.50	0.001*	–0.04	0.40
Tumor regression	–0.26	0.04*	–0.24	0.05*
Tumor stage	0.33	0.01*	0.12	0.20
Lymph node involvement	0.29	0.02*	–0.04	0.40
Pigmentation	–0.26	0.04*	–0.24	0.05*

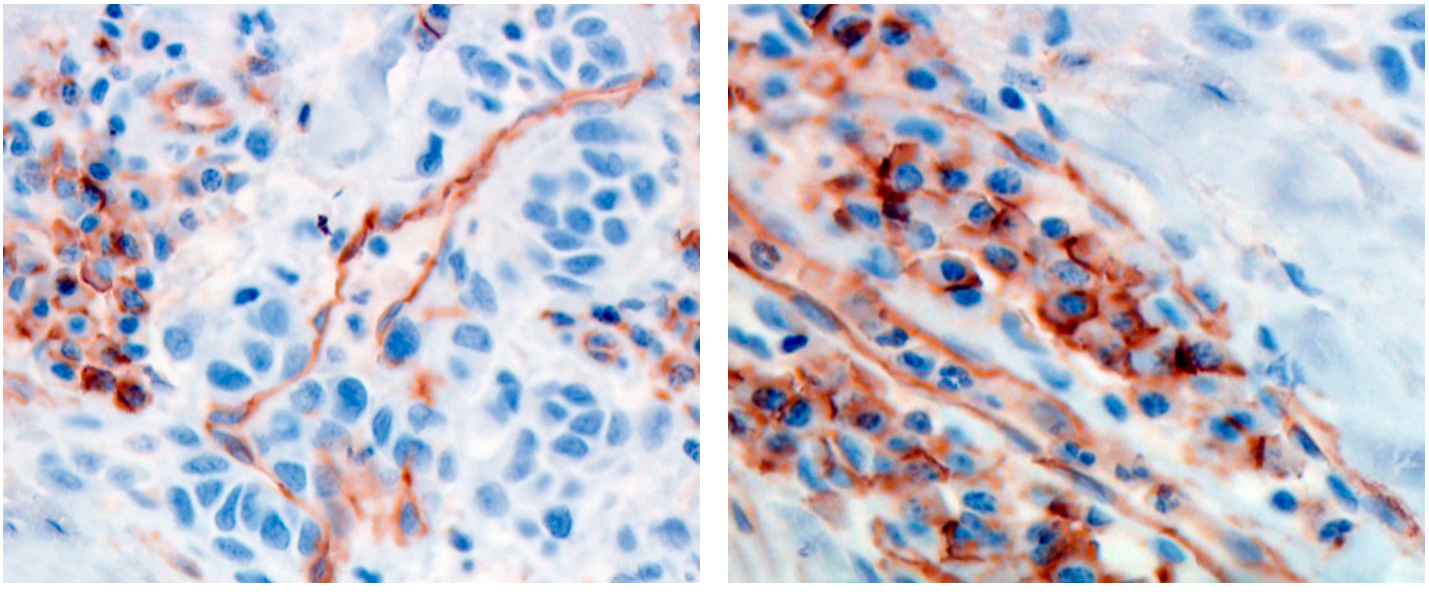
Note: r – correlation coefficient. The asterisk (*) indicates statistically significant results ($p < 0.05$).

A significant positive correlation was identified between LVI and tumor thickness ($r = 0.54$, $p < 0.001$) as well as Clark level ($r = 0.46$, $p < 0.001$). LVI also demonstrated significant associations with microsatellitosis ($r = 0.50$, $p < 0.001$), tumor stage ($r = 0.33$, $p = 0.01$), and lymph node involvement ($r = 0.29$, $p = 0.02$). A moderate, inverse correlation was observed between LVI and pigmented melanomas ($r = –0.26$, $p = 0.04$), suggesting that lymphovascular infiltration tends to occur less frequently in melanotic (pigmented) lesions, and is more often associated with amelanotic tumors. No significant correlation was found between LVI invasion and age, gender, or mitotic activity ($p > 0.05$). Overall, the pres-



a b

Fig. 1 Perineural and perivascular invasion of cutaneous malignant melanoma.
Note: *(A) Clusters of atypical melanocytic tumor cells infiltrating the perineurium, consistent with perineural invasion (HE, ×200). (B) Tumor cells surrounding and infiltrating the wall of a small dermal vessel, demonstrating perivascular/vascular-associated invasion (HE, ×400)*



c d

Fig. 2 Vascular invasion of cutaneous malignant melanoma.
Note: *(C–D) Angiotropic spread and intravascular invasion with hyperchromatic melanoma cells. CD31 immunohistochemistry confirms vascular invasion: endothelial cells stain brown (HRP/DAB), while infiltrating melanoma cells distort or penetrate the vascular wall (Mayer's hematoxylin counterstain, ×400).*

ence of lymphovascular invasion was associated with deeper, more advanced melanomas and with histopathological parameters reflecting poor prognosis.

Perineural invasion (PNI) was also associated with adverse prognostic features. Its presence correlated positively with lymphovascular invasion ($r = 0.28$, $p = 0.03$), indicating a tendency for co-occurrence of vascular and neural infiltration. Weak, non-significant correlations were found between perineural invasion and tumor thickness ($r = –0.03$, $p = 0.41$) and Clark level ($r = 0.11$, $p = 0.22$). No significant associations were observed between PNI and ulceration, mitotic activity, or lymphocytic infiltration ($p > 0.05$). A negative correlation with pigmented melanomas ($r = –0.24$, $p = 0.05$) approached statistical significance, suggesting that perineural invasion tends to occur less frequently in melanotic lesions and more commonly in amelanotic variants.

Discussion

This study examines the prognostic relevance of lymphovascular and perineural invasion in cutaneous malignant melanoma, emphasizing their association with ad-

vanced disease features and markers of aggressive tumor behavior. Our findings show that especially lymphovascular invasion correlate strongly with histopathologic indicators of progression, including increased tumor thickness, higher Clark level, the presence of microsatellitosis, and lymph node involvement.

Taken together, these relationships underscore the importance of lymphovascular and perineural invasion as meaningful markers of invasive potential in cutaneous malignant melanoma. The current findings are consistent with evidence in the literature suggesting that lymphovascular invasion represents a strong predictor of tumor aggressiveness and poor prognosis [5]. Previous studies have demonstrated that lymphovascular invasion correlates with increased tumor thickness, ulceration, and sentinel lymph node positivity, all of which are established prognostic factors in melanoma [5]. The observed correlations in our cohort between lymphovascular invasion and parameters such as Breslow thickness, Clark level, microsatellitosis, and lymph node involvement reinforce its prognostic significance. This aligns with the results reported by Egger et al. (2011), who found lymphovascular invasion to be an independent factor associated with shorter disease-free and overall survival [5].

Interestingly, we observed a negative correlation between lymphovascular invasion and tumor pigmentation, suggesting that amelanotic melanomas may represent a more aggressive biological subtype with enhanced invasive potential. This observation is in accordance with prior research showing that amelanotic or hypopigmented melanomas are often diagnosed at a more advanced stage due to delayed clinical recognition and tend to have worse outcomes [7]. The association between lack of pigmentation and the presence of lymphovascular invasion may therefore reflect a distinct biological phenotype with enhanced invasive potential [7]. Loss of pigmentation in melanoma is often associated with cellular dedifferentiation and the epithelial –mesenchymal transition, processes that increase cell movement and invasiveness [8, 9]. This dedifferentiation is accompanied by reduced expression of melanocytic antigens, such as microphthalmia-associated transcription factor and tyrosinase, and by increased secretion of molecules that promote the formation of new blood vessels, including vascular endothelial growth factor A and vascular endothelial growth factor C [8, 9]. These molecular alterations create a microenvironment that supports invasion into blood vessels and the spread of cancer to distant sites.

Perineural invasion, although less common, showed a positive correlation with lymphovascular invasion, suggesting that both processes may share similar mechanisms of invasiveness. Infiltration of nerve tissue is known to involve neurotropic signaling pathways mediated by nerve growth factor and its receptor tropomyosin receptor kinase A, also known as neurotrophic receptor tyrosine kinase 1, as well as glial cell line-derived neurotrophic factor [10]. These signaling molecules promote the migration of tumor cells along nerve sheaths and enhance their survival.

The observation that perineural invasion occurs more frequently in non-pigmented, or amelanotic, melanoma variants supports the hypothesis that dedifferentiated melanomas acquire neurotropic characteristics, which facilitate local recurrence and spread along nerves [11].

Beyond these morphological findings, recent molecular research provides mechanistic explanations for these invasive behaviors [10]. Tumors that display lymphovascular invasion or perineural invasion often show reduced numbers of tumor-infiltrating lymphocytes and increased expression of programmed death-ligand 1, creating an immunosuppressive environment that allows tumor cells to evade immune detection. The resulting "immune-cold" phenotype promotes the formation of new blood vessels and remodeling of nerve tissue while diminishing the cytotoxic activity of cluster of differentiation 8-positive T cells.

This interaction between immune evasion and stromal invasion has been confirmed by recent immunogenomic studies of melanoma, which demonstrate that overexpression of programmed death-ligand 1 and low tumor-infiltrating lymphocyte counts are associated with increased vascular invasion and poorer survival outcomes [3, 4].

From a pathological standpoint, the identification of lymphovascular and perineural invasion in melanoma specimens should prompt careful staging and follow-up, as both features may indicate a higher risk of regional or distant metastasis. Including these variables in histopathological reports could therefore improve risk stratification and guide treatment decisions, particularly regarding sentinel lymph node biopsy and adjuvant therapy considerations.

Our study may have some limitations. The retrospective design introduces potential selection bias, and the relatively small number of cases may limit the generalizability of our findings. Additionally, the lack of follow-up and survival data precludes the assessment of the prognostic impact of lymphovascular and perineural invasion on patient outcomes. Another limitation is the restriction of our analysis to superficial spreading melanoma, which limits the extrapolation of results to other histological subtypes such as nodular or acral lentiginous melanoma.

Despite these limitations, our study adds to the growing body of evidence emphasizing the prognostic value of lymphovascular and perineural invasion in cutaneous malignant melanoma. The integration of these histopathologic features into routine diagnostic evaluation could enhance prognostic accuracy and ultimately contribute to more individualized management strategies for melanoma patients. Future research incorporating molecular profiling and long-term clinical follow-up is warranted to clarify the biological pathways underlying these invasive patterns and their potential therapeutic implications.

Conclusions

Lymphovascular invasion emerges as a robust histopathologic marker of aggressive biological behavior in superficial spreading melanoma. Its significant associations with tumor thickness, Clark level, microsatellitosis, and lymph node involvement underscore its prognostic impor-

tance and support its routine inclusion in pathology reports. The inverse relationship with pigmentation further suggests that amelanotic melanomas may possess greater invasive potential, likely reflecting tumor dedifferentiation and the activation of angiogenic pathways that promote vascular dissemination.

Perineural invasion, although less common, showed a positive correlation with lymphovascular invasion, implying partially overlapping mechanisms of tumor spread involving both vascular and neural structures. This observation aligns with the hypothesis that neurotropic signaling and microenvironmental remodeling contribute to melanoma progression, particularly in amelanotic variants.

Collectively, these findings position lymphovascular and perineural invasion as complementary indicators of tumor aggressiveness in melanoma. Their combined assessment –ideally integrated with molecular parameters such as tumor-infiltrating lymphocyte density and PD-L1 expression – has the potential to enhance prognostic precision and inform more individualized therapeutic strategies.

Competing interests

None declared.

Authors' contribution

AC: Resources, Investigation, Formal Analysis, Writing – Original Draft Preparation; EF: Conceptualization, Methodology, Writing & Editing; EC: Data Curation, Literature Review; DB: Assistance in the acquisition of histological specimens, Investigation, Formal Analysis; VD: Methodology, Data Curation, Validation; LŞ: Supervision, Validation; VF: Project Administration, Supervision, Final Approval of the Manuscript. All authors approved the final version of the manuscript.

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Ethics approval

This study was conducted with the approval of the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova (Minutes No. 4, dated 12.09.2025). All procedures adhered to institutional and national ethical standards for biomedical research and were performed in accordance with the principles outlined in the Declaration of Helsinki.

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RESEARCH ARTICLE

Contemporary insights into diagnosis and treatment of gastrointestinal non-Hodgkin lymphomas

Larisa Musteata[1], Maria Robu[2], Vasile Musteata[1,2*], Dumitrita Urescu[1,2], Irina Cebanu[1,2], Alina Capanji[1]

[1]Discipline of Hematology, Department of Internal Medicine, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[2]Institute of Oncology, Chisinau, Republic of Moldova

ABSTRACT

Introduction. The gastrointestinal tract (GIT) is the most common site of extranodal primary non-Hodgkin lymphoma (NHL), accounting for 20% to 40% of all extranodal lymphomas. The advanced stages at diagnosis and complications remain significant issues in NHL management, imposing a substantial disease burden on patients and healthcare systems.

Material and methods. We performed a descriptive cross-sectional and cohort study of patients with gastrointestinal NHL and a narrative review of the literature in the Discussion section. This study included 50 prospective and retrospective patients with NHL treated between 2015-2024 in the Institute of Oncology in Moldova. A bibliographic search was conducted using databases such as *PubMed, Hinari, SpringerLink, the National Center for Biotechnology Information, and Medline*. The final bibliography included 18 relevant sources deemed to be representative of the literature published on the topic of this article.

Results. According to the International Clinical Classification, most patients (22, 44.0%) were diagnosed with clinical stage IV. B symptoms occurred in 38 (76.0%) patients. The overwhelming predominance of diffuse large B-cell lymphomas (46 cases - 90.2%) was observed. The complete blood count, bone marrow aspiration and biopsy of the iliac crest did not detect any specific changes in cases without bone marrow involvement, with the exception of a decrease in hemoglobin and erythrocyte counts observed in cases of posthemorrhagic anemia. The overall survival (OS) of all patients with gastrointestinal NHL was 78.1% at 1 year, 59.4% at 3 years, and 35.9% at 5 years. In patients with stage IE NHL, the 1-, 3-, and ≥5-year OS was 93.4%, 76.5%, and 69.9%, respectively. In patients with stage IIE, the OS was 91.2% at 1 year, 71.4% at 3 years, and 63.8% at ≥5 years. In patients with stage IIIE-IV, the OS was 75.1% at 1 year, 54.8% at 3 years, and 28.5% at ≥5 years with combined chemotherapy.

Conclusions. Our study demonstrated that non-Hodgkin lymphomas with primary involvement of the gastrointestinal tract exhibited distinct histopathological, clinical-evolutionary and hematological features, which influenced treatment outcomes. The aggressive histological types and the advanced stages IIIE and IV prevailed within the structure of non-Hodgkin lymphomas with primary gastrointestinal involvement, and, thus, negatively impacted the survival and prognosis.

Keywords: non-Hodgkin lymphomas, gastrointestinal tract, aggressive histological types, chemotherapy, survival.

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Corresponding author: **Vasile Musteata**, PhD, MPH, associate professor
Discipline of Hematology, Department of Internal Medicine,
Nicolae Testemiţanu State University of Medicine and Pharmacy
165, Stefan cel Mare si Sfant blvd., Chisinau, Republic of Moldova, MD-2004
e-mail: vasile.musteata@usmf.md

Key messages

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

Despite the continuous improvement in outcomes for patients with non-Hodgkin lymphomas due to the increasing availability of efficient treatment options, advanced stages at diagnosis and complications remain significant issues, imposing a substantial disease burden on patients and healthcare systems. Therefore, the early diagnosis and prevention of these complications are of utmost im-

Authors' ORCID IDs
Larisa Musteata – https://orcid.org/0000-0001-7162-6391
Maria Robu – https://orcid.org/0000-0002-3228-7566
Vasile Musteata – https://orcid.org/0000-0002-9471-7170
Dumitrita Urescu – https://orcid.org/0000-0002-6711-0402
Irina Cebanu – https://orcid.org/0009-0000-8980-5988
Alina Capanji – https://orcid.org/0000-0001-7650-2718

portance for the successful management of non-Hodgkin lymphomas.

The research hypothesis

The original research and synthesis of contemporary literature will reveal a clear association among clinico-evolutionary and hematological features, histological types of non-Hodgkin lymphomas and treatment outcomes. The updated diagnostic patterns and treatment outcomes in gastrointestinal non-Hodgkin lymphomas will help to improve the management approaches.

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

This article provides the updated results of a prospective study and a synthesis of recent international publications concerning the diagnostic insights and treatment approaches in gastrointestinal non-Hodgkin lymphomas. The study's findings will contribute to the improvement of diagnosis management and therapeutic options in patients with non-Hodgkin lymphomas.

Introduction

The gastrointestinal tract is the most common site of extranodal primary non-Hodgkin lymphomas, accounting for 20% to 40% of all extranodal lymphomas [1, 2]. Advanced stages at diagnosis and complications remain significant issues in non-Hodgkin lymphomas management, imposing a substantial disease burden on patients and healthcare systems. The majority of these are generalized processes secondarily involving the gastrointestinal tract. Primary gastrointestinal lymphomas are less common, accounting for approximately 10% to 15% of all non-Hodgkin lymphomas [3-5]. Most non-Hodgkin lymphomas involving the gastrointestinal tract are of B-cell lineage, of which diffuse large B-cell lymphoma is the most common type, irrespective of location [2, 6-9].

The few studies and publications on primary non-Hodgkin lymphomas affecting the gastrointestinal tract in Moldova have led to the writing of this manuscript.

Material and methods

We performed a descriptive cross-sectional and cohort study of patients with gastrointestinal non-Hodgkin lymphomas and a narrative review of the literature in the Discussion section. This study included 50 prospective and retrospective patients with non-Hodgkin lymphomas treated between 2015-2024 in the Institute of Oncology in Moldova. The diagnosis of non-Hodgkin lymphoma was confirmed by morphopathological and immunohistochemical examinations of the post-biopsy material. The type of lymphomas was classified according to the 2022 Revision of WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. Staging at diagnosis was performed according to the Ann Arbor staging system and Lugano Classification. The study included both ambulatory and hospitalized patients.

The participant inclusion criteria comprised: age over 18 years, confirmation of a non-Hodgkin lymphoma diagnosis by bone marrow examinations, histological and immunohistochemical investigations of the post-biopsy specimens, patient's consent and adherence to participate in the study and the possibility of dynamic monitoring.

The exclusion criteria were as follows: patients aged <18 years, patients diagnosed with chronic lymphocytic leukemia, only cytological confirmation of diagnosis, the absence of patient's consent and adherence to participate in the study.

Descriptive statistics were used: qualitative data were presented as numbers and percentages, and quantitative data were presented as means. Overall survival was calculated according to the Kaplan-Meier estimate.

A bibliographic search was conducted using databases such as *PubMed*, *Hinari, SpringerLink*, *the National Center for Biotechnology Information*, *and Medline*. Articles published between 2000 and 2025 were selected using the following keywords: "non-Hodgkin lymphoma" in combination with terms such as "histologic types", "gastrointestinal", "chemotherapy" and "surgical treatment" in order to maximize search yield. Based on the established search criteria, a total of 90 full-text articles were identified. The final bibliography (References) included 20 relevant sources deemed to be representative of the literature published on the topic of this article.

The research project was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes №. 3 from 17.06.2022).

Results

The clinical course, complications and treatment outcomes of non-Hodgkin lymphoma with primary involvement of the gastrointestinal tract were studied in 50 patients aged 19-78 years (mean age 57.3 years), who were treated under the supervision of hematologists during the period 1998-2023. The distribution of patients by age and sex is presented in Table 1. There were 20 males (40.0%) and 30 females (60.0%).

The diagnosis of non-Hodgkin lymphoma with primary involvement of the gastrointestinal tract was more frequent-

ly established in patients aged 50-69 (64%) years (mean age 57.3 years). The average duration from the onset of the first clinical manifestations to the confirmation of the diagnosis of non-Hodgkin lymphoma ranged from 3 to 14 months. In most patients (33 cases,66.0%) the diagnosis of the disease was established within the first 6 months. In 9 (18.0%) patients, the diagnosis of non-Hodgkin lymphoma was confirmed only after 1 year. The distribution of patients according to the duration of the disease from the first clinical signs to the establishment of the diagnosis is presented in Table 2.

Table 1. Distribution of patients with gastrointestinal non-Hodgkin lymphomas according to the age and sex

Age groups, years	Number of patients, abs. (%)	Sex	
		Males, abs. (%)	females, abs. (%)
20-39	5 (10.0)	3 (15.0)	2 (6.7)
40-49	8 (16.0)	4 (20.0)	4 (13.3)
50-59	18 (36.0)	7 (20.0)	11 (36.7)
60-69	14 (28.0)	4 (30.0)	10 (33.3)
70-79	5 (10.0)	2 (10.0)	3 (10.0)
Total:	50 (100.0)	20 (40.0)	30 (60.0)

Note: *Descriptive statistics were used: qualitative data were presented as numbers and percentages, and quantitative data were presented as means.*

Table 2. Distribution of patients with gastrointestinal non-Hodgkin lymphomas according to the duration of the disease from the onset to diagnosis

Number of patients	Distribution of patients according to the duration of the disease (months)		
	3-6, abs. (%)	7-12, abs. (%)	≥13, abs. (%)
50	33 (66.0)	8 (16.0)	9 (18.0)

Note: *Descriptive statistics were used: qualitative data were presented as numbers and percentages, and quantitative data were presented as means.*

The study of the location of the primary focus in patients with non-Hodgkin lymphoma with primary involvement of the gastrointestinal tract showed that in 40 (80.0%) patients the stomach was affected, in 6 (12.0%) – the small intestine and in 4 (8%) – the large intestine (Table 3).

According to the International Clinical Classification, most patients (22, 44.0%) were diagnosed with clinical stage IV (Table 3). Stage IE was established in 12 (24.0%) cases and stage IIE in 14 (28.0%) cases. Stage IIIE was diagnosed in 2 (4.0%) patients. B symptoms occurred in 38 (76.0%) patients, mainly in stage IV disease (17, 34.0%).

Table 3. Distribution of patients with gastrointestinal non-Hodgkin lymphomas according to the stage of the disease

Primary site location	Number of patients, abs. (%)	Number of patients, abs. (%)			
		Stage IE	Stage IIE	Stage IIIE	Stage IV
Stomach	40 (80.0)	10 (25.0)	13 (32.5)	2 (5.0)	15 (37.5)
Small intestine	6 (12.0)	2 (33.3)	-	-	4 (66.7)
Large intestine	4 (8.0)	-	1 (25,0)	-	3 (75.0)
Total:	50 (100.0)	12 (24.0)	14 (28.0)	2 (4.0)	22 (44.0)

Note: *Descriptive statistics were used: qualitative data were presented as numbers and percentages, and quantitative data were presented as means. Staging was performed according* to *the Ann Arbor staging system and Lugano Classification.*

By distributing patients with non-Hodgkin lymphoma with primary involvement of the gastrointestinal tract according to the histological type of the tumor, we revealed the overwhelming predominance of diffuse large B-cell lymphomas (46, 90.2%). The small lymphocytic (2, 4.9%) and lymphoplasmacytic (2, 4.9%) types were rarely encountered.

The study of the clinical picture of non-Hodgkin lymphoma with primary involvement of the gastrointestinal tract showed that patients with primary localization of the tumor site in the stomach most often presented with pain in the epigastric region (36, 90%), weight loss (31, 62%), anorexia (18, 45%), nausea and vomiting (17, 42.5%). Vomiting with coffee grounds content occurred in 2 (5%) patients, and dysphagia and the feeling of a tumor formation in the abdomen occurred in 1 (2.5%) case. Fever occurred in 5 (10.0%) patients, and profuse night sweats – in 38 (76.0%).

Patients with primary small intestine involvement had abdominal pain (4, 66.0%) and vomiting (2, 33.0%). Patients with non-Hodgkin lymphoma with primary colon involvement had abdominal pain in 4 (100.0%) cases, anorexia in 2 (50.0%) cases.

The complete blood count, bone marrow aspiration and biopsy of the iliac crest did not detect any specific changes in cases without bone marrow involvement, with the exception of a decrease in hemoglobin and erythrocyte counts observed in cases of posthemorrhagic anemia.

In 20 patients in local stages (IE and IIE), the diagnosis of non-Hodgkin lymphoma was confirmed by surgical intervention with morphopathological and immunohistochemical examinations of the removed sector of the gastrointestinal tract, in the other cases, it was confirmed by endoscopic examination of the affected site with tumor biopsy and investigation of the removed material.

Patients with localized clinical stages (IE and IIE) underwent surgical treatment (gastrectomy), followed by 2-3 cycles of standard CHOP, R-COP and R-CHOP combined chemotherapy, subsequent radiotherapy with a total dose of 36-38 Gy to the involved sites, and then 3-4 additional cycles of combined chemotherapy using the aforementioned regimens. For patients in stages IIIE and IV, only the aforementioned combined chemotherapy was administered in 6-8 cycles.

The short-term responses to treatment in patients with non-Hodgkin lymphoma with primary involvement of the gastrointestinal tract were studied (Table 4).

The treatment proved to be effective in 41 (82%) of 50 patients. Complete responses were achieved in 24 (48.0%)

patients and partial responses in 17 (34.0%) patients. In stage IE, complete responses occurred in all 12 (100%) patients, in stage IIE – in 42.85%, and in stages IIIE and IV only in 25.0% of patients.

The long-term results of treatment of patients with primary involvement of the gastrointestinal tract are presented in Table 5.

Table 4. Short-term results of treatment in patients with gastrointestinal non-Hodgkin lymphomas according to the stage of the disease

Clinical stage	Number of patients, abs. (%)	Total response, abs. (%)	Type of responses, abs. (%)		Response failure, abs. (%)
			Complete	Partial	
IE	12 (24.0)	12 (100.0)	12 (100.0)	-	-
IIE	14 (28.0)	12 (85.7)	6 (42.85)	6 (42.85)	2 (14.3)
IIIE – IV	24 (48.0)	17 (70.8)	6 (25.0)	11 (45.8)	7 (29.2)
Total:	50 (100.0)	41 (82.0)	24 (48.0)	17 (34.0)	9 (18.0)

Note: *Descriptive statistics were used: qualitative data were presented as numbers and percentages, and quantitative data were presented as means. Staging was performed according* to *the Ann Arbor staging system and Lugano Classification.*

Table 5. Overall survival of patients with gastrointestinal non-Hodgkin lymphomas according to the stage of the disease

Clinical stage	Number of patients	Overall survival (%)		
		1 year	3 years	≥5 years
IE	12	93.4	76.5	69.9
IIE	14	91.2	71.4	63.8
IIIE – IV	24	75.1	34.8	28.5
Total:	50	78.1	59.4	35.9

Note*: Overall survival was calculated according to the Kaplan-Meier estimate. Staging was performed according* to *the Ann Arbor staging system and Lugano Classification.*

The overall survival of all patients with gastrointestinal NHL was 78.1% at 1 year, 59.4% at 3 years, and 35.9% at 5 years. In patients with stage IE NHL, the 1-, 3-, and ≥5-year overall survival was 93.4%, 76.5%, and 69.9%, respectively. In patients with stage IIE, the overall survival was 91.2% at 1 year, 71.4% at 3 years, and 63.8% at ≥5 years. In patients with stage IIIE-IV, the overall survival was 75.1% at 1 year, 54.8% at 3 years, and 28.5% at ≥5 years under the combined chemotherapy, and thus significantly lower ($p < 0.05$).

Adverse events were evaluated in patients with primary gastrointestinal involvement following treatment. The most common adverse event, observed in 34 (68.0%) patients, was leukopenia, which did not interfere with the planned treatment schedule. Peripheral neuropathy occurred in 28 (56.0%) patients with non-Hodgkin lymphomas and primary gastrointestinal involvement. The administration of appropriate medications allowed continuation of both chemotherapy and radiotherapy in standard doses and regimens.

Discussion

Non-Hodgkin lymphomas develop and disseminate at different rates, being divided according to histopathological and clinical-evolutionary characteristics into indolent and aggressive [10]. Tumors originating in extranodal tissue are identified as primary extranodal lymphomas, while hematogenous and lymphogenous spread of the disease from lymph nodes to the extranodal sites is termed secondary extranodal lymphoma [11]. The most common diagnoses are diffuse large B-cell lymphoma and marginal zone lymphoma (MALT), but many other lymphomas may be found in the gastrointestinal tract [2]. The most frequent sites of occurrence are the stomach, followed by the small intestine and ileocecal region. In the last 2 decades, there has been a rapid development in the diagnosis, staging and management of gastrointestinal lymphomas, but some of these lymphomas, especially T-cell ones, constitute a therapeutic challenge. Globally, non-Hodgkin lymphomas caused 6.8 million DALYs (disability-adjusted life-years) in 2016 [12]. Despite the development of new antineoplastic agents, the short- and long-term results of treatment of the aggressive non-Hodgkin lymphomas remain modest, with frequent relapses and primary refractory forms [13]. Patients' survival differs depending on the stage and histological type of malignant lymphomas at diagnosis, the presence of signs of intoxication, the age, and concomitant pathologies [14]. According to the study conducted in the United Kingdom between 2004 and 2016, 60 out of 100 patients with diffuse large B-cell lymphomas survived 5 years or more after diagnosis, while 55 out of 100 patients with Burkitt lymphoma survived 5 years, and only 35 out of 100 patients with T-cell lymphomas survived 5 years after diagnosis [14].

The incidence of extranodal lymphomas has been continuously increasing in recent years. There are numerous factors that "favor" this increase: HIV/AIDS infection, the expanded use of immunosuppressive therapy, chronic inflammatory diseases and indolent viral infections (EBV, CMV, HCV) [15]. Primary gastric diffuse large B-cell lymphoma is commonly associated with HIV/AIDS, and MALT lymphoma is associated with *Helicobacter pylori* [16]. *Helicobacter pylori* eradication, thus, is recommended in cases of MALT lymphoma. More than 70% of the patients obtain remission following eradication of Helicobacter pylori using triple or quadruple therapy [16].

The increase in morbidity and disability in the working-age population, the high rate of late diagnosis of non-Hodgkin lymphomas and the modest results of treatment of the aggressive histopathological types [5, 17-19] remain an actual problem for clinical medicine and public health, requiring additional management and financial resources. According to the MarketScan® Commercial Claims and Encounters and Health and Productivity Management Databases, patients with non-Hodgkin lymphomas suffered more significant losses of productivity at work (31.99 days; 95% CI: 25.24 days, 38.73 days; $p < 0.001$) as compared to the control group [18]. In aggressive non-Hodgkin lymphomas, the average monthly costs of induction treatment ($10,970) and palliative care ($9,836) exceeded those associated with secondary treatment ($3,302). The average cost of treatment failure in respective histopathological types was $14,174 per month and $85,934 over the entire study period [20]. Therefore, it is important to recognize different lymphoid and solid tumors within the gastrointestinal tract

in conjunction with the clinical and endoscopic features as gastrointestinal biopsies are among the most common specimens in academic and private pathology practices [8]. The recognition of these lymphomas' morphology, immunophenotype, and genetic/molecular patterns ensures an efficient and reliable clinical management and treatment.

Conclusions

Our study demonstrated that non-Hodgkin lymphomas with primary involvement of the gastrointestinal tract exhibited distinct histopathological, clinical-evolutionary and hematological features, which influenced treatment outcomes. The aggressive histological types and the advanced stages IIIE and IV prevailed within the structure of non-Hodgkin lymphomas with primary gastrointestinal involvement, and, thus, negatively impacted survival and prognosis. The response rates and overall survival of patients with gastrointestinal non-Hodgkin lymphomas are consistent with the short- and long-term outcomes observed in the cases of other localizations of aggressive malignant lymphomas and necessitate additional management and financial resources in order to improve life expectancy and quality of life.

Competing interests

None declared.

Authors' contributions

LM conceived the study, participated in study design and drafted the manuscript. MR revised the methodology and draft of the article. VM participated in the study design, performed the statistical analysis and helped drafting the manuscript. DU, IC and AC collected research data, summarized and systematized data from the published studies and revised the draft of the manuscript All the authors reviewed the work critically 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 №. 3 from 17.06.2022).

Patient consent

The informed consent was obtained from all identifiable study participants.

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RESEARCH ARTICLE

From prolonged premature rupture of membranes to bronchopulmonary dysplasia: the role of chorioamnionitis in the respiratory outcomes of preterm infants

Liuba Dascaliuc[1*], Larisa Crivceanscaia[1], Ludmila Oclanschi[1], Ninel Revenco[1], Angela Cracea[1], Zinaida Sârbu[2]

[1]Department of Pediatrics, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

[2]Department of Obstetrics and Gynecology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

ABSTRACT

Introduction. Prolonged premature rupture of membranes predisposes to intrauterine infection and chorioamnionitis, both of which have significant implications for neonatal outcomes. While chorioamnionitis has been linked to accelerated surfactant production and reduced respiratory distress syndrome, it is also associated with long-term pulmonary injury, including bronchopulmonary dysplasia and pulmonary hypertension. The objective of the study is to investigate the association between prolonged premature rupture of membranes, chorioamnionitis, and respiratory outcomes among preterm infants ≤34 weeks of gestation.

Material and methods. A prospective cohort of 108 preterm infants admitted to the Neonatal Intensive Care Unit of the Mother and Child Institute, Chișinău, between October 2023 and July 2024, was divided into two groups: infants born to mothers with clinical/histological chorioamnionitis (n = 54) and controls (n = 54). Maternal risk factors, incidence of prolonged premature rupture of membranes incidence, Apgar scores, type and duration of respiratory support, and pulmonary complications were analyzed. Statistical significance was tested using chi-square and logistic regression.

Results. Prolonged premature rupture of membranes was significantly more frequent in chorioamnionitis group (67% vs. 22%, $p<0.001$). Infants exposed to chorioamnionitis had lower 1-minute Apgar scores, greater need for invasive ventilation (5.9 ± 10.6 vs. 2.2 ± 4.8 days, $p<0.05$), and prolonged hospitalization. BPD incidence was higher in the chorioamnionitis group (25.9% vs. 3.7%, $p<0.05$). Mortality did not differ significantly between groups (27.8% vs. 22.2%).

Conclusions. Prolonged premature rupture of membranes is strongly associated with chorioamnionitis, which in turn significantly increases the risk of long-term pulmonary complications in preterm infants. Early recognition of prolonged premature rupture of membranes, antibiotic prophylaxis, antenatal corticosteroids, and interdisciplinary obstetric–neonatal management are essential to reduce the burden of bronchopulmonary dysplasia.

Keywords: premature rupture of membranes, chorioamnionitis, bronchopulmonary dysplasia, preterm infants, pulmonary hypertension.

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***Corresponding author:** Liuba Dascaliuc, MD, assistant professor, Department of Pediatrics
Nicolae Testemițanu State University of Medicine and Pharmacy
165, Stefan cel Mare și Sfânt, Chisinau, Moldova
e-mail: liuba.dascaliuc@usmf.md

Key messages

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

Although chorioamnionitis has been known to act as both a potential protective factor against respiratory distress syndrome and a risk factor for long-term pulmonary injury, its precise role in the evolution of bronchopulmonary dysplasia among preterm infants, particularly in relation to prolonged premature rupture of membranes, remains incompletely clarified.

Authors' ORCID IDs
Liuba Dascaliuc – https://orcid.org/0009-0002-0567-1762
Larisa Crivceanscaia – https://orcid.org/0000-0003-4388-374X
Ludmila Oclanscaia – https://orcid.org/0000-0001-7541-840X
Ninel Revenco – https://orcid.org/0000-0002-5229-7841
Angela Cracea – https://orcid.org/0000-0002-5283-1178
Zinaida Sârbu – https://orcid.org/0000-0003-3916-5630

The research hypothesis

We hypothesized that preterm infants exposed to maternal chorioamnionitis following prolonged premature rupture of membranes have a higher risk of developing bronchopulmonary dysplasia pulmonary complications compared to those not exposed.

The novelty added by manuscript to the already published scientific literature

This prospective cohort study is the first in the Republic of Moldova to systematically evaluate the relationship between prolonged premature rupture of membranes, chorioamnionitis, and neonatal pulmonary outcomes. Our findings provide regional evidence that maternal chorioamnionitis significantly increases the risk of BPD, reinforcing the importance of early recognition, preventive strategies, and interdisciplinary obstetric–neonatal management.

Introduction

Bronchopulmonary dysplasia (BPD) remains one of the most challenging complications of prematurity, affecting up to 40% of infants born before 28 weeks of gestation and associated with significant long-term morbidity [1]. Globally, prolonged premature rupture of membranes (PPROM) complicates approximately 2–3% of all pregnancies and up to 30% of preterm deliveries, while chorioamnionitis affects 10–25% of these cases. However, there are limited regional data from Eastern Europe, particularly Moldova, on how PPROM-related chorioamnionitis influences neonatal lung outcomes [1]. The pathogenesis of BPD is multifactorial, involving immaturity, oxygen toxicity, ventilator-induced trauma, and antenatal inflammation [2].

Prolonged premature rupture of membranes is a leading obstetric risk factor for intrauterine infection, allowing ascending microbial invasion and triggering an inflammatory cascade [3]. Chorioamnionitis, whether clinical or histological, has been associated with increased risk of neonatal sepsis, pulmonary hemorrhage, and chronic respiratory disease. However, its relationship with respiratory distress syndrome (RDS) and BPD remains complex: some studies suggest that antenatal inflammation accelerates surfactant production and reduces RDS, while others highlight its detrimental role in alveolar and vascular development, predisposing to BPD [4, 5]. This duality – where intrauterine inflammation may transiently enhance surfactant synthesis but also impair lung development – has been termed the *chorioamnionitis paradox*.

This study aimed to evaluate the relationship between PPROM, chorioamnionitis, and neonatal respiratory outcomes, particularly BPD and pulmonary hypertension (PH), in a cohort of preterm infants ≤34 weeks' gestation in Moldova.

Materials and methods

We conducted a prospective cohort study including 108 preterm infants ≤34 weeks of gestation admitted to the Neonatal Intensive Care Unit (NICU) of the Mother and Child Institute, Chişinău, between October 2023 and July 2024. The study protocol was approved by the Research Ethics Committee of *Nicolae Testemiţanu* State University of Medicine and Pharmacy (Approval No. 72, 28 October 2022). The representative study sample was calculated using the EpiInfo 7.2.2.6 software, "StatCalc – Sample Size and Power" module, for an analytical observational cohort study. With a 99.9% confidence interval, the study population was divided into two groups: the chorioamnionitis (CA) group included 54 preterm infants under 34 weeks of gestation born to mothers with chorioamnionitis, and the control group included 54 preterm infants under 34 weeks of gestation born to mothers without chorioamnionitis,

Inclusion criteria were: gestational age ≤34 weeks, inborn status (born in this hospital), and NICU admission. We excluded preterm newborns greater than 34 weeks of gestation, preterm newborns transferred from another hospital and those with congenital malformations incompatible with life.

For diagnoses of chorioamnionitis were used the criteria adopted from American College of Obstetricians and Gynecologists (ACOG) (2017) – maternal temperature greater than or equal to 39.0°C or when the maternal temperature of 38.0–38.9°C and at least one additional clinical risk factor is present [6]. Clinical risk factors included: maternal tachycardia (>100 beats per minute), fetal tachycardia (>160 beats per minute), uterine tenderness on palpation, the presence of purulent or foul-smelling amniotic fluid, maternal leukocytosis (>15,000/mm^3 in the absence of corticosteroid therapy), PROM >18 hours. Histological chorioamnionitis was related to the presence of neutrophilic infiltration within the fetal membranes and umbilical cord.

The subjects were categorized based on gestational age and birth weight, as described in medical literature [7]. Gestational age classification included: (a) extremely preterm (<28 weeks), (b) very preterm (28–32 weeks), and (c) moderate preterm (32–34 weeks).

Clinical data were extracted from medical records and collected to an Excel database. These included demographic data, maternal risk factors (including PPROM), neonatal characteristics (gestational age, birth weight, Apgar scores),

type and duration of respiratory support (CPAP, VAP, HFOV), and pulmonary complications (RDS, BPD, pulmonary haemorrhage, PH).

Analyzed maternal risk factors were history of preterm birth, abortions, stillbirths, amniotic sac infection, fever during labor, IUGR (Intrauterine growth restriction), oligohydramnios, PROM >18 hours, MSAF (Meconium-Stained Amniotic Fluid), TORCH (Toxoplasmosis, Rubella, Cyto-megalovirus, Herpes simplex, and Other agents like syphilis or HIV), anemia, preeclampsia, c-section. BPD diagnosis was based on clinical and radiological criteria.

Obtained data were analyzed using Microsoft Excel statistical tools. Continuous variables were expressed as mean ± standard deviation (SD) and 95% confidence intervals. Categorical variables were summarized as frequencies and percentages. Associations between maternal risk factors, chorioamnionitis, and neonatal pulmonary outcomes were evaluated using the Pearson chi-square test. Effect sizes for chi-square associations were quantified using Cramér's V. To examine the independent relationship between chorioamnionitis and respiratory complications such as BPD and PH, binary logistic regression was applied. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Examined confounders were gestational age and birth weight. Analyses were performed using the logistic regression module available in Microsoft Excel. No data were missing. A p-value <0.05 was considered statistically significant.

Results

A total of 108 preterm infants born at ≤34 weeks of gestation were included in the study, divided equally between the chorioamnionitis (CA) group (n = 54) and the control group (n = 54). The distribution by gestational age and sex was relatively uniform between the two groups, ensuring comparability. However, the CA group included a higher proportion of extremely preterm infants (<28 weeks) compared to controls (25.9% vs. 11.1%, $p<0.05$). The proportion of female infants was slightly higher in the control group (59.3% vs. 48.1%, not significant) (Table 1).

Table 1. Characteristics of the study population

n (54)		CA		Control		Total	
		%	n (54)	%	n (108)	%	
Term of gestation	<28 weeks	14*	25.9	6	11.1	20	18.5
	28-32 weeks	31	57.4	31	57.4	62	57.4
	>32 weeks	9	16.7	17	31.5	26	24.1
Gender	Female	26	48.1	32	59.3	58	53.7
	Male	28	51.9	22	40.7	50	46.3

Note: Data are expressed as number and percentage [n (%)]. Statistical comparison between groups was performed using the Chi-square test; p < 0.05 was considered significant (*). CA – chorioamnionitis; n – number of subjects.

Analysis of maternal and obstetric characteristics revealed several significant differences between groups (Table 2). Prolonged premature rupture of membranes (PROM > 18 hours) was markedly more frequent among mothers with chorioamnionitis (66.7% vs. 22.2%, $\chi^2 = 21.6$, $p<0.001$), confirming a strong association between PPROM and intrauterine infection. Meconium-stained amniotic fluid (MSAF) was also significantly more frequent in the CA group (20.4% vs. 1.9%, $\chi2 = 9.38$, $p = 0.002$).

Although other maternal factors, including maternal history of preterm birth was more prevalent in the CA group (14,8% vs 11,1%), abortion (37.0% vs 25.9%), stillbirths (9.3% vs 1,9%), these differences were not statistically significant. The incidence of amniotic sac infection was notable in our population 26,9%, but statistical comparison was not possible as this finding was exclusive to the CA group. Also, maternal fever was observed in 5.6% of the CA group.

Table 2. Maternal obstetric and infectious risk factors and their association with chorioamnionitis

Maternal obstetric and infectious risk factors	CA		Control		Total	
	n (54)	%	n (54)	%	n (108)	%
History of preterm birth	8	14.8	6	11.1	14	13.0
Abortions	20	37.0	14	25.9	34	31.5
Stillbirths	5	9.3	1	1.9	6	5.6
Amniotic sac infection	29	53.7	0	0.0	29	26.9
Fever during labor	3	5.6	0	0.0	3	2.8
IUGR	9	16.7	12	22.2	21	19.4
Oligohydramnios	13	24.1	7	13.0	20	18.5
PROM >18 hours (p < 0.001)	*36	66.7	12	22.2	48	44.4
MSAF (p = 0.002)	*11	20.4	1	1.9	12	11.1
TORCH	5	9.3	1	1.9	6	5.6
Anemia	28	51.9	25	46.3	53	49.1
Preeclampsia	15	27.8	16	29.6	31	28.7
C-section	30	55.6	36	66.7	66	61.1

Note: Data are presented as number and percentage [n (%)]. Statistical analysis between groups was performed using the Chi-square test; *p < 0.05* considered significant (*). CA – chorioamnionitis; PROM – premature rupture of membranes; MSAF – meconium-stained amniotic fluid, n – number of infants, IUGR – intrauterine growth restriction.

Infants born to mothers with chorioamnionitis demonstrated poorer adaptation at birth (Table 3). At 1 minute, 74.1% of CA-exposed infants had Apgar scores between 4–6, compared with 50.0% in controls, while only 9.3% achieved scores ≥7 compared to 42.6% in controls ($p < 0.05$). By 5 minutes, although most infants improved, 85.2% of the CA group had Apgar scores 7–8 vs 37.0% of controls, confirming delayed neonatal adaptation ($p < 0.05$).

Table 3. Apgar Score at 1 and 5 minutes of the subjects included in the study

n (54)		CA		Control		Total	
		%	n (54)	%	n (108)	%	
1 minute	1-3	9	16.7	4	7.4	13	12.0
	4-6	*40	74.1	27	50.0	67	62.0
	7-8	5	9.3	*23	42.6	28	25.9
5 minutes	1-3	0	0.0	3	5.6	3	2.8
	4-6	8	14.8	*31	57.4	39	36.1
	7-8	*46	85.2	20	37.0	66	61.1

Note: Data are presented as number and percentage [n (%)]. Statistical analysis was performed using the Chi-square test; *p < 0.05* considered significant (*). CA – chorioamnionitis; n – number of infants.

The need for respiratory support was universal among preterm infants but differed in intensity and duration between groups (Table 4). Infants in the CA group required significantly longer invasive ventilation (5.9 ± 10.6 days vs. 2.2 ± 4.8 days, p < 0.05) and had longer total hospital stays (32.6 ± 28.2 days vs. 25.0 ± 16.6 days). The duration of CPAP and HFOV use did not differ significantly, although a higher mean use was observed in the CA group.

These findings indicate that exposure to maternal infection was associated with more severe respiratory compromise and prolonged hospitalization.

Pulmonary complications were common in both groups (Table 5). Respiratory distress syndrome (RDS) occurred in almost all infants (99%), with no significant difference between groups. However, bronchopulmonary dysplasia (BPD) was significantly more frequent among infants exposed to chorioamnionitis **(**25.9% vs. 3.7%, $p < 0.05$). The odds ratio for developing BPD in the CA group was approximately 9.1 (95% CI 1.9–42.3, $p < 0.05$)**,** confirming a strong association between antenatal infection and chronic lung disease.

Table 4. Days on respiratory support (HFOV, VAP, CPAP)

		n	Mean	Std. Deviation	Std. Error Lower Bound	95% Confidence Interval for Mean		Minimum	Maximum
						Upper Bound			
Days of hospital stay	CA	54	32.59	28.200	3.838	24.90	40.29	1	162
	Control	54	24.98	16.609	2.260	20.45	29.51	3	70
	Total	108	28.79	23.349	2.247	24.33	33.24	1	162
HFOV (days)	CA	54	.37	.917	.125	.12	.62	0	4
	Control	54	.22	.664	.090	.04	.40	0	3
	Total	108	.30	.800	.077	.14	.45	0	4
VAP (days)	CA	54	*5.94	10.609	1.444	3.05	8.84	0	63
	Control	54	2.24	4.825	.657	.92	3.56	0	30
	Total	108	4.09	8.411	.809	2.49	5.70	0	63
CPAP (days)	CA	54	4.65	7.159	.974	2.69	6.60	0	38
	Control	54	3.48	4.041	.550	2.38	4.58	0	24
	Total	108	4.06	5.815	.560	2.96	5.17	0	38

Note: Data are presented as mean ± standard deviation (SD). Group comparisons were assessed using the independent-samples t-test; p < 0.05 considered significant (*). HFOV – high-frequency oscillatory ventilation; VAP – invasive ventilation; CPAP – continuous positive airway pressure; CA – chorioamnionitis, n – number of infants.

Pulmonary hypertension (PH) was also more prevalent in the CA group **(**20.4% vs. 9.3%), although this difference did not reach statistical significance ($p > 0.05$). Other complications such as atelectasis and pneumothorax occurred rarely and with similar frequency between groups.

Chi-square and Fisher's exact tests confirmed the statistically significant correlation between chorioamnionitis and BPD ($p = 0.001$, Table 6, Figure 1). This relationship remained consistent after adjusting for gestational age and birth weight in logistic regression analysis, suggesting that intrauterine inflammation independently contributes to long-term pulmonary morbidity in preterm infants.

Table 5. Pulmonary complications of the subjects included in the study

	CA		Control		Total	
	n	%	n	%	n	%
Pneumonia	54	100.0	54	100.0	108	100.0
RDS	54	100.0	53	98.1	107	99.1
BPD	14 *	25.9	2*	3.7	16	14.8
PH	11	20.4	5	9.3	16	14.8
PHT	4	7.4	4	7.4	8	7.4
Atelectasis	2	3.7	2	3.7	4	3.7
Pneumothorax	1	1.9	3	5.6	4	3.7

Note: Data are presented as number and percentage [n (%)]. Statistical analysis was performed using the Chi-square test; *p < 0.05* considered significant (*).CA – chorioamnionitis; RDS – respiratory distress syndrome; BPD – bronchopulmonary dysplasia; PH – pulmonary hypertension; PHT – persistent pulmonary hypertension of the newborn, n – number of infants.

When stratified by gestational age (Table 7), RDS was similar in both groups (>98%). BPD occurred most frequently among very preterm infants (28–32 weeks, 16.1%), while PH was also predominant in this gestational subgroup (21.0%). These findings support the concept that both immaturity and antenatal inflammation contribute to disease severity.

Table 6. Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	10.565[a]	1	0.001	0.002	0.001	
Continuity Correction[b]	8,878	1	0.003			
Likelihood Ratio	11,694	1	0.001	0.002	0.001	
Fisher's Exact Test				0.002	0.001	
Linear-by-Linear Association	10.467[c]	1	0.001	0.002	0.001	0.001
N of Valid Cases	108					

Note: Statistical output showing association between chorioamnionitis and BPD. Pearson Chi-square and Fisher's exact tests were applied; *p < 0.05* considered statistically significant. CA – chorioamnionitis; BPD – bronchopulmonary dysplasia.

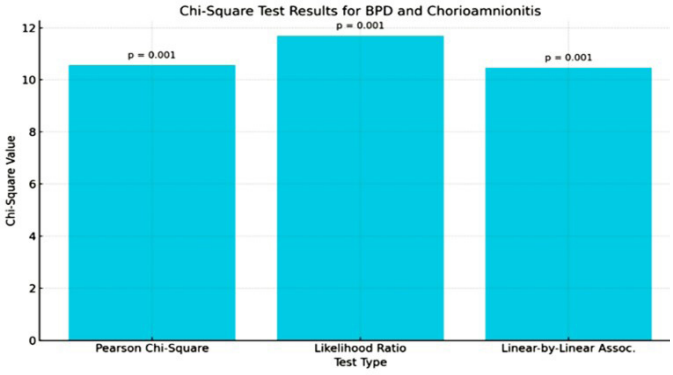

Fig. 1 Chi-square test for BPD and CA

Note: Figure 1 represents association between chorioamnionitis and bronchopulmonary dysplasia in preterm infants. The figure demonstrates a significantly higher proportion of BPD in the CA-exposed group compared with controls (*p < 0.05*, Chi-square test). CA – chorioamnionitis; BPD – bronchopulmonary dysplasia.

Overall mortality did not differ significantly between groups (27.8% vs. 22.2%, *p*>0.05; Table 8). The majority of deaths occurred during the early neonatal period (<7 days), accounting for 55.6% of all deaths, followed by late neonatal deaths (40.7%). Only one death occurred in the post-neonatal period (>28 days). Although mortality rates were comparable, infants in the CA group showed a trend toward more severe respiratory morbidity and prolonged ventilation prior to death.

Table 7. Pulmonary complications in the study subjects according to gestational age (weeks of gestation)

	<28		28-32		>32		Total	
	n	%	n	%	n	%	n	%
Pneumonia	20	100.0	62	100.0	26	100.0	108	100.0
RDS	20	100.0	61	98.4	26	100.0	107	99.1
BPD	3	15.0	10	16.1	3	11.5	16	14.8
PH	2	10.0	13	21.0	1	3.8	16	14.8
PHT	1	5.0	7	11.3	0	0.0	8	7.4
Atelectasis	0	0.0	2	3.2	2	7.7	4	3.7
Pneumothorax	0	0.0	3	4.8	1	3.8	4	3.7

Note: Data are presented as number and percentage [n (%)]. Statistical analysis was performed using the Chi-square test; *p < 0.05* considered significant (*). RDS – respiratory distress syndrome; BPD – bronchopulmonary dysplasia; PH – pulmonary hypertension; PHT – persistent pulmonary hypertension, n – number of infants.

Table 8. Death of the subjects included in the study

	CA		Control		Total	
	n	%	n	%	n	%
No	39	72.2	42	77.8	81	75.0
Yes	15	27.8	12	22.2	27	25.0
<7 days	7	46.7	8	66.7	15	55.6
8-28 days	7	46.7	4	33.3	11	40.7
>28 days	1	6.7	0	0.0	1	3.7
Total	15	100.0	12	100.0	27	100.0

Note: Data are presented as number and percentage [n (%)]. Statistical analysis was performed using the Chi-square test; *p < 0.05* considered significant (*). CA – chorioamnionitis; n – number of infants.

Discussion

Our findings highlight a strong association between chorioamnionitis and adverse respiratory outcomes in preterm infants, particularly bronchopulmonary dysplasia (BPD). In our cohort, infants exposed to chorioamnionitis had a nearly nine-fold higher risk of BPD compared with controls, alongside lower Apgar scores, longer need for invasive ventilation, and prolonged hospitalization. Although the incidence of respiratory distress syndrome (RDS) was similar in both groups, the risk of long-term pulmonary morbidity was significantly increased in the chorioamnionitis group.

These results are consistent with international evidence from large systematic reviews and meta-analyses [5, 8-10]. A comprehensive meta-analysis including more than 244,000 infants found that both clinical and histological chorioamnionitis were associated with an increased risk of BPD, supporting the hypothesis that intrauterine inflammation predisposes the preterm lung to chronic injury [9]. Similarly, a 2024 systematic review and meta-regression confirmed that exposure to chorioamnionitis significantly increases the odds of BPD in preterm infants, irrespective of the specific diagnostic criteria used [5]. Another meta-analysis of 27 studies (~6,099 preterm infants) demonstrated that histologic chorioamnionitis (HCA) increases the risk of BPD (RR ~1.68; 95% CI 1.19–2.36). This

supports our observation that histologic or more severe/inflammatory CA is more strongly linked to BPD [10].

Mechanistically, these associations can be explained by the concept of the "fetal inflammatory response syndrome." Intrauterine infection induces the release of pro-inflammatory cytokines, which may stimulate surfactant production and transiently reduce RDS severity but simultaneously disrupt alveolar and vascular development. This dual effect has been termed the "chorioamnionitis paradox" [3]. Our findings reflect this paradox: although nearly all infants developed RDS regardless of exposure, those with chorioamnionitis progressed more frequently to BPD and pulmonary hypertension, suggesting that antenatal inflammation amplifies vulnerability to long-term respiratory complications.

Despite the strong association between CA and BPD in our study we did not observe a significant difference in mortality rates between groups. This contrasts with other studies, such as [4], which identify CA as a risk factor for combined adverse outcomes. In that study, infants born to mothers with *acute and severe HCA* had significantly higher rates of the composite outcome of BPD or death (60% vs 27%, $p = 0.012$) than infants without HCA. This finding helps illustrate the high burden when CA is severe.

Taken together, these data underscore the importance of early recognition and management of prolonged premature rupture of membranes and chorioamnionitis in obstetric practice, as well as the need to minimize invasive ventilation in affected neonates. Preventive strategies such as timely maternal antibiotic therapy, antenatal corticosteroids, and coordinated obstetric-neonatal management remain essential to reduce the burden of BPD [11].

Conclusions

Prolonged premature rupture of membranes is strongly associated with maternal chorioamnionitis, which significantly increases the risk of bronchopulmonary dysplasia and pulmonary hypertension in preterm infants. Early recognition, preventive strategies, and interdisciplinary management are essential to mitigate long-term respiratory morbidity.

Competing interests

None declared.

Authors' contributions

LC contributed substantially to the conception and design of the study and also approved the final version to be published. LD contributed to acquisition of the data, substantial contributed to the analysis and interpretation of the collected data, drafting the article and taking responsibility for all aspects of the work. AC and LO also contributed to the analysis and interpretation of the data. ZS reviewed the article for intellectual content. The final approval was provided by NR and LC. All authors critically reviewed the work and approved the final version of the manuscript.

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Ethics approval.

The study protocol was approved by the Research Ethics Committee of *Nicolae Testemiţanu* State University of Medicine and Pharmacy (decision No. 4/3.4 dated June 28, 2023).

Patient consent

Obtained.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Limitations of the study.

This study has several limitations that should be acknowledged. First, the sample size was relatively small, and although all eligible preterm infants ≤34 weeks were included, the limited number of cases may reduce the statistical power, particularly for subgroup analyses. Second, this was a single-center study conducted in a Level III perinatal facility in Moldova, which may limit the generalizability of the findings to other settings or populations. Additionally, important inflammatory biomarkers (e.g., IL-6, IL-8, TNF-α) were not assessed, which limits the mechanistic interpretation of the association between chorioamnionitis and pulmonary outcomes. Potential confounding factors—including birth weight, gestational age distribution, ventilation strategies, nutritional practices, and timing of antenatal corticosteroid administration—may have influenced the risk of BPD despite adjustment attempts. Finally, follow-up was restricted to the NICU hospitalization period, and long-term respiratory or neurodevelopmental outcomes were not evaluated.

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RESEARCH ARTICLE

Exploring the clinical spectrum of DiGeorge syndrome

Cristina Tomacinschii[1,2*], Victoria Sacara[2], Alexandr Dorif[2], Marodi Laszlo[3,4], Svetlana Sciuca[1]

[1]Department of Pediatrics, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[2]Human Molecular Genetic Laboratory, Institute of Mother and Child, Chișinău, Republic of Moldova

[3]Primary Immunodeficiency Clinical Unit and Laboratory, Department of Dermatology, Venereology and Dermato-Oncology, Semmelweis University, Budapest, Hungary

[4]*St. Giles* Laboratory of Human Genetics of Infectious Diseases, Rockefeller University, NY, USA

ABSTRACT

Introduction. DiGeorge syndrome, known also as 22q11.2 deletion syndrome, is a rare multisystemic disorder characterized by a wide range of clinical features and may include thymic aplasia and subsequent immunodeficiency, conotruncal cardiac anomalies, typical facial features, palatal abnormalities, and hypocalcemia due to hypoparathyroidism.

Material and methods. Data were collected for 10 patients genetically confirmed with DiGeorge syndrome at the Institute of Mother and Child. This included general information, laboratory results, and clinical features.

Results. The mean age at diagnosis was 74.6 months (3 months – 28 years). Most cases were sporadic, with only 2 patients having a history of DGS (n=1), or close relatives with cardiac malformations (n=1). The most common symptoms that led to diagnosis were congenital heart defects (90%), and facial dysmorphism (90%). Common clinical features included recurrent infections (40%) and ENT disorders (20%). Weight was within normal percentiles for the entire group, but a delay in height growth was noted. Regarding the immunological characteristics: lymphopenia was recorded in 20% of patients, and thrombocytopenia in 2 patients.

Conclusions. Given the diverse array of symptoms associated with DiGeorge syndrome, physicians should be knowledgeable about both typical and less common characteristics of the syndrome to facilitate optimal treatment and potentially enable early diagnosis.

Keywords: DiGeorge syndrome, children, infections, congenital heart defects.

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***Corresponding author: Cristina Tomacinschii,** MD, PhD fellow
Department of Pediatrics
Nicolae Testemițanu State University of Medicine and Pharmacy
165, Ștefan cel Mare și Sfânt, Chișinău, Republic of Moldova
e-mail: cristinatomacinschii@gmail.com

Authors' ORCID IDs
Cristina Tomacinschii – https://orcid.org/0000-0001-6351-5659
Victoria Sacara – https://orcid.org/0000-0001-9200-0494
Alexandr Dorif – https://orcid.org/0000-0003-4269-4066
Marodi Laszlo – https://orcid.org/0000-0002-9692-3402
Svetlana Sciuca – https://orcid.org/0000-0003-1091-9419

Key messages

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

Despite extensive descriptions of DiGeorge syndrome, little is known about its presentation in Eastern European populations. This study adds new data on the clinical and immunological features of children with genetically confirmed DiGeorge syndrome in Moldova.

The research hypothesis

We hypothesized that children with DiGeorge syndrome in Moldova present a heterogeneous clinical profile, with both typical features and less common manifestations, and that systematic genetic confirmation can reveal underdiagnosed immunological and developmental abnormalities.

The novelty added by manuscript to the already published scientific literature
This is the first study of children with genetically confirmed DiGeorge syndrome in Moldova, offering new insights into their clinical and immunological features and helping clinicians recognize and manage both typical and less common manifestations.

Introduction

The DiGeorge syndrome (DGS) is a collection of signs and symptoms associated with the faulty development of the pharyngeal pouch system. Most cases are caused by a heterozygous chromosomal deletion at 22q11.2 discovered in the 1980s. The 22q11.2 chromosomal region contains approximately 30–40 genes that are critically involved in the embryologic formation of the third and fourth pharyngeal arches, thereby influencing the development of the cardiac outflow tract, great vessels, thymus, parathyroid glands, and craniofacial structures [1-4].

A comprehensive study conducted on a large population has shown that heterozygous chromosome 22q11.2 deletions are relatively widespread in the general population, establishing 22qDGS as the most common microdeletion syndrome. According to this study, approximately 1 in 5950 live births exhibited a deletion in this chromosomal region, with 83 percent of these infants also presenting a cardiac defect [5]. Another population-based study reported an incidence rate of 22qDGS as high as 1 in 4000 live births [6, 7]. Its frequency is 2.8-5% for congenital cardiac malformations. In conotruncal defects, this rate is reported as 10-19.4% in various studies [1,8]. The vast majority of DiGeorge syndrome cases (approximately 90%) occur spontaneously, although autosomal dominant inheritance has been reported in a minority of patients (8–28%) [7, 9].

The deletion of chromosome 22q11.2 presents a wide spectrum of clinical features, including heart and major blood vessel defects, underdevelopment of the parathyroid glands, deficiency in parathyroid hormone, hypocalcemia, seizures, aplasia and hypoplasia of the thymus, immunological disorders, growth hormone deficiency, anomalies of the facial skeleton and structure of the larynx, pharynx, trachea, inner ear, esophagus, teeth, kidney malformations, gastrointestinal and central nervous system anomalies, delayed speech and motor development, hyperactivity syndrome, and schizophrenia [10, 11].

In a study conducted on a group of postmortem-diagnosed DGS patients, alongside severe immune system alterations, these children exhibited various stigmata of dysmorphogenesis, such as low-set auricles, hypotelorism or hypertelorism, micrognathia, high palatal vault, cleft palate, saddle nose, etc., as well as various congenital malformations [12, 13].

In DGS, immunological defects vary from extended and recurring sinopulmonary infections (often referred to as partial DGS) to congenital athymia (a phenotype resembling severe combined immunodeficiency [SCID], termed complete DGS). The level of immunodeficiency severity correlates with the extent of thymic functionality [14].

Individuals with DGS exhibit increased susceptibility to autoimmune conditions, such as autoimmune cytopenia and juvenile idiopathic arthritis [15].

The objective of this study was to outline the clinical characteristics observed in a cohort of genetically confirmed DGS patients, with the aim of enhancing awareness and comprehension of DGS.

Material and methods

This descriptive observational study was conducted in the Human Molecular Genetics Laboratory and the Department of Pediatrics of the Institute of Mother and Child and included 10 consecutive patients investigated for suspected 22q11.2 syndrome. Participants were recruited based on clinical criteria formulated by Tobias et al. [16, 17], according to which inclusion was possible in the presence of a major conotruncal heart defect (such as tetralogy of Fallot, interrupted aortic arch, truncus arteriosus, or major aortopulmonary collaterals), two or more core features – characteristic facial anomalies, non-conotruncal heart defects, developmental delay or learning disabilities, hypocalcemia, immunodeficiency, or thymic hypoplasia – or a core criterion associated with complementary manifestations, such as long and thin fingers, short stature, hypotonia, palatal malformations (cleft palate, velopharyngeal insufficiency, difficulty swallowing), renal abnormalities, family history of congenital heart disease, or psychiatric disorders, especially bipolar disorder. For each patient, clinical data were collected in a standardized manner. The assessment of height and weight development was performed using SD scores and age-appropriate growth charts, and recurrent respiratory morbidity was assessed according to criteria described in the specialized literature [18].

All patients underwent a complete interdisciplinary evaluation, which included cardiological examination with Doppler echocardiography to identify conotruncal or non-conotruncal malformations, endocrinological evaluation consisting of the determination of total and ionized serum calcium and thyroid hormone levels (ELISA method), as well as ENT examination (including audiogram). The neurological examination was completed by neuropsychological evaluation, and the immunological profile included complete blood count, absolute lymphocyte count, platelet count, serum immunoglobulin levels (ELISA method), and lymphocyte subset analysis (performed by flow cytometry).

The genetic diagnosis of the 22q11.2 deletion was established using high-accuracy molecular methods, according to internally validated protocols, including multiplexed QF-PCR using FAM channels for internal controls, VIC/HEX for target regions, and LIZ for the size marker [13, 19, 20],

as well as fluorescence in situ hybridization (FISH), chromosomal microarray analysis (CMA), or droplet digital PCR (ddPCR) in cases that required additional confirmation. All analyses were performed under standardized conditions to ensure reproducibility of the results.

Data processing and descriptive statistical analysis were performed using Microsoft Excel (Microsoft Corporation, Excel, version 2010, WA, USA) for data organization, and IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), which was used to investigate the descriptive characteristics of the entire cohort.

The study was approved by the Research Ethics Committee of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy (No. 13/15.03.2019), and informed consent was obtained from all legal guardians of the participating minors.

Results

The mean age of patients was 9.3 years (2–30 years) at the time of the study and 74.6 months (3 months – 28 years) at diagnosis. In the study group, 30% of the patients were male, while 70% were female. Demographic and clinical features of the cohort are described in Table 1.

Table 1. Demographic and clinical features of patients with 22q11.2 deletion syndrome

Patient	Current age (years) / gender	Age of onset (months)	Cardiac defect	Other abnormalities	Family history
1	5/female	3	Common truncus	Facial dysmorphism Growth retardation	None
2	4/male	48	Tetralogy of Fallot	Facial dysmorphism	Great-grandmother and uncle with cardiac malformation
3	8/female	24	Tetralogy of Fallot	Facial dysmorphism Growth retardation Psychomotor, cognitive and language delay, attention-deficit hyperactivity Upper and lower respiratory tract infections, otitis Renal agenesis Swallowing difficulty	Father with DGS
4	30/male	28 years	Unspecified	Facial dysmorphism	Father of P3
5	8/female	72	None	Upper and lower respiratory tract infections	None
6	5/female	10	Tetralogy of Fallot	Facial dysmorphism Growth retardation Laryngomalacia	None
7	9/female	60	Patent foramen ovale (PFO)	Facial dysmorphism Cognitive and language delay	None
8	2/female	6	Tetralogy of Fallot	Facial dysmorphism Growth retardation	None
9	2/female	7	Right aortic arch	Upper and lower respiratory tract infections Facial dysmorphism Growth retardation	None
10	20/male	15 years	Tetralogy of Fallot	Facial dysmorphism Upper and lower respiratory tract infections	None

Note: DGS - DiGeorge syndrome; PFO - patent foramen ovale; P3- patient number 3

Most cases were sporadic, with only 2 patients having a history of DGS (n=1) or close relatives with cardiac malformations (n=1). During the neonatal period, subjects P3 and P9 experienced complications such as seizures, hypotonia, and sucking difficulties.

Cardiac malformations were the most common manifestation, accounting for 90% of cases (n=9). Among these, 77.7% exhibited conotruncal anomalies (5 had tetralogy of Fallot, 1 had right aortic arch, 1 – common truncus), while 33.3% had non-conotruncal anomalies. All patients with cardiac malformations required surgical correction, during which thymic hypoplasia was described in all cases.

Nine of ten patients (90%) exhibited facial dysmorphism, with microstomia being the most prevalent feature (9/10), followed by micrognathia (4/10), dental anomalies (1/10), hypertelorism (3/10), and low-set ears (3/10).

Forty percent of the patients presented recurrent infections, predominantly in the form of pneumonia. Following surgical correction, the number of infections decreased in most patients.

ENT anomalies were recorded in 20% of the patients, with one presenting laryngomalacia and one exhibiting swallowing difficulties and palatal anomalies. Other alterations observed in patients with DGS included speech delay in 2 patients and developmental delay in 2 patients. Regarding congenital renal conditions, renal agenesis was identified in P3.

Weight was within normal percentiles for the entire group, but a delay in height growth was noted. Most of the cohort experienced a decrease in height (between -1 and -2 SD), particularly during childhood. There were no instances of short stature (height < -2 SD) recorded. Subject P4 was excluded due to a lack of reported measurements. P3 presented with neonatal hypocalcemia.

From the immunological characteristics, lymphopenia was recorded in 20% of patients (Fig.1), with a mean val-

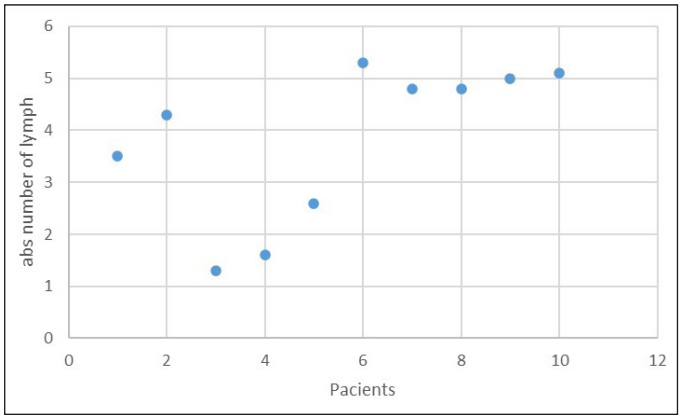

Fig.1 The lymphocyte count in the DGS group

Note: *Absolute lymphocyte count was calculated by multiplying the percentage of lymphocytes by the total leukocyte count (×10^9/L). Lymphopenia was defined as less than 3 ×10^9/L for children under 3 years of age and less than 1.5 ×10^9/L for those over 3 years of age.*

ue of 3.83 ± 1,48; low platelet counts (100-150,000) were observed in 2 patients. IgA was below normal levels in 1 patient. Elevated levels of IgE were noted in 2 patients.

Taking into account the presence of recurrent infections, predominantly pneumonia, patients underwent chest X-rays, which consistently showed the absence of a thymic shadow, a characteristic sign of DGS.

Discussion

The present study provided a clinical characterization of a group of patients with DGS. Considering the high clinical heterogeneity and the state of underdiagnosis in DGS, elaborating upon the clinical features of DGS may be helpful for improving timely diagnoses and diagnosis rates. In this group of patients, typical characteristics associated with 22q11.2DS were identified. These included congenital heart defects, distinct facial features, recurrent infections, and abnormalities in the immune system.

All patients included in the study were diagnosed with 22q11.2 deletion. In approximately 90% of cases, the 22q11.2 deletion arises de novo, while in 6–25% of patients it is inherited in an autosomal dominant manner [1, 21]. In line with previously published data, our cohort included one patient (10%) with a paternally inherited deletion, as well as one patient with a family history of congenital cardiac anomalies. These findings underscore the importance of comprehensive genetic counseling for affected families, including a clear discussion of recurrence risk and available prenatal diagnostic options [1].

As anticipated, congenital heart defects were a prominent feature, presenting in 90% of our patients. This prevalence aligns with prior findings, where 75% of individuals with the 22q11.2 deletion exhibit cardiac anomalies, predominantly conotruncal defects or aortic arch anomalies [1, 22], or 49–83%, according to other sources [23]. Among these, tetralogy of Fallot and interrupted aortic arch are the most common (27-62.3%; 14-53%, respectively). Transposition of the great arteries is infrequent. Non-conotruncal structural cardiac anomalies are also documented, with deletions potentially implicated in 5% of all newborns with cardiac defects [1, 24].

Another prominent characteristic of DiGeorge syndrome observed in our group was recurrent infections, which occurred in 40% of the patients. Jawad et al. and Oskarsdottir et al. reported recurrent infections in 60–65% of their study populations [25, 26]. Subsequent studies by Giardino et al., Cancrini et al., and Nissan et al. revealed even higher rates of recurrent infections in individuals with DiGeorge syndrome (64%, 54%, and 78%, respectively) [27-29]. These disparities between our cohort and previous studies may be attributed to the focus on other criteria, such as the documentation of recurrent infections.

Distinctive facial dysmorphic characteristics, including microstomia, narrow palpebral fissures, and micrognathia, were noted in all study patients. Additionally, less common traits, such as low-set ears, dental anomalies, and hypertelorism were also observed. The findings may differ based on race and ethnicity [30, 31]. In this study, the majority of patients with the 22q11.2 deletion exhibited the characteristic facial features of the syndrome. This underscores the significance of visual inspection in clinical assessment.

In our cohort, 20% of patients exhibited speech and developmental delays, which is lower than the prevalence reported in earlier studies [32].

Hematologic abnormalities, particularly thrombocytopenia and lymphopenia, have been extensively documented in individuals with DiGeorge syndrome in the literature. Thrombocytopenia was described in some cases as having an autoimmune etiology, but in most cases, its etiology is unknown [32, 33].

No severe immunodeficiency resembling leaky SCID was observed in our cohort. The immunological abnormalities detected may reflect thymic hypoplasia, commonly reported in 22q11.2DS [28, 34].

Conclusions

In conclusion, DiGeorge syndrome is a condition that impacts various organ systems. Prompt detection of this syndrome is key to proper care. Given the diverse array of symptoms associated with DiGeorge syndrome, physicians should be knowledgeable about both typical and less common characteristics of the syndrome to facilitate optimal treatment and potentially enable early diagnosis. This marks the initial report of DGS patients in our country. Despite the relatively high frequency of DGS, only a small number of patients with a confirmed diagnosis are currently under follow-up.

Competing interests

None declared.

Authors' contributions

TC conceived the conceptualization, methodology, data collection, analysis, and interpretation, and was responsible for writing – original draft preparation; VS, DA, and LM – co-

ordination of genetic analysis; SS – research coordination, conception, review writing, editing, and validation. All authors read and approved the final manuscript.

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Ethics approval

The study protocol was approved by the Research Ethics Committee of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy (No.13 from 15.03.2019).

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RESEARCH ARTICLE

Emergency care for trauma patients in the red zone: clinical experience from 2024

Tatiana Malacinschi-Codreanu*, Larisa Rezneac, Raed Habach, Eugenia Ciubotaru, Natalia Mocanu

Gheorghe Ciobanu Department of Medical Emergencies, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

ABSTRACT

Background. The traumatized patient with signs of shock remains a major cause of morbidity and mortality, requiring rapid diagnosis and multidisciplinary management. This study evaluates clinical, paraclinical, and therapeutic aspects of trauma patients admitted with shock signs in the red zone of the Emergency Department of the Institute of Emergency Medicine during 2024.

Material and methods. A prospective analysis of 60 polytrauma patients in shock was performed. Demographic data, trauma mechanisms, Glasgow Coma Score, vital signs, imaging, emergency interventions, and outcomes were evaluated. The patients were managed according to an experimental protocol developed at the institutional level, which provides for a multidisciplinary approach to the traumatized patient. This protocol was developed based on the international protocol for Advanced Trauma Life Support. It will later be implemented at the institutional level.

Results. The cohort included 79% males, with a mean age of 49.7 ± 15.2 years; 75% urban residents. Grade III and IV shock were diagnosed in 40% and 28.3%, respectively. Causes of trauma included physical aggression (29.5%), road traffic accidents (28.5%), and accidental trauma (21.0%), falls from height (21.0%). Radiological lesions were detected in 63.3%, positive findings on Focused Assessment with Sonography for Trauma in 40%, and cranial lesions identified by computed tomography in 60%. Intraglotic intubation was required in 80% of patients, vasopressor support was administered in 50%, antibiotic therapy in 95%, and emergency surgery was performed in 30%. Mortality was 1.7%.

Conclusions. Protocol-driven multidisciplinary care facilitated effective stabilization of traumatized patients presenting with shock. Early diagnosis, the use of advanced imaging techniques, and timely therapeutic interventions significantly contributed to improved patient outcomes. These findings underscore the critical role of structured trauma life support protocols in emergency medical settings.

Keywords: trauma, shock, emergency department, resuscitation, Advanced Trauma Life Support.

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***Corresponding author:** Tatiana Malacinschi-Codreanu, MD, PhD, associate professor,
Gheorghe Ciobanu Department of Medical Emergencies
Nicolae Testemițanu State University of Medicine and Pharmacy
165 Stefan cel Mare și Sfant blvd, Chisinau, Republic of Moldova, MD-2004
e-mail: tatiana.codreanu@usmf.md

Authors' ORCID IDs
Tatiana Malacinschi-Codreanu – https://orcid.org/0000-0001-9098-9470
Larisa Rezneac – https://orcid.org/0000-0001-7545-1728
Raed Habach – https://orcid.org/0000-0002-0471-5340
Eugenia Ciubotaru – https://orcid.org/0000-0002-1169-3821
Natalia Mocanu – https://orcid.org/0000-0001-5989-4553

Key messages

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

There is limited published data on the real-time clinical management of polytraumatized patients in shock in Eastern European emergency departments. In particular, the epidemiological profile, temporal distribution, clinical outcomes, and adherence to internationally recognized protocols such as Advanced Trauma Life Support in Moldova remain insufficiently explored.

The research hypothesis

The implementation of a multidisciplinary, protocol-driven approach – based on Advanced Trauma Life Support principles – in the emergency department leads to improved early stabilization,

more efficient therapeutic interventions, and reduced in-hospital mortality among polytraumatized patients presenting with clinical signs of shock.

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

This is the first prospective clinical analysis of polytraumatized patients in shock managed in the Red Zone of the Institute of Emergency Medicine in Moldova. The study offers novel insights into trauma epidemiology, time-of-day incidence, and demonstrates the effectiveness of standardized, protocol-based emergency care – including Advanced Trauma Life Support – in a resource-limited Eastern European setting.

Introduction

One of the causes of increased morbidity and mortality globally is trauma accompanied by shock, requiring rapid diagnosis and a multidisciplinary approach [1, 2]. This study evaluates the clinical and paraclinical characteristics, as well as therapeutic outcomes, of traumatized patients presenting with shock in the red zone of the Institute of Emergency Medicine (IEM) during 2024.

Adherence to evidence-based protocols, such as the Advanced Trauma Life Support (ATLS), has been demonstrated to improve survival by prioritizing life-threatening injuries and streamlining patient care [1, 3, 4]. Understanding the epidemiology and management of traumatic shock is crucial for optimizing emergency medical response and resource allocation. Historically, management evolved from rapid problem-solving to focused stabilization of vital functions, significantly reducing mortality [1, 5, 6].

While early approaches focused on solving as many problems as quickly as possible, current practice emphasizes prioritizing life-threatening factors, a strategy that has proven effective in reducing patient mortality. Managing a traumatized patient with signs of shock poses not only medical challenges but also has long-term implications for patient quality of life and outcomes in emergency medicine [1, 7].

It is crucial to identify and analyze the main causes that lead to the clinical manifestation of shock in trauma patients. This would support a more precise understanding of how shock evolves across various patient groups and improve strategies for early recognition and intervention.

The aim of the research was to clinically and paraclinically evaluate cases of shock in patients involved in trauma situations, who were treated in the Emergency Department (ED) of IEM, and to assess the quality of care provided.

Material and methods

A prospective observational study was conducted between January 1 and December 31, 2024, at the Institute of Emergency Medicine (IEM), including 60 adult polytraumatized patients presenting with signs of shock admitted to the red zone.

Study design and participants. Inclusion criteria were patients aged 18 years or older, with confirmed trauma and clinical signs of shock according to ICD-10-CM classification, and complete clinical documentation available. Patients presenting with non-traumatic shock or incomplete medical records were excluded from the study.

Recruitment and ethical approval. Patients were consecutively recruited upon admission to the emergency department's red zone. The study protocol was approved by the Institutional Ethics Committee of IEM (Protocol No. 1, dated January 12, 2024). The chairperson of the Ethics Committee was Dr. Diana Manea, Director of the Institute of Emergency Medicine. All participants or their legal representatives provided informed consent in accordance with the Declaration of Helsinki.

Data collection. Data collected comprised demographic characteristics (age, sex, residence), trauma mechanisms, Glasgow Coma Scale (GCS) scores at admission, vital signs (blood pressure (BP), heart rate, respiratory rate, oxygen saturation), imaging findings, laboratory parameters, and emergency interventions.

Imaging and paraclinical assessment. All patients underwent targeted radiological examinations based on suspected injury patterns. Radiography was performed on the thorax, pelvis, spine (cervical, thoracic, and lumbar), as well as the upper and lower limbs using a digital radiography system. In 90% of cases, three anatomical regions were examined; in 10% of cases, only one region was imaged. Computed tomography (CT) was conducted using a 64-slice scanner for whole-body assessment in all patients. Focused Assessment with Sonography for Trauma (FAST) was performed bedside using convex and phased-array probes to assess for free fluid in the thoracic and abdominal cavities. Electrocardiographic monitoring was applied to all patients to assess for acute cardiac abnormalities.

Laboratory evaluations included haemoglobin, lactate, arterial pH, and base deficit values. Monitoring of vital signs and hemodynamic status was conducted continuously throughout emergency department management.

Interventions and clinical management. The patients were managed according to an experimental protocol developed at the institutional level and approved by the institution's ethics committee. The protocol provides for a multidisciplinary approach to the traumatized patient and was developed based on the international Advanced Trauma Life Support (ATLS) protocol. This protocol will be implemented at the institutional level. At the national level there is no protocol for Advanced Trauma Life Support. Emergency interventions included airway assessment and management (oxygen therapy, oropharyngeal airway placement, endotracheal intubation), intravenous fluid resuscitation with crystalloids and blood products, administration of vasopressors, broad-spectrum antibiotic therapy, analge-

sia, and surgical intervention where indicated. A multidisciplinary team including emergency physicians, surgeons, anaesthesiologists, traumatologists, and neurosurgeons was involved in case management. The treatment approach followed internationally accepted trauma protocols.

Statistical analysis. Descriptive statistics were performed using mean ± standard deviation (SD) for continuous variables and percentages for categorical variables. Data analysis was conducted using SPSS version 25 (IBM Corp., Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

Results

Demographics aspects. The prospective study conducted during 2024 included the evaluation of 60 patients involved in a trauma case with a diagnosis of shock established in the ED of the IEM, who were subsequently treated in the intensive care units and in the specialized wards within the IEM. Of the patients included in the research, 13 subjects were female, representing 21%, and 47 were male, representing 79%. Thus, the ratio between men and women in case of traumatic shock is found to be 3.6 to 1. The average age was 49.7 ± 15.2 years, which highlights the involvement of this serious clinical condition in the working-age population. Of the subjects included in the research, 75% were urban, while 25% were rural. All patients included in the study had medical insurance.

It was important to assess the time of onset of shock in traumatized patients, being reported to the time of day, the so-called diurnal. The day was divided into 3 time periods. The research results showed that the highest incidence of traumatic shock in the ED, 54%, was during the day from 16:00 to 23:00, a finding likely corresponding to the period when adults are free from work and are more likely to be exposed to trauma, accidents or physical aggression. The lowest incidence was found between 00:00-07:59, representing 17.5%, this low incidence is likely due to the night time when most of the population is inactive.

Where it was possible to collect the anamnesis, it was found that all patients included in the research had certain pre-existing health problems. The presence of arterial hypertension in the anamnesis, which was being treated by the family doctor, was confirmed in 30 cases (50%); obesity grade II-III was present in 24 patients (40%); ischemic heart disease for which they received treatment in the past in 9 patients (15%), stroke in the anamnesis in 3 patients (5%), bronchial asthma in 3 patients (5%), allergic reaction in the anamnesis in 4 patients (6.67%), renal pathology in 1 patient (1.67%). Any coexisting chronic pathology in a trauma patient, including the medications they patient receive, has an impact on the evolution of the patient's condition.

Clinical presentation. All patients included in the study were transported by the 112 service to the IEM, upon arrival, they were triaged according to the criteria for shock, which is an imminent vital risk for the traumatized patient, and were directed to the red zone (Table 1). At triage, the severity criteria were confirmed in all patients according to the vital parameters. Systolic arterial hypotension less than 80 mm Hg was confirmed in all cases. Parameters indicating respiratory compromise, such as respiratory rate less than 8 per minute was confirmed in 4 cases (6.66%), and respiratory rate greater than 30 per minute was confirmed in 93.34% of cases (in 56 patients). Oxygen saturation less than 90% was confirmed in all analyzed cases, which had an essential impact on airway management. The Glasgow Coma Scale (GSC) score of 12-9 points was confirmed in 49 patients (81.66%), and the GCS score of less than 8 points was established in 11 patients (18.34%).

Table 1. The degree of distribution of hypovolemic shock in traumatized patients based on clinical/paraclinical triage data: *Vital Signs at Admission*

Shock Grade (hypovolemic shock)	Number of Patients	Percentage (%)
Grade I	2	3.3
Grade II	17	28.3
Grade III	24	40.0
Grade IV	17	28.3

Note: *(Mean ± SD): Systolic BP: 82.4 ± 14.1 mmHg, Heart rate: 114 ± 18 bpm, Respiratory rate: 26.2 ± 4.9 rpm, Temperature: 35.1 ± 0.5°C, GCS: 10.8 ± 3.4*

Craniocerebral trauma was identified in 45% of cases, followed by polytrauma in 36% of cases, abdominal trauma in 12% of cases, and thoracic trauma complicated with traumatic shock was present in 7% of cases.

The circumstances of the trauma are important in the development of the trauma mechanism. The research found that most frequently patients suffered physical aggression, (29.5%), followed by road accidents (28.5%), accidental trauma (21%), which included patients involved in workplace trauma, and falls from height (21.0%) (Table 2).

Table 2. Distribution of trauma mechanisms and diagnostic interventions

Mechanism of Injury	Frequency (n)	Percentage (%)
Road traffic accidents	17.1	28.5
Accidental trauma, workplace trauma	12.6	21.0
Physical aggression	17.7	29.5
Falls from height	12.6	21.0

Note: *Data are presented as absolute values (n) and percentages (%) out of the total number of patients (n=60). Descriptive statistics were applied using SPSS version 25 (IBM Corp., Armonk, NY, USA). No abbreviations were used in this table.*

The evaluation of mortality in the ED of the IEM trauma patients in shock included in the research was inevitable. Of the 60 patients who received qualified emergency medical care in the red zone ED of the IEM, one died, which constituted 1.7%. Prompt management of the trauma patient with shock in the red zone ensures this acceptable mortality rate.

Patients with traumatic shock benefited from a multidisciplinary approach, and were consulted by specialist doctors (surgeon, neurologist, neurosurgeon, traumatologist, internist, intensive care specialist).

Diagnostic workup. All patients underwent laboratory tests (hemoglobin, lactate, pH etc.). Paraclinical findings

were as follows: Hemoglobin 9.2 ± 2.1 g/dL; Lactate 4.1 ± 1.6 mmol/L; Base deficit 7.8 ± 3.2; Arterial pH 7.25 ± 0.09.

The paraclinical examination by applying imaging investigations was important for diagnostic purposes (Table 3). *The radiological examination* was indicated in 60 cases, it was performed on different anatomical regions depending on what the specialist suspected. In some patients, a single anatomical region was examined, in others several. Thus, in 54 patients (90%) 3 anatomical regions were examined radiologically, and in 6 cases (10%) – only one anatomical region. Radiological examinations confirmed the presence of fractures in 30 cases (50%), tension pneumothorax in 5 cases (8.33%), and massive hemothorax in 2 cases (3.33%), damage to the abdominal cavity organs was confirmed in one case, constituting 1.67%. In total, in 38 patients (63.33%) various lesions were confirmed during the radiological examination (Table 3).

Bedside FAST ultrasound – *E-FAST* (Focused Assessment with Sonography for Trauma) ultrasound was performed on all patients included in the study, and was positive in 24 patients (40%), confirming the presence of free fluid in the abdominal cavity in 22 patients (36.67%) and in the thoracic cavity in 2 patients (3.33%).

Full body computed tomography (CT) allowed for detailed assessment of trauma in any anatomical region and is considered the investigation that provides the best result on both the condition of hard tissues and fluid accumulations, organ lesions. CT was performed on 60 patients, being applied in 100% of cases. Computed tomography confirmed craniocerebral injuries in 36 patients (60%), and in 6 cases (10%) intervention for urgent neurosurgical treatment was necessary. Damage to parenchymal organs on CT was recorded in 4 patients (6.67%), damage to cavitary organs in 2 cases (3.33%), and the presence of retroperitoneal hematoma in one case (1.66%).

Table 3. Diagnostic Interventions in Emergency Department

Intervention	Patients (%)
Radiological lesions	63.33
Positive FAST ultrasound	40
Cranial CT lesions	60

***Note**: Data are presented as percentages (%) of the total number of patients (n=60). Radiological examinations included thoracic, pelvic, spinal, and limb X-rays, based on clinical suspicion. FAST = Focused Assessment with Sonography for Trauma; CT = Computed Tomography. Descriptive statistical analysis was conducted using SPSS version 25 (IBM Corp., Armonk, NY, USA). No inferential statistical tests were applied for this table.*

Electrocardiographic monitoring was recorded in all patients in the study and did not confirm the presence of acute injuries.

Management in emergency department. All patients in the study were initially treated in the red zone. The emergency physician was the specialist who managed the case from a clinical and paraclinical point of view, requesting specialist consultations and having a decision-making role in determining treatment tactics (Table 4).

In accordance with the ABCDE (A-airway; B-breathing; C-circulation; D-disability; E-exposure) approach, the emergency physician ensured the appropriate treatment of immediately life-threatening injuries at each stage. In addition to continuous clinical examination and reassessment after each manipulation performed, monitoring of vital parameters was ensured: respiration, oxygen saturation, systolic and diastolic blood pressure, heart rate monitoring, capillary refill time, evaluation of pupil appearance and photo reaction, reassessment of the state of consciousness (AVPU (A-alert, V-verbal, P-reactive to pain, U-unconscious) / GCS), blood glucose assessment and temperature.

With reference to the treatment of the pain syndrome, if it was not initiated at the pre-hospital stage, or if it was ineffective, it is continued in the ED. Analgesic-tranquilizer therapy is a mandatory component in the treatment of shock, which must be initiated at the prehospital stage by the 112 service. The indications for this treatment were the state of compensated shock, when the patient is conscious, with persistent pain syndrome and obvious discomfort; stable hemodynamics, balanced respiration. It is welcome to perform a lesion assessment and assess the presumptive diagnosis, and the actual treatment is done in accordance with the blood pressure (BP) values, the stability of vital functions, and the patient's age. In the treatment of the traumatized patient with signs of shock, pain control and patient sedation are essential for reducing physiological stress, preventing hypoxia and managing anxiety. From the clinical cases evaluated, it was found that this treatment was administered to 55 patients (91.7%), who presented with states of agitation, anxiety and complained of severe pain. The analgesics used were Ketamine (in analgesic doses), Fentanyl, and Tramadol. According to the data from the medical records, the sedatives used were Diazepam and Propofol.

In all patients included in the study, airway patency was assessed, and the liquid content of the oral cavity was aspirated in 11 patients (18.34%), who underwent upper airway prosthesis by applying a Guedel tube. Oxygen was administered by applying an oxygen mask, as all initially presented with oxygen saturation below 90%.

In 48 patients (80%), who weer refractory to oxygen therapy, ventilation and preparation for definitive lower airway prosthesis by intraglotic intubation by inserting an endotracheal tube were indicated. Premedication was performed beforehand, after which the emergency physician performed the infraglottic airway prosthesis. In case of identifying the characteristic signs of tension pneumothorax or massive hemothorax, thoracocentesis was performed.

In point C-circulation of the ABCDE assessment, it is mandatory to ensure a central venous access (jugular vein, subclavian vein), continue volemic resuscitation and drug treatment according to the patient's needs. The research found that volemic resuscitation initiated at the prehospital stage continued in all patients in shock, both micromolecular (crystalloid) and macromolecular solutions were administered. In 45 patients (75%), erythrocyte mass/plasma group I rhesus factor negative (O(I) Rh negative) were administered.

Vasopressors, such as Noradrenaline and Dopamine were required by 30 traumatized patients in shock (50%).

According to the recommendations of Advanced Trauma Life Support, in case of traumas where there is a risk of infections, such as open wounds, gunshot wounds, abdominal traumas, broad-spectrum antibiotics are administered according to existing national protocols, for example ceftriaxone 1g and metronidazole 500 mg to prevent secondary infections. According to the information from the ED files examined in 95% of the cases (57 patients), this treatment was administered.

In any state of shock, there is a risk of cardiorespiratory arrest, which most frequently occurs through a pulseless electrical activity rhythm. Cardiorespiratory and cerebral resuscitation is performed in the ED in accordance with international protocols for Advanced Cardiac Life Support, ensuring medication depending on the identified heart rhythm. Cardiorespiratory arrest occurred in 11 cases (18.33%) and advanced resuscitation was initiated in accordance with –protocol for pulseless electrical activity. In one case, cardiorespiratory and cerebral resuscitation was ineffective and biological death was determined.

Table 4. Therapeutic interventions in Emergency Department

Intervention	Number of Patients	Percentage (%)
IV fluid resuscitation	60	100
Oxygen therapy	60	100
Analgesia (opioids or NSAIDs)	55	91.7
Vasopressor support (e.g., norepinephrine)	30	50
Antibiotic administration	57	95
Emergency surgery (laparotomy, thoracotomy)	18	30
Endotracheal intubation	48	80

***Note**: Data are presented as absolute numbers and percentages (%) of the total study population (n=60). Descriptive statistical analysis was performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). NSAIDs = Non-steroidal Anti-inflammatory Drugs; IV = Intravenous.*

Trauma patients who developed shock were stabilized in the ED, IEM red zone, and subsequently treated in the intensive care unit, preliminarily, if necessary, being treated surgically according to the indications of specialists (surgeon, traumatologist, neurosurgeon). The transfer from the ED to the department where the patient was to be treated was performed by the emergency physician in the red zone (Table 5).

Table 5. Patient outcomes after stabilization in Red Zone

Outcome	Number of Patients	Percentage (%)
Stabilized and transferred	36	60
Transferred directly to OR	23	38.3
Death in ED	1	1.7

***Note**: Data are presented as absolute numbers and percentages (%) relative to the total number of patients (n=60). Descriptive statistics were applied using SPSS version 25 (IBM Corp., Armonk, NY, USA). OR = Operating Room; ED = Emergency Department.*

Discussion

The present study highlights a predominance of male patients (79%) with a mean age of approximately 50 years, which aligns with findings from regional trauma registries indicating that the working-age population is primarily affected by severe trauma leading to shock [3, 7, 8]. The mechanisms of injury identified – road traffic accidents, physical aggression, and accidental trauma – are consistent with international data underscoring these causes as the leading contributors to traumatic shock worldwide [9-11]. These epidemiological patterns emphasize the ongoing need for targeted public health interventions focused on trauma prevention and injury reduction.

Our analysis revealed a peak incidence of traumatic shock during late afternoon and evening hours (16:00–23:00), a time frame associated with increased risk activities and social interactions. This temporal distribution corroborates other epidemiological studies reporting similar patterns and suggests that emergency services should anticipate higher patient influx during these periods [11]. Such data should guide staffing and resource allocation to improve response times and care delivery.

Imaging modalities, particularly Focused Assessment with Sonography for Trauma (FAST) and computed tomography (CT), played a pivotal role in early and accurate diagnosis, facilitating timely surgical decision-making. These findings are in line with Advanced Trauma Life Support guidelines recommending the integration of bedside ultrasound and CT scanning in the diagnostic algorithm for polytrauma patients [1, 3, 12]. The timely use of these imaging tools contributed significantly to the stabilization of patients and reduced the interval to definitive care.

The recorded mortality rate of 1.7% within the red zone is indicative of the effectiveness of the implemented emergency protocols and is comparable to international trauma centers with similar infrastructure, where mortality rates range between 1.5% and 5% [7, 11, 13]. However, the high proportion of patients requiring airway management (80% intubation) and vasopressor support (50%) reflects the critical condition of this cohort, underscoring the importance of continuous monitoring and specialized intensive care capabilities.

Despite these successes, challenges remain, particularly in the timely transfer of patients from prehospital settings to the emergency department and in the optimal allocation of resources. Such issues have been recognized in global trauma care literature as significant factors influencing patient outcomes [11, 12]. Addressing these challenges requires concerted efforts to strengthen prehospital care systems, improve triage protocols, and enhance ongoing training programs in ATLS for all emergency personnel.

Furthermore, adherence to evidence-based protocols, including antibiotic prophylaxis and effective pain management, is essential for reducing complications and improving survival in trauma patients presenting with shock [1, 7, 12, 13]. Continuous monitoring of vital signs, hemodynamic parameters, and urine output was critical in early detection of

secondary complications and informed timely therapeutic adjustments. The multidisciplinary and protocol-driven approach adopted in the red zone of the Institute of Emergency Medicine has demonstrated its value in managing complex trauma cases with signs of shock. Continued efforts to refine prehospital transfer systems, reinforce ATLS training, and optimize resource use are imperative for further improving patient outcomes and reducing trauma-related mortality and morbidity.

Conclusions

A multidisciplinary, protocol-driven approach in the IEM red zone effectively stabilized traumatized patients presenting with shock. The integration of advanced monitoring, timely fluid resuscitation, and comprehensive imaging significantly improved short-term outcomes. Early recognition, complete resuscitation, and prompt surgical intervention remain essential in managing traumatic shock. Our findings support continued implementation and reinforcement of ATLS protocols, alongside enhanced prehospital transfer systems and ongoing training, to further reduce trauma-related morbidity and mortality.

Competing interests

None declared.

Authors' contributions

TMC, LR, and RH conceived the study and participated in its design. TMC and RH contributed substantially to the acquisition, analysis, and interpretation of data. TMC and LR drafted the manuscript. EC and NM critically revised it for important intellectual content. LR approved the final version to be published. In addition, TMC, LR, RH, EC and NM assume full responsibility, and accountability for all aspects of the work.

Ethics approval

The research project was approved by the Research Ethics Committee of the Institute of Emergency Medicine (Minutes no. 1, from January 12, 2024).

Patient consent

Obtained.

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Provenance and peer review

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RESEARCH ARTICLE

Development and testing of a questionnaire: assessment of occupational risk factors in surgeons

Ana Vîlcova[1,2*], Alexandru Ferdohleb[2], Larisa Spinei[3], Angela Paraschiv[4], Raisa Deleu[1], Elena Ciobanu[1]

[1]Hygiene Discipline, Department of Preventive Medicine, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[2]*Timofei Moșneaga* Republican Clinical Hospital, Chișinău, Republic of Moldova

[3]*Nicolae Testemițanu* Social Medicine and Management Department, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[4]Epidemiology Discipline, Department of Preventive Medicine, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

ABSTRACT

Introduction. Surgery exposes professionals to significant physical and psychological risks, including intense exertion, prolonged static postures, and repetitive gestures, often leading to musculoskeletal pain. International studies report prevalence rates above 90%, linked to long procedures and poor ergonomics, alongside exposure to biological and chemical hazards, radiation, toxic smoke, and chronic stress. In the Republic of Moldova, occupational health in the medical sector is underexplored, with no tools tailored to surgeons. This study aims to develop and validate the first nationally standardized questionnaire to assess these risks and support public health policies.

Material and methods. The study used a sequential mixed-methods design: a qualitative phase to identify domains and indicators, followed by a quantitative phase for psychometric testing. Four domains were defined: working conditions, occupational factors, general health, and psycho-emotional state. The questionnaire was refined from 70 to 47 items after expert review and validation, with internal consistency (Cronbach's α) and content validity (I-CVI, modified Kappa) assessed. It was pre-tested on 52 surgeons, and data were analyzed using SPSS 27 ($p<0.05$).

Results. Content validation revealed S-CVI/Ave values ranging from 0.934 to 1.00 and S-CVI/UA values from 0.738 to 1.00, with all domains exceeding the accepted threshold for relevance and clarity, except for one domain, which fell slightly below the recommended level for unanimous agreement. I-CVI values ranged from 0.857 to 1.00 (relevance) and 0.847 to 1.00 (clarity), while κ* indices were all rated as "excellent". Based on expert feedback, 24 questions were revised and 15 were removed. Overall internal consistency was very good (α = 0.808), with section values ranging from 0.769 to 0.864, the highest being for "Psycho-emotional state" (α = 0.864). The pre-test sample comprised 52 surgeons, mostly male (57.7%), with a mean age of 44.92 years, predominantly from urban areas (92.3%). Respondents generally found the questions clear but noted some lengthy formulations, repetitiveness, and sensitive items. These observations contributed to optimizing the final version of the instrument.

Conclusions. The final questionnaire meets the initial theoretical dimensions and shows strong psychometric properties, with high validity and internal consistency. The tool is comparable to established instruments and suitable for assessing surgeons' health and occupational risks, with potential for wider use.

Keywords: questionnaire, occupational risk factors, surgeons, occupational health, internal consistency, content validity.

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***Corresponding author: Ana Vîlcova**, PhD fellow
Hygiene Discipline
Nicolae Testemițanu State University of Medicine and Pharmacy

Key messages

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

The long-term effects of occupational risk factors specific to surgeons on their physical and mental health remain insufficiently

165, Ștefan cel Mare și Sfânt, Chișinău, Republic of Moldova,
e-mail: ana.vilcova1@gmail.com

Authors' ORCID IDs
Ana Vîlcova – https://orcid.org/0009-0004-9739-6378
Alexandru Ferdohleb – https://orcid.org/0000-0003-3290-8139
Larisa Spinei – https://orcid.org/0000-0002-5370-9801
Angela Paraschiv – https://orcid.org/0000-0003-0817-8316
Raisa Deleu – https://orcid.org/0000-0001-8922-2491
Elena Ciobanu – https://orcid.org/0000-0002-8969-922X

documented. Furthermore, no validated national instrument currently exists to evaluate these risks in a comprehensive and standardized manner.

The research hypothesis

The questionnaire developed for surgeons is a valid and reliable tool for assessing occupational risk factors and their potential impact on physical and mental health.

The novelty added by manuscript to the already published scientific literature

The study presents the development and validation of a novel questionnaire for assessing occupational risk factors among surgeons, representing a significant contribution to the scientific literature. This is the first standardized instrument tailored to the specific professional context of the Republic of Moldova, enabling comprehensive evaluation and facilitating international comparability.

Introduction

Despite its reputation for technical precision and operative control, surgery consistently exposes professionals to both physical and psychological occupational risks, many of which remain insufficiently recognized. International research indicates that these risks are not isolated events but recurrent features of daily practice: intense physical strain, prolonged maintenance of static postures, and the repetitive execution of identical surgical gestures are frequently associated with musculoskeletal pain, particularly in the cervical and lumbar regions [1, 2]. Bibliometric studies published over the past decade reveal growing scientific interest in this issue, with a marked increase since 2016 [3]. Research conducted in Spain reported a prevalence exceeding 90% for musculoskeletal pain among surgeons, with some cases requiring medical treatment that led to temporary absenteeism or presenteeism. Identified contributing factors include excessive duration of surgical procedures, elevated body mass index, and inappropriate positioning of laparoscopic screens [4].

In addition to physical discomfort, surgical practice involves other categories of occupational risks with a significant impact on health. Biological and chemical hazards, exposure to radiation, and surgical smoke, which may contain toxic compounds such as phosgene, combine with chronic stress and psychological pressure, requiring a more sophisticated assessment than simple direct observation [5].

In daily practice, surgeons face a set of complex risks, often masked by professional pressure. The application of a well-designed questionnaire, tailored to the specifics of their practice, enables the transformation of subjective perceptions into quantifiable data, with both clinical and preventive value.

Over the past decade, the questionnaire has emerged as a refined tool capable of capturing subtle aspects of surgeons' professional activity [6]. One line of research employs predictive algorithmic models to estimate the individual risk of developing injuries, integrating objectively measured ergonomic parameters and providing personalized physiotherapeutic recommendations [4]. Recent literature also describes initiatives to design questionnaires tailored to operating theatre staff, psychometrically validated and rigorously tested for reliability and internal consistency [7, 8].

In the Republic of Moldova, occupational health remains largely focused on workplaces in the real sector, while the healthcare sector is insufficiently explored. The absence of specialized institutions, the lack of dedicated indicators, and the inexistence of a coherent monitoring system significantly reduce prevention capacity [9]. In this context, the initiative to develop a rigorous questionnaire for surgeons is not merely a research exercise but a strategic step towards strengthening occupational health at the national level.

Data published in recent years indicate worrying trends: both occupational mortality and morbidity among healthcare workers are on the rise, resulting from exposure to physical, chemical, biological, and psychosocial factors [10-13]. For surgeons, daily exposure to surgical smoke and intense stress constitutes a distinct risk profile; under such conditions, the use of well-calibrated assessment tools enables the objective quantification of exposures and early intervention [14].

At the national level, the creation and validation of such a questionnaire would open concrete prospects: it could become the first tool tailored to the specifics of surgical practice in the Republic of Moldova, with immediate applicability in hospital settings; it could serve as the basis for a standardized register of occupational risks, useful both for epidemiological surveillance and for informing public policies; and it could be integrated into the training of occupational medicine specialists, providing a practical approach to real rather than purely theoretical risks.

The aim of the research is to develop and validate a tool tailored to assess occupational risk factors among surgeons.

Material and methods

A sequential mixed-methods design was used, comprising a qualitative phase to explore the dimensions of the work environment and occupational risk factors present in

surgeons' workplaces. The data obtained were thematically analyzed to identify relevant domains and indicators, which formed the basis for drafting the questionnaire items.

Subsequently, a quantitative phase was conducted with the aim of psychometrically testing the pilot version of the questionnaire. This involved administering the instrument to a sample of active surgeons selected through convenience sampling. Sociodemographic data were collected, along with responses to items addressing working conditions, exposure to occupational factors, general health status, and psycho-emotional state.

The first version of the questionnaire, developed by the working group, comprised 70 items. This version underwent scientific review by the research team, resulting in a second version containing 62 items. The latter was then submitted to experts for content validity and clarity assessment. Consequently, the third version of the questionnaire consisted of 47 items and was pre-tested on a sample of 52 surgeons (Fig. 1).

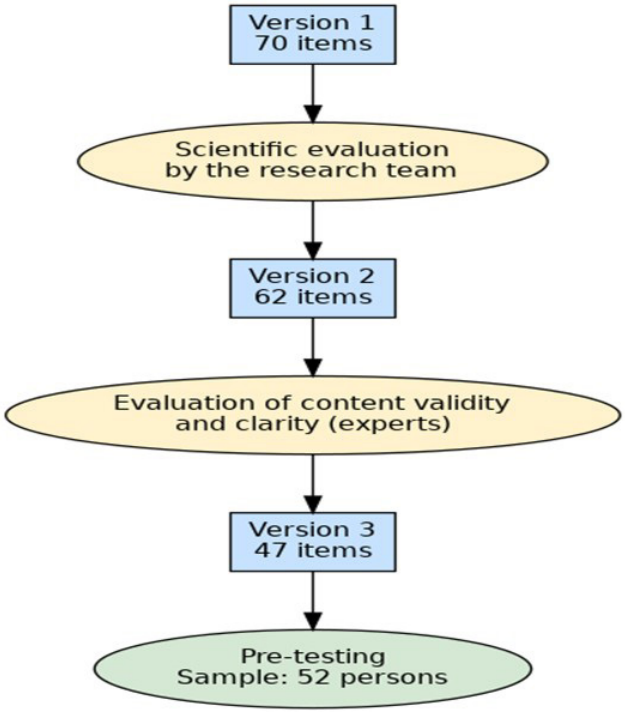

Fig. 1 Stages of the questionnaire evaluation.
Note: item = question.

The results formed the basis for developing items that measure the perceived impact of occupational factors on health status. The quantitative phase aimed to examine the reliability and validity of the measurements in a subset of practicing surgeons employed at the time of the study, conducted between December 2024 and May 2025 in the Republic of Moldova. The instrument was developed according to the following multi-stage process:

1. Literature review and identification of key components

In the initial stage of developing the questionnaire, our aim was to gain a thorough understanding of the occupational risk factors that may affect surgeons' health. To this end, we reviewed the current scientific literature, selecting relevant studies from the fields of occupational health, ergonomics, work psychology, and preventive medicine.

Analysis of these sources revealed that surgeons are frequently exposed to a range of occupational factors with a negative impact on both physical and mental health. The most commonly reported include uncomfortable posture maintained for prolonged periods, constant stress linked to decision-making responsibilities, long working hours, lack of sleep, exposure to biological or chemical agents, and difficulty maintaining a work–life balance [5, 14, 15]. Consequently, we identified four key domains considered essential for assessing occupational risks and their effects on health: working conditions, occupational risk factors, general health status, and psycho-emotional state.

Based on these domains, an initial version of the questionnaire was drafted, formulating items intended to reflect as accurately as possible the daily reality of surgeons. At this stage, we relied both on data from the literature and on the observations and recommendations of specialists with experience in practical surgery and occupational hygiene.

2. Conceptualization and development of the instrument

After identifying the key domains relevant to the study's objective, we proceeded to develop the questions and scales for each domain, taking into account the realities and specific features of the professional context in the Republic of Moldova. At this stage, our aim was to cover as accurately as possible the essential aspects of surgeons' work, ensuring that the instrument reflected their real experiences and challenges.

Initially, a total of 70 items were formulated, structured as follows: (i) 8 questions on respondents' sociodemographic data; (ii) 38 items relating to working conditions and occupational factors (e.g., work environment, schedule, physical and mental demands); (iii) 11 items on general health status; and (iv) 13 items on psycho-emotional state.

The construction of this questionnaire was based on a multidimensional perspective on professional culture and on how psychosocial and occupational factors influence surgeons' behaviour and well-being. We drew inspiration from the international scientific literature [16] and adapted the concepts to the local context to ensure the instrument's cultural validity and practical relevance [17, 18].

The first version of the questionnaire was finalised in December 2024 as the result of a collaborative process between public health researchers and medical professionals from the Republic of Moldova. The items were designed to capture the behaviours, attitudes, and subjective perceptions experienced by surgeons in their daily practice, both at the individual level and in relation to the work environment.

3. Review by the research team and refinement of the instrument

The first version of the questionnaire underwent scientific review by the research team, resulting in a second version comprising 62 items. Accordingly, 8 items were removed due to ambiguous wording and redundancy.

4. Review by an expert panel and determination of the instrument's content validity

Content validation is one of the initial and essential stages in the process of validating a measurement instrument. Its purpose is to determine the extent to which the items included in the questionnaire adequately and representatively reflect the concepts or domains the instrument is intended to assess. Content validity relies primarily on expert evaluation, and the quality of this stage depends significantly on the selection and engagement of the experts.

After developing the initial version of the questionnaire, the research team conducted a preliminary item analysis to identify elements with weak psychometric properties. Items with over 10% non-responses, high rates of abstention, or ambiguous wording were eliminated. As a result, the questionnaire was reduced from 62 to 47 items, this version being used for content evaluation.

For this stage, a panel of seven experts was assembled in relevant fields such as social medicine, occupational hygiene, medical psychology, and surgery. The team comprised two specialists in social medicine, biostatistics, and health management; two experts in occupational hygiene; one specialist in general hygiene; one surgeon; and one medical psychologist.

All selected experts had extensive experience in professional practice, higher education, and research. Their selection was based on demonstrated expertise in health services, occupational health, and work psychology.

Each expert was provided with the updated version of the questionnaire, and item evaluation was carried out independently. Experts were invited to assess the relevance of each item using a four-point ordinal scale, as recommended by Polit et al. [19, 20]: 1 = not relevant; 2 = somewhat relevant; 3 = quite relevant; 4 = highly relevant, and to assess the clarity of each item using a four-point ordinal scale: 1 = item is not clear; 2 = item requires some revisions; 3 = item is clear but needs minor revisions; 4 = item is very clear.

For the quantitative analysis, the content validity index for each item (I-CVI) was used. The rating scale was dichotomized: scores of 3 and 4 were considered "relevant" and "clear", while scores of 1 and 2 were classified as "not relevant" and "not clear". The I-CVI was calculated as the proportion of experts who rated the item as relevant and clear relative to the total number of experts.

To account for the possibility of chance agreement between evaluators, the modified Kappa coefficient (K*) was also calculated using the formula: $K^* = (\text{I-CVI} - p_a) / (1 - p_a)$, where p_a represents the probability of chance agreement, calculated according to the binomial distribution.

According to the scientific literature, for a panel of more than six experts, a minimal degree of disagreement is acceptable. The accepted CVI threshold is at least 0.83 [19, 21].

In addition to the scores provided, the experts offered valuable suggestions regarding question formulation, language clarity, and questionnaire structure, most of which were incorporated.

The outcome of this stage was a revised version of the instrument, comprising 47 items, formulated to reflect as accurately and comprehensively as possible the professional reality of surgeons in the Republic of Moldova.

5. Data collection and evaluation of the instrument's psychometric properties

To evaluate the psychometric properties of the instrument, we conducted a comprehensive statistical analysis focused on two main directions: item analysis and internal consistency (α), as well as content validity (CVI/Kappa) of the questionnaire.

5.1. Internal consistency (reliability)

Internal consistency reflects how well the items measuring the same concept are correlated. For this stage, Cronbach's α coefficient was used, which is considered the standard in assessing the reliability of questionnaire-type instruments. In general, an α value ≥0.70 is considered acceptable, indicating good internal coherence among items [22-24].

5.2. Pilot testing

The questionnaire was tested on a subset of 52 surgeons, selected through convenience sampling from among those working in public healthcare institutions in the Republic of Moldova, taking into account their accessibility and willingness to participate in the study. The study population is specific, given that, according to statistical data [25], the total number of surgeons was 460. Participants were employed in public healthcare institutions (republican and municipal) in the Republic of Moldova. The inclusion criteria were as follows: (i) active status as a physician in a surgical department; (ii) informed consent to participate.

All participants signed an informed consent form. The questionnaires were anonymous, printed, and distributed individually, and completion was carried out in paper format. To ensure confidentiality, the questionnaires and consent forms were collected separately.

Ethical approval. The study was approved by the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, approval no. 2 of 07.10.2024.

Data analysis. The results were presented as mean ± standard deviation (SD) for continuous variables and as absolute frequencies and percentages for categorical variables. Sociodemographic characteristics were described using frequency and percentage. Correlation was considered statistically significant when the p-value was less than 0.05. Cronbach's alpha coefficient was also used to determine internal consistency. Statistical analyses were performed using SPSS Statistics 27 (SPSS, Inc., Chicago, IL, USA).

Results

Content validation of the instrument. The scale-level content validity index (S-CVI) was assessed using two complementary measures. The average score across all items (S-CVI/Ave) reflects the mean level of content validity for the instrument, whereas the universal agreement index

(S-CVI/UA) indicates the proportion of items for which all experts provided concordant ratings. These indicators collectively capture the overall validity of the questionnaire in terms of both relevance and clarity (Table 1). The S-CVI/Ave values varied between 0.934 and 1.00 across the evaluated domains, with all domains exceeding the threshold for the S-CVI/Ave. For the S-CVI/UA, three domains scored ≥0.80, while the domain of occupational factors obtained 0.738 (relevance) – acceptable, but below the recommended threshold for unanimous agreement.

Table 1. S-CVI/Ave and S-CVI/UA for the four questionnaire domains of interest

Questionnaire domains	S-CVI/Ave*		S-CVI/UA**	
	Relevance	Clarity	Relevance	Clarity
Data relating to working conditions	0.976	0.988	0.833	0.917
Data relating to occupational factors	0.934	0.971	0.738	0.885
Data relating to health status	0.971	0.967	0.80	0.80
Data relating to psycho-emotional state	0.986	1.00	0.90	1.00
Total	0.959	0.966	0.817	0.824

Note: *S-CVI/Ave = Scale Content Validity Index, average method – the mean of the item-level content validity indices (I-CVI) for all items in the domain; values ≥0.80 indicate good content validity. **S-CVI/UA = Scale Content Validity Index, universal agreement method – the percentage of items in the domain for which there was complete agreement (I-CVI = 1.00) among experts; values ≥0.80 indicate acceptable unanimous agreement.

As shown in Table 2, the item-level content validity index (I-CVI) for relevance varied between 0.857 and 1.00. The corresponding kappa coefficients (κ*) indicated an excellent level of agreement among experts (κ* = 0.75–1.00). For the clarity assessment, I-CVI values ranged from 0.847 to 1.00, with κ* values confirming a similarly high level of consistency (κ* = 0.75–1.00). Following expert feedback, 24 questions were revised, and 15 items were removed to improve the instrument's overall precision.

Table 2. I-CVI and Kappa* for the relevance and clarity of the questionnaire domains of interest

Questionnaire domains	ICVI Relevance	κ* Relevance	ICVI Clarity	κ* Clarity
Data relating to working conditions	1.00	1.00	1.00	1.00
Data relating to occupational factors	0.857	0.848	0.847	0.849
Data relating to health status	1.00	1.00	1.00	1.00
Data relating to psycho-emotional state	0.868	0.851	0.857	0.889

Note: *κ represents the modified kappa agreement index; κ 0.75–1.00: excellent, κ* 0.60–0.74: good, κ* 0.40–0.59: acceptable, and κ* <0.40: poor. **I-CVI indicates the item-level content validity index

Internal consistency analysis. The internal consistency of the questionnaire was assessed using Cronbach's α coefficient, which was applied to evaluate the homogeneity of the responses and the coherence of the research instrument. The overall Cronbach's α coefficient for the entire questionnaire was 0.808, indicating a very good level of internal reliability and confirming that the items consistently measure the intended constructs.

At the section level, the coefficient ranged between 0.769 and 0.864 (Table 3). The sections "Working conditions" (α = 0.715) and "Occupational risk factors" (α = 0.782) demonstrated good and, respectively, high internal consistency, while "Psycho-emotional state" (α = 0.864) showed very good internal reliability. In contrast, the "Health status" section recorded a lower value (α = 0.769), still indicating good consistency, which may have been influenced by the diversity of aspects assessed.

Table 3. Internal consistency of the questionnaire and its sections

Questionnaire section	Number of items	Cronbach's α	Interpretation*
Working conditions	7	0.715	Good internal consistency
Occupational risk factors	15	0.782	High internal consistency
Health status	10	0.769	Moderate internal consistency
Psycho-emotional state	7	0.864	Very good internal consistency
Entire questionnaire	39**	0.808	Very good internal reliability

Note: *Interpretation of Cronbach's α coefficient according to George and Mallery (2003): α≥0.9 – excellent; α≥0.8 – very good; α≥0.7 – good; α≥0.6 – acceptable; α<0.5 – inadequate. **The eight questions from the „Sociodemographic data" section were not analyzed.

Pilot test

Sample characteristics. The socio-demographic characteristics of the participants involved in the pre-testing stage of the questionnaire are presented in Table 4. The respondents were aged between 32 and 58 years, with a mean age of 44.92 ± 6.36 (SD) years.

Table 4. Demographic and educational profile of respondents: pilot testing stage

Characteristics	n	%
Gender		
- Male	30	57.7
- Female	22	42.3
Marital status		
- Single	2	3.8
- Married	44	84.6
- Divorced	6	11.5
Place of residence		
- Urban	48	92.3
- Rural	4	7.7
Work experience		
- Up to 5 years	2	3.8
- 6-10 years	14	26.9
- 11-15 years	14	26.9
- 16-20 years	20	38.5
- 21-25 years	2	3.8

Type of healthcare institution		
- Republican	40	76.9
- Municipal	8	15.4
- District	4	7.7

Note: n - absolute number; % - percent.

As shown in Table 4, the majority of the 52 respondents in the sample were male (57.7%). It is noteworthy that most participants were from urban areas (92.3%) and were married (84.6%).

The questionnaire also included three open-ended questions, to which the respondents provided qualitative answers (Table 5).

Table 5. Results of responses to the three open-ended questions in the questionnaire at the face validity stage (n = 52)

	n (%)		
	Yes	No	Excluded from analysis
1. Please indicate the serial numbers of the questions in this questionnaire that are unclear or difficult to understand.	38 (73.1)	6 (11.5)	8 (15.4)
2. Please indicate the serial numbers of the questions that are clear.	16 (30.8)	28 (53.9)	8 (15.4)
3. Please suggest ways to improve the unclear or difficult-to-understand questions in this questionnaire.	30 (57.7)	14 (26.9)	8 (15.4)

Note: n - absolute number; % - percent.

The respondents provided feedback on the open-ended question regarding the clarity and comprehensibility of the questionnaire items. Most respondents considered the questions clear and easy to understand. Some participants, however, noted that certain questions were personal, direct, and sensitive. It was also mentioned that some questions contained multiple sub-questions.

From the responses provided regarding ways to improve unclear or difficult-to-understand questions in the questionnaire, some participants offered valuable suggestions for refinement, such as adding the response option "not applicable", rephrasing certain questions, clarifying specific technical terms, and reducing or simplifying lengthy formulations. A frequent observation concerned the repetitiveness of some questions, particularly those related to occupational risk factors.

Of the 52 individuals who participated in the questionnaire, 8 did not comment on any item; therefore, these responses were excluded from the analysis.

Discussions

The results of the study highlighted a high content validity of the questionnaire developed to assess the working conditions, occupational risk factors, health status, and psycho-emotional state of surgeons. For all investigated domains, the S-CVI/Ave values exceeded the 0.80 threshold, while the I-CVI and κ indices were, for the most part, classified as "excellent". The level of agreement obtained among the evaluators is comparable to that reported by Rapisarda et al. (2020) during the validation of the "Mental Health Professional Culture Inventory" (MHPCI) [26]. In that study, the involvement of a multidisciplinary panel, combined with the application of standardized criteria, allowed for the exclusion of items with reduced psychometric properties and the strengthening of the relevant dimensions. Similarly, Beyera et al. (2020), in validating a questionnaire designed to analyse determinants of healthcare service utilization for low back pain, reported high values of content validity indices, emphasizing the essential role of rigorous item selection and formulation [27].

The overall internal consistency of the questionnaire (α = 0.808) indicates a very good level of reliability, comparable to the values reported for other multidimensional instruments. For example, Jafari-Golestan et al. (2024), in the validation of the "Post-Stroke Self-Care Activities" (PSCA), reported a general α of 0.901, with variations between 0.734 and 0.948 across domains [28]. Similarly, the Spanish adaptation of the "Self-Efficacy for Ostomy Adjustment Scale" (SE-OAM-SV) achieved a high α of 0.96, while the Arabic validation of the "Long-Term Conditions Questionnaire" (LTCQ) conducted by Al-Qerem et al. (2025) reported α values of 0.90 for the factor "Empowerment and Functional Wellbeing" and 0.83 for "Health-Related Psychosocial Distress" [29]. In the present questionnaire, α values ranged between 0.715 and 0.864 across domains, an interval that remains within the accepted limits. This variation reflects, as in the LTCQ, the diversity of the constructs assessed, with certain domains demonstrating greater homogeneity than others.

The pilot stage confirmed both the face validity and the level of acceptability among the target population. Most participants considered the wording of the questions clear and easy to understand; however, some suggestions for adjustments were also made, a finding consistent with other studies. In research within the fields of nursing and nutritional management, linguistic and cultural adaptations have been identified as essential factors for enhancing the comprehensibility and relevance of instruments [30, 31].

From a structural perspective, the results confirm a coherent organization of the questionnaire domains, comparable to the multidimensional architecture reported in other validation processes. This convergence suggests that, regardless of cultural context or the characteristics of the population studied, the multidimensional approach remains relevant for assessing experiences related to health and professional activity.

A distinctive feature compared with many instruments described in the literature, including the LTCQ, PSCA, and SE-OAM-SV, is the specificity of the target group. While those questionnaires were primarily designed for patients with chronic conditions, our instrument is aimed exclusively at a professional population exposed daily to well-defined occupational risk factors. This particularity justifies the in-

clusion of domains focused on the assessment of the working environment; an aspect rarely encountered in clinical instruments with a general applicability.

Nevertheless, the applied methodology, which encompasses content validity assessment, internal consistency analysis, and piloting, remains consistent with that used internationally, thereby confirming the universal nature of psychometric principles.

In terms of applicability, the literature emphasizes that rigorously validated instruments can be integrated into clinical practice, research, and public policy development. In the case of the "Long-Term Conditions Questionnaire" (LTCQ), the authors recommended its use for monitoring quality of life and for planning patient-centered interventions, including its direct integration into electronic health systems. A similar framework could be adapted for our questionnaire, enabling the periodic assessment of the health status of medical professionals and supporting the development of occupational prevention and protection measures based on objective data.

The parallel with recent research in public health, clinical nutrition, and chronic disease prevention is relevant [31-35]: those studies demonstrated that personalized interventions, designed on the basis of detailed initial assessments, yield superior outcomes. Similarly, our questionnaire could support the personalization of strategies for preventing burnout syndrome and other occupational conditions, optimizing preventive interventions according to the identified risk profile.

Limitations

This study has several limitations that should be taken into account when interpreting the results. First, the investigated population is specific, consisting of surgeons actively working in public healthcare institutions in the Republic of Moldova. The total number of surgeons at the national level is 460, which means that the results cannot be directly extrapolated to other professional categories or contexts.

Second, the relatively small sample size (n = 52) did not allow for the performance of either a confirmatory factor analysis (CFA) or an exploratory factor analysis (EFA). The literature recommends, for such analyses, a minimum ratio of 5-10 participants per item, a condition that could not be met at this stage. Consequently, this phase of the study focused exclusively on content validity assessment, item analysis (discrimination, directionality), and internal consistency (Cronbach's α), with EFA and CFA planned to be conducted on a larger sample in a future study.

Conclusions

The final structure of the questionnaire fully corresponds to the initially defined theoretical dimensions and demonstrates robust psychometric properties, as evidenced by the high values of the content validity indices (I-CVI, S-CVI/Ave, S-CVI/UA, Kappa*)* and the very good overall internal consistency (Cronbach's α = 0.808). The domain-level analysis confirmed that each section consistently measures the intended constructs, with α values ranging from 0.715 to 0.864, indicating a balance between item homogeneity and the coverage of the diversity of aspects assessed.

The adjustments made based on expert feedback and observations from the piloting stage, such as the removal of items with low relevance, the rephrasing of unclear questions, and the clarification of technical terms, contributed to improving the clarity and practical applicability of the instrument. These results are consistent with data reported in the international literature for similar instruments, confirming that the applied methodology is robust and yields a tool comparable in psychometric performance to established questionnaires.

The resulting questionnaire is suitable for assessing health status and occupational risk factors among surgeons and can be applied both in research and in periodic institutional evaluations. Extending validation to larger samples and multicentric contexts, as well as adapting it to other medical specialties, will enhance its applicability, international comparability, and utility in informing occupational health policies.

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.

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Ethics approval.

The study protocol was approved by the Research Ethics Committee of *Nicolae Testemiţanu* State University of Medicine and Pharmacy (Protocol No. 02 of 07.10.2024).

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RESEARCH ARTICLE

Evolution of maxillary expansion in patients with cleft lip and palate

Silvia Railean[1], Cristina Poştaru[1*], Svetlana Melnic[2], Gheorghe Bordeniuc[3]

[1]*Ion Lupan* Department of Pediatric Oral-Maxillofacial Surgery and Pedodontics, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

[2]*Pavel Godoroja* Department of Propaedeutics Stomatology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

[3]Department of Therapeutical Stomatology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

ABSTRACT

Introduction. The craniofacial orthodontist who is part of the multidisciplinary team evaluating children with cleft lip and palate plays an important role in examining the development of dental occlusion. Early surgical interventions in children with cleft lip and palate frequently cause occlusion disorders with a prevalence of anterior crossbite in 62%. The expansion of the maxilla is important for normalizing the morphology and correct symmetrical tooth eruption. The aim is to evaluate the evolution of maxillary expansion in patients with cleft lip and palate.

Material and methods. This study included 20 patients with cleft lip and palate, including 8 girls and 12 boys aged 6-12 years, with a mean age of 9.35 years. Unilateral cleft lip and palate was present in 16 patients, bilateral cleft lip and palate – 2 patients, and clefts of the hard and soft palate – 2 patients. The study models were scanned, and the maxillary dimensions were examined using a 3D Dolphin Imaging program before and after maxillary slow expansion over an average of 12.2 months.

Results. Clinical evaluation of patients with cleft lip and palate showed crossbite occlusion. Of these patients, anterior and posterior crossbite occlusion was present in 11 (55%) patients, anterior crossbite occlusion in 3 (15%) patients, unilateral posterior crossbite occlusion in 3 (15%) patients and bilateral in 3 (15%) patients. In the transverse plane, the size of the upper jaw increased statistically significantly ($p = 0.002$), but in the sagittal plane we found a statistically significant elongation of the upper jaw ($p < 0.05$) following slow expansion treatment with a removable orthodontic appliance over an average of 12.2 months in patients with cleft lip and palate.

Conclusions. Slow maxillary expansion treatment during the mixed dentition period in patients with cleft lip and palate was found to be more effective in the sagittal plane than in the transverse plane.

Keywords: cleft lip and palate, dental occlusion, removable dental appliances, patients.

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***Corresponding author**: Cristina Poştaru, PhD, associate professor
Ion Lupan Department of Pediatric Oral-Maxillofacial Surgery and Pedodontics,
Nicolae Testemiţanu State University of Medicine and Pharmacy
165, Stefan cel Mare si Sfant blvd., Chisinau, Republic of Moldova, MD2004
e-mail: cristina.postaru@usmf.md

Key messages

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

Growth and development of the maxilla in children with cleft lip and palate slows down due to primary early surgery. Orthodontic treatment of malocclusion in children with cleft lip and palate should begin in an early period. The issue addressed is the effectiveness of upper removable orthodontic appliances in maxillary expansion used in children with cleft lip and palate during mixed dentition.

Authors' ORCID IDs
Silvia Railean – https://orcid.org/0000-0002-8919-3317
Cristina Poștaru –https://orcid.org/0000-0002-7101-6443
Svetlana Melnic – https://orcid.org/0009-0000-9507-582X
Gheorghe Bordeniuc – https://orcid.org/0000-0002-4013-9918

The research hypothesis
Maxillary expansion with orthodontic removable appliances during mixed dentition is effective in children with unilateral cleft lip and palate before alveolar bone grafting.

The novelty added by the manuscript to the already published scientific literature
Orthodontic treatment of malocclusions with removable orthodontic appliances in children with cleft lip and palate during mixed dentition.

Introduction

Cleft lip and palate are congenital deformities with a multifactorial determinism resulting from the interaction of genetic or environmental factors [1]. The worldwide incidence of cleft lip and palate is 1:700, with the highest in Europe being in Finland at 2.36 cases per 1000 newborns [2].

In the Republic of Moldova, among the most common clefts of the lip and palate are cleft palates [3]. Patients with cleft lip and palate present dento-maxillary anomalies of skeletal and dental form due to deformations of the maxillary bones and imbalance between facial muscle forces [4]. Consequently, the functions of the dento-maxillary system, such as mastication, phonation and facial appearance, may be negatively affected [5]. The craniofacial orthodontist, who is part of the multidisciplinary team, plays an important role in the assessment and treatment of the development of dental occlusion and facial appearance during the main stages of growth [6, 7].

During the development of dental occlusion, the orthodontic treatment of malocclusion goes through 2 phases: the interceptive orthodontic treatment phase, which represents the preparation of the dento-maxillary apparatus for bone grafting surgery, and the complete orthodontic treatment phase with or without orthognathic surgery and possible orthopedic and dental restorative treatments [8].

Reconstruction of the maxillary bone defect in the preadolescent period has become a critical period in the complete treatment of patients with cleft lip and palate after the period of primary reconstruction of the lip, nose, and hard and soft palate. In patients with unilateral and bilateral cleft lip and palate involving alveolar defects, bone grafting surgery has several benefits. It provides bone support for tooth eruption, prevents impaction of the upper canines, and facilitates safe orthodontic treatment [9].

Compression of the maxilla is frequently encountered in patients with cleft lip and palate, leading to malocclusion with a prevalence of anterior reverse occlusion of 62% [10].

Bone grafting surgery is performed after orthodontic treatment by expanding the maxilla. Expansion of the maxillary dental arch during the mixed dentition period is very important because it normalizes the morphology and induces correct and symmetrical eruption of the canine on the dental arch [11, 12].

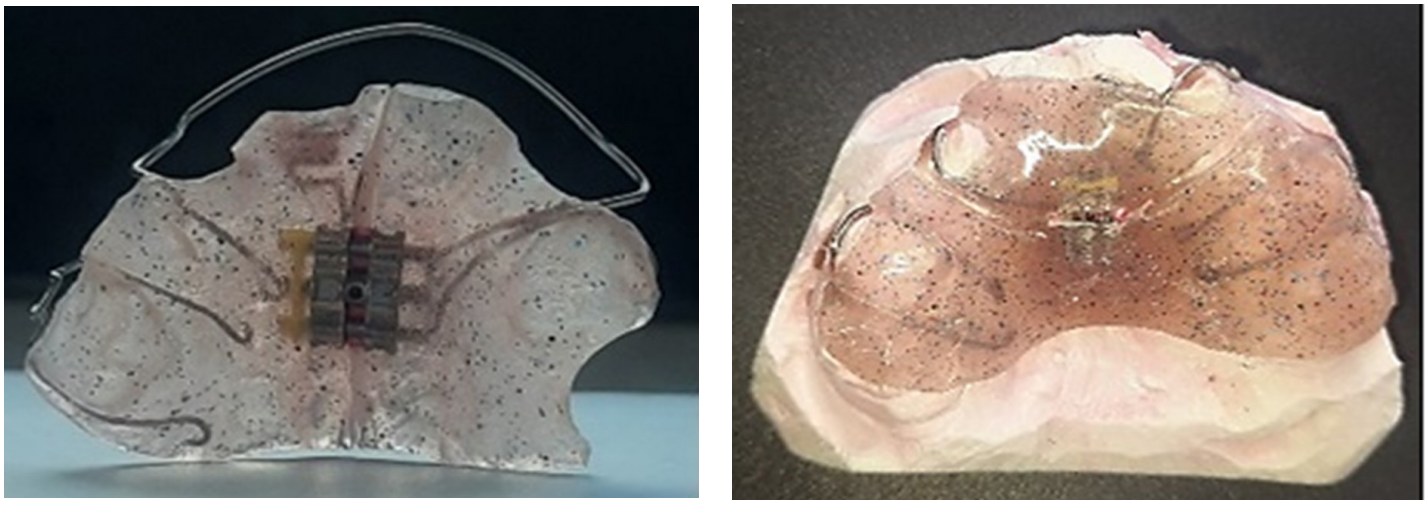
The aim of the study is to evaluate the size of the upper jaw in children with an average age of 9.35 years with unilateral cleft lip and palate in the transverse and sagittal plane over a period of 9 months and the preparation for bone grafting surgery.

Material and methods

The study included 20 patients with cleft lip and palate, including 8 girls and 12 boys aged 6-12 years The mean age of children was 9.35 years which include unilateral cleft lip and palate, bilateral cleft lip and palate and cleft palate only. All patients underwent primary lip surgery at the age of 3 months and primary hard and soft palate surgery in one stage at the age of 12 months. No formal *a priori* sample size calculation was performed. The sample consisted of all consecutive children aged 6–12 years with cleft lip and/or palate who met the inclusion criteria and received early interceptive orthodontic treatment at our center during the mixed dentition stage, prior to secondary alveolar bone grafting. All patients were treated and assessed under a uniform clinical workflow at the same institution, to reduce procedural variability. Measurement reproducibility (intra- and inter-examiner reliability) was not formally tested and is acknowledged as a limitation.

For the evaluation of this group of 20 patients with malocclusion in children with unilateral cleft lip and palate during the mixed dentition period, the following research methods were collected: clinical examination, photometric examination, biometric analysis of study models before treatment (T1) and after sagittal/transverse maxillary expansion (T2). The average treatment interval was 12.2 months.

The slow maxillary expansion with plate appliances was performed in the transverse and sagittal planes. The removable orthodontic appliance was fabricated in the dental laboratory from self-curing acrylate material containing a classic monomaxillary orthodontic transverse screw to produce symmetrical widening of the maxillary dental arch or sagittal screw to produce elongation of the maxillary dental arch. The expansion was achieved by activating the screw ¼ turn per week, which caused the two half-frames to move apart by 0.2-0.25 mm per week (Fig. 1). Of all the patients with unilateral cleft lip and palate, 11 patients underwent interceptive orthodontic treatment with a slow expansion orthodontic removable plate appliance in the transverse plane and 9 patients underwent interceptive orthodontic treatment with a slow expansion orthodontic removable plate appliance in the sagittal plane depending on the severity of crossbite in the anterior or posterior region.



a b

Fig. 1 Removable orthodontic appliances on the upper jaw

Note: A - *in the transverse plane; B - in the sagittal plane*

The plaster models were obtained immediately before and after 12.2 months of slow maxillary expansion orthodontic treatment.

All study plaster models of the upper arch were scanned using the Virtuo Vivo intraoral scanner (Fig. 2) and converted to STL format by an experienced laboratory technician to minimize procedural variability [13]. The measurements were made for both transverse and sagittal dimensions according to Pont and Korhaus indexes [14]. Maxillary expansion was evaluated using the Dolphin Image Software before and after 12.2 months of treatment.

The development of the upper dental arch before and after 12.2 months of interceptive orthodontic treatment was determined using the biometric method of study model analysis. The measurements included the inter-permanent-molar width. The distances with a tooth as a landmark were measured from the central fossa of the right and left upper first permanent molars before and after 12.2 months of orthodontic treatment (Fig. 3 A and B).

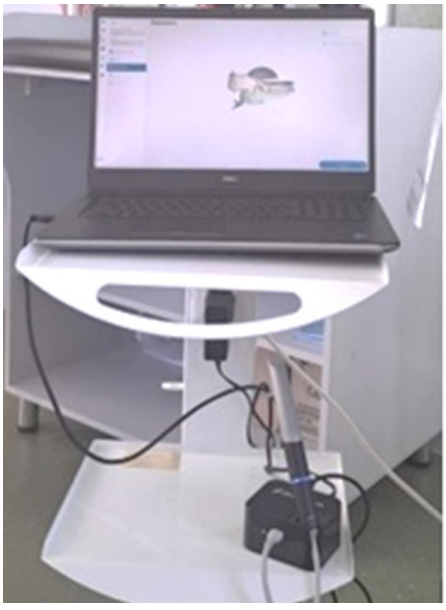


Fig. 2 Virtuo Vivo Straumann intraoral scanner Software.

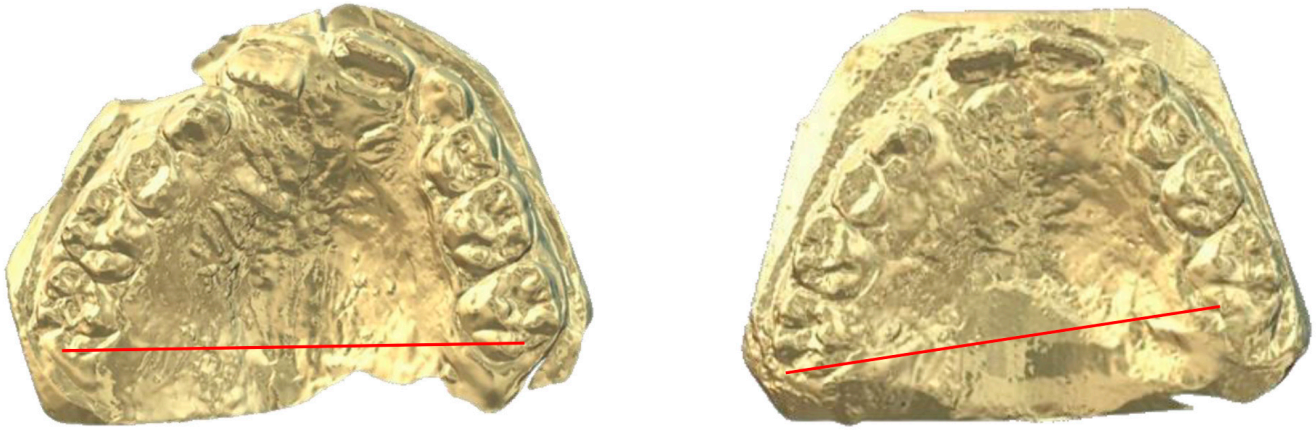


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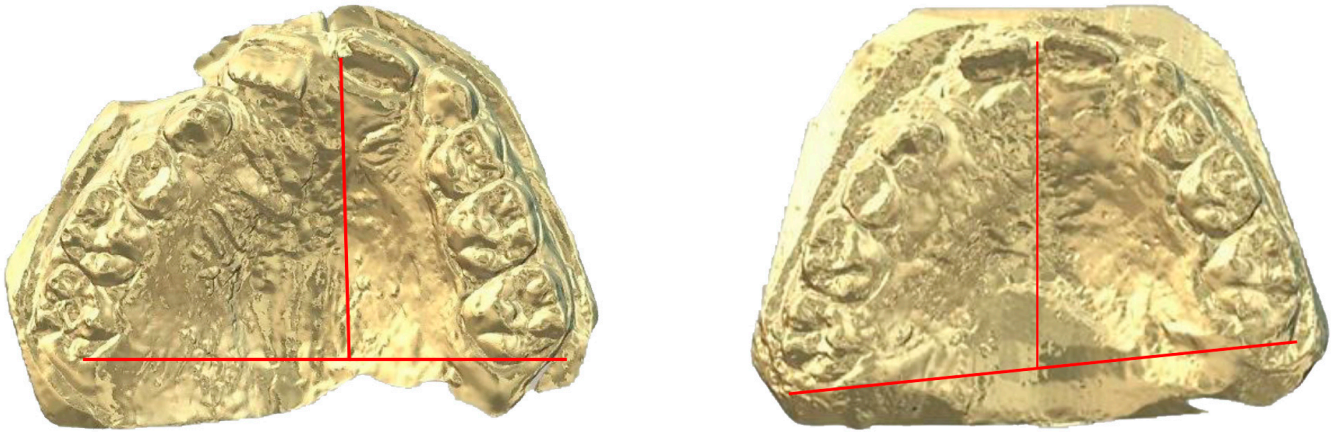
Fig. 3. Maxillary width of the dental arch measured on digital dental cast

Note: *A - before interceptive orthodontic treatment (T1); B - after interceptive orthodontic treatment (T2) for 12.2 months.*



a b

Fig. 4 Maxillary length of the dental arch measured on digital dental cast

Note: *A - before interceptive orthodontic treatment (T1); B - after interceptive orthodontic treatment (T2) for 12.2 months.*

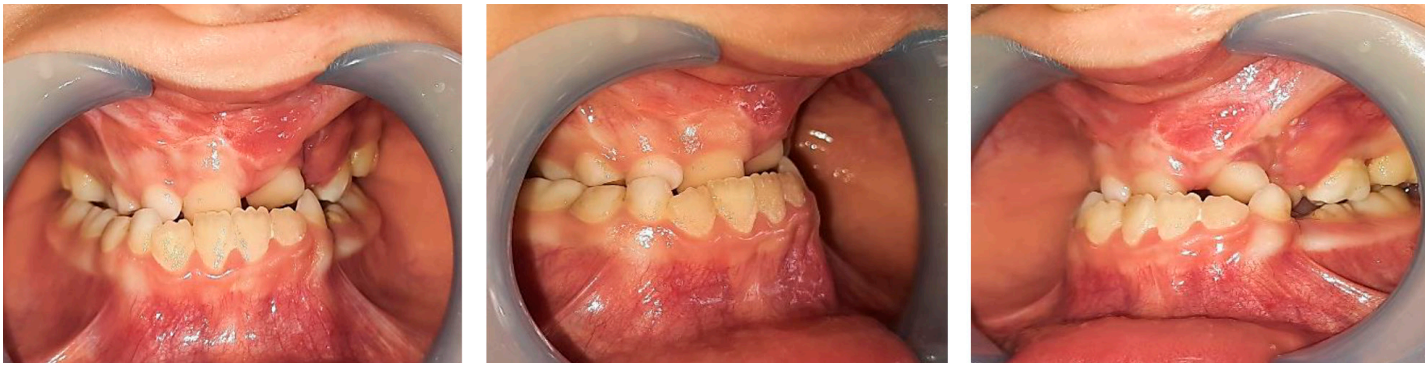
For sagittal dimensions, we analyzed on the cast the length of the upper dental arch which was measured from the point between the upper incisors perpendicular to the junction of the distal surfaces of the first permanent molars before and after 12.2 months of orthodontic treatment (Fig. 4A and B).

The data were analyzed using *SPSS* ver.18 for Windows platform (*SPSS* Inc., Chicago, Illinois, USA) and MS. Excel (Microsoft Office, Windows 2007, USA).

Descriptive statistics for the quantitative variables were obtained using mean and standard, deviation values for maxillary width and length before and after 12.2 months of orthodontic interceptive treatment. To evaluate differences in width and length of the dental arch before and after orthodontic treatment of 12.2 months paired *t*-tests with interaction effects were used to compare the maxillary arch dimensions before and after orthodontic treatment of 12.2 months and to determine significant differences (*SPSS* ver.18) in children with cleft lip and palate. For the analysis, $p < 0.05$ was considered statistically significant.

Results

The scanned dental casts for this study were taken at a mean age of 9.35 years. Boys slightly outnumbered girls in the study population (n = 12 vs n = 8). The most common cleft in this study was unilateral cleft lip and palate (n = 16), which clinically presented with anterior and posterior crossbite (Fig. 5).



a b c

Fig. 5 Anterior and posterior reverse occlusion in patient AC, 10 y. o. with unilateral cleft lip and palate.

A -frontal view; B- lateral right view; C- lateral left view

Frequency of crossbite in children with cleft lip and palate

During the clinical evaluation of patients with cleft lip and palate, we analyzed the frequency of crossbite at the beginning of the treatment, in which anterior and posterior crossbite was determined in 11 patients (55%) and anterior crossbite - in 3 patients (15%), unilateral posterior crossbite - in 3 patients (15%) and bilateral - in 3 patients (15%) (Fig. 6).

In 11 children with cleft lip and palate who underwent interceptive orthodontic treatment with a removable appliance in the transverse plane (Fig. 7), the following values were observed between T1 (pre-treatment) and T2 (12.2

months post-treatment) – the mean dimension in transversal plane has statistically significantly increased from 43.40 ± 2.94 to 45.77 ± 3.55 (p = 0.002), but in the sagittal plane, the post-treatment mean values (25.27 ± 2.64) did not change significantly (p = 0.711) from the pre-treatment mean values (25.13 ± 2.59) (Table 1).

In sagittal plane (Fig. 8) the results showed that in 9 children with cleft lip and palate who underwent interceptive orthodontic treatment with a removable appliance, the following values were observed between T1 (pre-treatment) and T2 (12.2 months post-treatment) – the mean dimension in the transverse plane slightly increased from 45.83 ± 2.92 to 45.88 ± 2.96 (p = 0.347), but in the sagittal plane, the post-treatment mean value (26.44 ± 4.71) modified significantly (p = 0.05) from the pre-treatment mean values (24.83 ± 3.89) (Table 2).

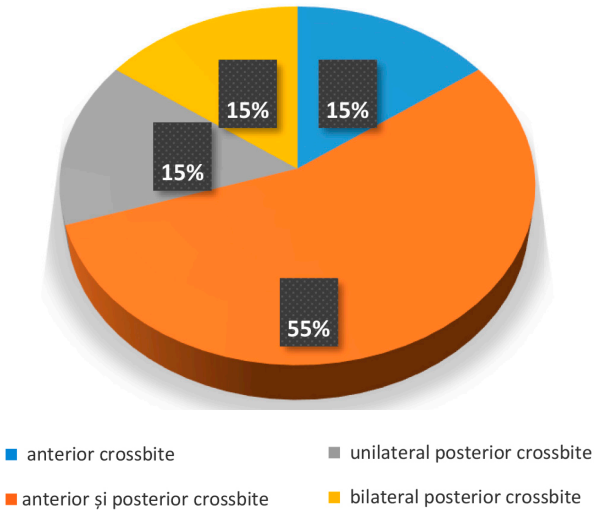
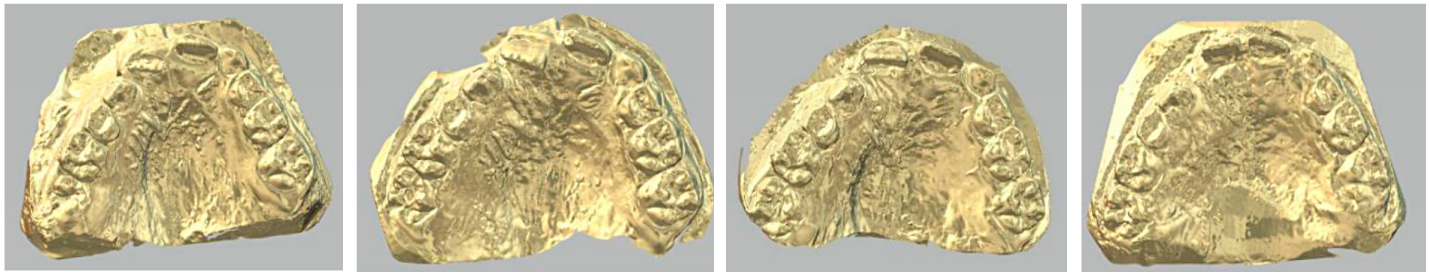


Fig. 6 Clinical forms of crossbite in patients with cleft lip and palate

Table 1. Biometric parameters of the maxillary dental arch in transverse and sagittal plane pre- and post-treatment for 12.2 months during transverse expansion

Comparisons (pre- vs. post-treatment)	t	*p*	Mean difference	SE difference	95% CI		Effect Size (Cohen's d)
					Lower	Upper	
Dimension in transverse plane	-4.280	0.002	-2.364	0.552	-3.594	-1.133	-1.291
Dimension in sagittal plane	-0.382	0.711	-0.136	0.357	-0.933	0.660	-0.115

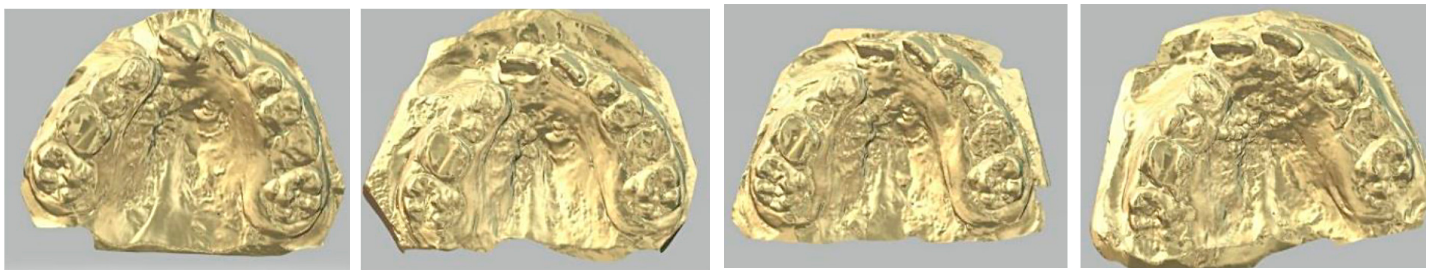
***Note**: t- t-student test; p - probability; SE – Standard Error; CI -confidence interval; Cohen effect size coefficient method*



a b c d

Fig. 7. Evolution of transverse expansion of maxillary dental arch in children with cleft lip and palate

***Note**: A – initial dental cast; B – 3 months of treatment; C- 6 months of treatment; D -12 months of treatment.*



a b c d

Fig. 8 Evolution of sagittal expansion of maxillary dental arch in children with cleft lip and palate

***Note**: A – initial dental cast; B – 3 months of treatment; C- 6 months of treatment; D -12 months of treatment.*

Table 2. Biometric parameters of the maxillary dental arch in transverse and sagittal plane pre- and post-treatment during sagittal expansion in average 12.2 months

Comparisons (pre- vs. post-treatment)	t	*p*	Mean difference	SE difference	95% Confidence Interval		Effect Size (Cohen's d)
					Lower	Upper	
Dimension in transverse plane	-1.00	0.347	-0.0556	0.0556	-0.184	0.0726	-0.333
Dimension in sagittal plane	-3.88	0.05	-1.6111	0.4148	-2.568	-0.6546	-1.295

***Note**: t -t-student test; p -probability; SE – Standard Error; CI -confidence interval; Cohen effect size coefficient method*

Discussion

The results of this study showed that interceptive maxillary expansion orthodontic treatment for 12.2 months in children with cleft lip and palate, using a removable orthodontic appliance, resulted in a greater increase in the sagittal plane than in the transverse plane. The growth of the maxilla in children with cleft lip and palate is important for the development of the dento-maxillary system and the eruption of permanent teeth. Surgical intervention at a very young age slows the growth and development of the maxilla, which is demonstrated by several studies [15-17].

Early orthodontic rehabilitation of children with cleft lip and palate leads to improved aesthetic, morphological and social appearance. The specific features of interceptive orthodontic treatment in children with cleft lip and palate aim to prepare the dental arches for secondary bone grafting before the eruption of the upper canines.

In different countries of the world there are different protocols for surgical rehabilitation of cleft lip and palate. A study that evaluated the growth of the maxilla following surgical treatment of the hard and soft palate in two stages and orthodontic treatment during the period of mixed, early and late permanent dentition showed that the width, length and surface of the hard palate increase satisfactorily during the period of mixed dentition. It was also found that the expansion of the maxilla occurs more in patients who underwent primary surgical rehabilitation in two stages compared to primary surgical rehabilitation in one stage [18].

Interceptive orthodontic treatment with slow or rapid maxillary expansion has been evaluated by several authors in both patients with and without cleft lip and palate [19, 20].

The results of one study showed that the duration of slow expansion treatment compared to rapid expansion treatment depends on the activation protocol of the dental appliance. Thus, both slow and rapid expansion resulted in significant increases in maxillary width and dental arch perimeter, but dental arch length and palatal depth decreased insignificantly after slow expansion and significantly after rapid maxillary expansion [21, 22].

Another study demonstrated the therapeutic effects with plate appliance in children without cleft lip and palate which showed that the increases in width, height and surface differed only slightly between the patient groups and did not relapse: the morphological changes were comparable in patients of different ages, with the greatest effects being observed in the posterior molar region [23].

The present study showed that the increase in length of the maxilla was greater than the width in children with cleft lip and palate. This may be due to early surgical intervention on the palate in children with cleft lip and palate and the plate appliances cannot generate considerable forces that can lead to stimulation of the midpalatal suture.

This study has several limitations. It is a single-center observational case series of 20 children with cleft lip and/or palate, aged 6–12 years, followed for about one year. No formal a priori power or sample size calculation was performed; instead, we included all consecutive eligible patients treated at our clinic during the mixed dentition stage, before planned secondary alveolar bone grafting. Cleft lip and/or palate occurs in roughly 1 in 600–800 live births worldwide (around 0.7–1.3 per 1,000 births in European countries) and shows substantial variation in presentation (unilateral, bilateral, isolated palate), requiring long-term multidisciplinary care. Because management strategies differ not only between centers but also between surgeons, creating a larger, homogeneous, prospectively followed cohort within a single institution is realistically difficult. The present analysis should therefore be interpreted as exploratory and descriptive rather than confirmatory.

A second limitation is the lack of a parallel control group (for example, unaffected peers or cleft patients managed with a different or delayed protocol). As a result, the post-treatment increases in maxillary arch dimensions cannot be attributed with certainty to the expansion protocol alone. Part of the change may reflect normal craniofacial growth during the mixed dentition period, when the maxilla continues to widen and lengthen. Maxillary and midfacial growth in cleft patients is also influenced by how and when the palate was surgically closed; early one-stage palatal repair has been associated with greater midfacial restriction, whereas staged or later closure tends to preserve maxillary growth more effectively. Because these surgical choices vary widely, they represent an additional confounder.

Third, all patients were treated in one institution using the same interceptive orthodontic workflow, and all maxillary measurements were obtained using the same digital workflow. This standardization helps reduce internal procedural variability, but we did not formally test intra- or inter-examiner reproducibility, so measurement bias cannot be excluded. External validity is also limited. Different cleft teams prepare the maxillary arch before secondary alveolar bone grafting in different ways: some use slow expansion, others use rapid maxillary expansion, and some perform preliminary anterior alignment before grafting. The timing of expansion relative to grafting also differs. These choices can influence arch form, segment position in the cleft area, and even graft performance, so our findings may not trans-

late directly to centers using other biomechanics, activation schedules, or timelines.

Despite these limitations, the findings are clinically relevant in a coordinated cleft care pathway. Interceptive orthodontic expansion during the mixed dentition is commonly used as preparation for secondary alveolar bone grafting: it helps open and derotate collapsed maxillary segments, improves transverse and sagittal arch form, creates bony support for eruption and alignment of teeth adjacent to the cleft (especially the canine and lateral incisor), and facilitates surgical closure of the alveolar defect. In most cleft protocols, the alveolar bone graft is placed in the mixed dentition, usually shortly before eruption of the permanent teeth bordering the cleft and often within a few months after expansion, with the aim of stabilizing the maxillary segment and supporting future occlusion, facial balance, nasal base support, and speech. Early orthodontic–surgical coordination is therefore regarded as part of functional rehabilitation, not just an aesthetic procedure. Prospective controlled studies with larger samples and standardized follow-up are still needed to determine whether the dimensional changes observed here persist and translate into long-term functional and aesthetic benefit after grafting and comprehensive orthodontic treatment.

Conclusions

(1) Slow maxillary expansion treatment during the mixed dentition period in children with cleft lip and palate was found to be more effective in the sagittal plane than in the transverse plane. Overall, the treatment-related changes were more pronounced in length than in width.

(2) The practical application of orthodontic treatment techniques in patients with cleft lip and palate demonstrated a favorable therapeutic evolution and an advantageous recovery of maxillofacial structures for subsequent stages of treatment.

Competing interests

None declared.

Authors' contributions

SR conceived the study and help drafting the manuscript, SM helped in drafting the manuscript, GB participated in the study design and performed the statistical analysis, CP performed the study design and performed digital model analysis. All authors have read and approved the final version of the manuscript.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes No. 42, dated 14.12.2016).

Patient consent

Obtained.

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RESEARCH ARTICLE

The role of odontogenic infection in the onset and evolution of focal disease

Sergiu Ciobanu*, Diana Marcu, Ion Roman, Olesea Musteaţă

Sofia Sîrbu Department of Odontology, Periodontology and Oral Pathology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chişinău, Republic of Moldova

ABSTRACT

Introduction. The focal disease is a pathological condition characterized by a wide variety of functional disorders and organic tissue alterations, due to chronic foci of infection, from which various microbes, microbial toxins, and toxic products of septic tissue disintegration originate. According to the percentage distribution, 90% of the foci of the body are located in the cephalic region, and 10% in the rest of the body [17]. Research has shown that on the list of foci of infection, those in the oral cavity are in first place, with 93% of active foci being caused by teeth and their pathologies. Important clinical criteria that mark this fundamental difference of the odonto-periodontal focal infection include the profile of local inflammation, the level of the tissue hypersensitivity process, the level of the microbial load in the focus and, no less importantly, the reactivity of the body.

Material and methods. The present study is a retrospective clinical observational study and included 87 patients with foci of odontogenic infection, classified according to the frequency of pathologies encountered and the virulence of microbial pathogens as follows: with periodontal disease - 35 patients (gingivitis - 11 patients and 24 patients with different stages of periodontitis); with endodontic pathologies - 27 patients (pulpitis - 5 patients and periapical lesions - periodontitis (Pt) - 22 patients); with dental caries of varying severity - 19 patients, and 6 patients with oral mucosa pathologies.

Results. Through clinical observations, it was found that with the removal of the foci of infection, the *"vegetative alarm symptoms"* begin to disappear, some of them even very quickly, such as causeless fatigue. At the same time, symptoms that have been present for a longer period, such as long-term depressive states and memory disorders in patients with periodontal disease–particularly severe periodontitis with a major microbial load–and lesions of the oral mucosa, decreased more slowly, over a period of 1-3 months. The 100% disappearance of symptoms in the case of carious lesions and oral mucosa lesions demonstrates the direct relationship between the foci of infection and the patient's general health. In relation to periodontal disease and periapical lesions (over 90% of symptoms have subsided), the remaining clinical signs are related to the increased bacterial load and the virulence of the pathogens.

Conclusions. The identification, evaluation, and elimination of foci of odontogenic infection play an important role in aggravating already existing systemic conditions, thus triggering focal disease. The role of the dentist in the prophylaxis of focal disease is primary in the detection and elimination of foci of odontogenic infection. In the prophylaxis of focal disease, doctor-patient cooperation is very important, and no less important is collaboration with general medicine specialists.

Keywords: focal disease, vegetative alarm symptoms, odontogenic focus, periodontal disease, infection, antigens.

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*__Corresponding author__: **Sergiu Ciobanu**, PhD, MD, university professor
Sofia Sîrbu Department of Odontology, Periodontology and Oral Pathology
Nicolae Testemiţanu State University of Medicine and Pharmacy

Key messages

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

The possibility of obtaining herbal reference standards (HRS) in the form of dry extracts using the same solvent for treating the plant material followed by salting-out extraction of target compounds.

165, Ştefan cel Mare şi Sfânt blvd, Chişinău, Republic of Moldova, MD 2004
e-mail: sergiu.ciobanu@usmf.md

Authors' ORCID IDs
Sergiu Ciobanu – https://orcid.org/0000-0002-7955-545X
Diana Marcu – https://orcid.org/0000-0002-3844-9175
Ion Roman – https://orcid.org/0009-0006-2241-6973
Olesea Musteaţă – https://orcid.org/0000-0002-2368-4930

The research hypothesis
Preparing the primary plant extract with an organic solvent that can be salted out in a subsequent technological stage should reduce time and solvent consumption, thereby simplifying the entire HRS preparation process.

The novelty added by manuscript to the already published scientific literature
Using the same water-organic mixture for preparing the primary plant extract and for subsequent salting-out extraction showed a good yield of medium-polar compounds, such as polyphenols, resulting in a very simple and inexpensive method for producing HRS.

Introduction

The focal disease is a pathological condition characterized by a wide variety of functional disorders and organic tissue alterations, due to chronic foci of infection, from which various microbes, microbial toxins, toxic products of septic tissue disintegration, and endo- and exogenous allergens are disseminated in the body via the blood, nervous system, or digestive tract, generating a vast array of dysfunctional and lesional manifestations [1]. Local infection combines particular features and emphasizes the main aspects, such as clinical manifestations, active or latent evolutionary character, and the existence of an antigenic tissue concentration of the type of a focus. The range of reactions caused by a microbial agent includes both significant general reactions and local changes related to the presence of the pathogen. Thus, we are talking about localized, circumscribed inflammatory reactions – "concentrated reactions" – in which, due to the maximum intensity of microbial aggression and tissue response, we can speak of a center, a nucleus, or, in well-established terminology, a focus of the infectious process [2]. "The mouth is probably the dirtiest place in the human body," noted Steve Kerrigan, a researcher at the Royal College of Surgeons of Ireland [3].

Illustrious scientists, doctors, and statistical consultants from many countries have demonstrated, through a series of clinical observations and statistics collected over the years, the importance, relevance, and topicality of this condition, presenting major values in terms of both prevalence and incidence. Focal infection appears as a disease of the whole organism, in which there is a chronic primary focus with latent evolution, unnoticed by the patient, and secondary lesions at a distance, which manifest differently. The term *focal*, in the clinical sense, refers to the presence in the body of an infectious process that is more active in a certain region [4-14].

According to the percentage distribution, 90% of the foci of the body are located in the cephalic region, and 10% in the rest of the body. Research has shown that, on the list of foci of infection, those in the oral cavity are in first place, with 93% of the active foci caused by teeth and their pathologies [15-18] (Table 1).

Table 1. Location of infection outbreaks expressed as a percentage (%)

Extracephalic foci	10%	Cephalic outbreaks	90%
Adnexites	2-7%	Dento-periodontal outbreaks	72%
Bronchi	0.8%	Tonsillitis	18%
Intestine	0.5%	Sinus foci	<18%
Extrahepatic bile ducts	0.5%	Middle ear	<18%

Note: *Focal infections occur predominantly in the area of the stomatognathic apparatus.*

Important clinical criteria that mark this fundamental difference of odonto-periodontal focal infection include the profile of local inflammation, the level of the tissue hypersensitivity process, the level of microbial load in the focus, and, no less importantly, the reactivity of the organism.

The anatomical features of the tooth create particularly favorable conditions for chronic, latent infections. The infectious process develops in tissues that do not have the possibility of recovery (enamel, dentin) and in locations (root canal) inaccessible to the body's antimicrobial defense mechanisms [1, 13, 15, 16].

In turn, the oral mucosa forms a non-specific (mechanical) defense zone. Thus, a complex barrier is created, consisting of local structures and biological factors that regulate local permeability to bacteria, fungi, viruses, and toxins. However, this physiological barrier is fragile and, under certain pathological conditions, becomes permeable, serving as an entrance gate to the interior of the body [19]. Studying and critically analyzing the specialized literature, from a historical point of view, it is observed that focal disease, with foci of infection at the level of the teeth, marginal periodontium, and tissues of the oral cavity (such as the mucosa, salivary glands, etc.), has been researched since ancient times. The first deductions regarding focal disease, on the relationship between dental infections and some pathological manifestations of the human body, date back several millennia. The Ebers Papyrus described the disappearance of some organic suffering after the extraction of affected teeth. The Nineveh cuneiform manuscript of 650 BCE mentions a cure for joint pain in King Anapper Essa, also after extraction of affected teeth. A cuneiform inscription on a stone found in Nineveh has been deciphered, showing that the pain in the limbs of the Assyrian king Sardanapalus ceased only after, on the advice of his court physician Araa Nana, he extracted all his teeth [1].

The first communications on the correlation of dental diseases with other pathological manifestations in the body were described in 1900 by Steffel. As early as the ninth century AD, an Arab physician wrote that, "the mouth is only a part of the human body, and what happens to it happens to the whole body". In 1828, Kocher drew attention to the pathogenic relationship of the dental system with the rest of the human body, describing the connection of nervous and rheumatic diseases with dental diseases. At the end of the 19th century, certain theories were developed, claiming that, following the extraction of teeth with a focus of infection, a cure for general diseases could occur. In 1906, Possler drew attention to the cause-and-effect relationship between chronic dental diseases and remote diseases. Later, at the beginning of the 20th century, "focus disease" was discovered. The theory of "focal infection" was introduced into pathology by Billings in 1912 [20].

Experimental research undertaken by Roşenow, Berger, Hateganu, Goia I., and Moga (1934) showed that focal disease is a disease of the whole organism, comprising 2 pathological entities: a) chronic foci of infection with slow oligo- or asymptomatic evolution, which represent the primary manifestation; b) dysfunctional or lesional manifestations at a distance, with rich and varied symptomatology, which represent the secondary determinations. This framework outlines 5 etiopathogenic theories: microbial etiopathogenic, which considers focal disease as an "attenuated septicemia" due to the migration of microorganisms from the focus via the blood and lymph, and their fixation on organs and tissues; allergic, which states that microbes entering the body cause a change in reactivity with the production of hyperergic antibodies (Ab); toxic, which supports the idea that toxins from the focus migrate through the endo- and perineural lymph, producing irritation of nerve centers and nerve cells; vascular, in which the focus of chronic irritation acts on the central and peripheral vegetative nervous system, determining the response of the vessels through changes in tone: ischemia or vasodilation; and the theory of adaptation syndrome, which regards focal disease as an excessive defense reaction of the body, with the adaptation syndrome being the totality of non-specific manifestations of the body's defense as a result of the action of harmful agents of any nature. According to these theories, the main factors in the dissemination of the infection are decreased body resistance, exacerbation of microbial virulence, tissue hyperemia, and loco-regional trauma [1].

The delegation of the German Medical Association conducted scientific studies and demonstrated that there are interactions between teeth and organs of the human body. Through electropuncture, the relationship between teeth or groups of teeth and organs of the human body was demonstrated for the first time. Thus, some dental diseases can signal organic dysfunctions, and the disease of a tooth corresponding to a certain organ can affect that organ: the incisors are related to the urinary system and can generate chronic infections of the urinary bladder; the canines with the liver, gallbladder, eyes, and stomach; the upper premolars with the large intestine and lungs; the lower premolars with the lungs and spleen (t. 34, 35), and with the lungs and pancreas (t. 44, 45); the upper premolars with the lungs and large intestine; the upper molars (t. 16, 17) with the thyroid, stomach, and pancreas, and (t. 26, 27) with the spleen; the lower molars with the stomach and intestines, the immune system, the maxillary sinus, and the thoracic spine; and the wisdom teeth with the heart and small intestine. To the same extent, there are relationships between teeth and the spine [21].

From the above, we can state that a chronic infection localized in a focus–an organ such as the tonsils, teeth, sinuses, bronchi, or kidneys–is delimited by a fibro-connective membrane, which represents a barrier but is imperfect under certain conditions. With increased aggressiveness of microorganisms, decreased body resistance, exacerbation of microbial virulence, or loco-regional trauma of the oral mucosa, microbes and their toxins can "migrate and infiltrate" via the blood and lymphatic pathways away from the initial focus of infection and stop in another healthy organ, which, in turn, will be affected [22].

Thus, chronic infections in the oral cavity can cause true organic diseases, grouped by Veil under the term "vegetative alarm symptomatology", which includes fatigue, morning sickness without explainable causes, memory disorders, unexplained nervous irritation, adynamia, tachycardia without organic substrate, episodic or prolonged depressive states, intermittent subfibrillations during the day, chills, long-lasting fever, insomnia, headache, myalgias, arthralgias, and loss of appetite. It is important to note that the dentist is obliged to detect primary infection foci based on clinical, radiological, and, if necessary, laboratory signs, thus establishing a correct diagnosis, after which they remove or treat the foci. Establishing the link between the infection foci and the secondary disease is not only the dentist's task; interdisciplinary collaboration is necessary to prevent the development of the actual outbreak disease [23].

The purpose of the study is to highlight the role of foci of odontogenic infection in the onset and evolution of focal disease.

Material and methods

The present study is a clinical-observational and microbiological study and included 87 patients (with informed consent) with foci of odontogenic infection, classified according to the frequency of pathologies encountered and the virulence of microbial pathogens as follows: with periodontal disease - 35 patients (gingivitis - 11 patients and 24 patients with different stages of periodontitis); with endodontic pathologies - 27 patients (pulpitis - 5 patients and periapical/periodontitis (Pt) - 22 patients); with dental caries of varying severity - 19 patients; and 6 patients with pathologies of the oral mucosa. The patients were selected and examined clinically and paraclinically (radiographically - RVG and OPG) (Figure 1). In patients with periodontal disease, laboratory examinations were performed, including hemogram, leukogram, and microbiological examination of periodontal pockets (PPr) using the polymerase chain reaction (PCR) method (Table 2).

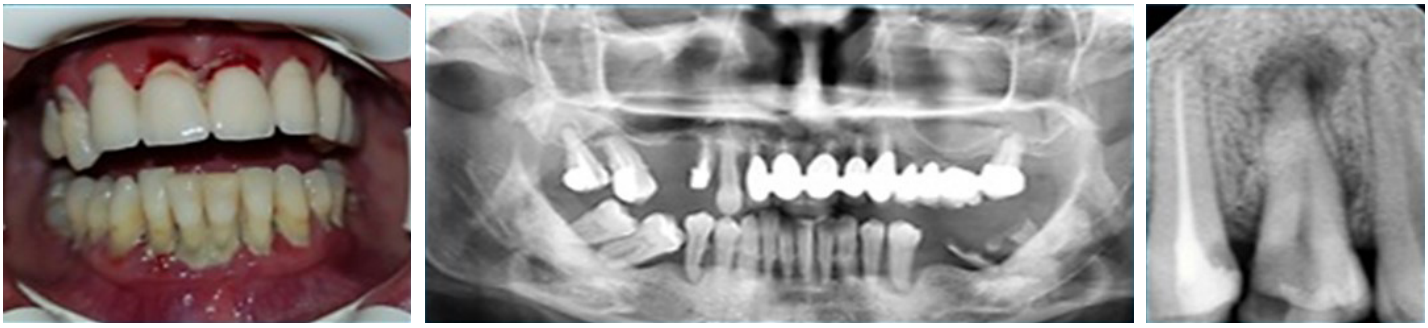

Fig. 1 Severe periodontitis, stage IV, Grade C, and chronic granulomatous apical periodontitis

Note: *Intraoral clinical and radiographic appearance.*

Table 2. Microbiological profile of a patient with severe periodontitis

Name of the microorganism	Result	Units of measurement	Reference values
Total mass of bacteria	8.5	Ig	<6.5
Actinobacillus actinomycetemcomitans	not detected	Ig	<4.0
Porphyromonas gingivalis	8.0	Ig	<5.0
Prevotella intermedia	6.6	Ig	<4.5
Tannerella forsythensis	6.9	Ig	<5.0
Treponema denticola	7.2	Ig	<3.5
Candida albicans	not detected	Ig	<4.5

Note: *Microbiocenosis – patient's laboratory medical bulletin.*

According to the data in Table 2, a high microbial load with periodontopathic agents is observed, the proteolytic enzymes of which are among the most important virulence factors, and their dissemination throughout the human body is a major risk factor in the onset of focal disease. Even if periodontopathic bacteria are only momentarily present in tissues, once the germs, their virulence factors, and/or inflammatory mediators reach distant organs, they can cause similar inflammatory reactions in the new environment, thus leading to tissue diseases or focal disease. Therefore, periodontal disease/periodontitis has a direct and reciprocal link to the development of systemic diseases such as cancer (including oral cancer), diabetes mellitus, cardiovascular diseases, and neurological pathologies, especially the neurodegenerative disease known as Alzheimer's disease [24].

Considering that the present study was largely a clinical-observational one, the patients were evaluated according to the Veil criteria of "vegetative alarm symptomatology". During patient examination and clinical symptom assessment, 8 of the 14 Veil criteria were used, specifically those to which patients could easily respond at the examination stage and after the removal/treatment of the foci of infection (Table 3).

Table 3. Evaluation of Veil criteria in patients with foci of odontogenic infection at presentation (presence of symptoms and number of patients)

Pathology/criteria	Fatigue without cause	Memory disorders	Nervous irritation without explanation	Adynamia	Depressive states, episodic or prolonged	Disorders	Arthralgia	Loss of appetite
Dental caries	-	-	-	-	-	-	± (3)	-
Endodontic pathologies (P, Pt)	+ (18)	-	± (10)	± (2)	± (13)	+ (21)	+ (16)	-
Periodontal disease (gingivitis/ periodontitis)	+ (27)	+ (29)	+ (19)	± (11)	+ (22)	+ (17)	+ (14)	± (9)
Pathologies of the oral mucosa	± (2)	-	+ (6)	± (1)	± (3)	+ (6)	-	+ (5)

Note: *Representation of clinical symptoms based on the evaluation at the examination stage - the criteria are indicated in the table.*

Based on the data in Table 3, we note that dental caries is one of the most widespread dental diseases; however, the presence of "vegetative alarm symptoms" is not critical, except for sporadic symptoms (in cases of multiple or decompensated caries), because the body's reactivity compensates. In contrast, in endodontic pathologies (chronic pulpitis, apical periodontitis), especially closed forms, residual cysts, etc., these symptoms are much more pronounced. Closed infections are more dangerous than suppurative ones, which have a drainage pathway (Figure 2). Periapical foci are the most frequent complications of dental caries, being represented by chronic pulpitis and chronic fibrous or granulomatous apical periodontitis, residual cysts, etc. [13].

At the same time, periodontal infection foci, having periodic escape routes, in terms of interaction with the entire organism, exert a much greater influence by affecting all periodontal tissues and by the presence of predominantly anaerobic (Gram-) periodontopathic microorganisms with increased virulence; thus, the expression of "alarm symptoms" is more pronounced (Table 3). A similar situation occurs in the case of foci located in the oral mucosa, taking into account the advanced absorption capacity of the oral mucosa. An important irritating action is also exerted by toxic environmental factors (toxic gases, dust, cigarette smoke, etc.). The virulence of germs is locally exacerbated due to particular anatomical conditions, episodic insufficiency

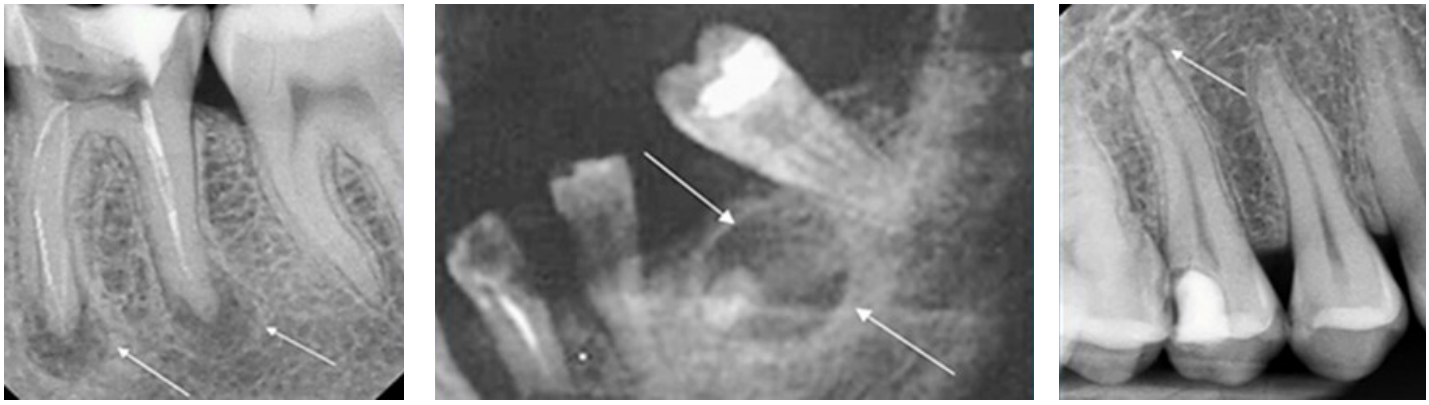

Fig. 2 Chronic closed odontogenic foci

Note: *Radiographic appearance of the periapical lesion and residual cyst (personal case study – S. Ciobanu)[4, 6]*

of local non-specific defense factors, and local circulatory disorders that reduce the contribution of general defense mechanisms. Serum concentrations of IgG, IgA, and IgM are lower in smokers, thus disrupting the body's defenses, and macrophages lose part of their adhesion and phagocytic capacity. Viral infections may also play a role in the onset and maintenance of autoimmune diseases [21].

All patients in the study underwent treatment to eliminate or remove foci of infection, according to protocols specific to the pathologies studied, as follows: treatment of dental caries, endodontic treatments, and complex treatment of periodontal disease and lesions of the oral mucosa, thus interrupting the spread of infection throughout the body.

Results

Along the course of treatment and especially at the end of the treatment of the studied pathologies, the patients were evaluated according to the same eight Veil criteria. Thus, through clinical observations, it was found that with the removal of the foci of infection, the "vegetative alarm symptoms" began to disappear, some even very quickly, such as causeless fatigue. At the same time, symptoms present for a longer period of time, such as long-term depressive states and memory disorders in patients with periodontal disease, in this case severe periodontitis with a major microbial load and lesions of the oral mucosa, diminished more slowly, over a period of up to 1-3 months (Table 4).

Table 4. Evaluation of Veil criteria after removal of odontogenic infection foci (presence of symptoms and number of patients)

Pathology/criteria	Fatigue without cause	Memory disorders	Nervous irritation without explanation	Adynamia	Depressive states, episodic or prolonged	Disorders	Arthralgia	Loss of appetite
Dental caries	-	-	-	-	-	-	-	-
Endodontic pathologies (P, Pt)	-	-	+ (1)	-	-	+ (2)	+ (1)	-
Periodontal disease (gingivitis/ periodontitis)	-	-	-	-	+ (2)	-	+ (4)	-
Pathologies of the oral mucosa	-	-	-	-	-	-	-	-

Note: *Representation of clinical symptomatology based on the evaluation of Veil criteria post-treatment – personal results [4].*

Therefore, by analyzing the symptomatology of patients with odontogenic foci, we find that the "vegetative alarm symptoms" subside significantly, which once again confirms that microbial germs, their virulence factors, and/or inflammatory mediators– such as IL-1, IL-6, IL-8, and TNF–are produced locally in odontogenic foci (periodontal pockets (PPr), periapical lesions, etc.), causing distant systemic inflammation, and that their removal restores the biological balance of the entire organism (Table 4). The 100% disappearance of symptoms in the case of carious lesions and oral mucosal pathologies demonstrates the direct relationship between the foci of infection and the patient's general health. In relation to periodontal disease and periapical lesions, over 90% of symptoms subsided; the clinical signs that were maintained are related to the increased bacterial load and the virulence of pathogens.

Discussions

The oral cavity has a complex microbial ecology, which represents an ideal habitat for the growth and reproduction of many microorganisms, ranging from bacteria to fungi, molds, and viruses. There are many opinions among researchers according to which focal disease is a condition caused by the simultaneous action of several factors that contribute to the decrease in the reactivity of the organism in general and at the level of the oral cavity in particular. According to the data of Ghicavîi et al. (2002), damage to an organ or a system depends largely on constitutional factors, individual receptivity, and the general condition of the organism [25]. An important role is also played by general conditions, and dysfunctional lesional manifestations, which in turn depend on the aggressiveness of the chronic focus, the number of foci, their content, the virulence of

microorganisms, etc. The onset of clinical manifestations of systemic disease, conditioned by the presence of odontogenic foci, is diverse and produces major changes in the immunological state of the entire organism. Thus, it is appropriate to place the qualification of an "infection focus" in a context corresponding to the current level of knowledge of local and general pathophysiological mechanisms, as well as immunological and microbiological processes. In this context, the idea of dividing morpho-clinical entities into active focal infections and latent focal infections appears of interest. The available literature suggests that the oral microbiota originating from odontogenic foci plays a significant role in the onset of focal disease, from the moment inflammatory mediators enter the bloodstream and exert a direct impact on distant tissues and organs, as shown by Miklossy et al. (2016) [24].

The study by Dominy et al. (2019) showed that over 90% of brain samples taken from patients with Alzheimer's disease contained gingipains as the most significant virulence factors. *P. gingivalis* or its virulence factors were repeatedly administered to animal subjects, which led to cognitive decline and an increase in pro-inflammatory mediators (β-amyloid) in their brains. Periodontal disease causes the spread of bacteria or toxins to the brain through peripheral nerves, with the trigeminal nerve being the main access point [26].

Solving dental problems is necessary in the presence of diseases of major organs. Thus, timely treatment, under the strict guidance of a specialist, has beneficial effects on the entire body, not only local effects in the oral cavity.

Conclusions

The identification, evaluation, and removal of foci of odontogenic infection have an important role in aggravating already existing systemic conditions, thus triggering focal disease. The role of the dentist in the prophylaxis of focal disease is a primary one in the detection and elimination of foci of odontogenic infections. In the prophylaxis of focal disease, doctor-patient cooperation is very important, and no less important is collaboration with general medicine specialists.

Competing interests

None declared.

Authors' contribution

All authors contributed to the conduct of this research.

Informed consent for publication

Obtained.

Ethics approval

The clinical cases in this study were selected from the list of patients treated in the dental clinic, based on informed consent and confirmed consent by signature in the medical record. Thus, approval of the Bioethics Committee was not required.

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Provenance and peer review

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RESEARCH ARTICLE

Developing and validating a questionnaire on knowledge and attitudes in health research ethics

Adriana Paladi[1*], Valentin Mîța[2], Lilian Şaptefraţi[3]

[1]Philosophy and Bioethics Department, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[2]School of Public Health Management, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[3]Department of Histology, Cytology, and Embryology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

ABSTRACT

Introduction. Research ethics and integrity are fundamental for safeguarding human participants and ensuring trustworthy scientific practices. Understanding researchers' knowledge, attitudes, and perceptions regarding ethical standards is important for all health researchers, but particularly relevant for early-career researchers. While several international instruments exist to evaluate specific aspects of research ethics, such as plagiarism, organizational climate, or responsible conduct of research, a multidimensional and contextually relevant tool is required.

Materials and methods. A comprehensive questionnaire was developed to assess ethical knowledge, attitudes toward research resources and institutional integrity measures, and self-perceived ethical competencies among doctoral students in the health sciences. Item formulation was guided by international standards, including the Declaration of Helsinki, the European Code of Conduct for Research Integrity, and Good Clinical Practice guidelines. The development process included a content validity assessment by 10 experts and a psychometric evaluation of the collected data from 274 doctoral students. Exploratory factor analysis (EFA) was applied to determine the latent structure of the questionnaire, and internal consistency was assessed using Cronbach's alpha.

Results. EFA revealed a six-factor structure explaining 64.5% of the total variance. The factors measured: (I) perceived importance of research resources, (II) self-perceived ethical competencies, (III) implemented institutional measures for research integrity, (IV) ethical principles and moral responsibilities, (V) perceived accessibility of research resources, and (VI) importance of institutional integrity measures. Factor loadings were generally high, and internal consistency was good to excellent, with Cronbach's alpha values ranging from 0.738 to 0.989. These findings indicate that the questionnaire captures multidimensional aspects of research ethics and integrity.

Conclusions. The developed questionnaire represents a robust, valid, and reliable instrument for assessing ethical knowledge and attitudes among researchers in health sciences. It can serve as an internal audit tool to evaluate research integrity climate, researcher satisfaction with available resources, and implementation of institutional policies. Moreover, it provides a foundation for designing targeted training programs and professional development initiatives aimed at improving ethical competencies.

Keywords: research ethics, human subjects, questionnaires, psychometrics, validation studies, medical education.

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[*]**Corresponding author**: **Adriana Paladi**, PhD, associate professor
Philosophy and Bioethics Department
Nicolae Testemițanu State University of Medicine and Pharmacy

Key messages

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

Although several international tools exist for assessing research ethics and integrity, there is a lack of psychometrically validated instruments tailored to the local research context.

165, Stefan cel Mare si Sfant blvd., Chisinau, Republic of Moldova
e-mail: adriana.paladi@usmf.md

Authors' ORCID IDs
Adriana Paladi – https://orcid.org/0000-0002-3655-0640
Valentin Mîța – https://orcid.org/0000-0002-5669-7455
Lilian Șaptefrați – https://orcid.org/0000-0003-2779-718X

The research hypothesis
It was hypothesized that a contextually adapted and psychometrically validated instrument can reliably capture the multidimensional aspects of researchers' ethical knowledge, attitudes, and perceptions.

The novelty added by the manuscript to the already published scientific literature
This study introduces the first psychometrically validated instrument tailored to the Moldovan research context, capturing multidimensional constructs of research ethics and integrity. The tool complements existing international instruments by providing a context-specific framework for evaluating ethical competence and integrity management.

Introduction

Research ethics in the field of health is not merely a formal obligation but constitutes the foundation for protecting the dignity, rights, and well-being of participants. Research ethics refers to an environment in which moral values, integrity, and transparency are more than just norms written in regulatory documents; they are embedded realities of current research practices. To support the production of ethical research and to foster research environments with a high level of integrity, training in the responsible conduct of research is essential [1]. To foster a culture of research integrity and adapt educational programs on the responsible conduct of research, it is essential to understand researchers' experiences and attitudes toward these issues, particularly among those in the early stages of their careers.

In many countries around the world, studies are conducted to assess the ethical research competencies of early-career researchers. One such example is the study carried out at the Faculty of Medicine of the University of Ljubljana, which evaluated the level of ethical knowledge and attitudes toward research among first-year doctoral students [2]. The findings revealed a very low level of expertise, with an average of only 18.9 correct responses ($p < 0.001$), compared to the expected score of 31 out of 39 questions. Those with prior research experience obtained higher scores. The authors concluded that, to ensure the responsible development of future researchers, more structured and rigorous educational programs in research ethics are necessary.

Another study conducted within a medical faculty in India aimed to assess the level of knowledge, attitudes, and practices related to research ethics among postgraduate students. The results indicated relatively modest average scores for knowledge, attitude, and practice as well. In conclusion, the authors propose the development of an integrated and comprehensive ethics curriculum, combining problem-focused programs with mentorship [3because of the need to improve healthcare. As medical research involves human participants, it has to be guided by fundamental ethical principles to ensure the protection of their rights and welfare. There are very few medical colleges in India with a standardised ethics curriculum, and with provisions for evaluation. This study was done to assess the knowledge, attitude and practices of Medical postgraduate students regarding research ethics. Methods - A one year Cross Sectional Study was done among 154 Postgraduate MD and MS students of medical college. An online questionnaire consisting of questions related to knowledge and attitude towards principles and practice of ethics in clinical research, informed consent, and role of the ethical committee in the institution was given to those who gave consent to participate in the study.Results The Mean ± SD score for Knowledge questions was 8.49 ± 1.65(11].

The study conducted by Hofmann et al. (2020) investigated knowledge, attitudes, and practices related to research integrity among doctoral students from medical faculties at three Scandinavian universities, using a questionnaire based on hypothetical scenarios and attitudes toward questionable research practices. The findings highlighted a diversity of perceptions and practices concerning research integrity, with variations across universities and fields of study. Key observations include: a significant number of respondents reported a willingness to fabricate, falsify, or omit contradictory data if they believed the overall conclusions of the study were correct; nearly one-third of respondents indicated that they had unjustly added one or more authors to their publications. The authors conclude that these results suggest that existing educational and research systems are partially failing to promote research integrity [4Oslo, Odense].

No similar studies have been conducted in the Republic of Moldova. In this context, we decided to develop and validate a questionnaire aimed at supporting the ethical management of research, which would enable the exploration of ethical knowledge regarding the protection of study participants, research integrity, the assessment of attitudes toward the research environment, and the evaluation of perceived ethical competencies. In designing the questionnaire, contextual aspects such as the type of existing training in research ethics, the regulatory framework, and applicable guidelines were taken into consideration.

Materials and methods

The process of developing the questionnaire followed several well-recommended [5, 6] successive stages, namely: 1) questionnaire development; 2) content validity testing; and 3) psychometric analysis, including construct validity testing and internal consistency assessment.

Stage 1. Questionnaire Development. The formulation of the questionnaire items was preceded by the establishment of its conceptual framework and theoretical dimensions. The items were developed based on the international literature on research ethics in the health field, including the World Medical Association Declaration of Helsinki [7] as well as other international guidelines on good research practices and academic integrity, such as the Good Clinical Practice Guideline (ICH GCP E6(R3) [8], and the European Code of Conduct for Research Integrity [9]. The first version of the questionnaire consisted of 79 closed-ended and multiple-choice questions.

Stage 2. Content Validity Testing. Ten experts reviewed the initial version of the questionnaire: four researchers in clinical studies, two researchers in basic science, two researchers in public health, one research manager in the health field, and one ethicist. A qualitative approach was employed, where experts provided a narrative evaluation of each item in terms of clarity, relevance, and domain coverage [10].

Stage 3. Psychometric Evaluation. The questionnaire was sent online (using the Google Forms platform) to doctoral students from the Doctoral School of Medical Sciences at the *Nicolae Testemițanu* State University of Medicine and Pharmacy in the Republic of Moldova, between March and July 2025. Out of 416 doctoral students, 274 responded to the questionnaire, representing a subject-to-item ratio of approximately 4:1. Although this ratio falls slightly below the commonly recommended 5:1 threshold for exploratory factor analysis [5], Kaiser-Meyer-Olkin (KMO) indicators supported the adequacy of the sample, where a KMO index ≥ 0.60 was considered acceptable, while values above 0.80 indicated excellent suitability of the data for factor analysis [13].

After data collection, the construct validity and internal reliability of the questionnaire were assessed. To identify the latent structure of the questionnaire, exploratory factor analysis (EFA) was applied. This method is recommended for the development and validation of psychometric instruments [11, 12]. The number of latent factors was determined based on the eigenvalue criterion (eigenvalues > 1) and examination of the scree plot [14]. Varimax rotation was applied to obtain a clear factor structure [15].

In the next step, the internal consistency of the questionnaire was assessed using Cronbach's alpha, along with the alpha-if-item-deleted analysis for each factor identified, in accordance with psychometric instrument development guidelines [16].

Statistical analyses were performed using Microsoft Excel (Microsoft 365) with the Real Statistics add-in, with a significance threshold set at $p < 0.05$.

Results

Questionnaire Development. A review of the literature in the field contributed to the formulation of 79 items, designed to cover four dimensions: (1) knowledge of ethical standards in research involving human subjects (14 items); (2) attitudes regarding research resources (importance versus accessibility) (21 items); (3) attitudes toward institutional measures aimed at ensuring research integrity (20 items); and (4) participants' self-assessed ethical competencies (14 items). The items were formulated as statements with responses on a 5-point Likert scale, except for questions intended to collect sociodemographic data from participants (10 items).

Content Validity Testing. The initial version of the questionnaire was sent to 10 experts who evaluated its content and provided recommendations and suggestions regarding clarity, relevance, and domain coverage. Based on the experts' feedback, 20 questions were reformulated, and additional items were included in the questionnaire: one question for the sociodemographic section, one open-ended question for each of dimensions 1–3, and two open-ended questions for dimension 4. This resulted in a revised version of the questionnaire consisting of 85 items.

Characteristics of the study participants. Analysis of the respondents' sociodemographic characteristics revealed a predominantly female profile (72.6% [95% CI: 67.1%–77.6%]), with a median age of 35 years and IQR = 32–39 (skewness > 1). Most participants were at an early stage of their research careers, with 0–5 years of research experience (73.4% [95% CI: 67.8%–78.5%]), and the majority had received prior training in research ethics (72.6% [95% CI: 67.1%–77.6%]), primarily through formal instruction sessions. This profile indicates that the sample represents early-career researchers with foundational exposure to ethical training, providing a relevant context for evaluating both the clarity and applicability of the questionnaire items.

Psychometric Analysis. The psychometric analysis was conducted on 70 items; open-ended questions and those aimed at collecting sociodemographic data were excluded from the evaluation. To identify the latent structure of the questionnaire, an exploratory factor analysis (EFA) was performed using the principal component analysis (PCA) extraction method. To assess whether the set of variables (questionnaire items) was suitable for identifying latent factors, the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was evaluated. The KMO values for individual items ranged from 0.60 to 0.95, except item 2 (which had a value of 0.33), indicating that, overall, the data were adequate for factor analysis.

According to the Kaiser criterion, 11 factors with eigenvalues values > 1were initially identified. However, to ensure a stable structure, only factors with eigenvalues > 2 were retained, resulting in six factors. The first factor explained 28.33% of the total variance, the second 14.36%, and the third 8.34%. Subsequent factors contributed smaller proportions (5.76%, 4.17%, and 3.57%, respectively). Together, these six factors accounted for approximately 64.5% of

the total variance, indicating a strong capacity of the questionnaire to capture the latent dimensions of the construct under investigation. The Scree Plot [14] confirmed this factor solution by highlighting an "elbow" after the sixth factor, where the slope of the curve levels off. Figure 1 presents the variance of the identified factors.

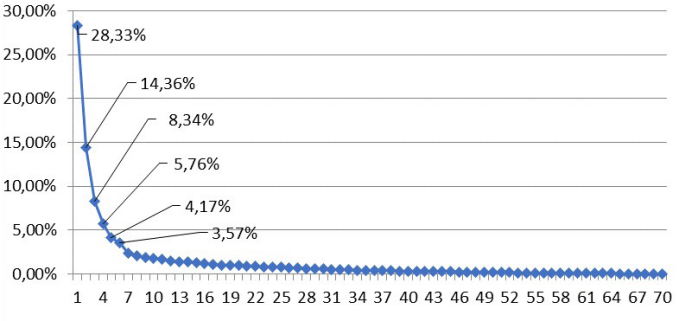

Fig. 1 Variance of the identified factors (Scree Plot).

Note: *The figure shows the eigenvalues for each extracted factor. The "elbow" after the sixth factor, where the curve levels off, supports the retention of a six-factor solution.*

The factor loading matrix was carefully examined to identify the items most strongly associated with each factor. To enhance the interpretability of the loadings for each item (Full Load Matrix), an orthogonal Varimax rotation was applied. Loadings greater than 0.40 were considered significant for inclusion in the factor structure [17, p.151]. Among the 70 items analyzed, 61 items met this threshold, while 2 items had slightly lower loadings of 0.35 and 0.37, respectively. Seven items exhibited loadings below 0.35 and were consequently excluded from further analysis. Overall, 63 items were retained for subsequent psychometric evaluation, ensuring a robust and reliable factor structure for the questionnaire.

For each identified factor, internal consistency was assessed using Cronbach's alpha, with a threshold of ≥ 0.70 considered indicative of satisfactory internal reliability [18]. Additionally, to evaluate the contribution of individual items to the overall coherence of each scale, the alpha-if-item-deleted analysis was conducted.

Analysis of Factor Loadings and Cronbach's Alpha by Factor.

Factor I – Important Research Resources. This factor encompasses items designed to assess participants' perceptions of the importance of various resources essential for conducting good health research. These include adequate funding, research infrastructure, qualified personnel, access to data and databases, clear institutional regulations, interdisciplinary collaborations, administrative and logistical support, sufficient time for research, research and ethics training programs, and mechanisms for study oversight. The items are presented in Table 1.

All items within this factor demonstrated very high factor loadings (ranging from -0.877 to -0.934), indicating a strong coherence and conceptual integrity of the construct. Internal consistency analysis confirmed excellent reliability, with a Cronbach's alpha of 0.989. Furthermore, the alpha-if-item-deleted analysis revealed values ranging from 0.987 to 0.990, with no significant fluctuations, thereby confirming that each item contributes positively to the overall consistency of the scale. These findings are summarized in Table 2.

Table 1. Factor I: *Important Research Resources*

Item	1	2	3	4	5
1. Adequate funding – for equipment, materials, and researchers' salaries.					
2. Research infrastructure – laboratories, analytical technologies, specialized software.					
3. Qualified personnel – researchers, technicians, bioethics specialists, and support staff.					
4. Access to data and databases – relevant sources, previous studies, health registries.					
5. Clear institutional research regulations, including ethical ones – compliant with international and national standards.					
6. Interdisciplinary collaborations – partnerships with other institutions and specialists from various fields.					
7. Administrative and logistical support – managing documentation, procurement, and team coordination.					
8. Sufficient time for research.					
9. Research and ethics training programs – by categories (for beginners, advanced, etc.).					
10. Oversight of study conduct (including research funding and resources).					

Note: *The subjects were asked to indicate the extent to which they agree with the following statements regarding important research resources, using the scale: 1 = not important, 2 = slightly important, 3 = moderately important, 4 = important, 5 = essential.*

Table 2. Statistical analysis of Factor I, including factor loadings and Cronbach's alpha values.

	Items	Loadings	Cronbach's alpha-if-item-deleted
Factor I	Item 1	-0,877	0,990
	Item 2	-0,928	0,987
	Item 3	-0,924	0,987
	Item 4	-0,932	0,987
	Item 5	-0,934	0,987
	Item 6	-0,907	0,988
	Item 7	-0,920	0,987
	Item 8	-0,929	0,987
	Item 9	-0,910	0,988
	Item 10	-0,906	0,988

Note: *Factor loadings represent the correlation between each item and Factor I, extracted through exploratory factor analysis using principal component extraction with varimax rotation. Cronbach's alpha-if-item-deleted indicates the internal consistency of the factor when the respective item is removed. All items were assessed on a Likert-type scale (1-5)*

Factor II – Perceived Ethical Competencies in Research

This factor encompasses items designed to evaluate researchers' self-perceived proficiency in research ethics and scientific integrity. It addresses competencies such as the application of international and national ethical regulations, protection of study participants, obtaining informed

consent, maintaining data confidentiality, responsible data management, and other related ethical practices. All items of the factor are presented in Table 3.

Table 3. Factor II: *Perceived Ethical Competencies in Research*

Item	1	2	3	4	5
1. Application of the principles of the Declaration of Helsinki in research.					
2. Application of national regulations and legislation regarding research on human subjects.					
3. Formulation of feasible study objectives and valid methodology.					
4. Adherence to the principle of equity in recruiting research participants.					
5. Identification and minimization of risks to study participants.					
6. Providing accurate and complete information to study participants and obtaining informed consent.					
7. Protection of the confidentiality of study participants' data.					
8. Completion of the research ethics committee submission dossier.					
9. Seeking advice from colleagues or the ethics committee in moral dilemmas.					
10. Reporting ethical and research integrity issues.					
11. Honest presentation of research results.					
12. Proper citation and avoidance of plagiarism in scientific publications.					
13. Application of ethical authorship principles in research.					
14. Management of conflicts of interest in research.					
15. Data management.					

Note: *The subjects are asked to evaluate their level of preparedness in the following areas/aspects of research, using the following scale: 1 = not at all prepared, 2 = slightly prepared, 3 = moderately prepared, 4 = prepared, 5 = very prepared.*

The factor loadings ranged from 0.614 to 0.857, reflecting a strong and consistent association between the items and the underlying construct. Internal consistency analysis confirmed a high level of reliability, with a Cronbach's alpha of 0.961. Additionally, the "Cronbach's alpha if item deleted" values varied between 0.956 and 0.960, indicating that each item contributes substantially to the overall scale and that none of the items compromises the coherence of the construct. This demonstrates that Factor II provides a robust measure of researchers' perceived ethical competencies in the context of scientific practice. These findings are summarized in Table 4.

Factor III – Measures Implemented to Ensure a Research Integrity Climate

This factor comprises items assessing researchers' attitudes toward the implementation of institutional measures aimed at ensuring research integrity. These include clear policies on integrity, periodic audits and sanctions, promotion of ethical behavior by leadership, management of conflicts of interest, prevention of favoritism and undue influence, plagiarism prevention, and related practices. The questions of Factor III are presented in Table 5.

Table 4. Statistical analysis of Factor II, including factor loadings and Cronbach's alpha values.

	Items	Loadings	Cronbach's alpha-if-item-deleted
Factor II	Item 1	-0,614	0,960
	Item 2	-0,682	0,959
	Item 3	-0,682	0,959
	Item 4	-0,783	0,957
	Item 5	-0,831	0,957
	Item 6	-0,841	0,957
	Item 7	-0,820	0,957
	Item 8	-0,751	0,959
	Item 9	-0,761	0,958
	Item 10	-0,804	0,958
	Item 11	-0,808	0,958
	Item 12	-0,850	0,957
	Item 13	-0,857	0,956
	Item 14	-0,833	0,957
	Item 15	-0,790	0,958

Note: *Factor loadings represent the correlation between each item and Factor II, extracted through exploratory factor analysis using principal component extraction with varimax rotation. Cronbach's alpha-if-item-deleted indicates the internal consistency of the factor when the respective item is removed. All items were assessed on a Likert-type scale (1-5)*

Table 5. Factor III: *Measures Implemented to Ensure a Research Integrity Climate*

Item	1	2	3	4	5
1. Ensuring research integrity through clear policies, monitoring, and strict sanctions.					
2. Promoting moral values in research through education and fostering an academic culture.					
3. Correct and transparent behavior of research leaders.					
4. Periodic checks to prevent data manipulation or falsification in research.					
5. Clear conflict of interest policies and their proper implementation.					
6. Clear and effective measures to prevent favoritism in research.					
7. Clear and effective policies for managing undue influences in research.					
8. Prevention of plagiarism through rigorous checks and clear, effective sanctions.					
9. Accessible and confidential mechanism for reporting incidents of research misconduct.					

Note: *The subjects were asked to indicate the extent to which the listed measures, aimed at ensuring the quality and integrity of research data, are implemented in their institution, using the scale: 1 = not implemented, 2 = limited implemented, 3 = moderately implemented, 4 = well implemented, 5 = fully implemented.*

Factor loadings ranged from 0.777 to 0.881, indicating that all items contribute significantly and coherently to defining the construct. The overall internal consistency for this factor was very high (Cronbach's alpha = 0.989), and the "Cronbach's alpha if item deleted" values ranged between 0.987 and 0.990, confirming that the removal of any single item would not substantially improve the reliability of the scale. These results demonstrate that Factor III provides a robust measure of researchers' perceptions of the implementation of institutional integrity measures. These findings are summarized in Table 6.

Table 6. Statistical analysis of Factor III, including factor loadings and Cronbach's alpha values.

	Items	Loadings	Cronbach's alpha-if-item-deleted
Factor III	Item 1	0,841	0,990
	Item 2	0,848	0,988
	Item 3	0,839	0,987
	Item 4	0,848	0,989
	Item 5	0,858	0,987
	Item 6	0,870	0,987
	Item 7	0,881	0,987
	Item 8	0,777	0,987
	Item 9	0,866	0,987

Note: *Factor loadings represent the correlation between each item and Factor III, extracted through exploratory factor analysis using principal component extraction with varimax rotation. Cronbach's alpha-if-item-deleted indicates the internal consistency of the factor when the respective item is removed. All items were assessed on a Likert-type scale (1-5)*

Factor IV – Ethical Principles and Moral Responsibilities in Research

This factor includes items assessing knowledge of ethical standards in research, such as the rights, safety, and well-being of participants, research integrity, and moral responsibility in human subjects' research. These items are presented in Table 7.

Table 7. Factor IV: *Ethical Principles and Moral Responsibilities in Research*

Item	1	2	3	4	5
1. The rights, safety, and well-being of study participants are the most important considerations in research and should take precedence over the interests of science and society.					
2. Any modification of the research protocol must be approved by the ethics committee before implementation.					
3. Researchers must inform potential study participants about the objectives, procedures, possible risks, and benefits of the study, including any additional information that may help them make an informed decision about participation.					
4. Once a person has been enrolled in a study, it is not advisable to provide information about newly identified risks to avoid causing stress.					
5. Researchers are responsible for protecting the personal data of study participants in accordance with data protection and confidentiality regulations.					
6. Researchers bear no moral responsibility for the well-being and rights of study participants if they are under scientific or financial pressure.					
7. It is impossible to ensure fair and equitable treatment for all participants in a study.					
8. Researchers are responsible for ensuring the transparency and integrity of the data obtained in studies.					
9. Undeclared conflicts of interest can compromise the integrity of research.					
10. Only individuals with professional and ethical competence should be authorized to conduct scientific research on human subjects.					

Note: *The subject is asked to indicate the extent to which they agree with the listed statements, using the scale: 1 = strongly disagree, 2 = partially disagree, 3 = neither agree nor disagree, 4 = partially agree, 5 = strongly agree.*

Factor loadings ranged from -0.353 to -0.692. Items 1 and 10 displayed relatively low loadings, indicating a weaker association with the latent construct; however, they were retained in the analysis due to their theoretical importance, as they address specific dimensions of ethical responsibilities. Overall internal consistency for this scale was satisfactory, with a Cronbach's alpha of 0.738. The "Cronbach's alpha if item deleted" values ranged from 0.697 to 0.733, which is acceptable, although some items had a lower impact on the overall homogeneity of the scale. These results suggest that Factor IV captures key aspects of ethical principles and moral responsibilities, even if certain items contribute less strongly to the latent construct. These findings are summarized in Table 8.

Table 8. Statistical analysis of Factor IV, including factor loadings and Cronbach's alpha values.

	Items	Loadings	Cronbach's alpha-if-item-deleted
Factor IV	Item 1	-0,374	0,725
	Item 2	0,572	0,715
	Item 3	-0,535	0,721
	Item 4	0,692	0,697
	Item 5	-0,452	0,733
	Item 6	0,673	0,698
	Item 7	0,639	0,701
	Item 8	-0,430	0,723
	Item 9	-0,436	0,722
	Item 10	-0,353	0,728

Note: *Factor loadings represent the correlation between each item and Factor IV, extracted through exploratory factor analysis using principal component extraction with varimax rotation. Cronbach's alpha-if-item-deleted indicates the internal consistency of the factor when the respective item is removed. All items were assessed on a Likert-type scale (1-5)*

Within the factor, some loadings are positive while others are negative, which can be explained by the existence of an inverse relationship between those items and the latent factor. In factor analysis, the sign of a loading is relative; what matters is the absolute magnitude of the loading, which indicates the strength of the relationship between the item and the factor.

Factor V – Accessible Research Resources

This factor comprises items assessing researchers' perceptions of the accessibility of resources necessary for conducting research. The factor items are presented in Table 9.

Factor loadings ranged from 0.586 to 0.739, indicating that all items contribute significantly to defining this construct. In comparison, Factor I, which evaluates the perceived importance of the same resources, exhibited substantially higher loadings. This discrepancy may be explained by the fact that researchers generally recognize the intrinsic value of essential research resources. In contrast, their perception of accessibility depends on individual experience and the institutional context in which they operate. Internal consistency for this factor was excellent, with a Cronbach's alpha of 0.912. The "alpha if item deleted" values ranged from 0.898 to 0.912, confirming that each item contributes meaningfully to the overall reliability of the scale. These findings are summarized in Table 10.

Table 9. Factor V: *Accessible Research Resources*

Item	1	2	3	4	5
1. Adequate funding – for equipment, materials, and researchers' salaries.					
2. Research infrastructure – laboratories, analytical technologies, specialized software.					
3. Qualified personnel – researchers, technicians, bioethics specialists, and support staff.					
4. Access to data and databases – relevant sources, previous studies, health registries.					
5. Clear institutional research regulations, including ethical ones – compliant with international and national standards.					
6. Interdisciplinary collaborations – partnerships with other institutions and specialists from various fields.					
7. Administrative and logistical support – managing documentation, procurement, and team coordination.					
8. Sufficient time for research.					
9. Research and ethics training programs – by categories (for beginners, advanced, etc.).					
10. Oversight of study conduct (including research funding and resources).					

Note: *Participants were asked to indicate the extent to which the listed research resources are accessible to them, using the scale: 1 = not accessible, 2 = limited access, 3 = moderate access, 4 = good access, 5 = full access.*

Table 10. Statistical analysis of Factor V, including factor loadings and Cronbach's alpha values.

	Items	Loadings	Cronbach's alpha-if-item-deleted
Factor V	Item 1	0,626	0,911
	Item 2	0,655	0,906
	Item 3	0,675	0,901
	Item 4	0,684	0,902
	Item 5	0,613	0,900
	Item 6	0,693	0,899
	Item 7	0,693	0,899
	Item 8	0,586	0,912
	Item 9	0,739	0,898
	Item 10	0,711	0,898

Note: *Factor loadings represent the correlation between each item and Factor V, extracted through exploratory factor analysis using principal component extraction with varimax rotation. Cronbach's alpha-if-item-deleted indicates the internal consistency of the factor when the respective item is removed. All items were assessed on a Likert-type scale (1-5)*

Factor VI – Important Measures to Ensure a Research Integrity Climate

This factor comprises items assessing the perceived importance of implementing institutional and organizational measures to ensure quality and integrity in research. The factor items are included in Table 11.

Factor loadings were strong, ranging from 0.812 to 0.868, indicating a robust association between each item and the underlying construct. Internal consistency for this factor was very high, with a Cronbach's alpha of 0.974. The "alpha if item deleted" values ranged from 0.969 to 0.973, demonstrating that the removal of any single item would not significantly improve the reliability of the scale. These findings are summarized in Table 12.

Table 11. Factor VI: *Important Measures to Ensure a Research Integrity Climate*

Item	1	2	3	4	5
1. Ensuring research integrity through clear policies, monitoring, and strict sanctions.					
2. Promoting moral values in research through education and fostering an academic culture.					
3. Correct and transparent behavior of research leaders.					
4. Periodic checks to prevent data manipulation or falsification in research.					
5. Clear conflict of interest policies and their proper implementation.					
6. Clear and effective measures to prevent favoritism in research.					
7. Clear and effective policies for managing undue influences in research.					
8. Prevention of plagiarism through rigorous checks and clear, effective sanctions.					
9. Accessible and confidential mechanism for reporting incidents of research misconduct.					

Note: *Participants were asked to indicate which of the listed measures can be considered important for ensuring the quality and integrity of research data, using the scale: 1 = not important, 2 = slightly important, 3 = moderately important, 4 = important, 5 = essential.*

Table 12. Statistical analysis of Factor VI, including factor loadings and Cronbach's alpha values.

	Items	Loadings	Cronbach's alpha-if-item-deleted
Factor VI	Item 1	0,812	0,971
	Item 2	0,823	0,970
	Item 3	0,840	0,970
	Item 4	0,842	0,970
	Item 5	0,859	0,970
	Item 6	0,856	0,971
	Item 7	0,862	0,969
	Item 8	0,868	0,973
	Item 9	0,861	0,970

Note: *Factor loadings represent the correlation between each item and Factor VI, extracted through exploratory factor analysis using principal component extraction with varimax rotation. Cronbach's alpha-if-item-deleted indicates the internal consistency of the factor when the respective item is removed. All items were assessed on a Likert-type scale (1-5)*

Overall, these results indicate that the questionnaire demonstrates robust psychometric properties, with high factor loadings and internal consistency across most factors, supporting its validity for assessing ethical knowledge, attitudes, and perceptions among early-career researchers.

Discussion

The exploratory factor analysis of the questionnaire revealed a multidimensional structure comprising six primary factors, collectively explaining approximately 64.5% of the total variance. Factor loadings were generally high, indicating strong associations between items and their respective constructs. Internal consistency across factors ranged from good to excellent, with Cronbach's alpha values between 0.738 and 0.989.

However, the literature notes that alpha values exceeding 0.95 may indicate potential item redundancy, suggesting that respondents might perceive certain items as overly similar [19]. For instance, within Factor I (Cronbach's alpha = 0.989), items such as adequate funding and administrative support could be interpreted by participants as closely related, reflecting overlapping aspects of the same construct. To minimize potential interpretation ambiguities, each item within the factor was accompanied by a clarifying description. For example, "Adequate funding" referred specifically to resources for equipment, materials, and researcher salaries, whereas "Administrative and logistical support" addressed management of documentation, procurement, and team coordination. While reducing the number of items could potentially decrease redundancy, maintaining a broad range of items ensures that multiple dimensions of the construct are captured. This approach aligns with the exploratory objective of the questionnaire, allowing for a comprehensive assessment of researchers' perceptions and experiences.

Several questionnaires have been developed to assess research ethics and integrity [20prerequisite to goals of subject protection and integrity in research practice. This article presents an update of a 2006 summary of measurement instruments in research ethics with psychometric information in the years 2008–2012. A review of 25 instruments identified seven used in the time period 2008–2012 and which had accumulated at least one study of its psychometric qualities beyond its developmental phase. Many of these instruments had been accumulating psychometric information over more than a decade. Two additional but still underdeveloped instruments addressing important bioethical issues – coercion and therapeutic misconception – are included because they address important issues in research ethics. Bioethicists use a wide range of methods for knowledge development and verification; each method should meet stringent standards of quality. Measurement instruments that meet these standards have the potential to greatly ease the work of institutional review boards and other regulatory bodies as well as to enhance empirical work on human research ethics."},"container-title":"Research Ethics","DOI":"10.1177/1747016114538963","ISSN":"1747-0161, 2047-6094","issue":"3","journalAbbreviation":"Research Ethics","language":"en","page":"141-150","source":"DOI.org (Crossref]. For example, Thrush et al. (2007) created a questionnaire to evaluate organizational culture in terms of research integrity, which demonstrated strong content validity (CVI = 0.90) after the removal of problematic items [21].

The SORC questionnaire is considered the first standardized instrument developed to assess organizational climate regarding research integrity [22]. Initially applied among academic researchers in the United States, it has subsequently been adapted and implemented in other cultural contexts [23, 24]. The instrument demonstrated strong reliability, with internal consistency coefficients (Cronbach's alpha) ranging from 0.80 to 0.87.

The questionnaire developed by Mavrinac et al. (2010) is an instrument designed to measure students' attitudes toward plagiarism across three factors: positive attitudes (e.g., perceived acceptability of plagiarism), negative attitudes (e.g., moral disapproval of plagiarism), and subjective norms (e.g., perceived social expectations). Internal consistency coefficients demonstrated good reliability for all three factors (0.83, 0.79, and 0.85), as confirmed through confirmatory factor analysis [25].

The Research Conduct Attitudes Scale is a two-factor instrument assessing (a) the perceived acceptability of ethically questionable research practices and (b) general attitudes toward unethical research behavior [26]. Both factors demonstrated good internal consistency, with Cronbach's alpha values exceeding 0.75.

A valid instrument for assessing researchers' knowledge and attitudes regarding participant rights in studies and research ethics education was developed in Saudi Arabia by Al-Madaney and Fässler [27]. The questionnaire's overall content validity indices exceeded 0.78 for all sections of the questionnaire; the split-half reliability coefficient was 0.755 for knowledge items, and Cronbach's alpha for the attitude scale was 0.77.

All of the studies mentioned share a common objective of operationalizing complex ethical constructs, such as knowledge and attitudes toward plagiarism, organizational climate, responsible conduct of research (RCR), and respect for human subjects' rights. At the same time, these instruments differ in their focus: some target specific domains (e.g., plagiarism), whereas others address broader constructs, such as organizational climate or research integrity culture.

Our study complements existing instruments and contributes to the field through its exploratory nature, targeting multidimensional aspects of research ethics, including the assessment of researchers' knowledge of ethical standards, their perceptions and attitudes toward the research environment, and their confidence in their own moral competencies. The internal consistency of the present questionnaire was very high for the majority of factors, which is comparable to previously published instruments. This questionnaire aligns with national trends in the development of validated instruments in the field of health sciences that are contextually relevant [28].

Even if the study presents a robust, multidimensional instrument for assessing researchers' knowledge and perceptions of ethics and integrity, its generalizability may be limited by the sample characteristics. Future research is recommended to validate the instrument in larger and more diverse populations and to conduct confirmatory factor analysis (CFA) to assess the factor structure of the scale. The questionnaire does not directly assess current ethical practices, highlighting the potential need for more specific instruments to assess distinct ethical behaviors in future studies.

Conclusions

The study demonstrated that the tested questionnaire is a robust and valid instrument for assessing knowledge and attitudes regarding research ethics and integrity in the health sciences. It can serve as an internal audit tool to evaluate the research integrity climate, researchers' satisfaction with available resources, and the implementation of institutional policies. Additionally, the instrument can inform the planning of targeted training programs and professional development initiatives, identifying areas where researchers exhibit insufficient knowledge or understanding of research ethics.

Competing interests

None declared.

Authors' contributions

All the authors participated in the study design, critically reviewed the work, and approved the final version of the manuscript**.**

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Ethics approval and participant consent

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy in the Republic of Moldova, Minutes No.70, dated August 08, 2024. Implicit consent was obtained.

Provenance and peer review

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Use of AI in manuscript preparation.

AI (GPT-4) was used solely to assist in drafting and refining the text; the authors conducted all analyses, interpretations, and conclusions.

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RESEARCH ARTICLE

The iCREATE registry: a model for strengthening injury surveillance in the Republic of Moldova

Angela Cazacu-Stratu[1*], Svetlana Cociu[1], Patricia Maria Marga[2], Dumitru Cartaleanu[1], Diana Dulf[2], Serghei Cebanu[1]

[1]Hygiene Discipline, Department of Preventive Medicine, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova
[2]Department of Public Health, Faculty of Political, Administrative and Communication Sciences, *Babeș-Bolyai* University, Cluj-Napoca, Romania

ABSTRACT

Introduction. Injuries represent a major public health issue, causing approximately 16,000 deaths globally each day (10% of all deaths), which is 32% more than the combined total caused by malaria, tuberculosis, and HIV/AIDS. Over the past 15 years, the WHO and regional initiatives have supported the piloting of trauma registries in low- and middle-income countries as essential tools for monitoring, planning, and prevention.

Objective. This article aims to assess the feasibility and utility of implementing a national trauma registry in the Republic of Moldova, in order to improve injury surveillance and emergency service planning.

Materials and methods. In 2018, the pilot iCREATE trauma registry was tested for the first time in three countries: Moldova, Armenia, and Georgia. The data collection instrument was developed based on WHO recommendations, ICD-10, and IDB-JAMIE standards under the guidance of partners from the University of Iowa and Babeș-Bolyai University, Cluj-Napoca. All trauma cases from the Institute of Emergency Medicine and the *Valentin Ignatenco* Municipal Clinical Children's Hospital in Chișinău were included in the registry.

Results. The analyzed sample consists of 7,942 individuals, predominantly male (57.3%). The most represented age groups were 19-29 years (17.8%) and 30-39 years (17.6%), while individuals aged 70 and above accounted for 11% of the total. Most incidents occurred in urban areas (76.9%). Of the total patients, 52.4% were treated and discharged, while 37.5% required hospitalization. Injuries occurred primarily at home (55.4%) and on public roads (24.7%). The leading mechanism of injury was falls (68.2%), followed by other causes (12.2%) and cut/pierce injuries (10.0%). The most frequently affected body regions were the head/skull (12.5%) and knee (11.8%), followed by the hip (6.7%) and wrist (6.4%). Fractures were the most common injury type (34.5%), followed by contusions (23.0%) and open wounds (14.7%). Several gaps in data collection and reporting were identified and should be considered in future efforts to enhance trauma surveillance.

Conclusions. The data highlight the need to develop a national trauma registry as an essential tool for monitoring, prevention, and effective intervention, alongside health promotion campaigns targeting vulnerable groups and the involvement of relevant stakeholders.

Keywords: registry, model, trauma, prevention.

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***Corresponding author: Angela Cazacu-Stratu**, PhD, associate professor
Hygiene Discipline, Department of Preventive Medicine
Nicolae Testemițanu State University of Medicine and Pharmacy

Key messages

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

Although injuries represent a major public health issue in the Republic of Moldova, to date, there have been no systematized and continuous national data on the types, causes, and consequences of trauma. Moreover, the impact of a national trauma registry on injury monitoring and prevention has not yet been evaluated.

165, Ştefan cel Mare si Sfânt blvd, Chişinău, Republic of Moldova, MD-2004
e-mail: angela.cazacu@usmf.md

Authors' ORCID IDs
Angela Cazacu-Stratu – https://orcid.org/0000-0002-0238-3063
Svetlana Cociu – https://orcid.org/0000-0001-8209-815X
Patricia Maria Marga – https://orcid.org/0000-0003-3614-4711
Dumitru Cartaleanu – https://orcid.org/0009-0009-9510-0007
Diana Dulf – https://orcid.org/0000-0002-8172-4961
Serghei Cebanu – https://orcid.org/0000-0002-2493-4882

The research hypothesis
The implementation of a national trauma registry using the iCREATE model will enable the collection of standardized, detailed, and comparable data on injuries, thereby improving surveillance, supporting evidence-based decision-making, and facilitating the development of effective prevention and intervention policies.

The novelty added by manuscript to the already published scientific literature
To our knowledge, this is the first study to describe the pilot implementation of an international trauma registry in Moldova, showcasing its role in strengthening national injury surveillance and prevention initiatives.

Introduction

Injuries are a major global public health concern, accounting for approximately 10% of the world's deaths each year and leaving millions with long-term disabilities [1]. Accurate and timely data are essential to understand the burden of injuries, identify risk factors, and develop effective prevention strategies. However, many countries, especially low- and middle-income countries, face significant challenges in collecting and analyzing high-quality injury data due to fragmented health information systems and underreporting [2].

An injury registry serves as a systematic and continuous data collection tool designed to capture detailed information on injuries, including their causes, severity, and outcomes. Registries play a crucial role in strengthening injury surveillance by providing comprehensive, standardized, and comparable data that can inform policy, guide resource allocation, and evaluate intervention effectiveness [3-5]. Moreover, they facilitate international comparisons and contribute to global efforts in injury prevention and trauma care improvement [6].

Injury registries represent a cornerstone solution by providing systematic, continuous, and standardized data collection on injury patterns, mechanisms, and outcomes [7]. Recent evidence underscores the transformative potential of registries in strengthening injury surveillance: in Brazil, the establishment of a national trauma registry in 2021 has enabled integration across hospitals, prehospital services, and law enforcement data. Initial evaluations have shown improved policy responsiveness and identification of regional injury trends [8]. The National Trauma Registry of Norway (NTR), covering all trauma-receiving hospitals, has demonstrated significant contributions to quality improvement and benchmarking through detailed tracking of more than 78,000 cases from 2015 to 2023 [9]. In the United States, linkage between the Iowa trauma registry and workers' compensation data has enhanced the identification of agricultural injuries and informed targeted prevention efforts [10].

Implementing robust injury registries represents a strategic model to enhance national and regional surveillance capacities. By integrating clinical, epidemiological, and demographic data, registries enable a better understanding of injury patterns and support evidence-based decision-making. This article explores the importance of injury registries as a cornerstone of modern injury surveillance systems and highlights best practices and lessons learned from successful implementations.

Material and methods

Data and study design. This study examines patients treated for injuries who presented to the Emergency Departments of two major tertiary care hospitals in Chişinău, the capital of the Republic of Moldova (population ~800,000), during 2018: the Emergency Medicine Institute (EMI) and the *Valentin Ignatenco* Municipal Children's Hospital (MHC). The data were sourced from the iCREATE Injury Registry, piloted for the first time in Moldova. The data collection instrument – a structured questionnaire – was developed in alignment with international standards, drawing on recommendations from the World Health Organization (WHO), the International Classification of Diseases, 10th Revision (ICD-10), the Injury Database-Joint Action on Monitoring Injuries in Europe (IDB-JAMIE) project, and the Iowa Emergency Department Registry. The pilot was nationally coordinated by the *Nicolae Testemiţanu* State University of Medicine and Pharmacy, and internationally supported by the Department of Public Health of *Babeş-Bolyai* University (Cluj-Napoca, Romania) and the College of Public Health at the University of Iowa (USA) to strengthen research capacity on injury and violence prevention in three low- and middle-income countries: Armenia, Georgia, and Moldova. The research project, "iCREATE: Increasing Research Capacity in Eastern Europe," was approved by the Ethics Committee of Nic*olae Testemiţanu* State University of Medicine, decision No. 43 of March 15, 2018.

Settings and population. A sample of 7,870 patients of all ages, with different types of injuries who received care at EMI and MHC in Chişinău between 1 March 2018 and 28 February 2019, was included in the analysis.

Data collection process. Data were manually extracted from patients' medical records by four resident physicians who had received specific training in data collection and coding protocols. The variables collected were defined ac-

cording to the iCREATE Injury Database Project framework. Following data extraction, the information was entered into electronic databases using the REDCap (Research Electronic Data Capture) platform. The study questionnaire comprised a core section and five supplementary modules, incorporating both open- and closed-ended items. The core section gathered general demographic data, medical care details, contextual information about the injury event, and specifics regarding injury type and anatomical location. The five supplementary modules addressed distinct categories of injuries, including those resulting from road traffic incidents, self-harm, interpersonal violence, sports activities, and traumatic brain injuries (TBI) within the preceding 12 months.

Statistical analysis. The statistical analysis was performed by evaluating the quantitative and qualitative characteristics of the patients enrolled in the study, according to the indicators provided in the iCREATE Registry Database. Data were analyzed using Microsoft Excel and SPSS 20.

Results

Sample characteristics. A total of 7,870 patients were included in the dataset. The age distribution, categorized by a binary variable, shows that 11.3% (n = 889) of patients were aged 0-17 years (children and adolescents), while the majority, 88.7% (n = 6,971), were aged 18 years and above (adults): adults aged 20-39 years (33.8%), followed by middle-aged adults (26.2%) and seniors aged 60 and above (25.9%).

Regarding sex distribution (sex variable), 57.2% (n = 4,504) of the patients were identified as male and 41.6% (n = 3,275) as female.

Nearly two-thirds of the patients were not employed at the time of data collection (64.0%, n = 5,036), while 21.3% (n = 1,679) were employed in skilled labor. Only 5.0% (n = 393) were employed in professional occupations, and 1.3% (n = 99) in manual labor. Regarding other social roles, 22.9% (n = 1,802) were classified as retirees, 11.1% (n = 877) were students, and 0.3% (n = 21) were homemakers (Table 1).

General characteristics of injuries among patients treated at the Emergency Department. Most injuries across all age groups occurred in urban areas, accounting for 6,049 cases (Table 2). The proportion of urban injuries was consistently high, particularly among adults aged 20-39 (n = 2,081) and middle-aged adults 40-59 (n = 1,526). Another 1,707 injuries were recorded in rural areas, and only 6 cases in metropolitan zones.

The overwhelming majority of injuries were unintentional (n = 7,123; 90.6%) across all age categories. Assault-related injuries were the most common intentional injuries (n = 548), particularly affecting teens (n = 47) and young adults (n = 285). Intentional self-harm was rare (n = 35), primarily occurring in the adult population. Other types of violence, as well as undetermined or unspecified intents, represented a small proportion of cases.

Falls were the leading injury mechanism, with 5,282 cases (67.1%), especially common among seniors (n = 1,627) and middle-aged adults (n = 1,388). Cut or piercing injuries (n = 845) and road traffic injuries (n = 370) were more prevalent among adults and teens. Other mechanisms (burns, poisoning) were relatively rare. Notably, 398 cases had an unknown mechanism, particularly among children and adolescents.

Table 1. Demographic characteristics of iCREATE Registry patients

Age in years		N	%
	0-5	236	3.0
	6-11	321	4.1
	12-17	332	4.2
	18-23	742	9.4
	24-29	750	9.5
	30-35	951	12.1
	36-41	662	8.4
	42-47	602	7.6
	48-53	553	7.0
	54-59	680	8.6
	60-65	751	9.5
	65+	1,287	16.4
	Total	7,867	100.0
	Missing	3	0.0
Total		7,870	100.0
Sex			
	Male	4,504	57.2
	Female	3,275	41.6
	Unknown	87	1.1
	Total	7,866	99.9
	Missing	4	0.1
Total		7,870	100.0
Employment			
	None	5,036	64.0
	Manual	99	1.3
	Skilled	1,679	21.3
	Professional	393	5.0
	Unknown	497	6.3
	Total	7,704	97.9
	Missing	166	2.1
Total		7,870	100.0
Another social role			
	Homemaker	21	0.3
	Retiree	1,802	22.9
	Student	877	11.1
	Not applicable	4,115	52.3
	Total	6,815	86.6
	System	1,055	13.4
Total		7,870	100.0

Note: *iCREATE: Increasing Research Capacity in Eastern Europe – abbreviation of the project;*
N –absolute number; % - percent. Specific statistical indicators (χ^2 (Chi-square) – Pearson's chi-squared test statistic; p-value – statistical significance indicator; Likelihood Ratio – alternative to Pearson's χ^2; Fisher's Exact Test – exact test used for small sample sizes) were applied in the five supplementary modules of the iCREATE Registry.

Table 2. General characteristics of injuries among patients treated at the Emergency Department

Injury particularities / Age groups		Infant (0-1 y/o)	Toddler (2-4 y/o)	Child (5-12 y/o)	Teen (13-19 y/o)	Adult (20-39 y/o)	Middle age adult (40-59 y/o)	Senior (60+)	Total
	N	N	N	N	N	N	N	N	
Injury occurrence	Urban area	63	106	385	398	2,081	1,526	1,490	6,049
	Rural area	7	10	30	72	548	509	531	1,707
	Metropolitan area	0	0	0	0	4	2	0	6
	Unknown	2	4	10	17	25	28	19	105
Total		72	120	425	487	2,658	2,065	2,040	7,867
Intent	Unintentional	72	116	412	424	2,273	1,875	1,951	7,123
	Intentional self-harm	0	1	0	2	26	2	4	35
	Assault	0	2	6	47	285	148	60	548
	Other violence	0	0	0	1	1	2	1	5
	Undetermined intent	0	0	3	4	4	2	3	16
	Other specified intent	0	0	0	0	2	1	2	5
	Unspecified intent	0	1	4	9	69	35	18	136
Total		72	120	425	487	2,660	2,065	2,039	7,868
Injury mechanism	Road traffic injuries	2	2	14	28	154	107	63	370
	Fall	61	90	295	261	1,560	1,388	1,627	5,282
	Cut/pierce	2	3	21	61	394	214	150	845
	Poisoning	0	0	0	1	1	2	0	4
	Thermal mechanism (burn/scald)	0	0	0	0	2	2	0	4
	Other	5	11	42	81	427	256	144	966
	Unknown	2	14	53	55	122	96	56	398
Total		72	120	425	487	2,660	2,065	2,040	7,869
Occurrence place	home	55	71	169	159	1,264	1,208	1,459	4,385
	institutions	1	7	47	43	23	20	12	153
	sports and recreational areas	2	8	37	68	227	50	24	416
	streets and highways	6	9	58	108	906	584	411	2,082
	commercial areas	1	2	2	2	25	19	8	59
	countryside	0	0	2	4	20	24	22	72
	other/unspecified	7	23	110	103	195	160	104	702
Total		72	120	425	487	2,660	2,065	2,040	7,869
Mode of transport	by ambulance	39	54	160	208	1,017	823	1,095	3,396
	by private transport	26	62	219	244	1,579	1,160	849	4,139
	other/unknown	7	4	46	35	64	82	96	334
Total		72	120	425	487	2,660	2,065	2,040	7,869
Treatment and follow-up	treated and released	2	8	33	208	2,086	1,401	1,094	4,832
	treated and admitted to the hospital	69	112	390	273	523	631	917	2,915
	other/unknown	0	0	0	1	26	17	10	54
Total		71	120	423	482	2635	2,049	2,021	7,801
Activity when injured	Paid work	0	0	1	3	48	46	21	119
	Unpaid work (includes domestic/ home construction, repair)	0	0	1	12	292	300	199	804
	Education	0	4	33	46	18	4	0	105
	Sports and exercise during leisure time or professional sports	0	2	10	56	179	32	3	282
	Leisure or play	14	36	109	79	263	139	219	859
	Vital activity	45	57	154	108	844	755	951	2,914
	Being taken care of	0	0	0	0	4	2	4	10
	Travelling not elsewhere classified	1	1	18	51	476	357	280	1,184
	Other	2	1	8	13	39	35	38	136
	Unspecified activity	10	17	89	110	491	385	305	1,407
Total		72	118	423	478	2,654	2,055	2,020	7,820

Note: *N –absolute number; specific statistical indicators (χ^2 (Chi-square) – Pearson's chi-squared test statistic; p-value – statistical significance indicator; Likelihood Ratio – alternative to Pearson's χ^2; Fisher's Exact Test – exact test used for small sample sizes) were applied in the five supplementary modules of the iCREATE Registry.*

Injuries occurring at home dominated (55.7%), especially among older adults (55+ years). The second leading location was the street or highway (26.5%), particularly among middle-aged adults (44-65 years). Sports and recreational injuries (5.3%) were more common among younger adults, while institutional injuries (schools, prisons, or long-term care) accounted for 1.9% of cases. The injury location was unspecified in 614 cases (7.8%).

Private transport (52.6%) was the most common mode of reaching medical care, followed by ambulance use (43.2%). Ambulance transport increased at the extremes of age, especially among young children (0-10 years) and older adults (65+ years). Middle-aged individuals (44-65 years) predominantly used private transport, possibly reflecting accessibility, self-reliance, or lower perceived severity of injury.

Most cases were treated and released (n = 4,832; 61.9%), while over one-third (37.4%) required hospital admission, suggesting a substantial burden of moderate to severe injuries. Children and young adults (0-32) were more likely to be hospitalized, with low proportions treated and released (under 8%). Middle-aged adults (44-54 years) had the highest rate of outpatient treatment (nearly 80%). Older adults (65+) had higher admission rates: 917 admitted vs. 1,094 released (approximately 46% admitted).

The most frequently reported activity during injury was "vital activity" (e.g., walking, moving around), with 2,914 cases (37.3%), especially common among seniors (n = 951) and adults aged 20–59 years. Leisure and play activities accounted for 859 cases, mostly among children and seniors. Unpaid work was reported in 804 cases, while paid work was less common (n = 119). Sports-related activities (n = 282) were more prevalent in the 20–39 age group. Travel-related injuries accounted for 1,184 cases (15.1%), and 1,407 cases had unspecified activity.

Injury particularities. The most frequent injury types across all age groups were fractures, with a total of 2693 cases (34.2%), especially prevalent among seniors (n = 951), middle-aged adults (n = 722), and adults aged 20-39 (n = 630) (Table 3). Contusions and bruises were recorded in 1,816 cases (23.1%), most commonly among adults aged 20-39 (n = 690). Sprains and strains accounted for 1,188 cases (15.1%), again mainly affecting working-age adults. Open wounds and abrasions represented 1,162 cases (14.8%), with the highest frequency in the 20-39 age group. Concussions or brain injuries occurred in 409 patients, more often in children and older adults. Injuries to muscles, tendons, or nerves were recorded in 190 cases, notably among adults and middle-aged individuals. Dislocations/subluxations were relatively rare (n = 51), as were multiple injuries (n = 88) and injuries to internal organs (n = 3).

Table 3. Injury particularities among age group

Type of injury/ age group	Infant (0-1 y/o)	Toddler (2-4 y/o)	Child (5-12 y/o)	Teen (13-19 y/o)	Adult (20-39 y/o)	Middle-aged adult (40-59 y/o)	Senior (60+)	Total
	N	N	N	N	N	N	N	N
Contusion, bruise	35	37	87	130	690	446	391	1,816
Open wound and abrasion	3	9	32	68	527	298	225	1,162
Fracture	9	47	199	135	630	722	951	2,693
Dislocation and subluxation	1	2	18	6	8	7	9	51
Sprain and strain	0	3	9	64	531	329	252	1,188
Concussion/brain injury	20	16	58	38	114	93	70	409
Foreign body	0	1	2	0	5	2	2	12
Burns and scalds	0	0	0	0	0	2	0	2
Injury to muscles, tendons, blood vessels, and nerves	2	3	13	28	59	64	21	190
Injury to internal organs	0	0	0	0	2	1	0	3
Poisoning	0	0	0	1	0	0	0	1
Multiple injuries	0	0	0	4	30	25	29	88
Other	2	2	6	13	59	74	88	244
Unknown	0	0	1	0	5	2	2	10
Total	72	120	425	487	2,660	2,065	2,040	7,869

Note: *N – absolute number; specific statistical indicators (χ^2 (Chi-square) – Pearson's chi-squared test statistic; p-value – statistical significance indicator; Likelihood Ratio – alternative to Pearson's χ^2; Fisher's Exact Test – exact test used for small sample sizes) were applied in the five supplementary modules of the iCREATE Registry.*

Referring to the injured body part, injuries to the head and skull were the most reported across all age groups, totaling 977 cases (12.4%), with a notably higher frequency among infants, toddlers, and young children. Injuries to the upper extremities, particularly fractures and trauma to the fingers (n = 508), hands (n = 392), and wrists (n = 388), were highly prevalent, especially among adolescents and working-age adults. Lower limb injuries also constituted a significant burden: the knee was involved in 527 cases, the ankle in 927 cases, and the lower leg in 318 cases. Other body regions also showed notable patterns but with fewer cases. Chest wall injuries (n = 329) were more frequent among adults. Pelvic (n = 48) and hip injuries (n = 249) showed an age-related increase, being disproportionately

higher among older adults and seniors. Multiple body part injuries were recorded in 96 cases, mostly in adults. Spinal injuries (thoracic/lumbar) were observed in 200 cases, with a gradual increase in frequency in older age groups.

Out of the 7,870 patients included in the dataset, the vast majority, 94.5% (n = 7,221), sustained a single injury treated in the emergency department.

Transport module. Of the total recorded cases, 348 (4.4%) contained valid information on the mode of transport involved. The most frequently reported mode of transportation was light motor vehicles, accounting for 43.1% of the cases. Pedestrians represented the second-largest group (27.3%), followed by users of two-wheeled motor vehicles (11.8%). All other transportation categories, such as bicycles, heavy motor vehicles, and non-motorized devices, each accounted for less than 3% of cases. Analyzing transportation mode by age group, children aged 0-10 years were most often involved as pedestrians or as passengers in light motor vehicles; adolescents and young adults (11-32 years) were more frequently associated with bicycles, two-wheeled motor vehicles, and light motor vehicles, and among older adults (65+ years), pedestrian involvement remained prominent. The results indicate a significant association between age group and transportation mode: χ^2 = 86.221, p = 0.015, Likelihood Ratio: p = 0.017, Fisher's Exact Test: p = 0.004, meaning that the distribution of transportation modes varies significantly across age groups and that age influences how individuals move through the environment and which type of transportation they are likely to use when involved in an incident.

The most frequently injured individuals were drivers, riders, or operators, accounting for 36.6% of the cases. Passengers represented 30.6%, and pedestrians made up 28.3%. When examining the injured roles across age groups, several age-related trends emerge: children (0-10 years) were more often injured as passengers (n = 9), with a small proportion as pedestrians; adolescents and young adults (11-32 years) were injured more often in driver/operator roles; middle-aged adults (33-54 years) had the highest involvement as drivers/riders, while pedestrians and passengers were more evenly distributed across age groups; older adults (65+ years) were less represented overall, but when involved, were primarily pedestrians or passengers. The role in transport-related injuries significantly varied by age group (χ^2 = 69.903, p < 0.001).

The most frequent counterpart was a light motor vehicle, involved in 52.5% of the incidents, and across all age groups (11 to 65+ years), it appeared as the most frequent counterpart.

Out of 333 valid cases analyzed for seatbelt use, only 25.5% (n = 85) of individuals were reported to have worn a seatbelt at the time of the incident, while 15.3% (n = 51) did not. Seatbelt use was highest among adults aged 22 to 43 years and lowest among children (0-10 years) and older adults (65+ years). The relationship between age and seatbelt use was statistically significant (χ^2 = 38.462, p = 0.000).

The overall use of child restraints was extremely low: only 7.3% (n = 21) reported using them, while 10.4% (n = 30) did not. Most cases of child restraint use were concentrated in the 0-10-year age group, with limited use among adolescents and young adults.

For helmet use, 309 valid responses were available. Of these, 20.1% (n = 62) of individuals reported wearing a helmet, while 12.6% (n = 39) did not. Helmet use was most common among individuals aged 11 to 32 years, a group likely to include cyclists, motorcyclists, and scooter riders.

Intentional self-harm module. Only 33 valid cases (0.4% of the total) had identified proximal risk factors. Most of the dataset (99.6%, n = 7,837) had missing data for this variable. Analyzing the proximal risk factors (individual-level circumstances close in time to an event, possibly a suicide attempt or other critical health event) in relation to age groups, over 40% of the identified risks were unspecified. Although the numbers are small, psychological conditions and unspecified risks tended to be reported more often in younger to middle-aged adults (22-43 years). There was no statistically significant association between age groups and the type of proximal risk factor in this dataset.

Among the 33 valid cases, only 1 individual (3%) had a recorded history of previous self-harm, and this person was aged 33-43 years. The majority of responses were unknown (75.8%), with the age group 22-32 having the most "unknown" responses.

Violence module. Only 525 cases (6.7%) had valid data regarding the victim's relationship to the perpetrator. Violence was most frequently perpetrated by strangers (57.9%) and acquaintances/friends (19.8%), while intimate partner or family violence represented a smaller but significant proportion. Age plays a decisive role: younger individuals (22-43 years) were predominantly victims of stranger violence, whereas older adults experienced more family- or intimate partner-related violence. There was a statistically significant association between age group and victim–perpetrator relationship (p<0.001).

Among 503 valid cases where the sex of the perpetrator was known, male individuals were overwhelmingly the primary aggressors, involved in 69% of cases, while female perpetrators represented only 3%. Statistical analysis showed no significant association between the perpetrator's sex and the age group involved (p > 0.05).

Adult perpetrators (25-64 years) were the primary aggressors, responsible for nearly 38% of recorded cases, across the 22-54 years victim/incident age range, peaking in the 33-43 years (n = 50) and 44-54 years (n = 39) groups. Adolescent perpetrators were most frequently associated with victims aged 11–21 years (n = 17) and 44-54 years (n = 3). Elderly and child perpetrators were rare, with a sporadic distribution across all age groups.

Among 533 valid cases with a recorded assault context, the vast majority (84.4%) were classified as physical assaults, with a marked concentration among young and middle-aged adults. Other contexts, such as robbery, gang violence, or sexual assault, were relatively rare and inconsistently distributed across age groups. There was no sta-

tistically significant association between the victim's age group and the context in which the assault occurred.

Sport module. Out of 7,870 total cases, only 271 cases (3.4%) had valid responses regarding the purpose of sport. Most reported injuries occurred during leisure-time activities (59%), particularly among youth and young adults (11-32 years). The purpose of sport was significantly associated with age group (χ^2 = 25.625, p = 0.004), with performance sports injuries being more prevalent in younger age brackets. No statistically significant association was found between age group and history of previous injury.

TBI module. Out of all injury cases, 2.6% of the sample (203 cases) had a recorded loss of consciousness. In nearly 41% of valid cases, loss of consciousness was confirmed, while in almost half, it was absent. Most episodes were brief (under 30 min), typical of mild TBI; only 2 patients (1%) had prolonged or severe loss of consciousness.

Out of 7,870 total cases, only 273 (3.5%) had documented GCS scores. Most patients presented with mild TBI (GCS 15), 218 cases (79.9%), while scores of 13 or below (moderate to severe TBI) were recorded in 14 cases (5.1%)

Among recorded TBI diagnoses (n = 174), mild traumatic brain injury (concussion) was the predominant form, representing more than 85% of valid cases. Severe forms, such as skull fracture, laceration/contusion, and other intracranial injuries, were extremely rare, each accounting for less than 3%.

Discussion

The present study highlights critical trends in injury epidemiology across different age groups, with clear implications for prevention and healthcare planning. Our findings are consistent with global data indicating that most injuries treated in Emergency Departments (EDs) are unintentional, fall-related, and occur in domestic environments or public spaces [1].

Falls, particularly among older adults, remain a leading cause of injury-related morbidity and hospitalization. In our dataset, seniors were disproportionately affected by falls and required more frequent hospital admissions, a trend also reported in previous studies across high-income and middle-income countries [11-13]. These findings reinforce the urgent need for age-friendly environments and fall-prevention strategies, such as home safety modifications, balance training, and medication review programs [7].

Fractures emerged as the predominant injury type across all age groups, with the highest prevalence in the elderly population. This aligns with data showing increased bone fragility due to osteoporosis in older adults, making them particularly susceptible to fractures following even minor trauma [6, 9]. In contrast, soft tissue injuries, including sprains, contusions, and lacerations, were more frequent among younger, active adults, often resulting from physical activity, road traffic incidents, or occupational hazards [9, 14].

Adults aged 20-59 constituted the largest proportion of injury cases. This may reflect their higher levels of mobility, occupational exposure, and engagement in physically demanding activities, which increase the risk of injury [15, 16]. Public health policies should thus not only focus on injury prevention in the elderly but also address risk factors among the working-age population, such as promoting workplace safety, road traffic regulation, and urban planning that encourages safe mobility.

While this study contributes valuable insights into injury patterns, it also highlights the need for robust injury surveillance systems and standardized reporting protocols, which would allow for more granular analyses by injury mechanism, severity, and long-term outcomes [4, 17]. Such registries are critical for informing targeted interventions and evaluating the effectiveness of prevention programs.

Head injuries, disproportionately observed among infants and toddlers, are in line with existing literature pointing to the anatomical and developmental vulnerabilities of young children, such as a larger head-to-body ratio, immature motor control, and limited environmental awareness [13, 18, 19]. These findings reinforce the urgent need for enhanced safety standards in homes, childcare settings, and playgrounds, including soft surface materials and parental education on fall risks.

In contrast, extremity injuries, particularly to the hands, wrists, knees, and ankles, were most prevalent in working-age adults. These injury types reflect high exposure to occupational hazards, manual labor, and recreational physical activities. Previous studies have similarly documented the high burden of upper and lower limb injuries in economically active populations, often linked to falls, overexertion, or machinery-related trauma [9, 20]. These findings support the ongoing need for occupational safety training, ergonomic interventions, and regulations targeting workplace hazards.

Our data further highlight distinct transportation-related injury patterns by age group, with implications for mobility and urban safety policies. Children, often injured as passengers or pedestrians, require age-appropriate transport safety strategies, such as school zone traffic control, child restraint systems, and road-crossing education programs [21-24]. In contrast, adults were more frequently injured as drivers or riders, pointing to the need for driver-focused risk mitigation, including alcohol and distraction prevention, fatigue management, and enforcement of speed limits.

Pedestrian injuries, although present across all age groups, showed a slight peak in older adults, likely due to slower gait, visual or cognitive impairments, and increased street-crossing difficulty. These results support targeted infrastructure improvements like longer crosswalk signal times, better lighting, and tactile paving, to protect vulnerable road users.

Furthermore, the analysis found that motorized vehicles, especially light motor vehicles, were the dominant agents in transport-related injuries, mirroring global road safety concerns [4]. Policies focusing on vehicle design improvements, occupant protection systems, and traffic law enforcement remain essential.

A notable limitation in our dataset was the scarcity of complete information on intentional injuries, particularly self-harm history, which limits a comprehensive understanding of mental health-related trauma. However, the available data reveal a concerning pattern of interpersonal violence, disproportionately involving young adult males as perpetrators. These findings echo broader global trends that link male gender and young age to elevated rates of aggressive behavior and injury infliction [6, 23]. This highlights the importance of early violence prevention, youth support programs, and mental health screening, especially for at-risk groups.

Limitations of the study. As with any observational study relying on registry data, our research faced several important limitations that should be acknowledged.

First, our analysis draws exclusively on data collected from the Emergency Departments of two major hospitals in Chișinău. This approach, while valuable for capturing a substantial number of injury cases, inevitably misses a wider spectrum of injuries, like those treated in primary care, minor injuries managed at home, or even severe cases that never reach the hospital.

Another challenge was the incomplete nature of several key variables. For example, information about the intent behind injuries, such as whether they resulted from self-harm or assault, was frequently missing or unknown. In the case of self-harm history, valid data were available for less than one percent of cases. Similar gaps appeared for contextual factors, like the activity at the time of injury or the use of protective equipment. These data limitations made it difficult to explore complex risk environments or to draw detailed conclusions about intentional injuries, mirroring challenges reported in other trauma registry studies.

The study's retrospective and observational design also brings inherent limitations. By relying on medical records completed after the fact, we were only able to observe associations, not establish direct cause-and-effect relationships between risk factors and injury outcomes. This means that while our findings reveal important patterns, such as the higher frequency of falls among older adults or the link between age and transportation-related injuries, prospective studies would be needed to confirm these relationships and to test the effectiveness of targeted interventions.

Lastly, it is important to recognize that the findings from this urban, hospital-based sample may not fully reflect the situation in rural areas or across the entire country. Differences in access to care, injury mechanisms, and reporting practices mean that caution should be used when generalizing our results beyond the study setting.

Conclusions

Our findings highlight that injuries vary significantly by age, with falls being most common among seniors, head injuries among young children, and extremity injuries among working-age adults. Transport-related injuries also differ by age and role, emphasizing the need for targeted prevention strategies. Improved data collection, especially on violence and self-harm, is essential to strengthen injury surveillance and guide effective interventions. The iCREATE Registry demonstrates the feasibility and potential impact of establishing a national trauma registry in Moldova. Addressing gaps in data completeness, expanding coverage to additional healthcare settings, and improving standardized reporting will be crucial for strengthening injury surveillance and prevention efforts in the future.

Competing interests

None declared.

Authors' contribution

The authors contributed equally to the elaboration and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version for publication.

Informed consent for publication

Obtained.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes No 43, from March 15, 2018).

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REVIEW ARTICLE

Micronutrient needs and supplementation strategies during pregnancy

Maria Cemortan*, Irina Sagaidac, Olga Cernetchi

Department of Obstetrics and Gynecology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

ABSTRACT

Introduction. Micronutrient deficiencies during pregnancy remain a major global public health concern, with implications for maternal health, fetal development, and long-term child outcomes. International organizations such as the WHO and FIGO have issued evidence-based guidelines on micronutrient supplementation, which are increasingly being adapted into national protocols.

Material and methods. This narrative review was based on literature searches in PubMed, Scopus, Web of Science, and Google Scholar, covering January 2000 to May 2025. Peer-reviewed studies, systematic reviews, and clinical guidelines from WHO, FIGO, and the Moldovan Ministry of Health were included. The review focused on iron, folic acid, iodine, calcium, vitamin D, and selected trace elements.

Results. Iron and folic acid emerged as the most consistently recommended supplements across guidelines, with proven efficacy in reducing maternal anemia and neural tube defects. Iodine and calcium are also emphasized, particularly in regions with documented dietary insufficiency. Moldova's antenatal care protocol largely aligns with WHO and FIGO recommendations, prioritizing targeted over universal supplementation for nutrients beyond iron and folate. Evidence on routine supplementation with multivitamin complexes remains inconclusive.

Conclusions. Evidence-based micronutrient supplementation is essential to optimizing pregnancy outcomes. Universal iron and folic acid supplementation remain the cornerstone of antenatal nutrition strategies. Context-specific approaches, as exemplified by the Moldovan model, can enhance implementation in resource-limited settings.

Keywords: micronutrient supplementation, pregnancy nutrition, antenatal care, iron deficiency anemia, folic acid, iodine.

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***Corresponding author: Maria Cemortan**, MD, PhD, assistant professor
Department of Obstetrics and Gynecology
Nicolae Testemițanu State University of Medicine and Pharmacy
bd. Ștefan cel Mare și Sfânt 165, Chișinău, Republic of Moldova, MD-2004
e-mail: mariacemortan@yahoo.com

Authors' ORCID IDs
Maria Cemortan – https://orcid.org/0000-0003-3137-7524
Irina Sagaidac – https://orcid.org/0000-0003-2491-9612
Olga Cernetchi – https://orcid.org/0000-0002-9229-8080

Key messages

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

Despite global recommendations, there is limited understanding of how international micronutrient supplementation strategies are adapted and implemented within national antenatal care protocols, including Moldova. The impact of context-specific modifications on maternal and fetal outcomes remains insufficiently explored.

The research hypothesis

Adherence to evidence-based, targeted micronutrient supplementation strategies, aligned with WHO and FIGO guidelines and adapted to local contexts, can optimize maternal and neonatal outcomes more effectively than non-individualized, multivitamin-based supplementation during pregnancy.

The novelty added by the manuscript to the already published scientific literature
This review uniquely integrates global guidelines with national policies from the Republic of Moldova to illustrate how targeted supplementation strategies are operationalized in practice. It highlights the clinical rationale for Moldova's tailored approach.

Introduction

Adequate nutrition during pregnancy is a cornerstone of maternal and fetal health, with micronutrients playing a critical role in ensuring optimal outcomes for both mother and child. Pregnancy increases metabolic demands and physiological needs, making women more susceptible to deficiencies in essential vitamins and minerals. These deficiencies can have significant short- and long-term effects on maternal health, fetal development, and the overall trajectory of child growth [1].

To address the high burden of micronutrient deficiencies, numerous international organizations, including the World Health Organization (WHO) and the International Federation of Gynecology and Obstetrics (FIGO), have developed evidence-based guidelines recommending both population-level and targeted supplementation strategies [1-3]. National authorities, such as the Ministry of Health of the Republic of Moldova, have adapted these strategies to their epidemiological context by integrating supplementation and counseling into routine antenatal care [4-6].

This narrative review explores the current evidence on the physiological roles and public health significance of key micronutrients in pregnancy. It also examines global and national recommendations on supplementation strategies, aiming to inform clinical practice and guide future interventions to improve maternal and neonatal health outcomes.

Material and methods

This article was designed as a narrative review, aimed at synthesizing current scientific evidence and relevant public health guidance concerning the physiological importance, intake requirements, and supplementation strategies for key micronutrients during pregnancy. The review places particular emphasis on iron, folic acid, iodine, calcium, vitamin D, and other essential micronutrients implicated in maternal and fetal health outcomes.

A comprehensive, non-systematic literature search was conducted using the databases PubMed, Scopus, Web of Science, and Google Scholar, covering publications from January 2000 to May 2025. Search terms included combinations of "pregnancy", "micronutrients", "maternal nutrition", "supplementation", "iron deficiency", "folic acid", "iodine in pregnancy", "antenatal care", "maternal outcomes", and "public health nutrition". The search strategy prioritized peer-reviewed literature, including randomized controlled trials, systematic reviews, meta-analyses, Cochrane reviews, and high-quality observational studies. In addition, official documents issued by international bodies such as the World Health Organization and the International Federation of Gynecology and Obstetrics, as well as national-level protocols and guidelines from the Republic of Moldova, were included to ensure the integration of global and local policy perspectives.

Inclusion criteria focused on studies involving human pregnancy and micronutrient intake or supplementation with defined maternal or neonatal outcomes. Eligible sources included clinical trials, systematic reviews, meta-analyses, Cochrane reviews, and national or international policy documents. Non-peer-reviewed materials, animal studies, case reports, and studies not addressing micronutrient-related outcomes in pregnancy were excluded.

Given the narrative nature of this review, the selection of sources may be subject to selection bias. No formal quality assessment tool was applied, and the inclusion of references was guided by relevance, citation frequency in guidelines, and alignment with the Moldovan clinical context. This approach may limit the reproducibility and objectivity of the findings. Additionally, the review does not include quantitative data synthesis or statistical comparison between interventions, which is inherent to the narrative design.

Despite these limitations, the narrative format allows for a comprehensive and policy-oriented synthesis of scientific literature, offering a practical and comparative overview of international and national strategies for micronutrient supplementation during pregnancy.

This review does not constitute a systematic review or meta-analysis and does not include quantitative data synthesis. Rather, it presents a qualitative integration of current knowledge and practice standards, with the aim of providing clinicians, public health professionals, and policy-makers with an up-to-date overview of the rationale, scope, and implementation of micronutrient interventions during pregnancy.

Results

The findings from the literature and national and international guidelines converge on the critical importance of specific micronutrients during pregnancy. Iron, folic acid, iodine, calcium, and vitamin D were consistently identified as the most essential micronutrients with significant clinical and public health implications [7].

Iron deficiency anemia is among the most prevalent conditions during pregnancy, affecting over 30% of pregnant women globally and up to 40% in low- and middle-income countries [8]. Iron deficiency anemia is recorded in 8-15% of women of reproductive age, while iron deficiency is found in every third woman. Approximately 50-60% of pregnant women suffer from iron deficiency anemia, and in 70% of these cases, iron deficiency is detected. In the Republic of Moldova, over 10,000 women give birth each year with diagnosed iron deficiency anemia, representing 56.4% of all births, and the incidence is increasing [9].

Iron-deficiency anemia (IDA) in pregnant women is increasingly recognized as a common and persistent problem, even in high-income countries. Several modern lifestyle, dietary, and physiological factors contribute to its rising prevalence today compared to past generations. Pregnancy significantly increases the body's iron requirements due to the expansion of maternal blood volume, fetal growth, and placental development. It is estimated that a woman needs approximately 1,000-1,200 mg of iron throughout pregnancy, with the majority required in the second and third trimesters. When iron stores are already depleted before conception, these demands quickly outpace supply, leading to iron deficiency.

Contemporary diets contribute substantially to this issue. Many pregnant women consume less red meat or follow plant-based diets, which are lower in heme iron – the most bioavailable form. Additionally, widespread consumption of highly processed foods, which are often poor in micronutrients, combined with inhibitors of iron absorption such as calcium, tea, and phytates, further reduces dietary iron availability. At the same time, short intervals between pregnancies and pre-existing conditions like heavy menstrual bleeding or chronic inflammation (e.g., obesity, infections) impair iron absorption or increase iron loss.

Lastly, health system practices play a role. In many settings, iron supplementation is offered only when anemia is detected, rather than as a preventive strategy. Moreover, preconception care often overlooks iron status, and antenatal screening may be delayed until mid-pregnancy, missing opportunities for early intervention.

In this context, rising rates of IDA reflect a mismatch between physiological needs and current dietary and healthcare patterns, highlighting the importance of timely assessment, individualized counseling, and context-sensitive supplementation strategies.

Insufficient iron intake is associated with fatigue, increased risk of infections, low birth weight, and preterm birth. Supplementation has been shown to reduce the risk of maternal anemia and improve perinatal outcomes when appropriately initiated and monitored [10]. Iron was universally recognized as a key nutrient for the prevention and management of maternal anemia. WHO, FIGO, and national guidelines recommend routine iron supplementation in all pregnant women, especially in regions with a high prevalence of anemia [1, 3]. The Moldovan national protocol mandates the administration of 100 mg elemental iron every second day starting from 12 weeks' gestation, aligned with WHO's recommendation of 30-60 mg daily where anemia is prevalent [1, 5, 6]. Iron supplementation has been shown to reduce the incidence of maternal anemia at term, lower the risk of preterm birth, and improve infant iron stores at birth.

International recommendations on iron supplementation during pregnancy vary significantly, reflecting differences in anemia prevalence, nutritional status, and healthcare infrastructure across regions. The World Health Organization and the International Federation of Gynecology and Obstetrics (FIGO) advocate for universal daily supplementation with 30-60 mg of elemental iron and 400 μg of folic acid for all pregnant women (Table 1).

Table 1. Summary of iron supplementation guidelines in pregnancy

Organization	Universal Supplementation?	Dose	Screening	When to Supplement
WHO	Yes	30-60 mg/day	Not emphasized	All pregnant women
ACOG	No	60-120 mg/day (if IDA)	Yes	Only if anemic
RCOG	No	100-200 mg/day (if IDA)	Yes	Only if anemic
NICE	No	Based on need	Yes	Only if anemic
CDC	Yes	30 mg/day	Yes	All pregnant women
FIGO	Yes (in most settings)	30-60 mg/day	Recommends screening but supports universal supplementation where anemia is prevalent	All pregnant women, especially in LMICs
Moldova	Yes	100 mg elemental iron every second day starting from 12 weeks' gestation	Yes	All pregnant women

Note: WHO – World Health Organization, ACOG – American College of Obstetricians and Gynecologists, RCOG – Royal College of Obstetricians and Gynaecologists, NICE – National Institute for Health and Care Excellence, CDC – Centers for Disease Control and Prevention, FIGO – International Federation of Gynecology and Obstetrics, IDA – Iron-deficiency anemia

This approach is particularly emphasized in low- and middle-income countries (LMICs), where iron-deficiency anemia is highly prevalent and screening services may be limited. FIGO also supports this universal strategy but acknowledges that in settings with well-developed health systems, targeted supplementation based on screening results may be appropriate.

In contrast, organizations based in high-income countries tend to recommend a screen-and-treat approach. The American College of Obstetricians and Gynecologists (ACOG) and the Royal College of Obstetricians and Gynaecologists (RCOG) both advise routine anemia screening during pregnancy, with iron supplementation reserved for women diagnosed with iron-deficiency anemia [11, 12]. Similarly, the National Institute for Health and Care Excellence (NICE) discourages routine supplementation for non-anemic women and emphasizes dietary management and monitoring. An exception is the Centers for Disease Control and Prevention (CDC) in the United States, which supports low-dose daily iron supplementation (30 mg) for all pregnant women, beginning at the first prenatal visit, regardless of anemia status.

This variation in recommendations illustrates a broader public health debate between preventive universal supplementation and individualized treatment, shaped by local epidemiology and healthcare capabilities. As global dietary patterns shift and concerns grow regarding declining nutrient density in modern diets, some experts suggest that even in high-income settings, universal or semi-universal supplementation strategies may merit reconsideration.

In low- and middle-income countries (LMICs), where iron deficiency is highly prevalent (>40% of pregnant women), organizations like the WHO and Centers for Disease Control and Prevention recommend universal daily iron supplementation during pregnancy [13]. This is seen as a cost-effective population-level strategy to reduce adverse outcomes like maternal mortality, preterm birth, and low birth weight.

In contrast, high-income countries (HICs) like the USA (ACOG), UK (RCOG, NICE), where IDA prevalence is lower due to better baseline nutrition and access to healthcare, promote a screen-and-treat approach. This minimizes unnecessary supplementation and potential side effects [13]. Routine supplementation is beneficial in settings with poor access to health services, as many women may not receive timely testing or follow-up. However, in settings where anemia screening is feasible, targeted treatment is preferred to avoid risks such as:

- Gastrointestinal side effects (nausea, constipation)
- Iron overload in women with adequate stores or undiagnosed conditions (e.g., hemochromatosis)
- Poor adherence due to side effects in women who do not need iron

In LMICs, universal supplementation simplifies logistics and ensures that iron reaches all women regardless of access to testing. In HICs, healthcare systems can afford individualized care based on blood tests (hemoglobin, ferritin), enabling personalized supplementation.

The debate between universal vs. targeted iron supplementation reflects a balance between population-level public health strategies and individualized clinical care. While global nutritional transitions and changes in food quality may suggest a need for broader supplementation, recommendations continue to be tailored based on local epidemiology, health system capacity, and risk-benefit assessments.

Folic acid is another critical micronutrient. Its periconceptional and early pregnancy supplementation is strongly associated with a reduced risk of neural tube defects (NTDs), as it supports DNA synthesis and cellular replication. A daily intake of 400 µg of folic acid, initiated before conception and continued through the first trimester, can reduce NTD risk by up to 85% [14, 15].

Despite strong recommendations from global authorities, many pregnancies remain unplanned, and supplementation is often delayed or insufficient [3]. Folic acid supplementation emerged as a cornerstone of periconceptional and early pregnancy care. Both WHO and FIGO recommend a daily intake of 400 µg folic acid, starting at least one month before conception and continuing through the first trimester, to prevent neural tube defects (NTDs) [1, 3]. In Moldova, standard practice includes 1 mg folic acid every second day throughout pregnancy for all women, and 5 mg/day up to the 12th week of pregnancy for those with elevated risk (e.g., history of NTDs, diabetes, antiepileptic use) [4-6]. Although this differs from the internationally recommended dose of 400 µg daily, it is likely influenced by the limited availability of low-dose folic acid formulations in pharmacies, making 1 mg tablets a more practical choice for routine use. Despite the strength of evidence, global adherence to preconceptional folic acid use remains suboptimal. This has led some countries to adopt mandatory folic acid food fortification programs, which have been associated with significant reductions in NTD prevalence. However, such programs are not universally implemented, and targeted supplementation remains a critical strategy (Table 2).

Table 2. Folic acid supplementation guidelines during pregnancy

Organization	Recommended Dose	Timing	High-Risk Groups
WHO	400 µg/day	Preconception to 12 weeks' gestation	4 mg/day if history of NTDs
FIGO	400-800 µg/day	Preconception to first trimester	Higher doses for high-risk groups
ACOG	400-800 µg/day	≥1 month before conception to 12 weeks	4 mg/day for high-risk women
RCOG	400 µg/day	Preconception to week 12	5 mg/day for high-risk groups
NICE	400 µg/day	From preconception to 12 weeks	5 mg/day if high-risk
Moldova National Protocol	400 µg/day	Start ≥1 month before pregnancy, continue through first trimester	4 mg/day if previous NTDs or other risk factors

Note: WHO – World Health Organization, FIGO – International Federation of Gynecology and Obstetrics, ACOG – American College of Obstetricians and Gynecologists, RCOG – Royal College of Obstetricians and Gynecologists, NICE – National Institute for Health and Care Excellence, NTDs – neural tube defects

Mandatory folic acid food fortification is a public health policy in which governments require the addition of folic acid (the synthetic form of folate) to certain staple foods – most commonly wheat flour, maize flour, or rice – to improve the population's folate status. The rationale is to reduce the incidence of neural tube defects (NTDs) by ensuring that all women of childbearing age, including those with unplanned pregnancies, receive adequate folic acid intake regardless of supplement use.

This strategy is particularly effective in addressing the limitations of preconceptional supplementation, such as lack of awareness, late initiation of prenatal care, and high rates of unplanned pregnancies.

As of 2023, over 85 countries have implemented mandatory folic acid fortification policies. These include the United States, Canada, Australia, Chile, South Africa, and several countries in Latin America and the Middle East. In these

countries, significant reductions in NTD rates have been reported—up to 30–70%, depending on baseline prevalence and adherence to fortification standards. For example, in the United States, mandatory fortification of enriched grain products began in 1998 and has been associated with a 36% decline in NTD prevalence. In Chile, after wheat flour fortification was introduced, NTD rates dropped by approximately 50%.

Despite this success, many European countries, including Moldova, have not adopted mandatory folic acid fortification, often due to concerns about overexposure in the general population, possible masking of vitamin B12 deficiency, and a preference for voluntary supplementation strategies.

Overall, mandatory fortification is considered a cost-effective and equitable intervention, especially in settings where supplement coverage is low and dietary folate intake is insufficient. The World Health Organization and FIGO support the implementation of food fortification as a complementary strategy to supplementation for the primary prevention of NTDs.

Folic acid supplementation is safe, inexpensive, and effective. Ensuring adequate intake before and during early pregnancy is a critical component of maternal and child health. Public health efforts must continue to promote awareness and access, particularly for populations with limited healthcare engagement or poor nutritional status.

Iodine is vital for fetal brain development and maternal thyroid function. Even mild-to-moderate iodine deficiency in pregnancy is linked to lower cognitive scores in offspring and increased risk of goiter and hypothyroidism, miscarriage, and cretinism in severe cases [16]. Strategies like universal salt iodization have improved iodine status globally, but gaps persist in some regions, especially in areas with limited dietary diversity [1, 16, 17]. Iodine was highlighted for its critical role in fetal neurodevelopment. Mild-to-moderate iodine deficiency remains a concern in many regions, including parts of Europe, despite the implementation of universal salt iodization programs.

During pregnancy, iodine requirements increase by approximately 50% due to increased maternal thyroid hormone production, enhanced renal iodine clearance, and fetal needs. The World Health Organization recommends a daily iodine intake of 250 µg for pregnant and lactating women, which often cannot be met through diet alone, especially in regions without universal salt iodization (USI). The Moldovan guidelines endorse iodine sufficiency but do not currently recommend routine iodine supplementation beyond dietary measures (Table 3).

Table 3. Iodine supplementation guidelines during pregnancy

Organization	Recommended Dose	Supplementation Approach	Target Groups
WHO	250 µg/day (total intake)	150 µg/day if salt iodization is inadequate	All pregnant/lactating women
FIGO	150-250 µg/day	Preferably via iodized salt or supplements	Women in low-iodine areas
ACOG	150 µg/day	As part of daily prenatal multivitamin	All pregnant and breastfeeding women
NICE	No specific guideline on iodine	Emphasizes dietary intake and public health measures	General population-level iodine sufficiency
Moldova National Protocol	Do not currently recommend routine iodine supplementation beyond dietary measures		

Note: WHO – World Health Organization, FIGO – International Federation of Gynecology and Obstetrics, ACOG – American College of Obstetricians and Gynecologists, NICE – National Institute for Health and Care Excellence

WHO, FIGO, and other international bodies recommend iodine supplementation (150–250 µg/day) for pregnant and breastfeeding women living in areas with inadequate iodine intake or where USI is not reliably implemented. Iodine is commonly provided through potassium iodide (KI) or potassium iodate (KIO_3) in the form of multivitamin supplements. The goal is to ensure adequate maternal and fetal thyroid function throughout gestation.

Calcium plays a vital role in fetal skeletal development, muscle contraction, vascular function, and neuromuscular signaling [18]. During pregnancy, calcium demands increase to support the growth of the fetal skeleton, especially in the third trimester, when fetal bone mineralization is most active. While maternal physiological adaptations (e.g., increased intestinal calcium absorption) help meet this demand, insufficient calcium intake can lead to maternal bone demineralization and elevated risks of hypertensive disorders, particularly preeclampsia.

The World Health Organization recommends daily calcium supplementation (1.5-2 g of elemental calcium) for all pregnant women in populations with low dietary calcium intake (<1,000 mg/day). This recommendation is based on evidence showing that calcium supplementation significantly reduces the risk of preeclampsia, especially in high-risk women and those with low baseline calcium intake. WHO also advises dividing the dose into 2-3 daily administrations to enhance absorption and avoid gastrointestinal discomfort.

Organizations in high-income countries, such as ACOG and NICE, do not universally recommend calcium supplementation for all pregnant women [11, 19]. Instead, they encourage meeting calcium needs through diet, unless deficiency is suspected or dietary intake is inadequate. Dietary sources include dairy products, fortified plant-based milk, leafy greens, almonds, and small fish with bones. Moldova's standard antenatal care package does not recommend routine supplementation with calcium, which instead emphasizes dietary counseling (Table 4).

Table 4. Calcium supplementation guidelines during pregnancy

Organization	Universal Supplementation?	Recommended Dose	Target Population
WHO	Yes (in low-intake settings)	1.5-2 g/day (divided doses)	All pregnant women in populations with <1,000 mg/day calcium intake
FIGO	Yes (in high-risk or low-intake settings)	1.5-2 g/day	Women at risk of preeclampsia or with low dietary calcium
ACOG	No universal supplementation	Through diet; supplement if needed	High-risk or insufficient intake
NICE	No universal supplementation	Emphasis on dietary intake	High-risk groups only
Moldova National Protocol	Yes (selected cases)	Typically 1 g/day	Recommended for women at risk of preeclampsia or low calcium intake

Note: WHO – World Health Organization, FIGO – International Federation of Gynecology and Obstetrics, ACOG – American College of Obstetricians and Gynecologists, NICE – National Institute for Health and Care Excellence

Vitamin D, vitamin B12, zinc, and other trace micronutrients were addressed more variably across sources. While deficiencies are associated with adverse pregnancy outcomes, such as impaired bone development, immune dysfunction, and intrauterine growth restriction, there is insufficient evidence to support universal supplementation in the absence of individual risk factors. The Moldovan antenatal protocol explicitly advises against routine supplementation with multivitamin or multimineral complexes, except in documented deficiency states.

Discussion

This review confirms that evidence-based micronutrient supplementation during pregnancy is essential to optimizing maternal and neonatal outcomes. Iron and folic acid remain the most consistently recommended supplements worldwide, backed by strong evidence demonstrating reductions in maternal anemia and neural tube defects. Iodine and calcium also play crucial roles, particularly in fetal neurodevelopment and preeclampsia prevention, respectively, though recommendations for their supplementation are more variable and context-dependent.

Global guidelines, including those from WHO and FIGO, generally support universal supplementation with iron and folic acid, especially in populations where deficiencies are prevalent. In contrast, iodine and calcium supplementation are often recommended only in populations with known insufficiencies or specific risk factors, such as low dietary intake, inadequate food fortification policies, or increased risk of hypertensive disorders. This stratification allows for efficient resource allocation and minimizes unnecessary supplementation [20].

The Republic of Moldova's antenatal care protocol reflects this targeted, pragmatic approach, aligning closely with international recommendations while tailoring interventions to local resource availability and population health data. The country mandates iron and folic acid supplementation – though in formulations and schedules that diverge slightly from WHO dosing, possibly due to formulation availability. Meanwhile, calcium and iodine supplementation are advised only under certain clinical conditions, with dietary counseling forming the foundation of care.

Despite clear clinical guidelines, several challenges persist. Adherence to preconceptional folic acid supplementation remains low globally due to high rates of unplanned pregnancies. Similarly, gaps in iodine sufficiency persist in regions without robust salt iodization programs. The limited use of calcium supplementation in Moldova may reflect an underestimation of preeclampsia risk or insufficient dietary assessment. There is also a lack of consensus on the use of broad-spectrum multivitamins, with Moldova explicitly discouraging their routine use except in cases of diagnosed deficiency.

Emerging tools, such as the FIGO Nutrition Checklist, may enhance individual risk assessment and improve the integration of micronutrient strategies into routine antenatal care [21]. Future research should evaluate the effectiveness and cost-efficiency of targeted versus universal supplementation approaches, as well as the long-term impacts of national protocols on maternal and child health indicators.

Conclusions

Adequate intake of key micronutrients during pregnancy – particularly iron and folic acid – is critical for maternal and neonatal health. While universal supplementation with these two nutrients remains a global standard, recommendations for iodine and calcium vary according to dietary sufficiency and risk factors. Moldova's antenatal care model offers a context-sensitive strategy that aligns with international guidelines while accommodating local resource constraints and nutritional realities.

This review underscores the importance of combining evidence-based protocols with individualized clinical judgment. Integration of supplementation, nutritional counseling, and screening within routine antenatal visits enables more effective and equitable care. Moving forward, strengthening adherence to periconceptional folic acid use, expanding iodine sufficiency programs, and tailoring calcium and vitamin D strategies based on risk will be essential for advancing maternal nutrition and birth outcomes.

Competing interests

None declared.

Authors' contributions

All 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.

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REVIEW ARTICLE

Thromboprophylaxis in pregnancy, delivery and puerperium: a review of literature and current guidelines

Corina Cardaniuc*, Irina Sagaidac

Department of Obstetrics and Gynecology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Republic of Moldova

ABSTRACT

Introduction. Venous thromboembolism is one of the leading causes of maternal morbidity and mortality. Pregnancy induces a hypercoagulable state as an adaptive mechanism to prevent hemorrhage during childbirth. These physiological changes significantly increase the risk of venous thromboembolism – by up to six-fold during pregnancy and up to 10-fold during the postpartum period compared to the non-pregnant population. Given these risks, proper identification of patients who may benefit from thromboprophylaxis is essential to improve maternal outcomes.

Material and methods. This manuscript reviews medical articles and current clinical guidelines on thromboprophylaxis in pregnancy and the puerperium, highlighting similarities, differences, and practical considerations in the management of at-risk patients. International guidelines developed to aid clinicians in venous thromboembolism risk stratification and prevention, including the Royal College of Obstetricians and Gynecologists, the American College of Obstetricians and Gynecologists, the American Society of Hematology, and the National Institute for Health and Care Excellence were analyzed.

Results. The incidence of Venous thromboembolism ranges from 1 to 2 per 1,000, with up to 80% attributed to deep vein thrombosis cases occurring during the antepartum period, and 20%-25% being pulmonary embolism cases. In contrast, the incidence of pulmonary embolism is significantly higher after childbirth, with 40% to 60% of all pulmonary embolism cases occurring during the postpartum period. The impact of venous thromboembolism is not limited to mortality. Acute venous thromboembolism and the need for long-term anticoagulant therapy are associated with a significant clinical and psychological burden, while potential long-term sequelae, such as pulmonary hypertension and post-thrombotic syndrome, can have lifelong consequences. Ensuring thromboprophylaxis is essential, and the primary responsibility lies with the obstetrician. Ultimately, effective thromboprophylaxis is about balancing efficacy and safety between the need to prevent a potentially life-threatening event and the cost of an increased risk of bleeding.

Conclusions. Effective thromboprophylaxis during pregnancy and the puerperium remains a critical component of maternal care. The national protocol aims to assist healthcare professionals in identifying women at increased risk of venous thromboembolism during pregnancy, childbirth, and the postpartum period, and in making evidence-based decisions regarding the use of thromboprophylaxis and anticoagulant agents.

Keywords: thromboprophylaxis, deep vein thrombosis (DVT), pulmonary embolism (PE), venous thromboembolism (VTE), pregnancy, puerperium, low molecular weight heparin.

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***Corresponding author**: **Corina Cardaniuc**, MD, PhD, associate professor
Department of Obstetrics and Gynecology
Nicolae Testemițanu State University of Medicine and Pharmacy,
165 Stefan cel Mare și Sfânt blvd, MD-2004, Chisinau, Republic of Moldova.
E-mail: corina.cardaniuc@usmf.md

Key messages

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

While thromboprophylaxis in pregnancy has advanced, significant knowledge gaps persist, particularly regarding personalized risk stratification, dosing, timing, and long-term outcomes. Addressing these issues will optimize care, reduce venous thromboembolism incidence, and minimize harm.

Authors' ORCID IDs
Corina Cardaniuc – https://orcid.org/0000-0003-3465-9666
Irina Sagaidac – https://orcid.org/0000-0003-2491-9612

The research hypothesis
Strengthening early detection of women at risk for venous thromboembolism in pregnancy or the postpartum period and prevention policies by effective thromboprophylaxis can reduce maternal morbidity and associated healthcare costs.

The novelty added by the manuscript to the already published scientific literature
The article systematically compares multiple international guidelines, highlights areas of consensus and points of divergence, especially around thresholds, dosing, and postpartum duration, and provides a side-by-side interpretation of risk stratification tools and their practical consequences. This comparative format helps clinicians working across borders or in multicultural health systems harmonize their decision-making, a rarely emphasized but clinically important angle.

Introduction

Venous thrombosis and pulmonary embolism remain the leading direct causes of maternal death during pregnancy or within the first six weeks postpartum [1]. Venous thromboembolism (VTE) complicates 1-2 per 1,000 pregnancies, and pregnancy increases the relative risk of VTE by 6 to 10 times compared to non-pregnant individuals.

Normal pregnancy is associated with major changes in hemostasis – specifically, hypercoagulability and hypofibrinolysis – with the purpose of maintaining placental function throughout pregnancy and preventing excessive bleeding during delivery [2]. While these hemostatic changes protect the mother from hemorrhage at birth, they also predispose her to thromboembolism during both pregnancy and the postpartum period.

Due to the prothrombotic state – characterized by hypercoagulability and venous stasis – pregnancy is associated with a fivefold increase in the risk of VTE compared to non-pregnant women, and with a 20-fold increase in VTE risk during the first three months postpartum [3].

The adaptive changes in hemostasis during pregnancy are primarily attributed to elevated estrogen levels and affect all components involved in coagulation: vascular capacity, vessel wall integrity, plasma levels of coagulation factors and fibrinolytic activity, platelet function, and plasma proteins [4, 5]. Anatomical changes related to the gravid uterus play a central role by causing venous stasis in the lower extremities.

The concentration of most coagulation factors increases, while levels of endogenous anticoagulant factors and fibrinolytic activity decrease [5, 6]. This imbalance in hemostasis can lead to fibrin deposition, resulting in placental ischemia and hypoxia, preeclampsia, intrauterine growth restriction, or miscarriage.

Physiological pregnancy is characterized by a progressive increase in the levels of coagulation factors VII, VIII, IX, X, and XII, as well as von Willebrand factor, fibrinogen, and tissue factor. The rise in factors VII and X, which can reach 120-180% of baseline levels, contributes to the shortened prothrombin time (PT) observed from mid-gestation through term [7].

Fibrinogen, a plasma glycoprotein, is a key component of the coagulation cascade and is involved in both primary and secondary hemostasis. In non-pregnant women, normal plasma fibrinogen levels range from 2.0 to 4.5 g/L [8, 9]. In pregnancy, fibrinogen levels increase progressively from the first trimester, reaching an average of 5 g/L by term. This suggests that fibrinogen values considered normal in the non-pregnant population may signal an underlying coagulopathy in pregnant women [8, 10].

During pregnancy, the braking mechanisms of coagulation are attenuated due to decreased levels of inhibitory coagulation factors.

Antithrombin (AT). AT levels remain unaffected by estrogen or progesterone; however, a moderate ~15% decline in AT III is observed in the late gestation and immediate postpartum periods. This is believed to reflect physiological activation of coagulation through intervillous placental thrombosis. Additional factors include hemodilution, altered synthesis, increased clearance, or consumption. James et al. reported a ~30% drop in AT levels at delivery, reaching a nadir 12 hours postpartum and normalizing by 72 hours [3].

Protein S (PS) and Protein C (PC). Total PS and PC levels remain stable during pregnancy and postpartum, but *free* PS and PS activity progressively decrease, accompanied by rising resistance to activated ***Protein C***, which also enhances von Willebrand factor. Free PS can drop by ~50% at term and remain low for two months postpartum, particularly during lactation. Therefore, PS quantification during pregnancy, even in early stages, is not recommended.

Protein C activity increases in the first and second trimesters, possibly offsetting decreased PS activity and increased thrombin generation to support fetal circulation. PC activity then declines in the third trimester, rebounding in the immediate postpartum period.

Pregnancy also elevates thrombomodulin, tissue factor pathway inhibitor, and heparin cofactor II. Concurrently, prothrombin fragments 1+2 and thrombin–antithrombin complexes rise, cumulatively heightening thrombotic risk.

Fibrinolytic activity progressively decreases throughout pregnancy, reaching its lowest point in the third trimester [11]. This hypofibrinolytic state contributes to the prevention of bleeding at the time of placental separation.

Fibrinolysis is suppressed due to increased synthesis of plasminogen activator inhibitor type 1 (PAI-1) by endothelial cells and substantial production of plasminogen activator inhibitor type 2 (PAI-2) by the placenta [12]. Tissue plasminogen activator (tPA) levels decrease significantly towards term, and this reduction persists for 6-8 weeks postpartum.

D-dimers are recognized as the most sensitive markers of secondary fibrinolytic activation. Paradoxically, despite elevated levels of PAI-1 and PAI-2 – which would be expected to lower D-dimer levels due to hypofibrinolysis – plasma concentrations of D-dimer increase progressively by two- to fourfold during pregnancy, reaching values of up to 198-266 ng/mL at delivery (compared to ~80 mg/L in non-pregnant women) [13]. A strong positive correlation exists between gestational age and D-dimer concentration. This progressive increase complicates the use of D-dimer levels in ruling out VTE in pregnant women with clinical suspicion [2, 14-17].

Fibrin degradation products also increase progressively, resulting from enhanced fibrin formation (through intravascular coagulation) and subsequent intensified fibrinolysis due to placental release of thromboplastic factors.

The hemostatic and coagulation changes described above develop gradually during normal pregnancy, reaching a peak state of hypercoagulability in the third trimester and resolving slowly during the postpartum period [7]. These changes represent an adaptive physiological mechanism to protect the pregnant woman from hemorrhage during delivery and typically do not carry clinical consequences. However, the risk of VTE and pulmonary embolism is known to be four to six times higher in pregnant women compared to non-pregnant women of similar age [18].

Blood flow changes in pregnancy. Endothelial trauma and venous stasis contribute to the increased thrombotic risk during pregnancy. Blood stasis is mediated by venous dilation, decreased venous tone, reduced venous flow, and compression of the iliac veins by the pregnant uterus, particularly the left common iliac vein. Additionally, vascular injury occurs following delivery [1, 19].

In conclusion, pregnancy represents a complex interaction of thrombosis-predisposing factors, aimed at achieving postpartum hemostasis. However, pregnancy simultaneously represents a condition that triggers the initiation of the pathological cascade of coagulation disorders and thrombotic events [20].

A proper understanding of the physiological changes in hemostasis during pregnancy is essential for recognizing coagulation-related pathologies and, consequently, for selecting the most appropriate therapeutic strategies [21].

Material and methods

A comprehensive literature search was conducted to identify current articles and clinical guidelines on thromboprophylaxis during pregnancy and the puerperium. The search was performed using electronic databases including PubMed, MEDLINE, the Cochrane Library, as well as professional society websites such as those of the American College of Obstetricians and Gynecologists (ACOG), Royal College of Obstetricians and Gynecologists (RCOG), American Society of Hematology (ASH), and the International Society on Thrombosis and Haemostasias (ISTH). Keywords and Medical Subject Headings (MeSH) used included combinations of "thromboprophylaxis," "pregnancy," "puerperium," "postpartum," "venous thromboembolism," "clinical guidelines," "anticoagulation," and "deep vein thrombosis." Inclusion criteria were: published medical articles, clinical guidelines, consensus statements, or position papers focusing on thromboprophylaxis in pregnant and postpartum women; Guidelines addressing risk assessment, pharmacological and non-pharmacological prophylaxis, and management strategies. Exclusion criteria included: guidelines not specifically addressing pregnancy or postpartum periods; primary research studies, case reports, or reviews not offering guideline-level recommendations. Relevant data were extracted systematically, including recommendations on risk stratification, types and doses of anticoagulants, timing and duration of thromboprophylaxis, and considerations for special populations. The findings were synthesized qualitatively to compare and contrast the approaches recommended by various professional bodies.

Results

All major guidelines agree on the importance of systematic VTE risk assessment. This should occur at multiple stages:

- At the first prenatal visit
- During any hospital admission
- At the onset of labor or before cesarean section
- Immediately postpartum

Key Risk Factors. The risk factors commonly identified include:

- Personal history of VTE
- Known thrombophilia (e.g., Factor V Leiden, antithrombin deficiency)
- Obesity (BMI ≥30)
- Age >35 years
- Multiparity
- Preeclampsia
- Assisted reproductive technology
- Prolonged immobilization
- Cesarean section, especially in emergency settings
- Smoking

RCOG employs a point-based scoring system to determine the necessity and duration of prophylaxis, while ACOG and ASH promote individualized approaches based on clinical judgment and shared decision-making.

RCOG developed a risk-scoring system [22], enabling individualized estimation of thrombotic risk during pregnancy, and permitting implementation of a risk-adapted strategy for antithrombotic prophylaxis during pregnancy and the puerperium (Table 1).

Table 1. Risk factors for VTE, [23].

Pre-existing risk factors	Score
Previous VTE (except a single event related to major surgery	4
Previous VTE provoked by major surgery	3
Known high-risk thrombophilia	3
Medical comorbidities e.g. cancer, heart failure; active systemic lupus erythematosus, inflammatory polyarthropathy or inflammatory bowel disease; nephrotic syndrome; type I diabetes mellitus with nephropathy; sickle cell disease; current intravenous drug user	3
Family history of unprovoked or estrogen-related VTE in first-degree relative	1
Known low-risk thrombophilia (no VTE)	1a
Age (> 35 years)	1
Obesity	1 or 2b
Parity ≥ 3	1
Smoker	1
Gross varicose veins	1
Obstetric risk factors	
Pre-eclampsia in current pregnancy	1
ART/IVF (antenatal only)	1
Multiple pregnancy	1
Caesarean section in labor	2
Elective caesarean section	1
Mid-cavity or rotational operative delivery	1
Prolonged labor (> 24 hours)	1
PPH (> 1 liter or transfusion)	1
Preterm birth < 37+0 weeks in current pregnancy	1
Stillbirth in current pregnancy	1
Transient risk factors	
Any surgical procedure in pregnancy or puerperium except immediate repair of the perineum, e.g. appendicectomy, postpartum sterilization	3
Hyperemesis	3
OHSS (first trimester only)	4
Current systemic infection	1
Immobility, dehydration	1
Covid -19	
Total:	

Note: *ART assisted reproductive technology; IVF in vitro fertilization; OHSS ovarian hyperstimulation syndrome; VTE venous thromboembolism.*
If the known low-risk thrombophilia is in a woman with a family history of VTE in a first-degree relative postpartum thromboprophylaxis should be continued for 6 weeks.
b. BMI ≥ 30 = 1; BMI ≥ 40 = 2

Risk assessment for venous thromboembolism (VTE), RCOG 2015, [22]:

- If total score ≥ 4 antenatally, consider thromboprophylaxis from the first trimester.
- If total score is 3 antenatally, consider thromboprophylaxis from 28 weeks.
- If total score ≥ 2 postnatally, consider thromboprophylaxis for at least 10 days.
- If admitted to hospital antenatally, consider thromboprophylaxis.
- If prolonged admission (≥ 3 days) or readmission to hospital within the puerperium, consider thromboprophylaxis.
- For patients with an identified bleeding risk, the balance of risks of bleeding and thrombosis should be discussed in consultation with a haematologist with expertise in thrombosis and bleeding in pregnancy.

Previous thromboembolic episode. Women with a history of venous thromboembolism (VTE) have an increased risk of recurrence during pregnancy and the postpartum period, with reported recurrence rates of 2-11%. The risk of recurrence appears to remain constant throughout the entire pregnancy [18, 22, 23]. Women with a history of VTE should receive pre-pregnancy counseling and have an individualized prospective management plan for thromboprophylaxis during pregnancy. Women who become pregnant before receiving such counseling should be referred as early as possible during pregnancy to a physician with expertise in thrombosis in pregnancy.

Prevention of VTE in pregnant women with thrombophilia and no previous VTE. Thrombophilias are conditions associated with an increased risk of thrombosis and can be either inherited or acquired. Most studies assessing the risk of VTE in pregnancy have focused on inherited thrombophilias. Although about 50% of pregnancy-associated VTE cases are linked to inherited thrombophilias, these abnormalities are quite common and are collectively present in at least 15% of the general population [24].

Inherited thrombophilias that have the potential to increase thrombotic risk, according to the Romanian Society of Obstetrics and Gynecology, include: factor V Leiden mutation, the G20210A mutation in the prothrombin gene, protein S deficiency, protein C deficiency, and antithrombin III deficiency. Screening for these mutations is recommended for all pregnant women with a history of venous thromboembolism [25].

Factor V Leiden mutation is the most common form of inherited thrombophilia, transmitted in an autosomal dominant manner with incomplete penetrance, and with variable prevalence ranging from 2-3% up to 10-15% across Europe [25, 26]. Data from reviews evaluating the association between thrombophilia and pregnancy-associated VTE show that the highest risks are associated with homozygosity for factor V Leiden or the prothrombin G20210A variant [24, 27]. Pregnant women who are heterozygous for factor V Leiden or the prothrombinG20210A variant have lower risks. Thrombosis due to the prothrombin gene mutation may present as deep vein thrombosis in the lower limbs or as pulmonary embolism. The prothrombin gene mutation is autosomal dominant and is responsible for up to 17% of venous thromboembolism events during pregnancy [28]. Deficiencies of antithrombin, protein C, and protein S have been associated with moderate increases in VTE risk. Antithrombin deficiency is a rare condition but

has a high thrombogenic potential [29, 30]. Nearly all types of antithrombin deficiency are heterozygous; the homozygous form is incompatible with life or results in a severe thrombotic phenotype. Protein C, together with protein S, contributes to the natural anticoagulation process by inactivating factors Va and VIIIa, thereby controlling thrombin generation. Protein S deficiency is inherited in an autosomal dominant pattern. Moderate protein S deficiency seems to affect about 1 in 500 people, while severe deficiency is extremely rare [29]. The prevalnce of severe protein C deficiency is rare (0.2%) [29]. The thrombotic risk of protein C deficiency increases if combined with factor V Leiden mutation. Estimated absolute VTE risks suggest a low thrombotic risk (0.5-1.2% of affected pregnancies) for most inherited thrombophilias, except possibly for homozygous carriers of factor V Leiden or prothrombin mutations, where the estimated risk is around 4%.

Classification of thrombophilias by risk

Low-risk thrombophilias:

- Heterozygous factor V Leiden mutation
- Heterozygous prothrombin gene mutation G20210A
- Activated Protein C Resistance
- Activated Protein S Resistance
- Hyperhomocysteinemia
- PAI (plasminogen activator inhibitor) abnormalities
- MTHFR mutations

High-risk thrombophilias:

- Homozygous factor V Leiden mutation
- Homozygous prothrombin gene mutation G20210A
- Compound heterozygosity for factor V Leiden and prothrombin mutation G20210A
- Protein C deficiency
- Protein S deficiency
- Antithrombin deficiency
- Antiphospholipid syndrome [31]

Based on recent studies, patients with low-risk thrombophilias are not routinely prescribed thromboprophylaxis but rather are closely monitored during pregnancy. The presence of low-risk thrombophilias, combined with other possible risk factors, may be sufficient to initiate thromboprophylaxis.

There is considerable disagreement among current guidelines regarding thromboprophylaxis during pregnancy in women with antithrombin, protein C, or protein S deficiencies. According to ACOG [29], anticoagulant prophylactic treatment should be started in patients with high-risk thrombophilias, specifically those with antithrombin, protein S, or protein C deficiency, and homozygous factor V mutation [23, 32, 33]. The RCOG recommends prophylactic anticoagulation for women with a history of venous thromboembolism and antithrombin deficiency during pregnancy and the puerperium [22]. The Society of Obstetricians and Gynaecologists of Canada advises testing for thrombophilia mutations after a venous thromboembolism episode [34].

The inconsistencies in recommendations likely stem from different risk thresholds for initiating prophylaxis and concerns raised by older studies suggesting these are high-risk thrombophilias (studies now considered to have methodological limitations).

The Royal College of Obstetricians and Gynaecologists recommends that the interpretation of hereditary thrombophilia test results be carried out by a clinician with specific expertise in this area. Generally, testing for hereditary thrombophilias is recommended in the presence of a medical history indicating previous venous thromboembolism (VTE).

Testing for thrombophilias should also be considered in pregnant women without a personal history or risk factors for VTE, but who have a first-degree relative with a history of unprovoked or estrogen-related VTE before the age of 50. Thrombophilia testing is especially indicated if a first-degree relative of the pregnant woman has a known thrombophilia (D) [22].

Acquired thrombophilias. The most common acquired thrombophilias during pregnancy are antiphospholipid syndrome (APS) and hyperhomocysteinemia. APS is associated with an increased risk of venous thromboembolism (VTE) [24] and obstetric complications such as preterm birth, recurrent pregnancy loss, fetal death in utero, premature placental abruption of normally implanted placenta, intrauterine growth restriction, severe early-onset preeclampsia, and HELLP syndrome. Approximately 1% of patients develop the catastrophic form of APS, characterized by multiorgan thrombotic complications during pregnancy or the postpartum period [35].

In general, primary thromboprophylaxis is not recommended for pregnant women who test positive for antiphospholipid antibodies but have no prior clinical history of thrombosis, due to limited data on the protective effects of low-dose aspirin or anticoagulants in this population. There are no strict treatment guidelines in this situation, and treatment options may include:

- no treatment,
- low-dose aspirin (around 150 mg/day),
- low-dose aspirin combined with a prophylactic dose of low molecular weight heparin.

However, most guidelines recommend low-dose aspirin therapy for pregnant women with positive antiphospholipid antibodies, especially those at high risk for preeclampsia. Treatment decisions should be individualized based on a careful risk-benefit assessment for each patient [36-38]. Long-term anticoagulant treatment, starting from the beginning of pregnancy, is essential for pregnant women with APS and thrombotic events diagnosed prior to pregnancy. Oral anticoagulant therapy should be switched to therapeutic doses of enoxaparin throughout the pregnancy, with a subsequent transition back to oral anticoagulants in the postpartum period [36].

Hyperhomocysteinemia. MTHFR mutations are a common cause of elevated homocysteine levels, which were previously considered a risk factor for venous thromboembolism (VTE). However, recent data suggest that it represents a weak risk factor, and MTHFR mutations have not been shown to increase the risk of VTE [39]. A meta-analysis

conducted by Robertson L. and colleagues failed to demonstrate an association between MTHFR mutations, hyperhomocysteinemia, and VTE during pregnancy [23]. Currently, ACOG recommends against screening for MTHFR mutations or measuring fasting homocysteine for thrombophilia evaluation and VTE risk assessment during pregnancy [29].

Age as a risk factor for VTE. Data on age are contradictory, but a modestly increased relative risk of less than twofold is suggested for women over 35 years old. In a large population cohort study from the United Kingdom, outside of pregnancy, women aged 35-44 years had a 50% higher rate of VTE compared to women aged 25-34 years. The VTE rate did not increase with age during the antepartum period; however, during the postpartum period, women aged ≥35 years had a 70% increased risk of VTE compared to women aged 25-34 years (corresponding to an absolute excess risk of 1.6 per 1000 person-years). The RCOG maintains age over 35 years as a thrombotic risk factor both antenatally and postpartum [22].

Obesity. The WHO defines obesity as an abnormal or excessive accumulation of fat that may be detrimental to health, operationally defined as a body mass index (BMI) ≥ 30 kg/m^2. The risk of VTE increases as BMI rises above 25 kg/m^2. This is the threshold defining overweight (BMI 25 to 29.9 kg/m^2), meaning the risk increases even at BMI values lower than those defining obesity. The association between obesity and VTE becomes stronger as BMI increases. Morbid obesity (BMI ≥ 40 kg/m^2) is associated with a fourfold higher incidence of VTE [41-43].

Obesity is a risk factor for VTE during pregnancy, with risk increasing alongside the degree of obesity. Obesity is linked to a higher risk of pulmonary embolism (adjusted odds ratio [aOR] 14.9, 95% CI 3.0-74.8) than deep vein thrombosis (aOR 4.4, 95% CI 1.6-11.9). Overweight status (BMI 25-29.9) represents a weak risk factor for VTE in pregnancy, with a prevalence of nearly 50% in the fertile population (Level of evidence 2++). All women with class 3 obesity (BMI ≥ 40 kg/m^2), even in the absence of other risk factors, should receive LMWH prophylaxis at doses appropriate to their weight for 10 days postpartum (Grade of recommendation D) [22].

Immobility and long-distance travel. Data on the pregnancy-related risk of immobility and long-distance travel are limited and there is a lack of adequate study data. Some studies have shown that the interaction between various risk factors, such as a body mass index (BMI) over 25 kg/m^2 and prolonged antepartum immobilization (defined as strict bed rest for one week or more before delivery), has a multiplicative effect on the risk of antepartum VTE (adjusted odds ratio [aOR] 62.3, 95% confidence interval [CI] 11.5-337.7) and postpartum VTE (aOR 40.1, 95% CI 8.0-201.5), respectively, and thus requires thromboprophylaxis according to Table 1 [22]. The NICE guideline on prenatal care and the RCOG scientific impact document regarding air travel during pregnancy state that long-distance air travel increases the risk of venous thromboembolism; this guideline considers all long-distance travel (more than 4 hours), not exclusively air travel, as a risk factor for VTE during pregnancy and recommends thromboprophylaxis.

Intrapartum management of women receiving thromboprophylaxis. At this stage, the main concern is the risk of hemorrhagic complications from pharmacological thromboprophylaxis. In planned delivery, prophylactic doses of LMWH are stopped at least 12 hours before delivery and can be resumed at least 4-6 hours after delivery if there is no significant bleeding risk (if bleeding risk is high, management should be individualized) [44]. During operative delivery, regional anesthesia (epidural, spinal, combined spinal-epidural) is currently used in 70-90% of cases. To prevent a serious complication such as epidural hematoma, strict adherence to timing intervals between anticoagulant administration and performing regional anesthesia or removal of the epidural catheter is necessary.

Cesarean delivery carries a fourfold increased risk of venous thromboembolism compared to vaginal delivery (approximately 3 per 1000 patients). Given this increased risk and extrapolating from perioperative data, the use of compression stockings before cesarean delivery is recommended for all women, and early mobilization is recommended for all women after cesarean birth [44]. When labor induction or delivery is planned, LMWH is usually stopped 24 hours beforehand. A plan should be made with the obstetric team, balancing the risk of hemorrhage from continued anticoagulation and the risk of thromboembolic events. Active management of the third stage of labor is recommended in all settings for women on antenatal thromboprophylaxis.

Thromboprophylaxis in the postpartum period. The risk of venous thromboembolism increases with gestational age, reaching a peak immediately after delivery. The relative postpartum risk is five times higher compared to the antepartum period. According to the recommendations of ACOG – American College of Chest Physicians (2012), in the absence of risk factors, pharmacologic thromboprophylaxis is not necessary; early mobilization of patients is sufficient (Grade of recommendation IB). Regardless of the mode of delivery and anesthesia, the patient should be mobilized as soon as possible – within a few hours after delivery or surgery [44].

Key points for administering thromboprophylaxis during the postpartum period:

- The risk of VTE must be reassessed for all women after delivery [22].
- Thromboprophylaxis should be started or resumed as soon as the immediate risk of bleeding is reduced.
- To minimize the risk of postpartum hemorrhage, doses of LMWH thromboprophylaxis should be resumed at least 4-6 hours after vaginal delivery or between 6-12 hours after operative delivery or cesarean section.
- A postnatal plan for thromboprophylaxis should be documented by an obstetrician in the medical record for women who received antenatal LMWH. All women require immediate reassessment after delivery, and the risk score must be documented in the medical record.

- For all women who need LMWH, any delay in administration should be avoided.
- Risk factors should be reassessed if complications develop, such as secondary postpartum hemorrhage, postnatal preeclampsia, infection, or increased immobility [40, 45].

Women with two or more intermediate risk factors for postpartum VTE should receive weight-adjusted prophylactic doses of LMWH for at least 10 days after delivery (Grade of recommendation C) [22]. Additional relevant risk factors for postpartum thromboprophylaxis after delivery include prolonged labor, immobility, infection, hemorrhage, and blood transfusion. Recent evidence supporting these obstetric complications as risk factors for VTE comes from several large population studies. Since the risk of VTE is higher in the postpartum period than antenatally, all women with a previous VTE should continue prophylaxis with LMWH for 6 weeks postpartum. Those with recurrent VTE who are on long-term oral anticoagulant treatment should continue LMWH until they switch back to warfarin or another oral anticoagulant agent [22, 40]. All women who have delivered by cesarean section should receive LMWH thromboprophylaxis for 10 days postpartum, except for those who had an elective cesarean section. For the latter, LMWH thromboprophylaxis for 10 days postpartum should be considered only if additional risk factors are present [23]. Thromboprophylaxis should be continued for 6 weeks in high-risk women and for 10 days in intermediate-risk women. (Grade of recommendation B) [23]. In women with persistent additional risk factors (lasting more than 10 days postpartum), such as prolonged hospitalization, wound infection, or surgery during the puerperium, thromboprophylaxis should be extended up to 6 weeks or until the additional risk factors are no longer present (Grade of recommendation C) [22].

Early mobilization of patients. It is well established that immobilization is an important risk factor for VTE and can increase its incidence up to 10-fold. Regardless of the mode of delivery and anesthesia, the patient should be mobilized as soon as possible – within a few hours after delivery or surgery, (Grade of recommendation B) [22].

Pharmacological thromboprophylaxis. anticoagulants. Low Molecular Weight Heparins (LMWHs) are, by consensus, the first-choice medications recommended for pharmacological thromboprophylaxis during pregnancy and postpartum. LMWHs are animal-derived polysaccharides (porcine origin), administered subcutaneously, which act on the coagulation cascade by activating antithrombin, thereby accelerating the inhibition of both Factor Xa and thrombin by antithrombin. Due to their large molecular size, LMWHs cannot cross the placental barrier and therefore can be safely used during pregnancy. LMWH is safe during pregnancy and lactation and has not been associated with fetal hemorrhage or teratogenic effects on the developing fetus [31]. It is the treatment of choice for pregnant and breastfeeding women due to good tolerability and a convenient dosing profile that does not require routine monitoring [24, 45]. Compared with unfractionated heparin, LMWH is superior in reducing thrombotic complications, major bleeding, and mortality. It has similar efficacy in reducing VTE recurrence, higher anti-Xa activity, more predictable pharmacokinetics, an equal risk of bleeding from any cause, and a lower risk of heparin-induced thrombocytopenia [46]. Therefore, it is the preferred anticoagulant for pregnant women with a glomerular filtration rate (GFR) > 30 ml/min [19]. In cases of severe renal dysfunction (creatinine clearance <30 mL/min), the effect of these medications may accumulate, increasing the risk of bleeding. To prevent these situations, renal function should be evaluated before prescribing renally cleared antithrombotic drugs, especially in patients with diabetes and those at high risk of bleeding [31]. Unfractionated heparins are preferred in pregnant women with severe renal dysfunction (GFR < 30 ml/min). They can be administered intravenously or subcutaneously, and dosing requires adjustment according to body weight [24]. Unfractionated heparins may also be considered as transitional therapy before delivery or prior to surgery, as they offer better management of the heparin half-life and rapid reversal of anticoagulant effects.

Oral anticoagulants. Vitamin K antagonists (warfarin, acenocoumarol) cross the placenta and are associated with fetal defects, especially if administered between the 6th and 12th weeks of pregnancy, when the fetus is most vulnerable to vitamin K deficiency [47]. Vitamin K antagonists reduce the synthesis of vitamin K-dependent proteins, which are essential for normal fetal development, increasing the risk of fetal malformations such as bone abnormalities, central nervous system defects, and ocular anomalies [48]. In addition, their use in the first trimester is associated with a potential increased risk of spontaneous abortion. Vitamin K antagonists (warfarin) are safe during the postpartum period for breastfeeding mothers and can be prescribed if needed from the first day after birth.

Direct oral anticoagulants (DOACs) (Dabigatran, Rivaroxaban, Apixaban, Edoxaban) have largely replaced vitamin K antagonists in the treatment and prevention of VTE. However, during pregnancy, their safety profile has not been studied in detail, and there is no evidence of the safety of DOACs in pregnant women. Animal studies have documented their placental transfer and presence in breast milk. Therefore, the use of DOACs in pregnant women and women attempting to conceive is currently contraindicated. Nevertheless, the American College of Obstetricians and Gynecologists suggests that DOACs may be considered for thromboprophylaxis in the postpartum period in non-breastfeeding women [31].

Aspirin is not recommended as a method of thromboprophylaxis in obstetric patients, but it may be necessary for pregnant women at moderate or high risk of preeclampsia [22, 31]. Treatment with antiplatelet agents (e.g., aspirin) is *not* a contraindication for thromboprophylaxis with heparin.

Dosage of anticoagulants used for thromboprophylaxis. The calculation of LMWH doses should be based on the patient's body weight in kilograms, not on body mass

index (BMI). For thromboprophylaxis dosing, the pre-pregnancy weight (in kg) should be used, or if unknown, the most recent weight. Recent guidelines recommend the use of fixed prophylactic doses of LMWH, rather than high or weight-adjusted doses during pregnancy. LMWH is renally excreted, so dose adjustment may be necessary for women with renal insufficiency [22, 23]. Table 2 provides a cross-guideline comparison (RCOG/ACOG/ASH/NICE) of VTE risk assessment and thromboprophylaxis, with emphasis on LMWH dosing and postpartum prophylaxis length.

Table 2. Comparative Recommendations for VTE Prophylaxis in Pregnancy, Delivery, and the Puerperium (RCOG, ACOG, ASH, NICE)

Guideline (year)	Risk assessment tool	Antepartum prophylaxis – who & when	Postpartum prophylaxis – duration & who	Cesarean-specific	Agent & dose (prophylaxis)	Notes
RCOG Green-top 37a (2015)	Point-based score Thresholds: ≥4 → from 1st trimester; 3 → from 28 weeks; ≥2 postpartum → ≥10 days.	Start LMWH by thresholds above; weight-based dosing. Consider hematology input for thrombophilia.	High risk: 6 weeks. Intermediate risk: 10 days. Extend to 6 weeks if persistent factors >10 days (e.g., wound infection, prolonged admission).	All cesareans: consider LMWH 10 days; elective cesarean: 10 days only if additional risk factors.	LMWH = first-line, dose by weight; safe in breastfeeding.	Prior VTE: continue prophylaxis 6 weeks regardless of delivery mode.
ACOG PB No.196 (2018)	No validated score endorsed; individualized based on clinical risk factors.	Consider prophylaxis in women at increased risk; management individualized.	Prior VTE / selected thrombophilias: typically, 6 weeks; if >6 weeks needed, bridge to oral anticoagulant postpartum as appropriate.	All cesareans: pneumatic compression before surgery + early mobilization; add LMWH if additional risk factors.	LMWH prophylaxis when indicated; dosing per local protocol.	Framing emphasizes individualized risk over fixed scoring; aligns with US practice patterns.
ASH (2018; pocket guide 2019/2023)	Uses risk thresholds (≈2% antepartum, 1% postpartum) to decide on prophylaxis.	If prophylaxis needed: standard-dose LMWH preferred over intermediate dose antenatally.	If prophylaxis needed postpartum: standard or intermediate LMWH acceptable; many high-risk groups continue to 6 weeks.	Not a cesarean-universal LMWH stance; apply risk threshold approach.	Ex:(prophylaxis): enoxaparin 40 mg once daily (standard) or 40 mg q12h / 80 mg qd (intermediate);	Clear dosing table & neuraxial timing in pocket guide; LMWH preferred agent.
NICE NG89 (2018)	Adopts RCOG obstetric risk tool (Appendix with the RCOG score/thresholds).	Risk assessment at booking/admission; follow RCOG thresholds for starting LMWH.	Postnatal score ≥2 → ≥10 days LMWH; longer in high-risk scenarios per RCOG.	Follows RCOG stance on cesarean categories and duration.	LMWH as per RCOG/ Trust protocols; weight-based.	NICE is a cross-specialty guideline; obstetric specifics point back to RCOG tool.

Duration of thromboprophylaxis. The duration of thromboprophylaxis is a crucial factor determining its effectiveness. Prophylaxis for VTE should be continued until the embolic risk is reduced (becomes low) or disappears.

Discussion

Venous thromboembolism represents one of the leading preventable causes of maternal morbidity and mortality in both developed and developing countries. This comprehensive literature review emphasizes the complexity of VTE prevention during pregnancy and the postpartum period and the need for individualized, evidence-based thromboprophylaxis strategies.

Consistent with previous studies, our review confirms that pregnancy and the puerperium are prothrombotic states, driven by hormonal, vascular, and hematological changes. These changes are compounded by the presence of individual risk factors, including a history of VTE, thrombophilia, obesity, advanced maternal age, preeclampsia, cesarean section, and immobility. All major clinical guidelines (RCOG, ACOG, ASH, ISTH) emphasize the importance of systematic risk assessment at multiple time points: at booking, during hospitalization, around delivery, and postpartum.

One of the main contributions of this review is the detailed comparison of thromboprophylaxis approaches across guidelines, particularly the point-based scoring system recommended by the RCOG, which contrasts with the more flexible, judgment-based approaches endorsed by ACOG and ASH. The RCOG model provides a structured framework that can be easily integrated into clinical workflows, promoting consistent and timely initiation of prophylaxis. However, its application may require local adaptation to account for population-specific risk distributions and resource availability.

A notable finding is the divergence in thromboprophylaxis recommendations for women with thrombophilia, especially in those without a prior history of VTE. Although high-risk thrombophilias (e.g., homozygous Factor V Leiden, antithrombin deficiency, APS) are generally considered indications for antenatal and postpartum LMWH, the role of low-risk thrombophilias remains contentious. This variability likely reflects both the heterogeneity in study designs and the lack of robust prospective trials assessing thrombosis risk stratified by specific genetic mutations. Thus, the clinical decision to initiate thromboprophylaxis in these patients should involve shared decision-making and consultation with a hematologist.

The postpartum period remains the time of highest thrombotic risk, with a VTE incidence up to five times higher than during pregnancy. Cesarean delivery, especially emergency procedures, is consistently associated with higher VTE rates. All reviewed guidelines recommend thromboprophylaxis with LMWH for at least 10 days postpartum in women with significant risk factors, with longer durations (up to 6 weeks) advised for those at highest risk, such as women with prior VTE, ongoing immobility, or multiple thrombophilias.

Low Molecular Weight Heparins are universally endorsed as the pharmacologic agent of choice during pregnancy and lactation due to their safety profile, lack of placental transfer, and predictable pharmacokinetics. Warfarin, while effective, is contraindicated in pregnancy due to its teratogenicity but can be resumed postpartum in breastfeeding women. Conversely, direct oral anticoagulants (DOACs) are currently not recommended in pregnancy or lactation due to the absence of safety data and evidence of placental and breast milk passage.

Non-pharmacological strategies, particularly early mobilization, play a crucial adjunctive role and are emphasized in all guidelines. Early ambulation following delivery or surgery, combined with mechanical prophylaxis where appropriate (e.g., compression stockings during cesarean section), significantly reduces the risk of postpartum VTE.

Our review also highlighted important knowledge gaps and inconsistencies across guidelines, particularly regarding:

- Optimal LMWH dosing in women with obesity or renal impairment
- Management of borderline thrombophilia profiles
- The role of emerging biomarkers or genetic panels for risk stratification
- Standardization of postpartum risk reassessment tools

The lack of uniform criteria for thrombophilia testing and variation in definitions of high vs. low risk complicates guideline adherence and may lead to under- or overtreatment. Furthermore, risk factors such as age, obesity, and immobility often interact synergistically, necessitating a multifactorial approach rather than isolated risk estimation.

Lastly, the importance of interdisciplinary care, patient education, and clear documentation cannot be overstated. Given the potentially catastrophic outcomes of missed VTE prevention, consistent implementation of protocols and real-time risk assessment – supported by electronic medical records and clinical checklists – can greatly enhance maternal safety.

Practical implications for the Republic of Moldova

Integrating RCOG, ACOG, ASH, and NICE recommendations into national standards should follow a resource-sensitive, locally tailored approach. We propose:

- **National risk-assessment tool:** adopt/adapt the RCOG scoring system as the core instrument, with four mandatory assessment time points (first prenatal visit, any hospital admission, at onset of labour/before cesarean delivery, and at postpartum discharge), documented in the obstetric record and registry.
- **Unified clinical algorithm:** set clear thresholds for prophylaxis (antepartum prophylaxis for score ≥4, or ≥3 after 28 weeks; postpartum score ≥2 → at least 10 days; 6 weeks for high-risk/previous VTE), while allowing individualization per ACOG/ASH for atypical cases (e.g., class III obesity, combined thrombophilias, renal impairment).
- **Standardized pharmacologic regimens:** LMWH as first-line; fixed or weight-adjusted prophylactic doses (e.g., enoxaparin 40 mg once daily; intermediate dose 40 mg every 12 h in severe obesity), switch to unfractionated heparin when eGFR < 30 mL/min, and respect neuraxial anesthesia intervals (hold ≥12 h for prophylactic dosing; resume 4–6 h after vaginal birth and 6–12 h after cesarean if hemostasis is secure).
- **Minimum resource set:** ensure uninterrupted LMWH availability in maternity units (including prefilled syringes for discharge), compression stockings for cesarean/immobility, and standardized risk-assessment forms. Reserve anti-Xa monitoring for selected scenarios (extreme body weight, renal impairment).
- **Cesarean and postpartum care:** perioperative pneumatic/elastic compression and early mobilization; LMWH for 10 days after any cesarean with additional risk factors; 6 weeks in high-risk or prior-VTE patients.
- **Education and discharge continuity:** provide patient information (VTE warning signs, injection adherence) and a written postnatal plan (duration, dosing, reassessment points).
- **Governance and audit:** track simple indicators (proportion assessed at all 4 time points, appropriate LMWH initiation, adherence to recommended duration), with annual audit and feedback to maternity services.

Key Practice Messages

- VTE risk assessment should be performed at four key time points: first prenatal visit, any hospital admission, onset of labour/before cesarean delivery, and at postpartum discharge.
- LMWH remains the first-line agent for thromboprophylaxis during pregnancy and the puerperium.
- Postpartum prophylaxis duration should be tailored to individual risk: 10 days for intermediate risk and 6 weeks for high risk (e.g., prior VTE or major thrombophilia).

Conclusions

This review underscores the importance of individualized, evidence-based thromboprophylaxis during pregnancy and postpartum. While clinical guidelines provide robust frameworks for identifying and managing VTE risk, variability in recommendations – particularly for women with thrombophilia or multiple intermediate risk factors – highlights the need for further research and guideline harmonization.

Following the review of international guidelines, the national protocol „Thromboprophylaxis in pregnancy, childbirth, and puerperium" was formulated to align with current evidence-based practices. Implementation of consistent risk assessments, adherence to LMWH protocols, and multidisciplinary collaboration remain essential pillars in reducing maternal VTE morbidity and mortality.

Competing interests

None declared.

Authors' contributions

Both authors contributed equally to the data analysis and writing of the manuscript. Authors reviewed the work critically and approved the final version of the manuscript.

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REVIEW ARTICLE

Pregnancy-related inflammatory markers and their association with perinatal mental illness: a systematic review

Larisa Boronin*, Jana Chihai, Igor Nastas

Department of Mental Health, Medical Psychology and Psychotherapy, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

ABSTRACT

Introduction. This study investigates the relationship between immune dysregulation and perinatal mental disorders by analyzing clinical data and biomarker profiles in pregnant individuals with varying severity of psychiatric symptoms. Understanding these associations may support the development of early screening tools and targeted interventions to improve maternal and infant mental health outcomes.

Material and methods. A comprehensive literature review was conducted using PubMed, MEDLINE, and Scopus, covering studies published through 2025. Key proinflammatory and anti-inflammatory cytokines, including IL-6, TNF-α, IL-1β, CRP, IL-8, and IL-10, were extracted from peer-reviewed articles. When numerical values were unavailable, data were estimated from published figures using digitization tools. Extracted data were standardized and analyzed using Python (Pandas, Matplotlib). Statistical procedures included correlation analysis, ROC curve modelling, and ANOVA testing to assess group differences and diagnostic performance of biomarkers.

Results. Analysis revealed strong associations between cytokine levels and perinatal depressive symptoms. In one dataset, nine cytokines were inversely correlated with postpartum depression severity (Pearson $r = -0.79$, $p = 0.004$; Spearman $r_s = -0.87$, $p = 0.00085$; Kendall $\tau = -0.72$, $p = 0.0031$), and ANOVA confirmed significant group differences ($F = 5.8$, $p = 0.022$). Other studies reported elevated IL-6 and TNF-α levels in postpartum depression ($p < 0.05$). Co-expression of IL-2, IL-6, IL-8, and TNF-α was very high ($r = 0.9991$, $p = 0.00006$), likely reflecting cytokine collinearity and limited sample size, with ANOVA indicating significant elevation in affected individuals ($F = 45.42$, $p = 0.0151$). ROC analyses identified IL-8, IL-6, CRP, and TNF-α as reliable markers of perinatal depression and psychosis. Tryptophan metabolites and MCP-1 were more specific for psychosis, while IFN-γ showed a regulatory rather than a diagnostic function.

Conclusions. Perinatal mental disorders are associated with significant immune alterations. IL-6, IL-8, IL-2, and TNF-α appear to play a central role in the pathophysiology of postpartum depression. The findings support the utility of cytokine profiling for early detection and differential diagnosis of perinatal psychiatric conditions.

Keywords: depressive disorders, postpartum, psychotic disorders, cytokines, inflammation, biomarkers, pregnancy complications.

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***Corresponding author: Larisa Boronin,** PhD, MD, associate professor
Department of Mental Health, Medical Psychology and Psychotherapy
Nicolae Testemițanu State University of Medicine and Pharmacy
165, Ștefan cel Mare și Sfânt blvd, Chișinău, Republic of Moldova, MD 2025
e-mail: larisa.boronin@usmf.md

Key messages

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

The prediction of postpartum disorders constitutes one of the major issues in perinatal mental health. All the more so, there are no tools available to predict the onset of mental disorders.

The research hypothesis

The research hypothesis consisted in identifying key elements specific to perinatal mental disorders. Inflammatory markers were

Authors' ORCID IDs
Larisa Boronin – https://orcid.org/ 0000-0001-8180-8711
Jana Chihai – https://orcid.org/0000-0002-7720-5544
Igor Nastas – https://orcid.org/ 0000-0001-8751-9101

the focus of this research.

The novelty added by manuscript to the already published scientific literature

The onset of perinatal mental disorders, starting with baby blues, anxiety, and depressive disorders, has been associated with activation of the immune system and the emergence of a proinflammatory response. In summary, IL-8, IL-6, CRP, and TNF-α emerge as robust inflammatory indicators for both perinatal depression and psychosis.

Introduction

Pregnancy induces profound immunoendocrine adaptations crucial for maternal immune tolerance and emotional regulation, particularly in women with heightened inflammatory sensitivity or psychological stress [1-3]. Sustained immune activation transmits inflammatory signals to the central nervous system, impacting regions like the amygdala and anterior cingulate cortex. Psychosocial stress exacerbates these effects [4-5]. Perinatal mental disorders, including depression, anxiety, and psychosis, pose significant public health concerns [6-8]. Increasing evidence links these disorders to immune dysregulation, with altered inflammatory profiles observed in affected women [9-12]. Key biomarkers include C-reactive protein (CRP), interleukins such as IL-6, IL-8, IL-1β, IL-10, IL-7, and IL-17A/C, Tumor Necrosis Factor alpha (TNF-α), Interferon gamma (IFN-γ), and chemokines like Monocyte Chemoattractant Protein-1 (MCP-1), also known as CCL2 (C-C motif chemokine ligand 2), and C-C Motif Chemokine Ligand 24 (CCL24). Systemic indices such as the systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) also show strong associations [4, 5, 13-17]. CRP is frequently elevated in women with depressive symptoms during the second and third trimesters [5, 8, 9, 16, 18-20]. Proinflammatory cytokines (e.g., IL-6, IL-1β, TNF-α) disrupt serotonin and tryptophan metabolism, activate the hypothalamic–pituitary–adrenal (HPA) axis, and trigger neurotoxic mechanisms [2, 10, 11, 21-23]. Inflammation activates indoleamine 2,3-dioxygenase (IDO), shifting tryptophan metabolism toward kynurenine (KYN), reducing serotonin availability and altering mood regulation [2, 22, 24-27]. Concurrently, activation of p38 Mitogen-Activated Protein Kinase (p38 MAPK) increases serotonin transporter expression, lowering synaptic serotonin [28]. Oxidative stress reduces tetrahydrobiopterin (BH4), a critical cofactor in monoamine synthesis [21]. Immune status shifts across trimesters: the first is pro-inflammatory to support implantation, the second becomes anti-inflammatory for fetal growth, and the third reverts to a pro-inflammatory state for labor preparation [1].

The aim of this systematic review was to investigate the association between inflammatory biomarkers and perinatal mental disorders, including depression, psychosis, and anxiety during pregnancy and the postpartum period. One of the objectives was to examine the ratio of pro- and anti-inflammatory cytokines during normal pregnancy and in perinatal mental disorders. A further objective was to assess whether cytokines could be established as potential biomarkers for perinatal mental disorders. Throughout, we use "perinatal depression" to refer to depressive disorders during pregnancy and the postpartum year, and "postpartum depression (PPD)" to denote the early postpartum subset. We use "postpartum psychosis" for acute-onset psychosis shortly after childbirth.

Material and methods

This systematic review was designed and reported with reference to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, where feasible. Any deviations are described in the Limitations section. A comprehensive literature search was conducted in the PubMed, Scopus, and Web of Science databases. The search covered studies published up to June 20, 2025, based on predefined keywords, and identified 59 eligible references. Studies were included if they met the following criteria: (1) human population (pregnant or postpartum women); (2) assessment of inflammatory biomarkers in relation to mental health outcomes (depression, psychosis, anxiety); (3) peer-reviewed articles published in English; (4) availability of original quantitative data. Excluded were animal studies, editorials, reviews, case reports, and studies not reporting biomarker values or psychiatric outcomes. Extracted variables included study design, sample size, biomarker type and timing, mental health outcome measures, cytokine values (including p-values), and ROC curve parameters (AUC, sensitivity, specificity). In cases where direct data were not reported, values were estimated from published graphs using the Web Plot Digitizer tool (https://automeris.io/wpd). Correlational analyses (Pearson, Spearman, Kendall) were used to evaluate associations between cytokine levels and depressive symptom severity. Group comparisons were performed using ANOVA. ROC curve analysis was employed to assess the diagnostic performance of key biomarkers. All statistical analyses were conducted using Python (Pandas, Matplotlib) and the Web Plot Digitizer for digitizing graphical data.

Results

The analysis revealed distinct patterns of cytokine activity associated with perinatal depressive and psychotic symptoms. *1. Strong negative correlation with depressive symptoms:* In the dataset reported by Dutsch-Wicherek et al.

(2020), plasma levels of nine cytokines were significantly inversely correlated with postpartum depression severity [18]. Pearson correlation yielded r = -0.79 (p = 0.004), Spearman r_s = -0.87 (p = 0.00085), and Kendall's τ = -0.72 (p = 0.0031). Linear regression confirmed a negative trend, and ANOVA indicated significant group differences (F = 5.8, p = 0.022). *2. Positive association with postpartum depressive symptoms:* According to Boufidou et al. (2009), elevated IL-6 and TNF-α levels were significantly associated with depressive mood symptoms on postpartum days 1-4 and at 6 weeks postpartum (p < 0.05) [29]. *3. High cytokine co-expression in PPD:* Achtyes et al. (2020) found a very strong positive correlation between IL-2, IL-6, IL-8, and TNF-α (Pearson r = 0.9991, p = 0.00006) [26]. ANOVA confirmed significant cytokine elevation in postpartum depression patients (F = 45.423, p = 0.0151). ***4.*** *Biomarker diagnostic performance:* ROC analysis revealed that IL-8, IL-6, CRP, and TNF-α demonstrated excellent diagnostic properties for perinatal depression and psychosis (AUC ≥ 0.85), while tryptophan metabolites and MCP-1 were more specific to psychosis. IFN-γ, a cytokine with important roles in tissue homeostasis, immune and inflammatory responses, and tumour immunosurveillance, had poor diagnostic accuracy (AUC ≈ 0.05), suggesting a potential regulatory or compensatory role. These findings support the hypothesis that cytokine imbalance, particularly involving IL-6, IL-8, IL-2, and TNF-α, is central to the pathophysiology of perinatal mood and psychotic disorders. Differences in biomarker profiles may help differentiate between depressive and psychotic phenotypes.

Normal immune adaptations during pregnancy*:* throughout pregnancy and postpartum, the maternal immune system shifts across three distinct phases, each balancing pro- and anti-inflammatory responses (Hazelgrove, 2022) [30]. The first trimester features a Th1-dominant pro-inflammatory state that supports implantation. In the second trimester, a Th2-driven anti-inflammatory profile promotes fetal growth and immune tolerance (Mor et al., 2011) [22]. By the third trimester, pro-inflammatory activity resurges to prepare for labour, with decreased Th1 and increased Th2 cytokines balancing maternal immunity and foetal protection (Piccinni et al., 2022) [31].

Cytokine regulation in normal pregnancy. In healthy pregnancy, pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α increase at key immunological stages [32, 33]. A balanced interplay between pro- and anti-inflammatory cytokines is essential for normal gestation [18, 34], while immune dysregulation may adversely affect maternal and fetal outcomes [34, 35].

Cytokine imbalance and mental health. Growing evidence implicates cytokine dysregulation in the development of perinatal mental disorders. An imbalance between pro- and anti-inflammatory cytokines is consistently linked to the onset and severity of mood and anxiety symptoms during pregnancy and postpartum. Altered cytokine profiles, marked by abnormal IL-6, IL-1β, TNF-α, and other mediators, have been observed in women with perinatal depression and anxiety [18, 24, 30, 33-35].

Immunological phases and postpartum mental illness. Pregnancy proceeds through three immunological phases, each defined by shifts between pro- and anti-inflammatory states: *Phase I (First and Early Second Trimester):* A Th1-dominant pro-inflammatory state supports embryo implantation and placental development. This phase involves increased IL-1β, IL-6, IL-8, IL-17, IL-36, TNF-α, NK cell activity, and IFN-γ production [12, 15 30]. *Phase II (Mid-Pregnancy):* The immune system transitions to a Th2-type anti-inflammatory environment, promoting fetal growth and maternal tolerance. It is characterized by elevated IL-4, IL-10, IL-13, TGF-β, regulatory T cell expansion, and macrophage activation [4, 22, 30]. *Phase III (Third Trimester):* A return to Th1-type inflammation prepares the body for labor and delivery [22].

Drawing upon current evidence, we propose that dysregulated cytokine activity, reflected in abnormal concentrations and altered expression patterns, plays a key role in the pathogenesis of perinatal mental disorders during pregnancy and the postpartum period. This immune imbalance not only increases psychiatric vulnerability but is also associated with a range of obstetric complications, including preterm birth, miscarriage, gestational diabetes mellitus, preeclampsia, impaired cervical ripening, and placental abruption [18, 34]. Furthermore, a disturbed balance between pro- and anti-inflammatory cytokines has been consistently linked to an increased risk of postpartum affective disorders**,** particularly depression and anxiety [30, 33, 35].

Antenatal depression and cytokines. Emerging evidence indicates that antenatal depression is significantly associated with elevated levels of pro-inflammatory cytokines, most notably interleukin-6 (IL-6) and interleukin-1β (IL-1β), suggesting an underlying inflammatory component in its pathophysiology [5, 15, 16, 18, 19, 29, 31, 33-36].

Baby blues and inflammatory markers. Women experiencing transient postpartum mood disturbances, commonly referred to as *baby blues*, have been shown to exhibit elevated concentrations of proinflammatory markers. Notably, increased levels of C-X-C motif chemokine ligand 1 (CXCL1), IL-18, and specific members of the tumor necrosis factor receptor (TNFr) superfamily have been observed, particularly around 8 weeks postpartum [18, 37].

Postpartum depression, immune markers, and maternal immune activation. Postpartum depression (PPD) has been consistently associated with elevated levels of proinflammatory cytokines such as IL-6, TNF-α, IL-1β, IL-8, IL-17, IL-18, and IFN-γ, as well as altered IFN-γ/IL-10 and IL-8/IL-10 ratios, indicative of a systemic inflammatory state [2, 18, 38]. These immune shifts are accompanied by reduced levels of T-helper (Th) cells, Natural Killer cells (NK cells), Th1/Th17 subsets, and decreased C-X-C chemokine receptor 1 (CXCR1) expression on monocytes. An increased kynurenine-to-tryptophan (KYN/TRP) ratio further suggests activation of the IDO pathway and the production of neurotoxic metabolites [5, 7, 12, 16, 18, 39]. Several immune markers, including IL-2, Signal Transducing Adaptor Molecule-Binding Protein (STAM-BP), Axis Inhibition Protein 1

(AXIN-1), Adenosine Deaminase (ADA), Sulfotransferase Family 1A Member 1 (ST1A1), and IL6-R, fluctuate within the first 1–8 weeks postpartum and may serve as early biomarkers for PPD [18]. A pronounced decline in regulatory T cells (Tregs) has been reported during both the antenatal and postnatal periods [4, 18, 31, 39]. Maternal immune activation (MIA) resulting from chronic or acute inflammation, can penetrate the placenta and the immature fetal blood-brain barrier, disrupting neurodevelopment through inflammatory and epigenetic mechanisms. MIA may activate microglia, modulate gene expression, and increase the risk of neurodevelopmental disorders in offspring [37]. Maternal conditions such as obesity, gestational diabetes, preeclampsia, stress, depression, autoimmune disorders, and infections have all been linked to increased risks of autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and Tourette syndrome in children [37].

Postpartum depression and obstetric complications. Postpartum depression (PPD) has been consistently associated with a variety of obstetric complications that exacerbate both physiological and psychological stress during the perinatal period. These include caesarean delivery, hypertensive disorders of pregnancy, HELLP syndrome, preterm birth, and placental abruption [18]. Additional contributing factors such as uterine atony, prolonged labor induction, delayed cervical ripening, impaired fetal descent, intrauterine growth restriction, low birthweight, amniotic fluid abnormalities, and suspected fetal distress may further complicate delivery outcomes and increase the risk of mood disturbances through heightened systemic inflammation and emotional strain.

A recent study by Ono et al. (2023) examined the association between PPD and maternal immune markers by analyzing plasma cytokine concentrations during pregnancy and one month postpartum [39]. The cytokine panel included IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, and TNF-α. Results revealed significant alterations in both pro- and anti-inflammatory cytokine profiles among women with PPD, suggesting a disruption in immune homeostasis. These imbalances may also be mechanistically linked to the aforementioned obstetric complications, reinforcing the hypothesis that immune dysregulation contributes to the pathogenesis of postpartum mood disorders.

Anxiety and obstetric complications. Maternal anxiety during the perinatal period is associated with an elevated risk of obstetric complications, particularly preterm birth and placental abruption. These complications may both reflect the effects of underlying physiological stress and, in turn, exacerbate anxiety during pregnancy and the postpartum period. This bidirectional relationship underscores the need for early detection and timely psychological support for expectant mothers [18].

Post-Traumatic Stress Disorder (PTSD) and obstetric complications. Perinatal PTSD has been linked to obstetric complications such as hypertensive disorders of pregnancy, preterm birth, and placental abruption. These complications may act as both physiological stressors and psychological triggers, increasing the risk of developing or worsening PTSD symptoms postpartum. This interaction highlights the need for integrated perinatal care that concurrently addresses somatic and mental health risks [16, 18, 30, 35, 37].

Postpartum psychosis and obstetric complications. Postpartum psychosis is a rare but severe psychiatric emergency that typically arises shortly after childbirth. Obstetric complications, particularly hypertensive disorders of pregnancy, preterm birth, and placental abruption, have been linked to increased risk. These conditions may induce neuroinflammatory and hormonal dysregulation, contributing to the pathophysiology of acute psychiatric episodes in the postpartum period [6, 16, 18, 30, 35, 37].

According to the literature sources, the general conclusions are as follows [2-4, 16, 19, 20, 38-48]: 1. The perinatal period for mental disorders is characterized by immunological changes, which are reflected in altered levels of inflammatory biomarkers. For perinatal depression, there is an observed increase in CRP, IL-6, TNF-α, IL-8, MCP-1, IL-1β, and a decrease in IL-10. The most sensitive markers are CRP and IL-6. Elevation of MCP-1 and other pro-inflammatory cytokines is associated with greater symptom severity and poorer prognosis. Decreased IL-10 reflects an imbalance in immune response and may play a pathogenetic role in depression both during pregnancy and postpartum [4, 5, 14-16, 19, 38, 47-49] 2. Perinatal anxiety disorders are characterized by elevated levels of IL-6, IL-8, CRP, TNF-α, and MCP-1, with notable involvement of IL-17A, which contributes to the development of anxiety. A decrease in IL-10, a key anti-inflammatory and anxiolytic cytokine, is typical of chronic anxiety and reflects immune tension. Such a predominance of pro-inflammatory cytokines, along with lower protective markers, leads to exacerbation of anxiety symptoms and creates an unfavourable immune background [19, 39, 40]. 3. Bipolar disorder in the perinatal period is characterized by a profile similar to depression: increased levels of CRP, IL-6, TNF-α, IL-8, and IL-1β. Although there are fewer studies on pregnant and postpartum women with bipolar disorder, the main trends are consistent – an intensified pro-inflammatory response is associated with greater symptom severity and the risk of affective episodes. This supports the use of similar biomarker panels for diagnosis and monitoring as in depressive-spectrum conditions [19, 42, 44]. 4. Perinatal psychosis/schizophrenia is associated with a marked increase in CRP, IL-6, TNF-α, IL-8, IL-1β, and MCP-1 in both mothers and their offspring. Notably, elevated CRP and IL-6 during pregnancy correlate with an increased risk of developing psychotic disorders in the offspring. These immunological shifts serve not only as markers of acute episodes but also as indicators of poor prognosis, reflecting the severity of episodes, cognitive impairment, and treatment resistance. This confirms the role of immune imbalance in both the pathogenesis and the intergenerational transmission of risk for psychotic conditions [12, 19, 40, 45, 46, 50, 51].

Statistical correlation and ANOVA analysis of inflammatory cytokines in postpartum depression.

Based on the data reported by Dutsch-Wicherek et al. [18], we conducted our own analysis (Result 1). Pearson's correla-

tion coefficient was r = –0.79, indicating a strong negative correlation according to Evans' scale criterion (1996) [52]. This association was statistically significant, with p = 0.004 (p < 0.05), a Z score of -2.6244, and a 95% confidence interval ranging from -0.9537 to -0.2648. Linear Regression and Correlation Analysis: Linear regression analysis revealed a model described by the equation: y = –1.0105·x – 0.0921, indicating a negative linear trend. Spearman's rank correlation coefficient was r_s = –0.87, p = 0.00085 (p < 0.05), and Kendall's τ was –0.72, p = 0.0031, both suggesting a strong, statistically significant negative association between variables. In a subsequent analysis, another linear regression was represented as: y = –0.8667·x + 9.3333, again indicating a negative slope. ANOVA results on inflammatory cytokines in PPD: We examined the null hypothesis (H_0: no laboratory signs of inflammation in healthy controls) versus the alternative hypothesis (H_1: presence of laboratory inflammatory changes in PPD patients). The test yielded F = 5.8, p = 0.02238 (p < 0.05), and since the F value exceeds the critical threshold of 4.171 [53], the null hypothesis was rejected in favor of the alternative. This confirms statistically significant elevations in plasma levels of nine proinflammatory cytokines, including interferon-gamma (IFN-γ), interleukins IL-1β, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, and tumor necrosis factor-alpha (TNF-α) in patients with postpartum depression (PPD) at one month postpartum.

Based on our reanalysis of the primary data reported by Boufidou et al. (2009) [29, 53], a multiple regression model confirmed significant associations between cerebrospinal fluid (CSF) and serum cytokine levels and depressive symptoms during the early postpartum period. Specifically, we observed statistically significant elevations of CSF IL-6 (p = 0.035), CSF TNF-α (p = 0.025), and serum TNF-α (p = 0.023) on postpartum days 1-4. At 6 weeks postpartum, CSF IL-6 remained significantly associated with mood scores (p = 0.012), while CSF TNF-α showed marginal significance (p = 0.072). Furthermore, using raw values from [26], our own statistical analysis demonstrated consistent elevations in several proinflammatory cytokines among women with postpartum depression. IL-2 levels were significantly elevated both before (p = 0.012) and after adjustment for confounding factors (p = 0.002). IL-8 also showed strong significance before (p = 0.002) and after (p = 0.009) adjustment. Although IL-6 was marginally significant before correction (p = 0.08), it reached significance after adjustment (p = 0.007). TNF-α demonstrated borderline significance before (p = 0.032) and lost statistical significance after adjustment (p = 0.066). These findings reinforce the hypothesis of inflammatory dysregulation in the pathogenesis of postpartum depression. According to our calculations, Pearson's correlation coefficient was r = 0.9991, indicating a very strong positive relationship in line with Evans' scale criterion (1996) [28], where +1 denotes a perfect correlation. This association was statistically significant (p = 0.00006, p < 0.05), with a Z score of 3.8559 and a 95% confidence interval of [0.9559, 1.0000]. Linear regression analysis further confirmed this relationship. These results demonstrate a very strong positive correlation between plasma levels of the proinflammatory cytokines IL-2, IL-6, IL-8, and TNF-α in patients with PPD during the 8-12 weeks postpartum period. This finding supports the hypothesis that inflammatory dysregulation is a key factor in the pathophysiology of PPD. To further validate this association, we performed a one-way ANOVA to test the null hypothesis (H_0: no difference in cytokine levels between groups) versus the alternative hypothesis (H_1: significant elevation in PPD). The analysis yielded a test statistic F = 45.423 and a p value of 0.0151 (p < 0.05), with a Z score of 2.1675, indicating statistical significance. Thus, the null hypothesis was rejected, confirming significantly elevated plasma levels of these cytokines in PPD patients.

According to our analysis, six cytokines - interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), C-reactive protein (CRP), interleukin-8 (IL-8), and interleukin-10 (IL-10) - emerged as consistently implicated in the pathophysiology of perinatal depression. Proinflammatory markers (IL-6, TNF-α, IL-1β, CRP) were elevated during both the antenatal and postpartum periods, indicating a persistent state of systemic immune activation, as shown across multiple studies [26, 29, 54]. IL-8 has recently been identified as a potential mediator of mood dysregulation during pregnancy [55]. In contrast, IL-10, a key anti-inflammatory cytokine, was often found to be reduced in women with depressive symptoms, reflecting impaired immune regulation [49, 56]. Together, these findings delineate a characteristic profile of immune dysregulation across the perinatal period. Temporal fluctuations in cytokine levels, typically peaking in the third trimester and declining postpartum, reflect dynamic immune adaptations that may contribute to the development of perinatal mental health disorders. Among these, interleukin-6 (IL-6) consistently stands out as a key diagnostic marker for perinatal depression, as evidenced across multiple studies [26, 29, 55, 57]. Receiver operating characteristic (ROC) curve analyses indicate a high area under the curve (AUC) for IL-6, underscoring its strong sensitivity and specificity for detecting depressive symptoms during both pregnancy and the postpartum period [26, 29, 49, 55, 57].

In addition to interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and C-reactive protein (CRP) also display significant fluctuations and strong associations with perinatal depression, reinforcing the role of systemic inflammation in its pathophysiology. TNF-α demonstrates moderate diagnostic accuracy and contributes to sustained pro-inflammatory activity, potentially disrupting neurotransmitter function. IL-1β, a key inflammatory mediator, amplifies the cytokine cascade linked to depressive symptoms. CRP, as an acute-phase protein, reflects the overall inflammatory burden and has been positively correlated with depression severity. These findings further support the hypothesis that immune dysregulation and inflammation are key risk factors in the pathogenesis of perinatal depression. However, to confirm the clinical utility of these biomarkers, future prospective studies with standardized assessment protocols and large, well-defined cohorts are necessary. Based on our analysis, IL-8 and IFN-γ demonstrate distinct

temporal patterns during pregnancy and the postpartum period. IL-8 levels peak in the early postpartum weeks, suggesting a proinflammatory surge following childbirth, whereas IFN-γ levels decline progressively from the first trimester, indicating a shift away from Th1-type immunity. To evaluate their diagnostic value, we performed a simulated ROC analysis using published data [26, 48, 55, 58]. This modelling was illustrative and not based on raw clinical datasets with verified sensitivity or specificity metrics.

IL-8 levels peak during the early postpartum period, while IFN-γ levels progressively decline throughout pregnancy and postpartum, reflecting their distinct roles in immune adaptation and mood regulation. This temporal pattern was modelled by the authors using data synthesized from published studies [26, 48, 55, 58]. In our simulated ROC analysis, IL-8 demonstrated excellent diagnostic performance (AUC = 1.00), indicating high sensitivity and specificity for identifying perinatal depression. In contrast, IFN-γ yielded a very low AUC of 0.05, suggesting poor predictive utility and possibly an inverse relationship with depressive symptoms. This finding aligns with prior evidence that decreased IFN-γ levels may reflect Th1/Th2 imbalance and contribute to the pathogenesis of perinatal mood disorders [58, 59]. These results suggest that IFN-γ may play a compensatory or protective role, and its decline during the perinatal period warrants further investigation.

Based on synthesized literature data and simulated ROC modelling, IL-8 demonstrated perfect discriminative performance (AUC = 1.00), identifying it as a highly promising biomarker for perinatal depression. IL-6 and CRP showed high to moderate diagnostic accuracy, while TNF-α and MCP-1 had fair utility. In contrast, IL-10 (AUC = 0.60) and IFN-γ (AUC = 0.05) exhibited low predictive value, the latter potentially reflecting a protective or inverse association with depressive symptoms. These values were derived from a theoretical ROC analysis using published data [26, 48, 49, 54-56, 58, 59]. This model was developed for illustrative purposes and is not based on original clinical datasets with empirically validated sensitivity or specificity.

Diagnostic utility of inflammatory biomarkers in perinatal depression (AUC-based analysis). The analysis of area under the curve (AUC) values reveals notable differences in the diagnostic performance of inflammatory biomarkers associated with perinatal depression (PD): IL-8 demonstrated perfect diagnostic accuracy (AUC = 1.00), underscoring its exceptional ability to discriminate between women with PD and healthy controls. This makes IL-8 one of the most promising biomarkers for early detection and screening of perinatal depression. IL-6 and C-reactive protein (CRP) showed high to good AUC values (0.85 and 0.78, respectively), confirming their reliable sensitivity to inflammation-related depressive symptoms. These biomarkers may be particularly valuable when integrated into multi-marker diagnostic panels. TNF-α and MCP-1 displayed moderate diagnostic capacity (AUC = 0.72 and 0.70), indicating their potential as complementary markers, especially when used alongside IL-8 to assess symptom severity or monitor clinical progression. IL-10 presented a limited AUC value of 0.60, aligning with its anti-inflammatory and protective role in immune regulation. While its reduction may reflect immune dysregulation in PD, IL-10 is not sufficient as an independent diagnostic marker. In contrast, interferon-gamma (IFN-γ) exhibited a very low AUC of 0.05, suggesting poor diagnostic performance and a possible inverse relationship with PD. Elevated IFN-γ levels may indicate a reduced risk of depressive symptomatology, supporting a compensatory or protective immune role, particularly in the context of Th1/Th2 imbalance. This receiver operating characteristic (ROC) curve illustrates the diagnostic performance of key tryptophan pathway metabolites and serotonin in predicting perinatal depression. The figure models the discriminative ability of three inflammation-associated biomarkers - quinolinic acid (QA), the kynurenine-to-tryptophan ratio (KYN/TRP), and serotonin, based on inflammation-mediated metabolic alterations.

Based on synthesized data [26], several inflammatory biomarkers demonstrated moderate diagnostic performance, with AUC values ranging from 0.63 to 0.74. While these values fall below the AUC > 0.90 threshold considered indicative of excellent discriminative power, they suggest a promising role for these biomarkers as prognostic indicators for identifying women at risk of perinatal depression. Further research should explore the combined application of multiple biomarkers to enhance diagnostic accuracy and clinical applicability. This study underscores the potential utility of inflammatory biomarkers, particularly interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), in the diagnosis and prognosis of perinatal psychoses. Evidence drawn from related psychiatric research suggests that these markers exhibit moderate diagnostic accuracy, with reported sensitivity and specificity values typically ranging from 65% to 80%, and area under the curve (AUC) values between 0.75 and 0.84. These findings highlight the relevance of systemic inflammation in perinatal mental health disorders and support the continued exploration of IL-6, TNF-α, and CRP as clinically informative biomarkers [26, 29, 55].

Particular attention should be paid to tryptophan metabolism and kynurenine pathway markers (such as KYN and the KYN/TRP ratio), which reflect inflammatory activation and disrupted serotonergic transmission, and may play a key role in the pathogenesis of perinatal psychoses [26, 54]. However, it is important to emphasize the limited availability of direct data on perinatal psychoses and the considerable heterogeneity across existing studies, including differences in measurement methodologies and diagnostic criteria [15, 48, 57]. These limitations highlight the need for well-designed prospective studies with larger cohorts and standardized assessment protocols.

Integrating multi-biomarker panels–such as the combination of biological markers with psychosis rating scales and structured clinical interviews–into diagnostic algorithms, supported by machine learning techniques, holds promise for significantly enhancing the accuracy of perinatal psychosis prediction and diagnosis. This approach could facilitate

more personalized treatment strategies and improve prevention of associated complications [26, 55]. The simulated ROC analysis revealed varying diagnostic accuracy of inflammatory and neurochemical biomarkers in detecting perinatal psychoses. IL-8 demonstrated perfect diagnostic performance (AUC = 1.00; 100% sensitivity and specificity), identifying it as the most promising standalone marker. IL-6 (AUC = 0.85; 82%/80%) and quinolinic acid (QA) (AUC = 0.84; 77%/70%) also showed high accuracy, supporting their integration into multi-marker diagnostic panels. Serotonin (AUC = 0.81) and CRP (AUC = 0.78) further confirmed the role of immune and serotonergic dysregulation. Markers like TNF-α (AUC = 0.72), MCP-1 (AUC = 0.70), and KYN/TRP (AUC = 0.73) demonstrated moderate accuracy and may be useful as adjunctive indicators. IL-10 (AUC = 0.60) had limited standalone value but remains relevant for understanding immune modulation. In contrast, IFN-γ showed very poor diagnostic capacity (AUC = 0.05), possibly reflecting a protective or inverse association with psychosis risk, aligned with known Th1/Th2 shifts during pregnancy.

ROC Curve for the diagnosis of perinatal psychosis. Based on results reported by Achtyes et al. [26], simulated ROC analysis assessed the diagnostic utility of quinolinic acid (QA), the KYN/TRP ratio, and serotonin in identifying perinatal psychosis. QA showed the highest accuracy (AUC = 0.84; 77% sensitivity; 70% specificity), reflecting its neurotoxic and immune-activating role via the kynurenine pathway. Serotonin also performed well (AUC = 0.81; 75%/74%), supporting the role of serotonergic dysregulation. The KYN/TRP ratio yielded moderate accuracy (AUC = 0.73; 69%/68%) but remains relevant due to its inflammatory and neurochemical associations. These findings support the potential of neuroimmune biomarkers for early diagnosis and risk stratification in perinatal psychosis.

Diagnostic utility of inflammatory biomarkers in perinatal psychoses: Integrated findings from simulated AUC analysis and literature evidence.

This analysis synthesizes simulated AUC data with clinical findings to assess the diagnostic utility of inflammatory biomarkers in perinatal psychosis. IL-8 showed perfect diagnostic accuracy (AUC = 1.00), aligning with clinical evidence from other data identifying it as the most promising standalone biomarker [26, 48]. IL-6 also demonstrated strong performance (AUC = 0.85), with consistent elevation in perinatal mood and psychotic disorders, as supported by other studies [26, 29]. CRP (AUC = 0.78) was moderately accurate and may serve as a reliable supplementary marker [26, 55]. TNF-α and MCP-1 showed moderate predictive power (AUC = 0.72 and 0.70), useful in multi-biomarker panels despite limited specificity [49, 55]. IL-10 had limited value (AUC = 0.60), reflecting its anti-inflammatory role; while reduced levels were reported in PPD, it lacks standalone diagnostic relevance [56]. IFN-γ exhibited very poor performance (AUC = 0.05), suggesting a protective or inverse association with psychosis risk [58, 59]. Tryptophan metabolites - QA (AUC = 0.84), serotonin (AUC = 0.81), and KYN/TRP (AUC = 0.73) showed moderate to high accuracy in identifying perinatal psychosis with other authors highlighting links to neuroinflammation and glutamatergic dysregulation [26]. IL-8 and IL-6 emerge as key candidates for early screening. CRP, TNF-α, and MCP-1 may complement multi-analyte panels. IL-10 and IFN-γ, while diagnostically limited, provide mechanistic insight into immune involvement in perinatal psychiatric conditions.

Discussion

The comparative evaluation of inflammatory biomarkers underscores the pivotal role of interleukin-8 (IL-8) in both perinatal depression and psychosis. IL-8 exhibited perfect diagnostic accuracy across both conditions (AUC = 1.00), highlighting its outstanding ability to differentiate affected individuals from healthy controls. These findings are consistently supported by multiple studies [26, 48], positioning IL-8 as a leading candidate for early screening and risk stratification in perinatal mental illness. Interleukin-6 (IL-6) and C-reactive protein (CRP) also demonstrated robust diagnostic performance, with AUC values of approximately 0.85 and 0.78, respectively, across both affective and psychotic presentations. These markers reflect systemic inflammation and have been validated in several independent cohorts indicating their potential as core components in multi-analyte diagnostic panels [29, 54, 55, 57]. Tumor necrosis factor-alpha (TNF-α) showed moderate diagnostic utility (AUC ≈ 0.72) consistently across both disorders [26, 29, 55]. Although TNF-α may not function effectively as a standalone biomarker, it holds value as a complementary indicator of inflammatory activity and symptom burden. In contrast, interferon-gamma (IFN-γ) demonstrated consistently poor diagnostic performance (AUC = 0.05) in both perinatal depression and psychosis [58, 59]. This low AUC suggests a lack of direct association or a possible inverse relationship, potentially reflecting a protective immune function or compensatory shift in Th1/Th2 balance. In the specific context of perinatal psychosis, additional markers of interest emerged. Monocyte chemoattractant protein-1 (MCP-1) exhibited moderate predictive accuracy (AUC = 0.70), as reported by Sawyer Kristi M. et al. (2021), supporting its role in neuroinflammation-related symptomatology [49]. Interleukin-10 (IL-10) yielded a lower AUC (0.60), as reported by Anjum S. et al. (2020), aligning with its regulatory anti-inflammatory profile and suggesting that IL-10 is more suitable for mechanistic insight rather than diagnostic application [56]. Importantly, tryptophan pathway metabolites provided additional diagnostic value in perinatal psychosis, with quinolinic acid (QA) showing an AUC of 0.84, the kynurenine/tryptophan (KYN/TRP) ratio an AUC of 0.73, and serotonin an AUC of 0.81, as reported by Achtyes et al. (2020) [26]. These biomarkers reflect neuroimmune activation and serotonergic disruption, hallmark mechanisms in psychotic disorders, and demonstrate promise in biomarker-informed clinical models. Biomarkers like QA and KYN/TRP are not specific to psychosis, highlighting the need for integrated diagnostic models combining biological and clinical data.

In summary, IL-8, IL-6, CRP, and TNF-α emerge as robust inflammatory indicators for both perinatal depression and psychosis, while tryptophan-related metabolites and MCP-1 may offer greater specificity for psychosis. Conversely, IFN-γ is unlikely to be diagnostically useful and may instead play a regulatory or protective immunological role. These findings support the development of biomarker panels and multi-level predictive models that integrate immunological data to improve diagnosis and enable early intervention in perinatal psychiatric disorders.

Conclusions

Inflammatory dysregulation is closely linked to perinatal psychiatric disorders, as supported by existing meta-analyses and our modelling. This dysregulation suggests the presence of a unique proinflammatory mechanism specific to these conditions. Exploring inflammatory biomarker-guided screening and prediction could inform perinatal mental health practice and early intervention. Future research could evaluate the specificity of these biomarkers according to the type of mental disorder.

Limitations of the study

Inflammatory biomarkers in perinatal mental disorders show substantial heterogeneity across studies. Because we did not assess between-study heterogeneity or harmonize cytokine-level measurement methods, the reliability of the extracted data is uncertain, and findings should be interpreted as hypothesis-generating. Correlations, particularly for cytokines such as TNF-α, vary due to factors including sample timing, assay methods, and diagnostic criteria. Although a proinflammatory trend is common in perinatal depression and psychosis, the specificity and consistency of individual biomarkers remain limited and variable across populations.

This review has several methodological limitations. First, ROC curves and AUC estimates were derived from simulated inputs based on published summaries rather than raw patient-level datasets, constraining empirical accuracy. Second, reliance on screenshot-derived figures and potential double digitization may introduce measurement error and reduce the precision of extracted values. Third, standardized diagnostic thresholds for key biomarkers (e.g., IL-6, IL-8, quinolinic acid, serotonin) are lacking, hindering clinical translation. Fourth, most included studies involve relatively homogeneous populations, limiting generalizability to more diverse settings.

Competing interests

None declared.

Authors' contribution

LB conceived and prepared the original draft and was responsible for the conception and design of the review. JC and LB were responsible for data acquisition. IN and LB were responsible for the collection and assembly of the published data, as well as their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and approved the final version of the manuscript.

Informed consent for publication

Not required for this article.

Ethics approval

No approval was required for this study.

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REVIEW ARTICLE

Advances in disease-modifying therapies for multiple sclerosis: global updates and a regional comparison between the Republic of Moldova and Romania

Anna Belenciuc[1,2*], Olesea Odainic[2], Marina Sangheli[1], Elena Manole[1], Carmen Adella Sîrbu[3,4,5], Vitalie Lisnic[1]

[1]Department of Neurology No 1, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[2]*Diomid Gherman* Institute of Neurology and Neurosurgery, Chișinău, Republic of Moldova

[3]Clinical Neuroscience Department, *Carol Davila* University of Medicine and Pharmacy, Bucharest, Romania

[4]Academy of Romanian Scientists, Bucharest, Romania

[5]Department of Neurology, Central Military Emergency University Hospital, Bucharest, Romania

ABSTRACT

Introduction. The therapeutic landscape of multiple sclerosis has undergone a remarkable transformation over the past two decades. The paradigm has shifted from reliance on moderate-efficacy, first-generation injectable therapies toward earlier adoption of high-efficacy disease-modifying treatments, particularly in relapsing forms of MS. This evolution reflects an increasing focus on early intensive treatment strategies aimed at preserving long-term neurological function and brain health.

Materials and methods. This narrative review synthesizes recent global evidence on progress in disease-modifying treatments across all multiple sclerosis phenotypes, drawing from randomized controlled trials, real-world studies, and expert consensus guidelines. In addition, it includes a comparative health policy analysis assessing DMT availability, access, and implementation in Romania and the Republic of Moldova, based on national formularies, reimbursement frameworks, and care delivery models.

Results. Globally, the MS treatment algorithm has been reoriented toward early intensive treatment, supported by emerging evidence favoring high-efficacy therapies in the early disease course. While many countries have aligned their protocols accordingly, regional discrepancies persist. Romania, as an EU member, has expanded patient access to 16 reimbursed therapies and biomarker-driven monitoring, and has developed a network of specialized Multiple Sclerosis centers. Conversely, the Republic of Moldova faces structural and economic barriers that restrict access to high-efficacy treatments, advanced diagnostics, and multidisciplinary care – factors contributing to delayed treatment and suboptimal outcomes.

Conclusions. Understanding both global innovations and regional realities is necessary to place current Multiple Sclerosis care in context. Further advancements in science, health policy, and infrastructure will ultimately determine how effectively different nations can convert therapeutic progress into actual improvements in patient outcomes.

Keywords: multiple sclerosis, disease-modifying therapies, Republic of Moldova, Romania.

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[*]**Corresponding author: Anna Belenciuc,** MD, MPH, PhD fellow, Department of Neurology No 1
Nicolae Testemițanu State University of Medicine and Pharmacy
2, Korolenko str., Chișinău, Republic of Moldova MD-2028
e-mail: belenciuc@gmail.com

Key messages

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

Despite progress in multiple sclerosis disease-modifying therapies, few studies synthesize global advances with regional comparisons in Eastern Europe or assess how health system disparities affect access and early treatment, particularly in Moldova's underreported multiple sclerosis program.

Authors' ORCID IDs
Anna Belenciuc – https://orcid.org/0000-0002-0904-1410
Olesea Odainic – https://orcid.org/0000-0003-0225-1009
Marina Sangheli – https://orcid.org/0000-0003-4064-9472
Elena Manole – https://orcid.org/0000-0003-0164-859X
Carmen Adella Sîrbu – https://orcid.org/0000-0002-1982-1066
Vitalie Lisnic – https://orcid.org/0000-0002-5432-8859

The research hypothesis

This review hypothesizes that, despite global consensus on early high-efficacy disease-modifying therapies, disparities in access and clinical practices between Romania and Moldova may contribute to unequal long-term outcomes in multiple sclerosis care.

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

This manuscript presents a comprehensive synthesis of recent therapeutic advancements in multiple sclerosis, integrating global developments with a unique comparative analysis of treatment access and policy implementation in Romania and Moldova, thereby exposing the contrast between international clinical guidelines and regional healthcare limitations.

Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated disorder of the central nervous system (CNS) characterized by inflammation, demyelination, axonal loss, and progressive neurological dysfunction. Affecting more than 2.8 million individuals globally as of 2020, MS represents a leading cause of non-traumatic neurological disability among young adults [1]. Although the disease is classically described as relapsing-remitting MS (RRMS), with alternating episodes of neurologic worsening and recovery, it is now recognized that progressive phenotypes – namely, secondary progressive MS (SPMS) and primary progressive MS (PPMS) – account for a significant burden of irreversible disability. These forms are particularly challenging to manage due to their relative resistance to conventional anti-inflammatory treatments.

Over the past decade, the therapeutic landscape for MS has expanded dramatically. The once-limited options – restricted primarily to injectable interferons and glatiramer acetate – have given way to a diverse arsenal of oral agents, monoclonal antibodies, and small-molecule inhibitors targeting specific immunological pathways. The availability of more than 20 disease-modifying therapies (DMTs) recognized by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) has reshaped expectations regarding long-term disease control and quality of life for patients living with MS [2]. This progress has been paralleled by an increasingly nuanced understanding of MS pathophysiology, including the recognition of progression independent of relapse activity (PIRA), which emphasizes the need for early intervention with high-efficacy therapies rather than delayed escalation [2].

Despite these global advances, regional disparities in access to diagnosis, treatment, and monitoring persist. Western Europe and North America benefit from a full-spectrum therapies, comprehensive care models, broad DMT reimbursement, and access to advanced biomarker technologies. In contrast, many Eastern European nations face infrastructural and economic constraints that hinder the implementation of modern MS care standards.

Romania, as a member state of the European Union, has made substantial progress in aligning with international guidelines. The country offers a wide range of reimbursed DMTs, including high-efficacy monoclonal antibodies, and supports specialized MS centers capable of MRI-based monitoring and multidisciplinary management [3]. However, significant urban-rural disparities persist, and access to novel biomarkers such as neurofilament light chain remains limited to research or academic centers.

The Republic of Moldova, by contrast, lags behind, remaining at an earlier stage of systemic MS care development. Although MS prevalence in Moldova is lower than the EU average, estimated at approximately 34 per 100,000, the actual burden may be underestimated due to diagnostic delays and underreporting [4, 5]. National medical insurance covers only a limited range of DMTs, and high-efficacy options, such as ocrelizumab, are available at a single national institution, with restricted patient access. Biomarker testing is unavailable, and longitudinal disease monitoring remains largely clinical, limiting timely therapeutic adjustments.

This article aims to provide a comprehensive review of recent advances in MS disease-modifying therapies and to highlight how these developments are reflected – or remain inaccessible – in two geographically close but systemically divergent countries: Moldova and Romania. By examining their respective challenges and capacities, we aim to identify gaps and evaluate opportunities for alignment with global standards to ensure equitable and effective MS care delivery in resource-limited settings.

Materials and methods

This review is based on a structured analysis of recently published peer-reviewed literature, including randomized controlled trials, network meta-analyses, consensus guidelines, and health policy reports published between 2020 and 2025. Databases such as PubMed and ClinicalTrials.gov were used to identify relevant studies on disease-modifying therapies (DMTs) in multiple sclerosis. The search strategy combined the terms "multiple sclerosis" and "disease-modifying therapies". From a total of 196 articles initially re-

trieved, we excluded articles available only as abstracts, narrative reviews providing only general information about treatments, and studies containing duplicate data. Regarding the situation in Romania and the Republic of Moldova, peer-reviewed literature is scarce. Therefore, the analysis was supplemented with official governmental sources and national reports to ensure a comprehensive overview of treatment access, policy implementation, and health system disparities between the two countries. A qualitative thematic analysis was performed to identify key patterns related to treatment updates and the implementation of early high-efficacy therapy strategies. No specialized software was used for data extraction or analysis. The collected data were synthesized to provide both global updates and regional health system perspectives.

Results and discussions

Contemporary DMT landscape: treatment paradigm shift

Since the approval of interferon-beta in 1993, the DMT landscape has expanded to include over 20 therapies with diverse mechanisms of action [6]. The therapeutic goals of DMTs are to reduce relapse frequency, delay disability progression, and limit radiological activity.

Historically, DMT usage has followed a conservative escalation model: starting with injectables (interferons, glatiramer acetate), escalating to orals (fumarates, teriflunomide), and reserving high-efficacy therapies (HETs) for refractory cases.

Table 1. Classification of DMT's by route of administration

Injectables	Interferons and glatiramer acetate
Oral DMTs	Fumarates, Teriflunomide, Cladribine, and Sphingosine-1phosphate modulators (e.g., Fingolimod, Ozanimod)
Infuzional therapies	Ocrelizumab, Ublituximab, Natalizumab, and Alemtuzumab

First-generation therapies, such as interferon beta (IFN-β) and glatiramer acetate (GA), have demonstrated modest efficacy [1]. Despite their widespread use, these agents exhibit limited capacity to prevent long-term disability, particularly in progressive forms [7].

HETs include monoclonal antibodies (e.g., natalizumab, alemtuzumab, ocrelizumab, ofatumumab, ublituximab) and sphingosine-1-phosphate receptor modulators (e.g., fingolimod, ozanimod, ponesimod) [1, 2]. These drugs target B-cell and T-cell pathways, CNS lymphocyte trafficking, and inflammation more effectively. Network meta-analyses reveal monoclonal antibodies, such as alemtuzumab, ofatumumab, and ocrelizumab, as the most efficacious options for reducing relapses and disability progression [8].

The initial classification of disease-modifying therapies based on treatment lines is now considered outdated. It has been largely replaced in modern practice by efficacy-based, administration-based (Table 1), or mechanism-based categorization (Table 2), which supports earlier use of high-efficacy DMTs for appropriate patients.

Table 2. The latest classification of DMT, based on their mechanisms of action

DMT Class	Examples
Monoclonal antibodies	Ocrelizumab, Ofatumumab, Alemtuzumab, Natalizumab, Ublituximab
Sphingosine-1-phosphate receptor modulators	Fingolimod, Siponimod, Ozanimod, Ponesimod
Anti-proliferative agents	Cladribine, Teriflunomide
Immunomodulators with unknown mechanism	Dimethyl fumarate (DMF), Glatiramer acetate, Interferons

The traditional stepwise escalation model – starting with low-efficacy DMTs – has been increasingly replaced by early intensive therapy (EIT). This shift is reflected in national and international guidelines [2].

In recent years, high-efficacy therapies (HETs) have gained traction as the favored strategy in MS treatment for treatment-naïve patients with active disease. The existing evidence suggests that it is desirable to start HET as early as possible to achieve maximum neuroprotection. Randomized clinical trials and observational studies have demonstrated lower relapse rates, reduced MRI activity, and decreased long-term disability when HETs are initiated within the first five years following the onset of symptoms [2]. Prospective research has repeatedly demonstrated that both early relapse and MRI lesion activity predict, to a large extent, disability accumulation in the years to come. Therefore, interventions at these levels are crucial for modifying the course of the disease.

High-efficacy DMTs, including ocrelizumab, alemtuzumab, cladribine, natalizumab, and ofatumumab, have shown substantial efficacy, with annualized relapse rates (ARR) reduced by up to 70%, which is well beyond the efficacy of traditional injectable therapies, such as interferon-beta or glatiramer acetate [9]. Apart from relapse control, HETs are also more efficient in preventing PIRA, a phenomenon that is now clearly recognized. The ASCLEPIOS studies have shown that ofatumumab robustly delayed PIRA compared to teriflunomide [10]. Additionally, the effect of HETs on preserving brain volume is relatively significant. Brain atrophy (a neurodegeneration marker strongly correlated with cognitive and physical decline) develops at a slower pace in those treated with high-efficacy drugs, with annual rates of brain volume loss reduced to ~0.2% [11]. Most critically, real-world data now indicate that starting with HETs early in the disease is more effective than proceeding stepwise in a classic escalation approach. A retrospective cohort study by Harding et al. reported that patients initiated on HETs had a 47% reduction in the hazard of developing significant disability compared to those started on moderate-efficacy drugs [12]. This evidence has contributed to a treatment paradigm that emphasizes aggressive therapy with high-efficacy treatments as early as possible to achieve optimal long-term functional outcomes for individuals with MS.

A recent network meta-analysis by Samjoo et al. (2023) offers valuable insights into the comparative effectiveness of current HETs [8]. In terms of reducing the ARR, alemtuzumab, ofatumumab, and ublituximab emerged as the most

effective agents. For delaying three-month confirmed disability progression (CDP), the highest efficacy was observed with alemtuzumab, ocrelizumab, and ofatumumab. Similarly, in the context of a six-month CDP, alemtuzumab, natalizumab, and ocrelizumab demonstrated superior outcomes. The safety profiles of these high-efficacy therapies, especially when administered early in the disease course, have been reported in multiple trials to be similar to those of moderate-efficacy DMTs, providing data relevant to considerations around timing and selection of treatment strategies [2].

Special considerations in progressive MS treatment

Progressive MS patients exhibit poor response to traditional anti-inflammatory therapies. Smoldering lesions, PIRA, and oxidative stress contribute to relentless disability accumulation [13]. Therapeutic strategies targeting microglia, mitochondrial protection, and remyelination are gaining interest.

PPMS remains challenging due to its insidious onset and lack of relapses. Ocrelizumab is the only FDA-approved agent for PPMS, based on the ORATORIO trial, which demonstrated reduced disability progression over 120 weeks [13].

Historically, the treatment of SPMS has been hindered by the limited efficacy of traditional DMTs. However, recent trials have demonstrated benefits with newer agents. Siponimod, a selective S1P1/5 modulator, showed a significant reduction in 3-month CDP in the EXPAND trial, particularly in active SPMS [14, 15]. Ocrelizumab has also shown promise in reducing PIRA in SPMS subpopulations [13].

Novel and investigational therapies

Recent advances in molecular immunology and neuroinflammation have led to the development of a new generation of DMTs that target pathways previously difficult to access in MS pathogenesis. Among these, Bruton's tyrosine kinase (BTK) inhibitors, Janus kinase (JAK) inhibitors, and other kinase-targeting molecules represent some of the most promising drug classes currently in development [16]. A common advantage of many of these agents is their ability to penetrate the CNS, enabling modulation of immune activity both systemically and within the CNS – a critical step in addressing progressive and treatment-refractory disease phenotypes.

Bruton's tyrosine kinase (BTK) inhibitors

BTK inhibitors are small-molecule oral agents designed to interfere with B-cell receptor signaling, ultimately modulating B-cell activation, survival, and antigen presentation. In the context of MS, these effects extend beyond peripheral immune suppression; many BTK inhibitors have demonstrated the capacity to cross the blood-brain barrier (BBB), targeting B-cell populations within the CNS. This is particularly important given the increasing recognition of CNS-compartmentalized inflammation, including meningeal B-cell follicles and smoldering lesions, as key drivers of disease progression in MS [6].

Among the BTK inhibitors under investigation, evobrutinib and tolebrutinib have progressed furthest in clinical development [16, 17]. Tolebrutinib, for instance, has shown efficacy in reducing the formation of new T2 lesions and has demonstrated potential in addressing non-relapsing secondary progressive MS in Phase III studies. In addition, tolebrutinib has recently shown a 31% reduction in disability progression in non-relapsing secondary progressive MS, earning breakthrough status from the FDA [18]. BTK inhibitors may offer a therapeutic advantage in patients with progressive disease forms, for whom current therapies offer limited benefit.

JAK inhibitors and kinase-targeting molecules

Janus kinase (JAK) inhibitors constitute another novel therapeutic class under evaluation for MS. By modulating the JAK-STAT signaling pathway – a key regulator of cytokine-mediated immune responses – these agents may suppress pro-inflammatory cascades in both adaptive and innate immune cells. While already approved for other autoimmune conditions such as rheumatoid arthritis and ulcerative colitis, their role in MS remains investigational. JAK inhibitors may be beneficial in MS subtypes with overlapping autoimmune features or treatment-resistant inflammation.

Researchers are also exploring other intracellular signaling targets, including the PI3K/Akt/mTOR and MAPK pathways, which regulate cell survival, proliferation, and metabolism. Modulators of these pathways may influence not only immune cell function but also glial responses and neurodegeneration, making them appealing candidates for progressive MS and neuroprotective therapy strategies [6].

Novel immunotherapies and viral-targeted approaches

Beyond kinase inhibition, entirely new immunological strategies are being developed. One of the most revolutionary concepts involves chimeric antigen receptor (CAR) T-cell therapy, an approach adapted from oncology. In MS, CAR-T cells are engineered to target and selectively eliminate autoreactive B or T lymphocytes [19]. These therapies remain in preclinical and early-phase trials but show promise in creating long-term immune tolerance and could benefit patients with aggressive or refractory disease.

The investigation of the Epstein-Barr virus (EBV) role in MS development represents a separate research direction. The available epidemiological and experimental data indicate that EBV infection serves as a necessary, although not sufficient, condition for MS development. The development of vaccine candidates targeting EBV proteins including gp350 has reached early-phase clinical testing [1]. This vaccine strategy focuses on preventing EBV infection or reducing its impact in individuals already infected. If proven effective, EBV vaccination could serve as a primary prevention method for at-risk groups or as an adjunct treatment for patients in the early stages of MS.

Comparative analysis: disease-modifying therapies in the Republic of Moldova and Romania

While global therapeutic advancements in MS have reshaped disease management paradigms, access to these therapies varies considerably across countries. This is especially evident in Eastern Europe, where health system resources, regulatory policies, and socioeconomic factors influence the availability and implementation of DMTs. A comparative overview of the Republic of Moldova and Romania provides critical insights into disparities in MS care within the region.

Romania, a European Union (EU) member state, has de-

veloped a national MS treatment program supported by the National Health Insurance House, enabling broad access to EMA-approved DMTs. In contrast, the Republic of Moldova – a non-EU country with limited healthcare funding – faces substantial barriers to ensuring comprehensive DMT coverage. Both countries report an increasing prevalence of MS, with Romania estimating 12,500-13,000 patients and Moldova approximately 2,000, although registry data in Moldova remain incomplete.

Availability of DMTs

As of 2025, Romania offers an extensive portfolio of 16 EMA-approved DMTs through its national program, including both moderate- and high-efficacy agents. Romanian patients typically have access to DMTs across all disease stages, including those with SPMS and PPMS, with established pharmacovigilance and MRI monitoring frameworks integrated into their clinical care (Tables 3 and 4).

In contrast, Moldova currently reimburses only three DMTs through its national insurance company. Two additional therapies, glatiramer acetate and cladribine, are included in the expanded list approved by the Ministry of Health and are expected to become available by the end of 2025 as part of a national MS initiative (Table 4). However, access remains dependent on centralized hospital supply, and high-efficacy agents are unavailable outside of clinical trials and private purchases abroad. This limited repertoire constrains physicians' ability to individualize therapy or apply early high-efficacy treatment strategies advocated by current international guidelines [2].

Table 3. DMT's availability in Romania and Republic of Moldova

Romania	Republic of Moldova
Interferon beta-1a	Interferon beta-1a
Interferon beta-1b	Interferon beta-1b
Glatiramer acetate	*Glatiramer acetate (approved in 2025)*
Dimethyl fumarate	n/a
Diroximel fumarate	n/a
Teriflunomide	n/a
Fingolimod,	n/a
Ozanimod	n/a
Ponesimod	n/a
Siponimod	n/a
Natalizumab	n/a
Alemtuzumab	n/a
Ocrelizumab	Ocrelizumab (introduced in 2023)
Cladribine	*Cladribine (approved in 2025)*
Ofatumumab	n/a
Ublituximab (approved in 2024, pending reimbursement listing)	n/a

Treatment guidelines and practice patterns

Romania adheres to international best practices and has developed national MS management protocols aligned with the recommendations of the European Committee for Treatment and Research in MS (ECTRIMS). Romanian neurologists frequently adopt the early use of high-efficacy therapies, especially in patients with poor prognostic indicators.

In Moldova, treatment often follows an escalation-based approach due to the limited therapeutic options available. Therapy initiation is frequently delayed because of diagnostic lags, out-of-pocket imaging costs, and the centralization of care at tertiary centers. The national protocol has not yet incorporated recommendations regarding HETs, and MS is not recognized as a priority condition under Moldova's chronic disease funding mechanisms.

Moldova's planned expansion of the DMT list marks a step forward. However, systemic challenges – including regulatory approval timelines, cost constraints, a limited neurologist workforce, and the absence of MS-specific rehabilitation services – continue to hinder equitable care. Collaborative efforts, including regional partnerships, telemedicine, and integration with EU-funded health programs, could help bridge this therapeutic divide.

Table 4. Comparative analysis of national MS peculiarities

Factor	Romania	Republic of Moldova
Number of reimbursed DMTs	15 (soon to be 16)	3 (soon to be 5)
Access to high-efficacy DMTs	Broad (ocrelizumab, alemtuzumab, natalizumab, ofatumumab etc.)	Limited (ocrelizumab)
Reimbursement system	National insurance with broad DMT coverage	Coverage via centralized procurement
Number of MS centers	20	1
Monitoring infrastructure	MRI every 6–12 months; registry-based	Irregular MRI access; limited registry
Protocol adherence	ECTRIMS-aligned, EIT-supported	Outdated, escalation-based
Pediatric MS therapies	available	limited to second-line therapy with interferons

Conclusions

The management of multiple sclerosis has undergone significant evolution in recent years, driven by a growing understanding of disease mechanisms and the long-term consequences of early inflammatory activity. According to current international standards and clinical guidelines, early initiation of HETs is now considered the preferred strategy for patients with active MS, offering better long-term outcomes in terms of relapse reduction, disability progression, and preservation of brain volume, across all disease phenotypes. In contrast, the traditional escalation model, once widely adopted, has been largely abandoned due to its inherent delays in achieving optimal disease control, or, in some cases, is reserved for a modest subgroup of patients.

Although the global landscape of MS treatment has evolved significantly, regional inequalities remain considerable. MS management practice in Romania has advanced to a modern standard, with unrestricted access to all available DMTs and adequate infrastructural resources for biomarker-guided treatment. Moldova, however, continues to grapple with systemic challenges and limited access to high-efficacy therapies, despite the availability of ocrelizumab for the past

2 years. Addressing these disparities requires not only increased resource allocation but also the adoption of current, evidence-based treatment paradigms that prioritize early, effective disease control in all individuals with MS.

Competing interests

None declared.

Authors' contributions

AB conceived the study, contributed to the design, and drafted the manuscript. OO participated in the literature review and contributed to data synthesis. EM assisted in the analysis of clinical data and contributed to manuscript writing. MS contributed to the organization of data and formatting of the manuscript. CAS provided critical input on the Romanian healthcare system and contributed to the regional policy analysis section. VL supervised the study, provided expert guidance throughout, and critically revised the final manuscript. All authors reviewed the work critically and approved the final version of the manuscript

Ethics approval

Not needed for this study.

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REVIEW ARTICLE

Correlation between pathogenetic factors and vascular endothelial damage in patients with rheumatoid arthritis

Eugeniu Russu[1,2*], Liliana Groppa[1], Lia Chişlari[1], Svetlana Agachi[1], Marius Semionov[1], Chiril Nartea[1], Iosif Leanca[1], Artemie Pastuhov[1]

[1]Discipline of Rheumatology and Nephrology, Department of Internal Medicine *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

[2]*Timofei Moşneaga* Republican Clinical Hospital, Chisinau, Republic of Moldova

ABSTRACT

Introduction. Systemic rheumatoid vasculitis accounts for 1 to 5% of complications seen in rheumatoid arthritis, while autopsy studies report an average of 23% incidence. This enormous difference in numbers emphasizes the rate of misdiagnosis or underdiagnosis of systemic rheumatoid vasculitis. It mainly affects people with a median age of 65 years. It is particularly noteworthy, as systemic rheumatoid vasculitis has a high mortality and relapse rate. Also, the multifactorial aetiology: cytokines/immune cells and other particles determines clinical complexity of this type of angiitis.

Materials and methods. A comprehensive literature search of articles published since 1996 was conducted using MEDLINE via PubMed and HINARI. The search included terms such as "rheumatoid vasculitis", "rheumatoid arthritis", and "endothelial dysfunction", focusing on mechanisms driving vascular damage. A total of 217 relevant sources were identified, including original studies, reviews, and book chapters. The study evaluated pathogenic factors like cytokines, immune complexes, and systemic inflammation, highlighting their roles in endothelial dysfunction and hypercoagulability.

Results. The main pathogenetic factors in systemic rheumatoid vasculitis were immune complexes, cytokines (IL-6/TNF-α and IL-17), immune and blood cells (CD20/TH17/Platelets) and others (microparticles, blood rheology modifications). Even though, taken separately, those factors appear to have little to no impact on vascular endothelium, their synergistic effect lead to significant endothelial damage.

Conclusions. To conclude, each disease has its own pathogenetic factors which determine the natural course of this pathology. Understanding these mechanisms plays an important role for clinicians helping them to diagnose and effective treatment. Taking into consideration the relationships between specific factors in rheumatoid angiitis, we can make more specific decisions for its diagnosis and treatment. We can also use the pathophysiology of systemic rheumatoid vasculitis as a foundation for developing prevention measures.

Keywords: rheumatoid vasculitis, rheumatoid arthritis, hypercoagulability, endothelial dysfunction, cytokines, immune complexes, systemic inflammation.

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Corresponding author: Eugeniu Russu, MD, PhD, associate professor, Discipline of Rheumatology and Nephrology, Department of Internal Medicine *Nicolae Testemiţanu* State University of Medicine and Pharmacy, 29 Nicolae Testemiţanu str., MD-2025, Chisinau, Republic of Moldova
e-mail: eugeniu.russu@usmf.md

Key messages

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

The precise interplay between immune complexes, cytokines, and endothelial dysfunction in driving vascular damage in rheumatoid arthritis and systemic vasculitis remains inadequately understood.

The research hypothesis

This study hypothesizes that immune-mediated mechanisms, particularly involving immune complexes and cytokines, play a pivotal in driving vascular damage in rheumatoid arthritis and vasculitis.

Authors' ORCID IDs
Eugeniu Russu – https://orcid.org/0000-0001-8957-8471
Liliana Groppa – https://orcid.org/ 0000-0002-3097-6181
Lia Chişlari – https://orcid.org/ 0000-0002-7088-568X
Svetlana Agachi – https://orcid.org/ 0000-0002-2569-7188
Marius Semionov – https://orcid.org/0009-0007-8749-710X
Chiril Nartea – https://orcid.org/0009-0004-6931-2173
Iosif Leanca – https://orcid.org/0009-0001-9335-3360
Artemie Pastuhov – https://orcid.org/0009-0005-5310-8650

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

This manuscript explores the interplay between rheumatoid arthritis and systemic rheumatoid vasculitis, emphasizing the roles of immune complexes, cytokines, and microparticles in perpetuating endothelial dysfunction and systemic inflammation. It identifies key molecular pathways, such as NF-κB and MAPK, as potential therapeutic targets to mitigate vascular damage and improve outcomes in autoimmune diseases.

Introduction

Rheumatoid vasculitis (RV), a severe systemic complication of rheumatoid arthritis (RA), is driven by multifactorial mechanisms linking autoimmune inflammation to vascular endothelial dysfunction. The study investigates the pathogenetic connections between hypercoagulability and systemic inflammatory responses in RA patients, emphasizing the pivotal roles of cytokines, immune complexes, and endothelial alterations. Elevated levels of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-17, alongside increased autoantibody profiles such as anti-citrullinated peptide antibodies (ACPA), underlie the immune-mediated damage to vascular walls. This damage is exacerbated by the deposition of circulating immune complexes, complement activation, and the recruitment of inflammatory cells, collectively leading to endothelial cell apoptosis, angiogenesis, and systemic hypercoagulable states [1]. At present, no single factor has been identified as solely responsible for inducing systemic rheumatoid vasculitis (SRV). This complication, as other extra articular manifestations of rheumatoid arthritis, has a multifactorial origin. It is believed that intimal and endothelial damage in SRV is associated with a combination of exogenous, immune and genetic specificities. Patients who had RA associated with p53 mutations were found to have a higher risk for SRV.

Another predisposing factor involves impaired immune tolerance, with the presence of HLA-DRB1*04/04 or HLA-C*03 alleles. Besides that, a connection between RV and KIR2DL3/HLA-C*0802 complex and cutaneous lesions were detected. Increased levels of macrophage migration inhibitory factor were found to enhance inflammation. Long standing RA, male sex, smoking, rheumatoid nodules and different alleles of HLA-I/HLA-II have been linked to an increased rate of RV. Other comorbidities predisposing to this type of connective tissue disease associated vasculitis are cerebrovascular and peripheral vascular disease, maybe due to already present vascular damage [2]. SRV is considered one of the most dangerous systemic complications of RA. The median age at presentation ranges from 62-68 years and the median duration of RA prior to SRV is 10-16 years [3]. Nowadays, the clinical prevalence is estimated from 1-5% (affecting predominantly one in 9 men and one in 38 women [4]), whereas autopsy studies reported 15-31% [1, 3]. It is strongly associated with smoking and advanced RA, as SRV was mainly found in patients with destructive, nodular, and long-standing RA.

Rheumatoid vasculitis usually affects medium and small size vessel, strongly resembling polyarteritis nodosa [5]. Currently, no definitive methods exist to distinguish SRV from other vasculitis, but a strong correlation between the presence of rheumatoid antibodies and SRV. In 90% of cases, patients with SRV are positive for ACPA (anti cyclic citrullinated peptide antibody), whereas only 40% test positive for ANA (antinuclear antibody) (showing a weaker correlation) [2, 6]. To better understand the importance of this study, we should assess the impact of SRV on the affected population. Its mortality rate is estimated to be around 30-50% and relapse in case of remission in 25% [7], while the treatment-related toxicity and disease complications often surpass these figures. This pathology involves skin (palpable purpura/nodules/ulcers/digital necrosis) [6] and peripheral nerve lesions (symmetric sensory polyneuropathy, mononeuritis multiplex) in more than 80% of patients. Rarely, major systems are affected: heart, kidneys and bowel, leading to complication including myocardial and bowel infarction as well as renal failure. These multi-organ manifestations are the main causes of death in SRV.

Material and methods

A search for scientific papers published since 1996 was conducted using the MEDLINE electronic database via the PubMed and HINARI (Health Internet Work Access to Research Initiative) search engines – part of the Research4Life program. Full-text articles available on these platforms were selected. The search terms used (in English) included: "rheumatoid vasculitis", "rheumatoid arthritis", "hypercoagulability", "endothelial dysfunction", "cytokines", "immune complexes", and "systemic inflammation". Original articles, meta-analyses, systematic reviews, and book chapters were included. No language restrictions were applied, but articles in English were prioritized. Additionally, the bibliographies of selected articles were reviewed to identify other relevant sources. The included studies focused on the mechanisms underlying the development of rheumatoid vasculitis.

Initially, the identification of relevant digital sources was based on a comprehensive review of the scientific literature, supplemented by other information sources, such as official reports and press articles. In total, 217 bibliographic sources were consulted and identified. The selection criteria included relevance to rheumatoid vasculitis, recent publication, and recognition in the scientific community.

Data on each identified pathogenic mechanism were collected from various sources, including official websites, scientific articles, and public information. Collected data included descriptions of mechanisms, functionalities, implementation methods, and reported outcomes.

Evaluation and comparison of the primary mechanisms and pathogenic factors were carried out using qualitative and quantitative methods. Each mechanism and factor was assessed based on its physiological and pathological characteristics, usability, data accuracy, and adaptability to different contexts. This process involved a thorough analysis of data and reported outcomes, comparing these against pre-established criteria relevant to the literature review's objectives.

The results were interpreted considering the specific requirements for addressing rheumatoid vasculitis, with a focus on its pathogenic factors (including cytokines, circulating immune complexes, and systemic inflammation). The study emphasized the methodological efficiency and effectiveness in managing endothelial dysfunction and hypercoagulability. Limitations and critical aspects of each pathogenic mechanism were evaluated in the context of practical needs and constraints.

Data validation was ensured by consulting and verifying information from multiple independent and reliable sources. This approach guaranteed the accuracy and reliability of the analyzed data, contributing to the study's validity and robustness.

Using these rigorous methods and diverse data sources, the study provided a detailed comparative analysis of the pathogenic mechanisms involved in rheumatoid arthritis that contribute to vascular damage. This facilitated the early identification and prediction of this severe systemic complication.

Results

Vascular endothelium relation with coagulation in inflammation

Endothelial cells have specific functions and adaptations aimed at preventing thrombus formation, maintaining blood fluidity and tissue perfusion. These functions are closely related to endotheliocytes activation and regulation of the extravasation of fluids, solutes, hormones, and macromolecules, as well as of platelets and blood cells during inflammation. There are two types of EC activation: Type 1, called stimulation and Type 2. Type 1 activation is independent of new gene expression, while Type 2 activation depends on gene transcription [8]. An example of Type 1activation is represented by inducing the histamine receptor. EC can be activated through GPCR (G-protein coupled receptor), for example by histamine coupling Histamine1-R (Gq). This process increases intracytosolic Ca2+ concentration, caused by PLC (Phospholipase C) activation. Further, this process stimulates PLA2 (Phospholipase A2) which cleaves phosphatidylcholine into arachidonic acid (substrate for Prostaglandin H2/Prostacyclin). Increased Ca2+ also activates NOS-3 (Nitric Oxide synthase 3). Besides that, Ca-calmodulin complex together with inhibited Myosin Light Chain Phosphatase determine increased contractility in actin filaments, part of tight junctions. This induces formation of intercellular gaps, which in addition to NO/Prostacyclin stimulates exudate formation. Using the same pathway, EC expresses both PAF (platelet activating factor) and P-selectin, providing tethering and activation of circulating neutrophils. Other adhesins, like CD99 (single chain type 1 glycoprotein) and CD31 (platelet-endothelial cell adhesion molecule 1) are essential for adherence and transmigration of monocytes and neutrophils. Type 2 EC activation is mediated by several cytokines. TNF-α (Tumour Necrosis Factor alfa) induces synthesis of E-selectin/VCAM1 (vascular cell adhesion molecule 1)/COX-1 and COX-2 (cyclooxygenase 1,2). Using the same pathway as TNF-α, IL-1 induces pro-inflammatory protein synthesis. These pathways typically act in a complementary manner, enhancing the inflammatory cell response. A specific feature for EC observed in chronic inflammation is the ability to present both MHC I and II (major histocompatibility complex I, II), thus activating T cells. It also stimulates angiogenesis by enhancing VEGF (vascular endothelial growth factor) transcription [9].

In addition, endothelial cells can secrete preformed vesicles (Weibel-Palade bodies) which store von Willebrand factor (VWF), P-selectin, and angiopoietin-2. These molecules are involved in platelet binding, leukocyte recruitment, and inflammation modulation. Furthermore, some endothelial receptors are involved in the generation of the important anticoagulant, activated protein C (aPC), which can interrupt the coagulation cascade by cleaving coagulation factors Va and VIIIa [10].

Pathophysiology of SRV

RA is an autoimmune disease, characterized by loss of tolerance to self-antigens, especially those containing citrulline residues generated by posttranslational modification. Coupling of Ab with Ag determines formation of IC (immune complexes). In addition, systemic inflammation determines a vascular reaction: increased permeability, upregulation of adhesion molecules, and enhanced expression of MMPs, PAD and pro-inflammatory cytokines. These adaptive immune responses determine formation of specific Ag (citrullinated/carbamylated proteins) which bind antibodies both at the site of inflammation and those in systemic circulation. Increased vascular permeability favours CICs (circulating immune complexes) deposition. Local IC formation and deposition determines progression and maintenance of inflammation through complement activation, coupling with specific receptor and activation of pro-inflammatory signalling pathways [11]. SRV, same as rheumatoid arthritis, has a multifactorial pathogenesis, with progressive discovery of new pathological linkages. The most important effectors are believed to be the immune complexes. Besides that, some cytokines tend to maintain and progress the inflammation. Immune and blood cells, through specific pathways (NET-osis, MET-osis, enzymes and cytokines secretion, and the release of cell-derived microparticles) induce and maintain the RV. Taken together,

IC's deposition, cytokine release and immune cell activation, determine blood vessel wall damage.

Effect on vascular endothelium

Immune complexes (ICs) play a critical role in the pathogenesis of any systemic immune mediated vasculitis, especially those affecting small blood vessels. RV is associated with small- and medium-vessel vasculitis in the context of connective tissue diseases. These types of angiitis mainly occur due to ICs deposition in the vascular endothelium, inducing or amplifies inflammation. Under normal physiological conditions, when the antigen-to-antibody ratio is close to 1, predominantly large ICs are formed. These are efficiently cleared through phagocytosis. In the condition of Ab excess, small ICs are formed, remaining in the serum and precipitating in the vascular bed, where they stimulate cytokine release and inflammatory pathways activation [12]. Once Ag-Ab complex transmigrated in subendothelial space, it will activate the inflammatory cells through Fc receptors (FcR). This will induce phagocytosis by activating ITAM (immunoreceptor tyrosine-based activation motif) and Cytokine transcription enhancement in all stimulated cells. Additionally, ICs bind to the C1q initiating the complement cascade, which determines chemotaxis (C3a, C5a), opsonization (C5b) and cytolysis (through membrane attack complex).

TNF-α, is a cytokine which mediates and promotes inflammation. There are two receptors responsible for its action: TNF-Receptor-1 (on nearly all cells) and TNF-Receptor-2 (on immune cells/heart). The binding of membrane-bound TNF-α to TNFR2 induces the expression of VEGFR2 via activation of endothelial tyrosine kinase (Etk). It also participates in activation of NF-kB (nuclear factor kB) by stimulating phosphorylation of IkBa (inhibitor of nuclear factor kB). This process facilitates translocation of the transcription factor in the nucleus. Besides stimulating NF-kB, TNFR1 also signals through p38MAPK (playing a role in rearrangement of cytoskeleton) and MAP3K/JNK (having a crucial role in pro-inflammatory molecules synthesis). Upon activation of the TNF-α–TNFR1 complex, JNK phosphorylates c-Jun and activating transcription factor-2 (ATF-2), further promoting inflammatory gene transcription. TNF-αAs a pro-inflammatory cytokine, TNF-α induces morpho-pathological changes in vascular endothelium. First of all, it provokes endothelial barrier dysfunction through several mechanisms. Activation of TNFR1 has been reported to induce p38MAPK/ERK pathway, which, through PKC-dependent rearrangement of the actin cytoskeleton, destabilizes microtubules and induces formation of intercellular gaps. It leads to an increased paracellular diffusion of plasma and macromolecules in endothelium. In parallel,

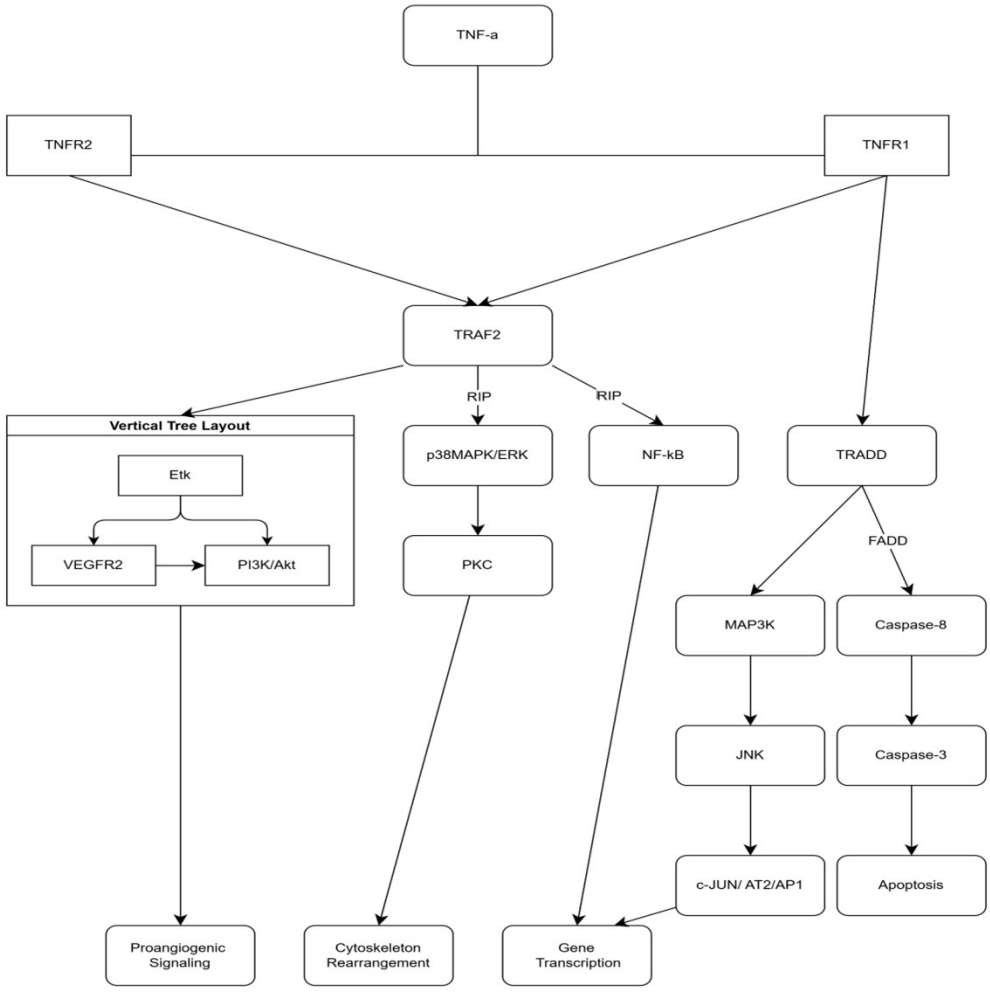

Fig. 1 Coupling both TNFR1/TNFR2, TNF-α induces activation of TRAF2, except TNFR1-TNF-α complex activates TRADD-pathway too. FADD and RIP are playing the role of co-stimulators, inducing and enhancing signals. These cascade-like processes, mainly stimulate gene transcription, cytoskeletal rearrangements and angiogenesis or apoptosis.

***Note**: TNF-α - Tumor Necrosis Factor-alpha; TNFR1 - Tumor Necrosis Factor Receptor 1; TNFR2 - Tumor Necrosis Factor Receptor 2; TRAF2 - TNF Receptor-Associated Factor 2; RIP - Receptor-Interacting Protein; NF-κB - Nuclear Factor-kappa B; TRADD - TNF Receptor-Associated Death Domain; FADD - Fas-Associated Protein with Death Domain; MAP3K - Mitogen-Activated Protein Kinase Kinase Kinase; JNK - c-Jun N-terminal Kinase; Caspase-8 - Cysteine-aspartic Protease 8; Caspase-3 - Cysteine-aspartic Protease 3; PI3K/Akt - Phosphoinositide 3-Kinase/Protein Kinase B; VEGFR2 - Vascular Endothelial Growth Factor Receptor 2; PKC - Protein Kinase C; p38MAPK/ERK - p38 Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase; Etk - Erythroblast Transformation Specific Kinase; c-JUN/AT2/AP1 - c-JUN/Activator Protein 1.*

TNF-α increases tyrosine phosphorylation of cadherin (activation of NF-kB) in vascular endothelium, decreasing intercellular adhesion. Secondly, TNF-α decreases NO levels (vasodilator) in endotheliocytes. Through TNFR1 stimulation, it suppresses the endothelial nitric oxide synthase (eNOS) gene promoter, inhibiting mRNA formation, consequently decreasing eNOS enzyme synthesis. Moreover, endogenous accumulation of ADMA (asymmetric dimethylarginine) directly inhibits eNOS by coupling to the active site. Thirdly, in a complementary manner, TNFR1 and TNFR2 stimulate the transcription of adhesion molecules (E-selectin/ICAM-1/VCAM-1) within 30-120 minutes of activation, mediating the recruitment and transmigration of circulating leukocytes into the vascular wall [13].

IL-6 is one of the main pro-inflammatory cytokines in rheumatoid arthritis manifesting dose-dependent effects. There are several cell types which secrete IL-6: lymphocytes, monocytes, fibroblast, osteoblasts, endotheliocytes and mesangial cells. Mainly, cells secreting IL-6 are activated through TLR-DAMP (toll-like receptor-damage associated molecular pattern) / PAMP (pathogen associated molecular pattern) complex formation. The signalling pathway of the TLRs and the expression of inflammatory cytokines (e.g., IL-1, TNFα, and IL-17) work as it determines activation of NF-kB, similar to TNF-α. The stimulation of TLRs, IL-1, IL-17 and TNF-α activates IkB, which determines recruitment of NF-kB to the promoter of IL-6 gene. Further interaction with CREB induces hyperacetylation. This permeative action determines IL-6 gene transcription. IL-6 also stimulates differentiation of naïve T cells into Th-17 cells by inducing STAT3 transcription. In RA this pathway is more prominent because of citrullinated proteins, having role of PAMPs/DAMPs [14]. In order to induce an effect, this interleukin needs the presence of glycoprotein130 (coupled with JAK-Janus Kinase) on the membrane of the cell, activating either classical (IL-6+IL-6R) or trans-signalling (IL-6+sIL-6R) pathways. Besides having membrane receptors, it can also bind to the soluble receptor sIL-6R, formed by enzyme cleavage in myeloid cells and hepatocytes. sIL-6R is generated through proteolytic cleavage by enzymes such as ADAM10 in myeloid cells and hepatocytes. Importantly, trans-signaling enables IL-6 to act on cells that lack membrane-bound IL-6R—such as endothelial cells—but express gp130. Formation of this complex determines the activation gp130 signal transducing molecule, inducing JAK in target cells. Further, MAPK/STAT3/ERK1,2/Akt enhances proinflammatory molecules transcription, resulting in osteoclasts activation, Lymphocytes B differentiation and proliferation, metalloproteinases / VEGF/monocytes chemoattractant protein-1 synthesis [11]. To be more specific, classical activation with membrane receptor poorly induces activation of STAT3 (Signal transducer and activator of transcription 3), while trans-signalling strongly stimulates this mechanism. The other transcription factors are stimulated equally. IL-6 also contributes to inflammation by inducing the expression of ICAM-1, CCL2/MCP-1, and CXCL8/IL-8, promoting monocyte and neutrophil recruitment and adhesion to endothelial cells (ECs) [15, 16]. Also, stimulating ADAM10 expression through IL-8 and GM-CSF, determines cleavage of EC membrane CD162, thus increasing circulating neutrophils [17]. On the other hand, induction of ICAM1 membrane expression in vascular EC determines leukocyte adhesion and transmigration by coupling with LFA (integrin) [18].

IL-17 is a family of cytokines (IL-17A to IL-17F) secreted for host protection, have a major pathogenetic role in autoimmune and inflammatory diseases. It is synthesized mainly by TH17 cells, together with IL-6, IL-8 and MMP. TH17 cell activation and subsequent IL-17 secretion is stimulated by IL-6, IL1 and TGF-β [19]. The biological activity of IL-17 is mediated through a group of receptors, IL-17RA to IL-17RE. The principal signalling pathway activated by the IL-17/IL-17R complex involves the adaptor protein ACT1, which stimulates downstream activation of NF-κB and MAPK (mitogen-activated protein kinase) pathways, enhancing pro-inflammatory cytokine transcription [20, 21]. IL-17 accelerates the inflammation in vascular endothelium by stimulating vWF secretion. Von Willebrand factor mediates platelet (PLT) aggregation and stabilization of factor VIII. Importantly, IL-17 determines its effect on vascular endothelium, mainly through inducing cell apoptosis. It can be stimulated through Caspase 3 and 9 activation or by inducing Bax pathway [22]. IL-17 induces secretion of pro-inflammatory cytokines (IL-6, GM-CSF, IL-8 and CXCL1). When combined with TNF-α, IL-17 significantly enhances the expression of adhesion molecules such as E-selectin and ICAM-1. This synergistic effect is supported by transcriptomic data: IL-17 alone stimulates the transcription of 45 genes, TNF-α alone stimulates 1,036 genes, but together they stimulate 10,873 genes [23]. IL-17 amplifies ADP-induces PLT activation. It also enhances vWF production by EC, mediating PLT adhesion. Another mechanism consists of activating TF and inhibiting CD-39/ Thrombomodulin [24].

CD-20 is a B-cell membrane protein which basically participates in controlled regulation of activating IgD B-Cell Receptors (IgD-BCR). Over the long term, CD-20 also participates in expression of several other membrane proteins: CD19, CD81, CD22, CD40 and IgM-BCR, which are part of IgD-BCR and participate in induction and regulation of B-cell antigen dependent activation. On the other hand, in case of short-term CD-20 loss, B-cell activation is significantly enhanced. This is due to the CD-20 role in down-regulation of several surface markers. Furthermore, we can also underline IL-6's role in differentiation and proliferation of B-cells. Together with IL-2 and IL-10, IL-6 induces loss of CD-20 in B-cells, emphasizing its role in initial activation of immune cells. Both processes determine increased plasmablasts proliferation, while IL-6 stimulates the process of differentiation in plasmocytes. IL-2 and IL-10 were also found to induce CD126 expression in plasmablasts [25].

TH17 cells represents a specific type of activated naive T cell. The process of differentiation is initiated by TGF-β and IL-21, while IL-6 and IL-1β allow the amplification of the lineage. Finally, IL-23 determines the pathogenetic role of TH17 cells by stimulating IL-17, IL-21 and IL-22 produc-

tion. In addition to conventional, antigen-induced Th17 cells—which express TCRs—there are also natural Th17 cells (nTh17), which lack TCRs and thus bypass the classical activation process. This factor denotes the importance of those immune cells in regulation of normal homeostasis. A common finding in human Treg genes is the absence of exon 2 in FoxP3, unbaling Treg to control IL-17+ T-cell proliferation [19, 22-24].

Another important pathogenetic factor is the production of cell-derived microparticles (MPs) by cells exposed to systemic inflammation and oxidative stress. They are released during both cellular activation and cell death, whether by apoptosis or necrosis. An important role in this process plays the Rho-associated kinase 1, which induces the blebbing process of the MP. Besides that, adrenaline, ADP, thrombin, Ca2+, collagen and complement were demonstrated to activate MP formation. It is also believed that their pathogenetic evolution starts with altered lipids distribution in cytolema bilayer. This disbalance is expressed through a low level of cholesterol and phospholipids, with a high expression rate of phosphatidylserine on the outer leaflet. Mostly, they contain cytoplasmic and cytolemic contents of the parent cell, which helps us deduce their origin. The presence of CD4, CD3 or CD8 on the MP surface indicates lymphoid origin while platelet MP are marked by the expression of glycoproteins IIb–IIIa, P-selectin/CD42a/ phosphatidylserine (binding annexin V) [26]. Similarly, endothelial MP displays surface CD31 or CD146. Their size varies between 50-800 nm which makes them detectable by scanning electron microscopy. According to Knijff-Dutmer *et al.* [27], MP's concentration in RA is significantly higher, correlating with disease activity. In rheumatoid arthritis, they mainly expose complement components or activator factors. An important point for connecting RA and MP is represented by the presence of citrullinated proteins in MPs, an important pathogenetic factor for rheumatoid arthritis. Platelet derived MP's have both inflammatory and pro-coagulative effects. On the one hand, secretory PLA2 determines formation of arachidonic acid which is converted in prostaglandins by COX-2, inflammation mediator. On the other hand, PAF is mainly found bound to the PMPs, inducing platelet activation, maintaining local and systemic inflammation/hypercoagulation. Furthermore, P-selectin, a receptor that mediates adhesion of platelets and endothelial cells to the monocytes and granulocytes, is induced by activation of platelets and is found on the surface of PMPs [28]. Additionally, MP interact with factors Va, VIII and IXa, thereby facilitating assembly of prothrombinase complex.

Microparticles role in pathogenesis of vasculitis can be supported by the fact that between 8% and 26% of the endothelial cells internalized MPs and MPs-ICs; a smaller proportion of these cells kept these vesicular structures on their surface (2% to 5%). Both internalization and membrane binding primarily involve the same set of receptors: ICAM-1, CD36 (a scavenger receptor), and CD93 (the C1q receptor). Microparticles complexed with CICs were observed to induce a stronger reaction compared to isolated MPs. This includes increased expression of ICAM-1/2 and supernatant accumulation of IL-6/8 was captured. Besides that, CCL-2/5, a group of chemokines responsible for recruiting and activating immune cells (especially monocytes) in peripheral tissues, were found to be hyper-expressed in endotheliocytes. In addition to the adhesion process to the endothelium being stimulated, intercellular connections between EC are destroyed. This is evidenced by reduced membrane expression of VE-cadherin and depolymerization of actin filaments, which together alter the structure, organization, and continuity of the endothelium in RA. Moreover, MPs are able to activate apoptosis of endothelial cells through unknown mechanisms. The fact that these manifestations are dose-dependent, makes them an important asset for clinicians in future [28].

During systemic inflammation, there are modifications in blood rheology, which also affect vascular endothelial function. Because of increased concentrations of platelets and plasma proteins, along with plasma loss volume, blood becomes more viscous. Additionally, disturbances in vascular smooth muscle tone in addition to increased roughness of endothelium, induce turbulent blood flow. It is believed that endothelial cells possess specialized mechanoreceptors—including components of the cytoskeleton, G-protein coupled receptors (GPCRs), junctional proteins, integrins, and ion channels—that can detect mechanical stimuli such as flow turbulence. Thus, stimulation of NF-kB pathway (c-JUN/AP-1) will determine an increase in adhesives (VCAM-1/E-selectin/ICAM-1) expression. Contrary to pro-inflammatory effects, TNF-α, along with IL-1b induces KLF2 (Kruppel-like factor 2) expression, normally stimulated by laminar flow shear stress. It inhibits the activation of p65 (transcription factor), downregulating VCAM1 and E-selectin transcription. It also upregulates thrombomodulin and NOS3 expression and inhibits transcription of VEGF receptor 2 [13].

Discussion

Due to multiple discoveries in RA and SRV pathogenicity and disease course, we can deduce several pathways leading to vascular damages in this type of vasculitis. All the effects described in this article could be applied both to the healthy and already damaged vessels. Based on RA pathogenicity, all the morphological changes develop at the same time, with rate of progression influenced by genetic predisposition, which causes certain processes to become exacerbated more rapidly than others. This is supported by higher incidence of SRV in RA patients who presented HLA-DRB1*04/04 or HLA-C*03 allele or p53 mutations. In the incipient forms, there is only local evidence of vessel modifications. Vascular endothelium is highly damaged at the site of affected articulations (PIP and MCP). It is favoured by intense inflammatory reactions of Fibroblast-Like Synoviocytes (FLS), ICs formation and deposition, as well as microparticle synthesis. FLS are known to secrete growth factors (TGF-β/FGF), adhesion molecules (VCAM-1, ICAM-1 and integrins) and cytokines (IL-6 and TNF-α) [29]. Entering the systemic circulation, they stimulate their targets, one of them being the endothelial cell of the vas-

cular wall. At this point, IL-6, IL-10 and IL-17 have additive effects. It was observed that all three inflammatory mediators have stimulated ICAM-1 expression, E-selectin was increased in IL-17 and TNF-α stimulation, VCAM-1 was associated with TNF-α. Both IL-6 and IL-17 enhanced cell adhesion through expression of other stimulating Interleukins and molecules. IL-1/CXCL1 and vWF are some of the effectors of the IL-17 mediated adhesion. In addition to that, IL-6 induces IL-8/CXCL8, MCP-1 and CCL2 transcription. Besides interleukins, these effects were observed in the MPs dependent cell activation, where increased ICAM-1/2, P-selectin and CCL-2/5 were observed. All these effects determine immune cells rolling, coupling and transmigration at the site of vascular endothelial cells. Further changes are induced by increased intercellular space between endotheliocytes. It is thought to be related with activation of p38MAPK/ERK pathway through TNFR1- TNF-α complex, which determines rearrangement and destabilization of microtubules. Also, the apoptosis induced by IL-17 and MPs, alongside Ca-CaM dependent MLCK inhibition, speeds up this step. MPs also inhibit VE-cadherin expression and enhance actin filament depolymerization. These changes are important for the translocating mechanism of the cells and ICs to the subendothelial space. Further processes can be more described as a destructive step. It is mainly connected to the ICs activity, as they have the ability both to form and precipitate in the subendothelial space. MET-osis, NET-osis, Citrullination and cell death are some of the pathways which can stimulate ICs. Through MET-osis and NET-osis, macrophages and neutrophils expel their intracellular (MMPs/PAD) enzymes in extracellular space. Besides their damaging action on self-cells, they degradation and citrullination of self-peptides, transforming them in Ag. Also, both necrosis and apoptosis induce Microparticles formation. They can also bind Ab as MPs contain citrullinated molecules. What's more, all the immune cells present at the site of inflammation express FcR on their membrane. All these factors determine ICs migration and synthesis in the subendothelial space. Lastly, coupling with specific receptors, induces stimulation of different cell processes, for example: ITAM activation increases the phagocytosis rate and cytokine transcription. Besides that, Ag-Ab complexes can bind C1q inducing complement cascade, which determines chemotaxis (C3a, C5a), opsonization (C5b) and cytolysis (through membrane attack complex). At this point, a self-sustaining inflammatory loop is established, wherein cytokines, immune cells, and ICs perpetuate each other's production and activity. A very important principle that we should not forget is that all those factors have reciprocal potentiating mechanisms, thus inhibition of one of those factors drastically decreases the inflammation.

Conclusions

The study provides a comprehensive analysis of the complex interactions between immune and vascular systems in RA and SRV. EC play a central role in regulating inflammation and coagulation, with activation pathways involving cytokines such as TNF-α, IL-6, and IL-17. These cytokines enhance the expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin), stimulate angiogenesis, and alter endothelial barrier integrity, leading to increased vascular permeability and immune cell recruitment. ICs, microparticles, and systemic inflammatory mediators contribute to the progression of vascular damage by amplifying pro-inflammatory cascades and inducing complement activation, phagocytosis, and cellular apoptosis.

In SRV, the interplay of IC deposition, cytokine release, and immune cell activation perpetuates endothelial dysfunction and hypercoagulability. Specific genetic predispositions, including HLA-DRB1 and HLA-C alleles, were identified as exacerbating factors, linking immune dysregulation to vascular pathology. Novel insights into the role of microparticles and immune cell-derived cytokines further emphasize their pathogenic contributions to endothelial disruption and thrombo-inflammatory processes.

This research highlights critical mechanistic pathways underlying vascular damage in RA and SRV, offering potential targets for therapeutic intervention. It underscores the importance of early detection and precise modulation of inflammatory and coagulative pathways to mitigate vascular complications in autoimmune diseases.

Competing interests

None declared.

Authors' contributions

Study conception and design: ER, AP, LG. Data acquisition: AP, MS, CN, IL. Analysis and interpretation of data: ER, LC and SA. Drafting of the manuscript: AP, ER. Significant manuscript review with significant intellectual involvement: LG, ER, SA, LC. Approval of the „ready for print" version of the manuscript: ER, LG, LC, SA, MS, CN, IL, AP.

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REVIEW ARTICLE

Rheumatoid arthritis worldwide: inequalities in epidemiology and care

Elena Deseatnicova[1*], Valeria Frunze[2], Rodica Usatii[3], Liliana Groppa[1]

[1]Discipline of Rheumatology and Nephrology, Department of Internal Medicine, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

[2]Department of Radiology and Imaging, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

[3]*Timofei Moșneaga* Republican Clinical Hospital, Chisinau, Republic of Moldova

ABSTRACT

Introduction. Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting approximately 0.5% of the global population. It represents a major cause of disability, reduced quality of life, and healthcare burden. The prevalence of RA is rising, especially in older populations and in low-income regions.

Materials and methods. A systematic literature search was performed in PubMed, ScienceDirect and Google Scholar for articles published between 2000 and 2025. Search terms included "rheumatoid arthritis", "epidemiology", "risk factors", "economic burden", and "healthcare disparities".

Results. RA prevalence ranges from 0.3% to 1.0%, with the highest values in Northern Europe (0.8–1.0%) and North America (0.7–0.9%), and the lowest in Africa (0.1–0.3%) and rural Asia (0.2–0.4%). Work incapacity has declined in several high-income countries, attributed to earlier diagnosis and the widespread use of disease-modifying antirheumatic drugs. Socio-economic status is a key factor for RA outcomes, with patients in the lowest income groups showing up to 50% higher disability rates. Other risk factors include female sex, HLA-DRB1 alleles, smoking, and environmental exposures. The economic burden is considerable, with direct and indirect costs disproportionately affecting low- and middle-income countries, where RA frequently results in early work disability.

Conclusions. RA causes disability and reduces quality of life. Its prevalence is rising worldwide, with higher detection and better outcomes in high-income countries, while low-income countries face underdiagnosis and limited access to modern therapies. Reducing these disparities requires stronger healthcare systems, early diagnosis, and affordable and accessible treatments.

Keywords: rheumatoid arthritis, epidemiology, prevalence, incidence, socio-economic factors, healthcare disparities.

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[*]**Corresponding author**: **Elena Deseatnicova**, MD, PhD, associate professor
Department of Rheumatology and Nephrology
Nicolae Testemițanu State University of Medicine and Pharmacy
165, Ștefan cel Mare și Sfânt blvd, Chișinău, Republic of Moldova, MD 2004
e-mail: elena.deseatnicova@usmf.md

Authors' ORCID IDs
Elena Deseatnicova – https://orcid.org/0000-0001-5029-2994
Valeria Frunze – https://orcid.org/0009-0000-1263-7963
Rodica Usatii – https://orcid.org/0009-0003-1486-5136
Liliana Groppa – https://orcid.org/0000-0002-3097-6181

Key messages

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

The role of economic and healthcare disparities in shaping rheumatoid arthritis prevalence and progression in different regions remains incompletely clarified.

The research hypothesis

Regional and socio-economic factors significantly influence the prevalence and management of rheumatoid arthritis worldwide.

The novelty added by manuscript to the already published scientific literature

This review combines recent data on RA epidemiology and emphasizes regional and socio-economic differences. It highlights practical aspects of health system inequalities and outlines future directions for improving global RA management.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by persistent synovial inflammation, progressive joint destruction, and extra-articular manifestations. Beyond joint involvement, RA is associated with substantial systemic effects and comorbidities, contributing to functional disability, impaired quality of life and increased mortality [1, 2]. Despite notable advances in early diagnosis and therapeutic strategies, RA continues to represent a major global public health challenge [3].

The global burden of RA has been rising, with considerable heterogeneity across regions in both prevalence and outcomes [3, 4]. Prevalence estimates range from 0.1% to 2.0% worldwide, reflecting not only true differences in disease occurrence but also variation in genetic susceptibility, environmental exposures, healthcare infrastructure, and diagnostic practices [4, 5]. High-income regions such as North America and Western Europe typically report higher prevalence rates and better clinical outcomes, largely attributable to improved case detection, timely referral to specialists and broader access to advanced therapies [6, 7]. In contrast, many low- and middle-income countries report lower prevalence figures, which may reflect underdiagnosis, scarcity of rheumatology specialists, and restricted availability of effective treatments [8, 9].

In addition to geographic variation, demographic and socio-economic disparities further shape the global distribution of RA. The disease affects all adult age groups, with peak incidence observed in middle age, and is two to three times more common in women than in men [10, 11]. Ethnic differences are also pronounced, with certain Indigenous populations exhibiting prevalence rates several-fold higher than those observed in the general population. These disparities underscore the complex interplay between genetic, hormonal, environmental, and socio-cultural determinants of RA epidemiology.

The consequences of RA extend well beyond individual health outcomes to impose a substantial socio-economic burden. Direct healthcare costs include specialist consultations, diagnostic imaging, pharmacological and non-pharmacological therapies, hospitalizations and rehabilitation, while indirect costs arise from work disability, productivity loss, and premature retirement [12]. Although the introduction of biologic and targeted synthetic disease-modifying antirheumatic drugs has led to substantial improvements in disease control, access to these therapies remains uneven worldwide, perpetuating inequalities in morbidity, disability and mortality [13, 14].

Given these challenges, a comprehensive understanding of current epidemiological patterns, risk factors, comorbidities and disease burden is essential to inform clinical practice, guide public health strategies and support evidence-based policy-making.

The objective of this review is to synthesize contemporary evidence on the global epidemiology of rheumatoid arthritis, with a particular focus on geographic and socio-economic disparities in prevalence and incidence, established and emerging risk factors and associated comorbidities and outcomes.

Material and methods

A narrative literature review was employed, using major databases such as ScienceDirect, PubMed, Google Scholar for articles published between 2000 and 2025. Keywords terms included “rheumatoid arthritis”, “epidemiology”, “social factors”, “economic burden”, and “risk factors”. Only articles published in English were included, with the addition of selected international publications in Romanian or Russian relevant to Eastern Europe.

Studies were eligible for inclusion if they: (i) examined the epidemiology, prevalence, or incidence of rheumatoid arthritis; (ii) discussed socio-economic or demographic factors associated with RA; or (iii) explored the economic burden of RA or disparities in treatment access. Eligible study types included original epidemiological studies (cross-sectional, cohort, or case–control), population-based reports or large registries, and systematic reviews or meta-analyses focused on RA epidemiology. Exclusion criteria included: case reports, case series with fewer than 50 patients, non–peer-reviewed sources, and purely experimental studies (in vitro or animal).

Data extraction and analysis. From each included study, data were extracted on: author, year of publication, country/region, study design, sample size, diagnostic criteria, prevalence and incidence estimates, reported risk factors, comorbidities and mortality data. Findings were synthesized descriptively and presented in summary tables. A narrative comparison was used to highlight geographic variation, socio-economic influences, and comorbidity trends.

Ethical considerations. Given the nature of the study as a review of existing literature, ethical approval was not required.

Quality considerations. This review is limited by the restriction to English, Romanian and Russian-language publications which may have led to the omission of relevant studies published in other languages. Potential heterogeneity in study designs and reporting standards was acknowledged. Limitations such as underdiagnosis in low-resource settings and variability in registry completeness were considered when interpreting the findings.

Results and discussions

Geographic and socio-economic disparities in prevalence and incidence. Global prevalence is estimated from around 0.1% to 2.0% across different populations [5, 15]. A 2021 meta-analysis found a global prevalence of about 0.46%, while the 2020 Global Burden of Disease study reported an age-standardized prevalence of ~0.21% [5, 15]. These differences highlight regional variation and disparities in estimation methods. Table 1 presents RA prevalence and incidence in selected countries to demonstrate global variability.

Table 1. Comparative prevalence and incidence of rheumatoid arthritis in different countries

Location	Prevalence (%)	Annual Incidence (per 100,000)
United States	~0.6% [5]resource allocation, and prevention. As part of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD	~20–50 (North America range) [5]resource allocation, and prevention. As part of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD
Canada	0.9%	-
France	~0.31% [16]	8.8 per 100,000 [16]
Romania	~1.0% [17]	–
Republic of Moldova	0.031% [18]	11 per 100,000 [18]
India	~0.75% [19]	0.5 to 1 per 1,000 [20]
Japan	~0.6–1.0% [16]	8 per 100,000 [16]
South Africa (rural)	0.0026%	– (extremely low, <1)

Note: Incidence "–" indicates not available or not reported in sources [5, 17–20].

High-income regions of North America and Northern Europe report a prevalence of around 0.5 –1%. (the United States – 0.6%, Canada – 0.9%) [5, 19]resource allocation, and prevention. As part of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD. In Romania, RA prevalence was estimated at approximately 1% of the population. In contrast, lower prevalence is found in some parts of Asia (South Korea - 0.26%) and Africa (South African rural community – 0.003%, Nigeria – 0%) [15, 19, 21]as epidemiologists do not study established RA separated from RA as a whole; especially no incidence studies can be found, as incidence refers to new cases (early RA. Reported RA prevalence in the Republic of Moldova is about 0.03% [18]. However, underdiagnosis and underreporting may contribute to this low prevalence. True RA prevalence in Moldova is expected to be closer to that of other Eastern European countries.

RA incidence also shows geographic variation. Incidence in North America and Northern Europe is estimated at around 20–50 per 100,000 per year; in Japan and France around 8–9 per 100,000 [15, 16, 22]. The annual incidence in the Republic of Moldova was reported to be approximately 11 per 100,000 in 2015 [18].

Urban–rural differences are observed as well. RA prevalence is often higher in urban populations than rural ones within the same country [16, 23, 24]. For instance, an urban area in one study showed 0.69% prevalence vs 0.54% in a rural area [25]. Urban living may increase exposure to risk factors like pollution or unhealthy diets. A north-south gradient in Europe has been noted, with Northern Europe historically having higher RA prevalence than Southern Europe. Studies from Germany and Sweden reported a prevalence of around 0.65%, while in other European countries such as France or Italy, the prevalence was lower (0.19% and 0.41%) [26].

Demographic disparities. RA affects all adult age groups and both sexes. The peak age is 49 ~ 74 years [27]. In 2020, global prevalence rates were lowest in young adults and highest in the 75–79 age group, reaching ~0.83% in that cohort [5]. RA is about 2–3 times more common in women than in men [28]. For example, a study in Argentina found a prevalence of 3.2% in women vs 0.6% in men. In a publication from Serbia, the rate was 0.29% among women compared to 0.09% among men. Some studies found no difference. The reasons are thought to include hormonal and genetic factors [28]. By 2050, an estimated 68.7% of rheumatoid arthritis cases will be in females [5]. However, men tend to develop RA at slightly older ages. They may experience more severe radiographic damage in some cases, though findings are mixed [29]. Additionally, healthcare utilization disparities by gender have been observed. Women with RA tend to seek medical care more often, possibly due to greater symptom burden or health awareness. This can influence apparent prevalence [30, 31]. Ethnic disparities are also significant in RA epidemiology, notably among Indigenous North American populations. Elevated RA prevalence has been documented in specific Indigenous groups such as the Pima, Chippewa, Blackfeet, Yakima, Tlingit, and Algonquin First Nations communities of Central Canada. The prevalence rates are approximately 5–6% (vs. 0.5–1% in the general population) [32, 33].

Risk factors and etiologic influences. RA develops through a combination of genetic predisposition and environmental triggers. The heritability of RA is estimated at 50–60%, with HLA-DRB1 being the main genetic risk factor [34, 35]. Cigarette smoking is the most established environmental risk factor for RA. Long-term smoking doubles the risk of RA and is especially linked to seropositive (RF or anti-CCP positive) RA [36-38]. Another emerging risk factor is silica dust exposure [39]. Obesity is associated with a modestly increased risk of RA or worse disease activity [40, 41]. Periodontal disease (chronic gum infection) caused by Porphyromonas gingivalis has been associated with RA, as it can citrullinate proteins and potentially trigger autoimmunity [42, 43]. Some studies suggest that moderate alcohol intake may even have a modest protective effect against RA [44, 45]. Geographic factors like climate and latitude have been posited (e.g. lower Vitamin D levels in northerly latitudes might contribute to autoimmunity), but data are inconclusive [33, 46, 47].

Disease outcomes and burden in RA. RA shows important geographic differences in prevalence and outcomes. High-income countries (for example, North American and Western European countries) generally report higher prevalence (around 0.5–1%). They achieve higher diagnosis rates and better outcomes, whereas many low-income countries report lower prevalence (often <0.3%). Part of this discrepancy is due to underdiagnosis in low-resource regions rather than true absence of disease [3, 5]. For example, in sub-Saharan Africa and rural South Asia, limited access to healthcare means many RA cases are never formally diagnosed. Nigeria has just 30 rheumatologists serving a population of about 200 million [48]. In India, there are approximately 100 registered rheumatologists for a population exceeding 1.3

billion people [49]. This shortage leads to many RA cases remaining undiagnosed, further contributing to the lower prevalence in these regions [23, 48, 50] In contrast, high-income countries have well-developed healthcare systems that can accurately detect and document most RA cases, leading to higher reported prevalence rates.

The countries with the highest number of rheumatologists in Western Europe are France (2,600), Italy (1,800), Spain (1,155), the UK (950), Germany (800), and the Netherlands (775) [51]. Nearly 70% of suspected RA cases in Canada are evaluated by a rheumatologist within three months [24]like availability, pricing/funding, and acceptability. In Latin America (LA. There are also public health initiatives (like anti-smoking campaigns and early arthritis referral programs) that have stabilized or slightly reduced RA incidence in recent decades [5, 52]resource allocation, and prevention. As part of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD.

Treatment disparities are significant as well. Advanced therapies like biologic disease-modifying antirheumatic drugs (DMARDs) are more accessible in North America and Western Europe, leading to better disease control and reduced disability rates. In lower-income countries, access to such treatments is limited due to cost and healthcare infrastructure. For example, in a study across 37 European countries, only about 59% of RA patients who are eligible for biologic therapy (by EULAR criteria) receive it. This inequity in care contributes to worse outcomes in those regions [14, 53, 54]. Indeed, RA mortality and disability rates are highest in regions with limited healthcare resources, whereas high-income regions have seen significant declines in RA disability and mortality due to early treatment [5, 55].

RA significantly reduces patients' quality of life compared with healthy populations. Patients commonly experience limitations in physical function, chronic pain, disability, and decreased mental well-being, with high rates of anxiety, depression, and impaired social, environmental, and sexual functioning [56, 57] . Without effective treatment, RA can rapidly progress, causing joint deformities within the first 1–2 years of onset, resulting in long-term functional limitations [58, 59].

Work disability is a major issue in RA. Approximately 30–40% of RA patients discontinue working prematurely, a rate notably higher than in the general population [7]. Specifically, a Romanian cohort study found that 27% of RA patients reported permanent work disability due to their condition, resulting in productivity losses [60]. Factors such as older age, longer disease duration, greater comorbidity burden, and higher disability scores significantly elevate the risk of work disability [7].

RA is frequently accompanied by multiple comorbidities driven by chronic systemic inflammation and immune dysregulation. Cardiovascular diseases (CVD), notably ischemic heart disease and stroke, are the leading comorbidities, accounting for 30–40% of RA-associated mortality [61-63]. Other significant comorbidities include interstitial lung disease, observed in approximately 10% of RA patients, osteoporosis, metabolic syndrome, lymphomas, and mental health disorders such as depression and anxiety [55, 63-65]. If the disease course is monitored, co-morbidities may be prevented during the course of RA [7].

RA is also associated with excess mortality. The Global Burden of Disease 2020 study reported about 38,300 deaths globally attributable to RA in 2020. The age-standardized mortality rate in RA has declined by ~24% since 1990, due to improved treatments. However, mortality improvements are uneven across regions [5, 66]. Mortality is higher for circulatory, respiratory, musculoskeletal, and digestive system diseases [67]. High-income regions saw the largest drop in RA mortality (a 44% decrease in RA-related deaths in Europe from 1990 to 2020), reflecting early use of effective therapies and better cardiovascular prevention [5].

These disparities in prevalence, incidence, comorbidities, and mortality trends underscore substantial global inequalities in the epidemiology and management of RA.

Socio-economic impact. The costs of RA can be categorized into direct medical costs and indirect costs (productivity losses) [40, 43]. Direct healthcare costs include inpatient care, outpatient services, diagnostic tests, long-term pharmacological and non-pharmacological treatments, laboratory and imaging studies, and various medical procedures. Additionally, costs extend to preventive programs, such as surgical interventions, rehabilitation, and therapeutic procedures. Expenses also cover salaries of healthcare professionals involved in patient care, as well as non-medical services like patient transportation, specialized dietary needs, and other logistical support [68]yielding frequency only to diseases of respiratory and cardiovascular systems. Given the fact that they are occurred in a young, workingage population, as well as people in older age groups, the treatment of these patients consumed large financial resources, which results in a high socio-economic importance of rheumatic diseases in general. The article focuses on the prevalence of these forms of pathology in Russia and several foreign countries, material costs of the medical care of such patients (including direct, indirect and additional costs.

A Medicare study in the USA found annual costs for RA patients to be $20,919, compared to $7,197 for non-RA individuals [69]10th Revision clinical modification codes were identified. Healthcare expenditures (inpatient care, outpatient care, emergency department, office visits, prescription medications, home health, and others. The mean estimated annual cost per patient in Europe with RA was €12,902 (Western Europe mean is €14,997 vs. Central/Eastern Europe mean €3,752) [22].

RA patients spend significantly more on healthcare, mainly due to prescription medications [69]10th Revision clinical modification codes were identified. Healthcare expenditures (inpatient care, outpatient care, emergency department, office visits, prescription medications, home health, and others. Treatment for RA focuses on the control and management of inflammation. Modern RA treatments (biologic and targeted synthetic DMARDs) are costly, contributing to higher treatment expenses. New RA patients or

those with less severe disease are usually prescribed less expensive non-biologic DMARDs. In contrast, patients who do not respond to DMARD treatment or exhibit more advanced disease are treated with biologics [70, 71].

However, these therapies have reduced other expenses by lowering rates of joint surgery, hospitalizations, and disability. For example, in Sweden, as biologic use increased from 2001 to 2010, RA patients saw significant declines in hospital admission rates and days on sick leave [23]. In developing countries, including Romania, healthcare system resources are limited and cannot cover the cost of treatment for all RA patients who would benefit from biological therapy [60].

According to EULAR recommendations, 32% of the total RA population in the European region is eligible for biologic DMARD treatment. However, only 59% of this population remains eligible after applying national reimbursement criteria (from 86% in 'high access' to 13% in 'low-access' countries) [53]partly owing to their high direct costs against a background of restricted healthcare budgets. This study compares the size of RA patient populations with access to reimbursed bDMARDs across 37 European countries, Russia, and Turkey, according to their treatment eligibility defined by European League Against Rheumatism (EULAR. The recent introduction of biosimilars has increased the availability and affordability of these medications in countries with limited resources [72].

In working-age populations, indirect costs from sick leave and permanent disability represent a major component of the overall RA burden. In Europe, work disability constitutes approximately 40–60% of the total RA-related costs. For example, annual indirect costs in England are estimated to reach up to €6.75 billion [69]10th Revision clinical modification codes were identified. Healthcare expenditures (inpatient care, outpatient care, emergency department, office visits, prescription medications, home health, and others. In Romania, the average annual indirect cost per RA patient was calculated at €3,968.71 [60]. In Sweden, RA patients experienced an increase in sick leave and disability pension days from an average of 43 days per year prior to diagnosis to approximately 147 days annually after diagnosis [73]. Similarly, in Taiwan, indirect costs related to productivity losses accounted for 61.6% of the total RA economic burden, which was estimated at approximately $224.9 million annually [74].

Trends and future projections. TThe Global Burden of Disease forecasts a significant increase in RA cases. The number of RA patients is expected to grow from ~18 million in 2020 to over 31 million by 2050. By 2050, nearly 70% of RA patients globally will be female over 60 years old. Not all regions will experience the same increase. By 2050, Central and Eastern Europe are expected to have stable or slightly lower RA case numbers due to declining populations and lower birth rates [5, 75].

Patient access programs and international collaborations are attempting to share expertise and improve care in low-resource areas. EULAR and ILAR are training rheumatologists in Africa and Asia and helping to adapt treatment guidelines to local needs [76].

Future projections must consider environmental changes. Modifiable risk factors, particularly smoking, remain central to future risk. Other preventable factors include obesity and environmental exposures [40, 77]. Public health improvements in oral health might reduce RA risk linked to periodontal disease [78]. Early detection remains crucial: joint damage often occurs within the first 1-2 years of disease onset. Ensuring treatment access, especially affordable biologic or biosimilar DMARDs, and strengthening specialist care networks are essential [79].

Finally, the increasing burden of RA-related disability highlights the need for robust rehabilitation and support services. Assistive devices, physical therapy, and occupational therapy can help RA patients remain functional.

Conclusions

Rheumatoid arthritis remains a global health problem with clear geographic and socio-economic inequalities. High-income countries have improved outcomes through early diagnosis, treat-to-target strategies and access to modern therapies, which reduced disability and mortality. In contrast, low-income regions continue to face underdiagnosis, limited treatment options and insufficient healthcare infrastructure, resulting in higher disease burden. Future efforts should prioritize earlier diagnosis, wider availability of affordable therapies, stronger healthcare systems, and preventive strategies. International initiatives such as EULAR and ILAR play an important role in reducing disparities and supporting equitable access to effective RA management worldwide.

Competing interests

None declared.

Authors' contribution

ED conceived the research idea; ED, VF, RU and LG developed the aim and objectives of the literature review; ED, VF, RU drafted the manuscript and realized the literature search; ED, VF, RU and LG designed the study and revised the manuscript critically. All authors have read and approved the final version of the manuscript.

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REVIEW ARTICLE

Current concepts in the surgical management of chronic suppurative otitis media

Iurie Noroc[1,2*], Sergiu Vetrican[1,2], Eusebiu Sencu[1,2]

[1]Department of Otorhinolaryngology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[2]*Timofei Moșneaga* Republican Clinical Hospital, Chisinau, Republic of Moldova

ABSTRACT

Introduction. Chronic suppurative otitis media represents a major public health concern in both children and adults, particularly in developing countries. The condition poses not only a medical challenge requiring complex clinical management but also a public health issue with significant socioeconomic implications and costs.

Material and methods. A review of selected literature from the PubMed, Hinari, SpringerLink, National Center for Biotechnology Information, and Medline databases was conducted. Articles published between 2000 and 2025 were selected using the following keywords: "chronic otitis media" and "chronic suppurative otitis media," combined in various ways with the terms "surgical treatment," "mastoidectomy," "canal wall-down," "canal wall-up," "canal wall-intact," "canal wall reconstruction," "tympanoplasty," and "ossiculoplasty" to maximize search efficiency. Based on the search criteria, 325 full-text articles were initially identified. The final bibliography included 66 relevant sources considered representative of the published material related to the topic of this review article.

Results. There are four traditional surgical procedures for the treatment of chronic suppurative otitis media: 1) simple (cortical) mastoidectomy, 2) radical mastoidectomy, 3) modified radical mastoidectomy, and 4) mastoidectomy with tympanoplasty. These surgical interventions can be classified into two categories: open cavity (canal wall-down – CWD) and closed cavity (canal wall-up – CWU) or canal wall-intact (CWI). The CWD technique is the most effective method for cholesteatoma eradication, as it allows a wide-angle view of the mastoid and middle ear structures. Currently, radical CWD mastoidectomy is rarely performed but may be indicated when complete excision of the cholesteatoma is not possible. To prevent complications associated with the mastoid cavity following CWD, surgeons may opt for CWU mastoidectomy or CWD mastoidectomy with mastoid cavity obliteration and reconstruction of the external auditory canal wall.

Conclusions. The current concept of managing patients with chronic suppurative otitis media involves developing a personalized approach based on anatomical, biological, radiological, and social factors. The selection of the surgical technique should be tailored to each patient according to the location and extent of the cholesteatoma, defects of the posterior canal wall, associated lesions, presence of complications, degree of hearing loss, and the patient's overall medical condition.

Keywords: chronic suppurative otitis media, mastoidectomy, canal wall-down, canal wall-up, canal wall-intact, canal wall reconstruction, tympanoplasty, ossiculoplasty.

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***Corresponding author: Iurie Noroc,** PhD fellow, assistant professor
Department of Otorhinolaryngology
Nicolae Testemițanu State University of Medicine and Pharmacy
Chișinău, Republic of Moldova MD-2004,
e-mail: iurie.noroc@mail.ru

Key messages

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

The inconsistent clinical outcomes and frequent complications of chronic suppurative otitis media have hindered the standardization of its surgical management. At present, many questions remain regarding the optimal management, whether medical and/or surgical, of these patients.

Authors' ORCID IDs
Iurie Noroc – https://orcid.org/0009-0007-8547-9362
Sergiu Vetricean – https://orcid.org/0009-0002-3168-9747
Eusebiu Sencu – https://orcid.org/0000-0001-7481-5649

The research hypothesis

It is hypothesized that a systematic analysis and synthesis of contemporary literature will demonstrate a significant association between specific surgical techniques applied in the management of chronic suppurative otitis media and their corresponding clinical outcomes, enabling the establishment of evidence-based indications and contraindications for each surgical method.

The novelty added by manuscript to the already published scientific literature

This article includes an analysis of the most recent international publications addressing the characteristics and effectiveness of contemporary surgical treatment methods for chronic suppurative otitis media. The findings of this study will contribute to the improvement of treatment protocols, focusing on the management and optimization of therapeutic approaches for patients with chronic suppurative otitis media.

Introduction

Chronic suppurative otitis media (CSOM) constitutes a major public health problem in both children and adults, particularly in developing countries. The condition represents not only a medical challenge requiring complex clinical management but also a public health issue with significant socio-economic implications and costs [1]. Globally, CSOM is one of the most common infectious diseases in childhood, likely due to an immature immune system and neglect of personal hygiene, and it is a frequent cause of hearing impairment, especially in resource-limited countries. According to the World Health Organization (WHO), approximately 3–4.7% of the population suffers from CSOM, with up to 60% presenting moderate to severe hearing loss [2]. Surgical intervention as a management strategy is considered a last-resort option and is performed in patients with CSOM who do not respond to systemic therapy for 3–4 weeks or to antimicrobial therapy for 3–4 days and otorrhea [2]. The primary goal of surgical management of CSOM with cholesteatoma is disease eradication and the creation of a dry, safe, self-cleaning ear with a low risk of recurrence. Secondary objectives include ossicular chain reconstruction to improve hearing and preservation of normal ear anatomy [3, 4]. A wide variety of surgical techniques exist for middle ear and mastoid diseases. Mastoidectomy and/or tympanoplasty (with or without ossicular chain reconstruction) are frequently used to achieve definitive healing of CSOM [1]. In this context, the aim of this article is a to provide a narrative synthesis of the most recent data regarding the characteristics and efficacy of modern surgical management methods for patients with chronic suppurative otitis media.

Materials and methods

To achieve the stated objective, an initial search of scientific publications was conducted using the databases PubMed, Hinari (Health Internet Network Access to Research Initiative), and SpringerLink,. Selection criteria for articles included contemporary data on surgical procedures such as mastoidectomy, tympanoplasty, and ossiculoplasty in the surgical treatment of patients with CSOM. The following keywords were used: "chronic otitis media" and "chronic suppurative otitis media", in various combinations with "surgical treatment", "mastoidectomy", "canal wall-down", "canal wall-up", "canal wall-intact", "canal wall reconstruction", "tympanoplasty", and "ossiculoplasty" to maximize search yield.

To refine the selection of bibliographic sources, the following filters were applied: full-text articles, articles in English, and publications from 2000 to 2025. After preliminary screening of titles, original articles, editorials, narrative and systematic reviews, and meta-analyses were selected if they contained relevant and contemporary information regarding the effectiveness of different techniques of mastoidectomy, tympanoplasty, and ossiculoplasty in the surgical management of CSOM patients. Additionally, reference lists of identified sources were screened to identify supplementary relevant publications not captured in the initial database search.

Information from the included publications was selected, classified, evaluated, and analyzed to highlight key aspects of contemporary perspectives on surgical treatment methods for patients with CSOM.

To minimize the risk of systematic errors (bias) in the study, exhaustive database searches were conducted to identify the maximum number of relevant publications for the study's purpose, only studies meeting validity criteria were evaluated, and strict exclusion criteria were applied to eliminate unsuitable articles from the review.

After processing the information retrieved from the databases , 325 articles addressing the management of patients with CSOM were identified. Following a preliminary analysis of titles, 78 articles were deemed potentially relevant for the present review. After full-text assessment of these sources, 66 publications were ultimately selected as relevant to the stated objective. The final bibliography of the study included these 66 articles, which were considered

representative of the published material on the topic of this review.

Results and discussion

The surgical management of chronic suppurative otitis media (CSOM) includes several traditional approaches, such as simple mastoidectomy, radical mastoidectomy, modified radical mastoidectomy, and mastoidectomy combined with tympanoplasty. These procedures are generally categorized into open cavity (canal wall down, CWD) and closed cavity (canal wall up/intact wall, CWU/CWI) techniques. The CWD approach allows extensive visualization of the mastoid and middle ear structures, making it highly effective for cholesteatoma eradication; however, it results in a large mastoid cavity with associated structural deficiencies. In contrast, the CWU technique preserves the anatomy of the external auditory canal and generally provides better auditory outcomes, although it carries a higher risk of residual or recurrent disease.

While CWD mastoidectomy is now infrequently performed, it remains indicated in cases where complete cholesteatoma removal is not achievable. To mitigate postoperative complications associated with CWD, alternative strategies include CWU mastoidectomy, CWD with cavity obliteration, canal wall reconstruction, or cavity ablation. Revision surgery represents an effective approach for managing post-radical ear pathology, aiming both at complete disease eradication and at preventing recurrence through procedures such as meatoplasty, canalplasty, mastoid cavity obliteration, posterior canal wall reconstruction, tympanoplasty, and ossiculoplasty.

Tympanoplasty is classified into multiple types based on the extent of reconstruction, ranging from simple myringoplasty to more complex procedures involving the ossicular chain and middle ear space. Successful outcomes are defined by full graft integration and measurable hearing improvement one year postoperatively, with primary tympanoplasty success rates reported between 60–99% in adults and 35–94% in children with CSOM. Ossicular reconstruction is guided by the Austin-Kartush classification, which describes the presence or absence of the malleus, incus, and stapes. Functional results following ossiculoplasty show that non-titanium prostheses achieve an air–bone gap closure of ≤20 dB in 37–65% of cases, while titanium prostheses achieve similar outcomes in 60–73% of cases, with overall prosthesis extrusion rates ranging from 0–4%.

Mastoidectomy. Mastoidectomy is a surgical procedure of the temporal bone that opens the postauricular pneumatic cells by removing the thin bony septa between them. The method remains the cornerstone therapy for malignant/atticoantral CSOM [1, 5-7]. A systematic literature review indicates that, overall, the incidence of "dry" ears is reported between 70–95%, while the incidence of ears without recurrent or residual cholesteatoma ranges from 55% to 97% of surgical cases [5]. There are four traditional surgical procedures: 1) simple (cortical, complete) mastoidectomy; 2) radical mastoidectomy; 3) modified radical mastoidectomy; 4) mastoidectomy with tympanoplasty. The goals of the last procedure, in addition to disease eradication, include preservation or reconstruction of the posterior-superior wall of the external auditory canal (EAC), the tympanic membrane (tympanoplasty), and the ossicular chain (ossiculoplasty) [8]. These procedures can be classified into two categories: open cavity (canal wall-down, CWD) and closed cavity (canal wall-up, CWU) or canal wall-intact (CWI), with or without middle ear reconstruction, each having its advantages and disadvantages [8-12]. These two techniques are mainly distinguished by EAC preservation. CWD is considered the most effective method for cholesteatoma eradication, as it allows wide-angle visualization of mastoid and middle ear structures. However, a small self-cleaning cavity cannot be achieved. These problems are avoided in CWU, as the EAC anatomy is preserved. Nevertheless, residual disease and recurrences are more common, whereas auditory outcomes are better in CWU compared to CWD [13].

Simple (cortical, complete) mastoidectomy or antrostomy involves removal of the mastoid cortex and some pneumatic cells, depending on the extent of the disease [14, 15].

Radical mastoidectomy is a CWD procedure with exposure of the middle ear and includes removal of the superior and posterior walls of the EAC, the tympanic membrane, and the ossicular chain without attempting reconstruction [14].

Modified radical mastoidectomy refers to the procedure introduced by Gustave Bondy in 1910, in which the disease is confined to the epitympanum, which is exteriorized by removing the posterior and superior walls while preserving the lateral epitympanic wall in whole or in part, without disturbing the intact tympano-ossicular system (pars tensa and ossicular chain). The posterior wall of the EAC is lowered to the level of the facial nerve, and the mastoid cavity floor continues with the EAC floor, thus creating a common cavity from the mastoid, epitympanum, and EAC. This method is indicated for epitympanic cholesteatoma with intact ossicular chain, normal pars tensa, and good hearing, aiming to achieve a safe ear and prevent intracranial complications. Advantages of this technique include the ability to perform the procedure in a single stage, lower incidence of residual/recurrent cholesteatoma, fewer revision surgeries, and better functional outcomes [14-20].

The method provides relatively safe surgical access for the removal of chronic middle ear and mastoid disease and yields reproducible results. However, it has been suggested that hearing outcomes may not be as good as after CWU (CWI) [20, 21].

*Canal Wall-Down (CWD) Mastoidectomy***.** CWD mastoidectomy, which includes radical and modified radical mastoidectomy, involves complete removal of the mastoid pneumatic cells, cortical margins of the mastoid, and the superior and posterior walls of the external auditory canal (EAC), combined with a meatoplasty to create an open cavity. This creates a single cavity encompassing the mastoid, middle ear space, and EAC, altering the anatomy and physiology of the middle ear and mastoid. Mastoid obliteration and meatoplasty should also be considered routine parts

of CWD mastoidectomy to achieve a complete surgical outcome [7-9, 11, 14, 16, 22, 23]. The CWD technique is usually performed in the presence of extensive infection, extensive cholesteatoma involving the mastoid and tympanic cavities, severe-to-profound hearing loss, ossicular chain erosion, and complications [24]. The main advantages of CWD procedures are excellent intraoperative exposure with access to the mesotympanum and epitympanum, facilitating more effective eradication of middle ear cholesteatoma, lower recurrence rates (2–17%), easy detection of recurrence, and reduced postoperative complication rates [6, 7, 11, 21-23, 25, 26]. A meta-analysis comparing recurrence cases (recurrent and/or residual cholesteatoma) found recurrence rates ranging from 5–17% for CWD techniques versus 9–70% for CWU techniques [27]. However, CWD tympanomastoidectomy has several well-known disadvantages, including cosmetic issues caused by an enlarged meatus, suboptimal functional outcomes, water intolerance and restrictions to prevent complications, vertigo (caloric or barometric), difficulty in fitting conventional hearing aids, absence of a self-cleaning mechanism (persistent otorrhea and granulation), which necessitates regular specialist visits for recurrence monitoring, mastoid cavity care, and medication for infection control [9, 11, 18, 22, 23, 26, 28, 29]. Even with proper care, a significant proportion of patients (20–60%) develop a defective mastoid cavity with persistent otorrhea, predisposing them to recurrent infections and additional complications, significantly impacting quality of life [11, 23]. Currently, CWD mastoidectomy is rarely performed but may be indicated in cases where cholesteatoma cannot be completely removed [14]. To avoid mastoid cavity issues after CWD, surgeons employ CWU mastoidectomy or CWD mastoidectomy with mastoid cavity obliteration, canal wall reconstruction (CWR), or cavity ablation (decavitation) [12]. Thus, the practice of performing an open cavity procedure without reconstruction, regardless of disease severity, is outdated. Optimal management should be determined on a case-by-case basis, influenced by the patient's goals and priorities, the surgeon's experience and technical skills, careful preoperative disease assessment, and intraoperative findings [25].

Mastoid Obliteration. Mastoid obliteration, introduced by Mosher in 1911, was proposed as an effort to avoid the disadvantages of both CWD and CWU mastoidectomy techniques. The main advantages of mastoid cavity obliteration are: (1) reduction of nitrogen-absorbing mucosa in the mastoid cavity, which prevents cholesteatoma recurrence in patients with Eustachian tube dysfunction, and (2) elimination of the mastoid cavity, preventing accumulation of squamous epithelium and mastoid cavity infection [9, 23, 28]. Currently, mastoid obliteration is the preferred treatment for eliminating mastoid cavities and can be performed during primary CWD mastoidectomy or as a secondary procedure during revision surgery [9, 11]. Various materials have been used for mastoid obliteration, including soft tissues (granulation methods, free soft tissue grafts), cartilage (autologous or homografts), bone (autografts, homografts, and heterografts), and biocompatible, non-resorbable synthetic materials (cement—hydroxyapatite, silicone, proplast, calcium phosphate, bioactive glass - Ceravital). Since no perfect solution exists for mastoid reconstruction or obliteration, a combination of different techniques is often used to achieve the most favorable outcome [9, 11, 23, 28, 30]. Several studies have reported acceptable outcomes with mastoid obliteration, with a high proportion (82%) of ears being dry, safe, and self-cleaning, with a small mastoid cavity. The most controversial aspect of mastoid obliteration is the risk of "silent" cholesteatoma recurrence within the obliterated cavity. Following CWD mastoidectomy with canal wall reconstruction, recurrence rates have been reported at 0–16.7%. Currently, imaging modalities such as diffusion-weighted MRI facilitate the detection of cholesteatoma in obliterated or reconstructed cavities [11].

CWU Mastoidectomy (Canal Wall-Up / Canal Wall-Intact Mastoidectomy). CWU mastoidectomy, also known as canal wall-intact (CWI) mastoidectomy, was introduced by Jansen in 1958. It is performed by removing pathological tissue while preserving the posterior and superior bony walls of the external auditory canal (EAC) [7, 8, 11, 14, 16, 19, 31]. The goals of this procedure are: (1) to avoid opening the mastoid cavity whenever possible, (2) to allow a staged procedure, if necessary (approximately 70% of patients), and (3) to enable re-exploration of the mastoid and middle ear for residual cholesteatoma when indicated [1, 7, 11, 31]. CWU mastoidectomy is indicated for patients with chronic suppurative otitis media (CSOM) with limited cholesteatoma confined to the epitympanum, an intact ossicular chain, good drainage of the mastoid and tympanic cavities, and no intracranial complications [24, 31]. As part of the CWU technique for attic cholesteatoma, procedures may include atticotomy, reconstruction of the lateral epitympanic wall, tympanoplasty using cartilage/perichondrium with or without ossiculoplasty, canalplasty, and cortical mastoidectomy [31, 32]. The major advantages of CWU mastoidectomy are preservation of the normal anatomical structure of the EAC, reduced structural damage, improved potential for hearing reconstruction, elimination of open cavity-related problems, facilitation of wound healing, and easier use of postoperative hearing aids. However, CWU mastoidectomy is associated with residual disease in 20–39% of cases and cholesteatoma recurrence in 3.8–50% of cases, predominantly located in the anterior attic, which is difficult to inspect. Therefore, a second-look surgery is typically planned, ideally 6–12 months after the initial procedure, to ensure complete disease removal and, when necessary, reconstruction of the conductive hearing mechanism [6, 8, 11, 21, 25, 26, 31, 33]. Contraindications for CWU mastoidectomy include revision or residual cholesteatoma, intracranial complications, non-reconstructible posterior EAC wall defect, poor patient compliance, and an unresectable matrix involving the labyrinth, facial nerve, dura mater, or tympanic sinus [31].

Atticotomy and Canal Wall Reconstruction (CWR). Atticotomy involves the removal of the lateral wall of the epi-

tympanum, improving access and visualization of the epitympanum and facilitating the removal of cholesteatoma or retracted tympanic membrane (TM). This technique allows excision of middle ear or attic space lesions without the need for mastoidectomy. Additionally, removal of the middle or lower portion of the medial aspect of the posterior EAC wall increases exposure to the hypotympanum and tympanic sinus [10]. Atticotomy techniques can also be performed during CWU mastoidectomy procedures if additional exposure is required to eradicate disease. Partial atticotomy, used during CWU, can significantly improve exposure and access to the middle ear, epitympanum, tympanic sinus, hypotympanum, and the round and oval windows. Furthermore, removal of cholesteatoma via attico-antrotomy with obliteration or reconstruction using bone or cartilage can significantly reduce recurrence rates [6, 32, 33]. The CWR technique (Canal Wall Reconstruction) combines the excellent exposure provided by CWD procedures with the preservation of the EAC wall seen in CWU, optimizing cholesteatoma eradication and obliterating the attic space, thereby reducing the risk of recurrence, complications, and retraction pocket formation. CWR was introduced by Mercke in 1987 and is performed with mastoid obliteration using osteoperiosteal flaps, alloplastic ceramic materials, bone cement grafts, and cartilage (tragal or costal) [1, 3, 11, 26, 30, 34]. CWR can be performed as a primary procedure in a single stage with CWD mastoidectomy, or secondarily during revision mastoidectomy [11]. Reconstruction of the EAC wall combined with tympano-ossicular allograft transplantation allows reconstruction of the mastoidectomy cavity, resulting in a near-normal ear with favorable outcomes: 76.25–85.0% anatomical surgical success, 9.5% functional surgical failure, and 67.0% improvement in overall hearing function [26, 34]. Thus, considering the above data, treatment of CSOM with cholesteatoma requires an individualized approach for each patient. The selection of the surgical technique should be based on anatomical, biological, radiological, and social factors. Currently, it is widely accepted that cholesteatoma surgery should be tailored to the location and extent of the cholesteatoma, defects of the posterior EAC wall, presence of complications, degree of hearing loss, and the patient's overall medical condition [15].

Revision Mastoidectomy. One of the most effective treatments for post-radical ear pathology is revision surgery. In addition to complete disease eradication, preventive measures are applied to reduce recurrence, including meatoplasty, canalplasty, mastoid obliteration procedures, wall reconstruction techniques, tympanoplasty, and ossiculoplasty [28, 29, 35-39]. Revision surgery is indicated after failure of any of the primary goals in CSOM surgery: (1) elimination of progressive disease to achieve a safe and dry ear, (2) modification of the tympanomastoid anatomy to prevent recurrent disease, and (3) reconstruction of the auditory mechanism. Additionally, revision mastoidectomy is performed in cases of recurrent cholesteatoma, recurrent suppuration, recurrent TM perforation, and residual or recurrent conductive hearing loss [29, 36-40]. According to several studies, the most common causes of previous surgical failure in CSOM were inadequately leveled facial ridge (43–98%), insufficient removal of mastoid cells (62–100%), recurrent or residual cholesteatoma (46–66%), inadequate meatoplasty with a narrowed meatus (24–84%), and the presence of the malleus head (40%) [40]. Specific surgical procedures relevant to revision mastoid surgery can be divided into: (1) procedures for complete disease removal and (2) modifications of soft tissue or bone to prevent future recurrence [37]. Surgical management in revision mastoid cavities initially focuses on removing recurrent and/or residual lesions, complications, and technical defects. When necessary, this is followed by meatoplasty, canalplasty, mastoid obliteration, posterior canal wall reconstruction, TM reconstruction, and ossicular chain reconstruction, all of which significantly improve patients' quality of life [28, 29, 26, 37-40].

*Tympanoplasty***.** Tympanoplasty, introduced by Zollner and Wullstein in 1952, is a surgical procedure performed to repair the TM with or without reconstruction of the ossicular chain (ossiculoplasty). The objectives of surgery for chronic otitis media are disease eradication, achieving a safe and dry ear with an intact TM, increasing the vibratory surface of the TM to restore hearing, and preventing reinfection [10, 41-47].

According to Wullstein's 1956 classification, types of tympanoplasty are distinguished based on the ossicular reconstruction method and rely on two parameters: 1) the remaining structures of the middle ear after disease eradication, and 2) the pathway by which sound is transmitted to the oval window [14, 43, 47, 48].

Currently, the most commonly used classification of tympanoplasty types is the modification of Wullstein's classical scheme by Nadol and Schuknecht, in which tympanoplasty types differ according to the method of ossicular reconstruction.

Tympanoplasty Types

- *Type I – Simple myringoplasty:* The middle ear and hearing are normal.
- *Type II – Tympanoplasty with minor ossicular reconstruction:* The TM graft or TM is in contact with the incus and stapes. This involves repair of the TM and middle ear due to small defects in the ossicular chain, which cause minimal hearing loss. The lever function is restored by placing a graft between the long process of the incus and the head of the stapes.
- *Type III – Myringostapediopexy:* The TM graft or TM is in direct contact with the stapes suprastructure (columella effect). It is divided into three subtypes of reconstruction:
  - *Columella stapes:* TM graft is placed on the intact stapes head.
  - *Minor columella:* A prosthesis is placed between the TM graft and the stapes head.
  - *Major columella:* A prosthesis is placed between the TM graft and the stapes footplate.
- *Type IV – Minor cavum:* The ossicular chain is ab-

sent, and the TM, in contact with the mobile stapes footplate, requires a CWD approach and a graft covering only the round window and auditory tube opening.

- *Type V – Fenestration:* A second-stage procedure following failure of Type III or IV tympanoplasty, in which the stapes footplate is fixed, a TM graft covers the hypotympanum, and a surgical window is created in the horizontal semicircular canal [14, 43, 47-49].
- *TM Perforation Size Classification:* Small (<50%), medium (50–75%), or large (>75%) [42].

The goal of tympanoplasty is to improve hearing by repairing the TM and/or ossicles and to prevent recurrent otorrhea. Any closure of the air–bone gap after surgery, which effectively measures conductive hearing loss, can be considered a clinical hearing improvement, although an air–bone gap closure to ≤10 dB is optimal [50-54]. Tympanoplasty is an effective procedure that can prevent middle ear infection, recurrent otorrhea, and cholesteatoma, while improving hearing function in 57–99% of cases [45, 55]. Since its introduction by Zollner and Wullstein in 1952, numerous techniques and graft materials have been described to reconstruct the TM: skin, fascia, vein, perichondrium, cartilage, and dura mater. Autologous graft material is readily available, biocompatible, cost-effective, stable, and well accepted by the body without extrusion. Among these, temporalis fascia, followed by tragal/conchal cartilage, remains preferred by surgeons due to availability, proximity to the surgical site, ease of harvesting and shaping, and handling, as well as acceptable hearing outcomes [14, 43, 44, 46, 56-58]. Over the years, various techniques have been proposed to improve tympanoplasty outcomes: overlay, underlay (subperforation), medio-lateral, cartilage shield, cartilage palisade, cartilage island, butterfly cartilage, and sandwich techniques [55, 58-60]. Each technique has its advantages and disadvantages, but two are fundamental in TM grafting: *overlay (lateral)* and *underlay (medial/substrate)* techniques [14, 41, 58].

Definition of Tympanoplasty Success. Correct anatomic placement of the graft, full integration, and hearing improvement one year after surgery [61]. Multiple studies report a primary tympanoplasty success rate of 60–99% in adults and 35–94% in children with CSOM [2, 45, 56, 59, 61]. However, complications after primary tympanoplasty may occur, the most common being graft failure (3.6–4.2%), conductive hearing loss (1.9%), TM perforation (1.0%), and the need for ventilation tube insertion [43].

Ossiculoplasty. Ossiculoplasty represents the reconstruction of the auditory mechanism (ossicular chain) using either an autologous graft (tragal cartilage or perichondrium) or a prosthesis, restoring the connection between the tympanic membrane (TM) and the oval window. The procedure can be performed with either partial ossicular replacement prosthesis (PORP) or total ossicular replacement prosthesis (TORP), depending on the status of the remaining ossicles after complete removal of diseased tissue [10, 41, 42, 48, 62-64]. A study on 279 ears after surgery for chronic suppurative otitis media (CSOM) found that the ossicular chain was eroded in 23.66% of cases. Erosion was more common in ears with cholesteatoma (69.3%) than in ears without cholesteatoma (13.9%). The most frequently affected ossicle was the incus (22.2%), followed by the stapes (11.1%) and the malleus (4.7%). The presence of cholesteatoma was associated with a higher prevalence of ossicular erosion (incus – 65.3%, stapes – 63.3%, malleus – 22.5%), including cases with two or more ossicles affected simultaneously [62].

Austin-Kartush Ossicular Classification:

- *Class 0*: Normal ossicles (M+I+S+)
- *Class A*: Absence of incus (M+S+)
- *Class B*: Absence of incus and stapes (M+S-)
- *Class C*: Absence of malleus and incus (M-S+)
- *Class D*: Absence of all ossicles (M-S-) [60]

Historically, ossicular chain reconstruction has been performed using various materials, including autologous bone grafts, ceramic, gold, hydroxyapatite, and polyethylene**.** Titanium prostheses were introduced in the early 1990s and quickly gained popularity. Cartilage and tragal perichondrium are recommended as graft materials due to easy availability, low toxicity, minimal extrusion, contraction, or lateralization, and cost-effectiveness for patients [48, 63]. A recent meta-analysis examining the impact of prosthesis material on outcomes did not find significant differences in audiometric results or extrusion rates between titanium and non-titanium prostheses. Nevertheless, titanium prostheses remain popular due to low weight, rigid profile, low acoustic impedance, small size, and design that facilitates visualization and accurate placement [63, 64]. In studies using non-titanium prostheses, the long-term mean postoperative air–bone gap ranges from 12 to 21 dB, with a success rate (air–bone gap ≤20 dB) of 37–65%. In studies using titanium prostheses, success is reported in 60–73% of cases. The overall prosthesis extrusion rate is 0–4%, indicating titanium's long-term stability in the middle ear [64]. Based on long-term outcomes (1–3 years), all four types of tympanoplasty improved hearing, measured by the mean reduction in the air–bone gap ($p<0.001$). Hearing improvement was less durable with PORP compared to TORP [65].

Long-term rates of significant complications (requiring further surgery) were reported as:

- 10.3% of patients experienced complications
- 8.2% required revision surgery
- 10.2% required secondary ventilation tube placement
- 3.6% had recurrent conductive hearing loss
- 4.1% experienced TM graft failure
- 1.5% had cholesteatoma recurrence [66].

Conclusions

Surgical management of chronic suppurative otitis media should be individualized according to disease extent and patient anatomy. The choice between open- and closed-cavity techniques must balance complete eradication of pathology with preservation of auditory function. Advances

in cavity obliteration, canal wall reconstruction, and ossicular prostheses have improved both surgical outcomes and hearing restoration. Revision procedures remain essential for managing residual disease and optimizing functional results. Continuous refinement of surgical methods and materials contributes to better long-term disease control and quality of life. While preoperative planning is crucial, the definitive surgical approach or operative technique is often determined during the surgical procedure based on the specific pathology encountered.

Competing interests

None declared.

Authors' contributions

IN, ES, SV made substantial contributions to the conception and design of the study, as well as to the acquisition, analysis, and interpretation of the data. ES were responsible for drafting the manuscript and critically revising it for important intellectual content. IN, ES, SV provided final approval of the version to be published and take full responsibility for the integrity and accuracy of all aspects of the work.

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CASE STUDY

Surgical treatment of dysphagia lusoria caused by an aberrant right subclavian artery: a case report and literature review

Dumitru Casian[1,2], Igor Maxim[1,3], Serghei Gutu[3], Alexandru Predenciuc[1,2*], Sergiu Danu[2], Ion Burlacu[1,3]

[1]*Nicolae Anestiadi* Department of General Surgery, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova
[2]Department of Vascular Surgery, Institute of Emergency Medicine, Chișinău, Republic of Moldova
[3]Department of Thoracic Surgery, Institute of Emergency Medicine, Chișinău, Republic of Moldova

ABSTRACT

Introduction. Dysphagia lusoria refers to dysphagia caused by an aberrant right subclavian artery (ARSA), a rare congenital anomaly of the aortic arch. This article presents a clinical case of symptomatic ARSA, accompanied by a comprehensive review of its anatomical, clinical, and therapeutic aspects.

Material and methods. We present the case of a 54-year-old female patient diagnosed with dysphagia lusoria due to ARSA, who underwent surgical intervention after clinical and imaging evaluations. The literature was reviewed through PubMed using the keywords: "aberrant right subclavian artery", "dysphagia lusoria", and "arteria lusoria".

Results. The patient presented with progressive dysphagia, morning cough, fatigue, and weight loss. Imaging revealed ARSA compressing the esophagus, and the presence of a bicarotid trunk. Surgical correction involved right subclavian-to-carotid transposition, with complete resolution of symptoms. The literature review showed a modest grade of evidence regarding the management of patients with dysphagia lusoria, mostly including case reports and limited case series. Treatment is usually indicated in symptomatic patients, with options including open surgery or hybrid approaches.

Conclusions. ARSA can cause significant esophageal symptoms in adults, representing a diagnostic challenge. Appropriate imaging and timely surgical intervention are crucial in symptomatic cases. Awareness of aortic anatomical variants is essential for both diagnosis and treatment planning.

Keywords: aberrant right subclavian artery, dysphagia lusoria, arteria lusoria, vascular anomaly.

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[*]**Corresponding author**: **Alexandru Predenciuc**, MD, PhD, assistant professor
Nicolae Anestiadi Department of General Surgery
Nicolae Testemițanu State University of Medicine and Pharmacy
1, Toma Ciorba str., Chișinău, Republic of Moldova, MD 2004
e-mail: alexandrupredenciuc@gmail.com

Authors' ORCID IDs
Dumitru Casian – https://orcid.org/0000-0002-4823-0804
Igor Maxim – https://orcid.org/0009-0002-9356-4368
Serghei Gutu – https://orcid.org/0000-0001-9583-0485
Alexandru Predenciuc – https://orcid.org/0000-0002-2730-8115
Sergiu Danu – https://orcid.org/0009-0008-3682-637X
Ion Burlacu – https://orcid.org/0009-0002-4160-9173

Key messages

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

Although dysphagia lusoria caused by an aberrant right subclavian artery is a recognized condition, there is still no consensus on the optimal diagnostic approach, timing of intervention, or long-term management strategies, particularly in adults. Additionally, the factors influencing symptom severity and the decision-making process between surgical and conservative treatment remain unclear.

The research hypothesis

An aberrant right subclavian artery can cause clinically significant dysphagia, and timely diagnosis through imaging and appropriate surgical management can lead to complete symptom resolution and an improved patient quality of life.

The novelty added by manuscript to the already published scientific literature

By presenting a rare case of dysphagia lusoria in an adult patient, highlighting the diagnostic challenges and a successful management strategy, this report emphasizes the importance of considering vascular anomalies in patients with unexplained dysphagia. The detailed imaging findings and clinical decision-making process offer practical insights for clinicians. Additionally, the literature review provides an updated synthesis of current evidence, addressing gaps in diagnosis and treatment approaches.

Introduction

The aberrant right subclavian artery (ARSA) was first discovered by Hunauld in the 18th century during an autopsy and represents the most common congenital anomaly of the aortic arch, with an incidence of 0.5-1.0% in the general population [1, 2]. The clinical importance of ARSA stems from its potential to cause symptoms through compression of adjacent mediastinal structures, primarily the esophagus, and a tendency to aneurysmal degeneration (Kommerell diverticulum). When esophageal compression by an ARSA leads to difficulty swallowing, the condition is referred to as "dysphagia lusoria" (from the Latin term "*lusus naturae*" – freaks of nature), a term introduced by Bayford in 1794 [1, 3, 4]. Since most individuals (more than 90%) with ARSA remain asymptomatic or undiagnosed with this anomaly throughout their lives, there is a paucity of data regarding the management of symptomatic cases. Current literature mostly includes case reports and case series, with significant heterogeneity of clinical presentations, diagnostic modalities, and curative approaches.

Clinical case

We report the clinical case of a 54-year-old female patient who presented to the outpatient clinic of the Division of Thoracic Surgery, complaining of progressive dysphagia, fatigue, and morning cough. The anamnesis also revealed two episodes of black-colored stools and a sensation of compression localized at the lower cervical level, with subsequent radiation to the thoracic region. The patient described a gradual onset of dysphagia approximately 6 months prior to presentation. These symptoms had been gradually worsening over the past month, especially after eating, particularly with solid and semi-liquid foods. Importantly, the patient reported an unintentional weight loss of approximately 8 kilograms over the past few months, attributed to reduced oral intake and food avoidance due to dysphagia. The patient was examined in several outside medical institutions with suspicion of malignancy, neurological, or even psychiatric diseases, none of which were confirmed.

The patient's past medical history includes chronic autoimmune thyroiditis (Hashimoto's) diagnosed approximately 10 years ago, stage II arterial hypertension, NYHA class II chronic heart failure, and grade I axial hiatal hernia associated with gastroesophageal reflux disease. The initial physical examination did not reveal cervical deformities, noting only the presence of slightly enlarged bilateral submandibular lymph nodes. The thoracic region showed no visible abnormalities. The patient denied any history of bodily injuries or trauma.

During the initial hospitalization, paraclinical investigations revealed microcytic hypochromic anemia (hemoglobin 88.0 g/L, erythrocytes 5.16 × 10^{12}/L, hematocrit 33.5%), associated with mild leukopenia (leukocytes 3.60 × 10^{9}/L). The blood coagulation test and biochemistry profile were within the normal range.

Ultrasonography of the cervical region showed a normal sonographic appearance of the musculo-tendinous, cutaneous, and subcutaneous structures, along with bilateral submandibular lymphadenopathy with ganglion hyperplasia up to 20 × 8 mm and findings suggestive of chronic autoimmune thyroiditis (Hashimoto's).

The presence of anemia, weight loss, and black-colored stool initially raised clinical suspicion for upper digestive tract pathology, and esophagogastroscopy was attempted. However, the procedure was stopped at the level of the first physiological narrowing of the esophagus, as the endoscope (with external diameter of 0.9 cm) could not be advanced further. Fibrobronchoscopy did not reveal any endobronchial pathologies. Barium swallow demonstrated evident esophageal narrowing at the level of Th3-Th4 vertebrae, with retention of the contrast media, suggestive of significant extrinsic esophageal compression (Fig. 1).

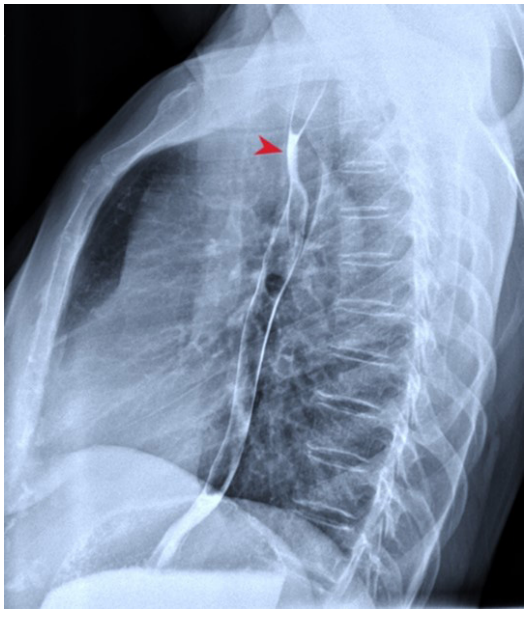

Fig. 1. Preoperative barium swallow image showing external compression of the esophagus at the T3-T4 vertebrae level.

Note: *The arrow indicates the level of esophageal compression.*

Computed tomography angiography with intravenous contrast (100 ml Iodixanol) identified an aortic arch anomaly – ARSA (diameter at the origin of 17 mm), originating distal to the left subclavian artery from the posterior surface of the aorta (Fig. 2) and passing to the right behind the esophagus, which was compressed between the artery and the trachea (Fig. 3). The presence of the common origin of the right and left carotid arteries – the so-called "bicarotid trunk" – and dextroposition of the thoracic lymphatic duct was also noted on the axial imaging. No other abnormalities were identified.

Cardiac ultrasonography revealed induration of the ascending aorta and the aortic and mitral valves, moderate dilation of the left atrium, and mild dilation of the right atrium, impaired left ventricular myocardial relaxation, with an ejection fraction of 63%, grade II mitral, grade I-II tricuspid, and grade I aortic insufficiency, along with signs of mild pulmonary hypertension (PASP 34 mmHg). Duplex ultrasonography of the extracranial vessels demonstrated non-diseased common and internal carotid arteries, a decreased diameter and moderate kinking of the right vertebral artery, with a peak systolic velocity of 60-70 cm/sec.

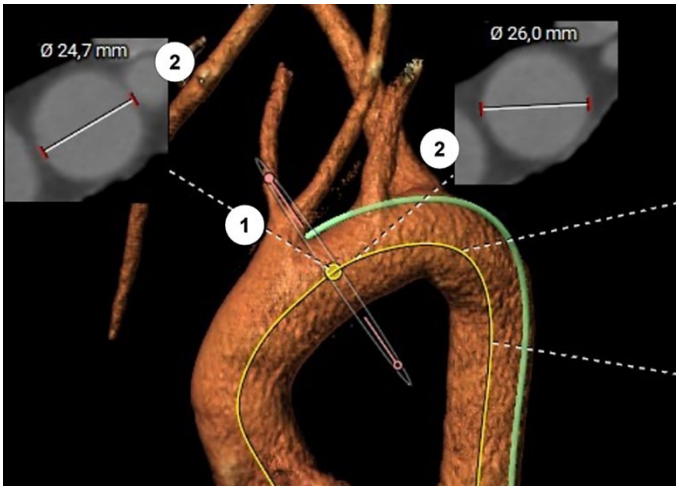

Fig. 2 Computed tomography angiography of the aortic arch and supra-aortic trunks.

Note: *bicarotid trunk (1) and aberrant right subclavian artery (2), Kieffer type III.*

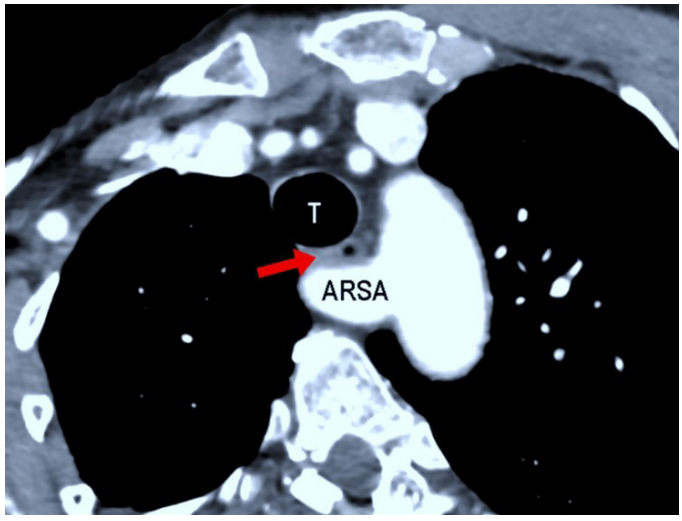

Fig. 3 Computed tomography angiography: compression of the esophagus between the aberrant right subclavian artery (ARSA) and the trachea (T).

Note: *The arrow indicates the esophageal compression.*

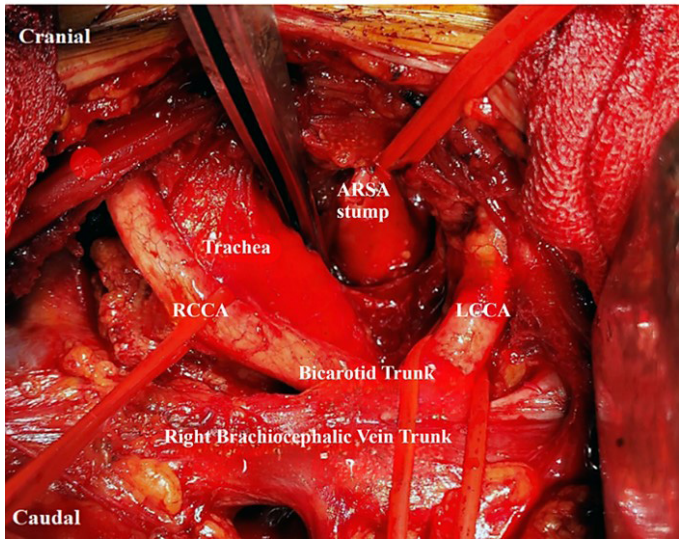

Fig. 4 Intraoperative image: surgically dissected supra-aortic trunks, suture-ligated proximal stump of the transected ARSA.

Note: *ARSA – aberrant right subclavian artery; RCCA – right common carotid artery; LCCA – left common carotid artery.*

Based on the results of clinical and paraclinical evaluation, the patient was diagnosed with esophageal obstruction (dysphagia lusoria) caused by ARSA type III Kieffer, with a Kommerell diverticulum, and was scheduled for surgical intervention. After a multidisciplinary discussion of possible treatment options, the decision was made to perform a right subclavian-carotid transposition surgery via a sternotomy approach.

Under general anesthesia with orotracheal intubation, a median sternotomy was performed, the thoracic cavity was explored, and the aortic arch and supra-aortic trunks were carefully dissected and controlled. The ARSA, originating from the posterior surface of the aorta in Ishimaru zone 3, was identified, mobilized in the para-tracheal and para-esophageal space, and followed down to the aorta. After systemic heparinization, the ARSA trunk (Kommerell diverticulum) was slowly double-clamped as close to the aorta as possible and transected, with suture-ligation of the proximal stump using 4/0 polypropylene suture, achieving a leak-proof closure with no bleeding noted (Fig. 4).

The distal segment of the ARSA, situated between the esophagus and thoracic vertebrae, was gradually dissected, retracted to the right side of the trachea, and transposed towards the right common carotid artery, avoiding any redun-

dancy or kinks. After clamping of the right common carotid artery, an arteriotomy was performed, and an end-to-side anastomosis with the transposed ARSA was performed using a running 5/0 polypropylene suture (Fig. 5). After clamp removal, pulsatile flow was present distal to the suture line. Surgical hemostasis was achieved, and a retrosternal drain was placed, followed by sternal wound closure in anatomical layers. The surgical intervention lasted 195 minutes, with an estimated blood loss of 600 ml.

The postoperative course was satisfactory, with no clinically relevant complications. The patient was mobilized early, resumed oral intake gradually, and demonstrated progressive improvement in swallowing function. She was discharged on the 12th postoperative day in satisfactory condition, reporting the complete resolution of dysphagia symptoms with liquid and solid food.

At the three-month follow-up, the patient reported a weight gain of 5 kilograms, reflecting improved nutritional status and confirming the favorable clinical outcome. She was able to consume solid foods without pain or difficulty during swallowing, and no residual compressive symptoms were reported. Upper digestive tract endoscopy and barium swallow confirmed the normal permeability of the esophagus and excluded any pathology of the stomach and duodenum.

Discussion

This review aims to synthesize the current evidence available primarily through the PubMed database concerning the anatomical variations, prevalence, diagnostic approaches, and management strategies in patients with ARSA and dysphagia lusoria.

The relationship of the ARSA to the esophagus and trachea during its mediastinal transit is clinically crucial. The most frequent course, observed in approximately 80-83% of cases, is retroesophageal, where the artery passes posterior to the esophagus, potentially compressing it against the trachea [5-7]. Less commonly – in approximately 15-17% of cases – the artery passes between the esophagus and the trachea (so-called interesophageotracheal course), compressing the esophagus against the vertebral bodies. Rarely, in around 5% of cases, ARSA may travel anterior to the trachea (pretracheal course) [8]. Imaging studies using CT have shown that ARSA is typically located anterior to the first to fourth thoracic vertebral bodies [9].

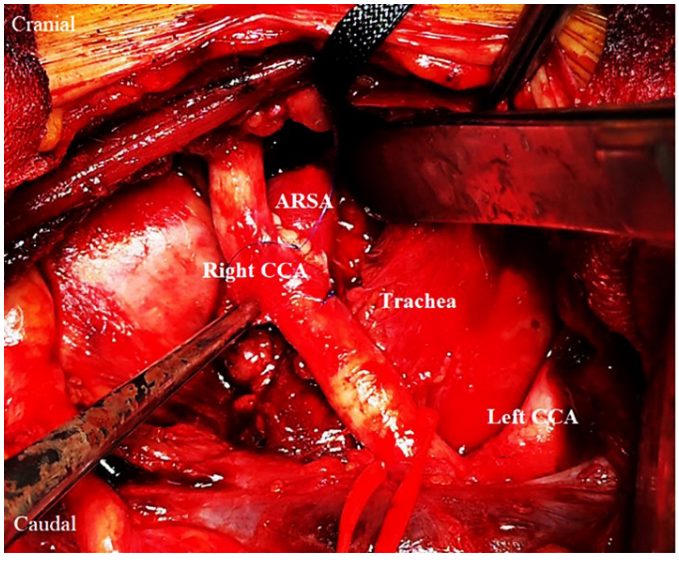

Fig. 5 Intraoperative image: end-to side anastomosis between the transposed ARSA and the right common carotid artery.

Note: *ARSA – aberrant right subclavian artery; Right CCA – right common carotid artery; Left CCA – left common carotid artery.*

Several classification systems exist to describe the anatomic variability of the ARSA, though they are not universally applied. The Adachi and Williams classification describes subtypes based on associated branching patterns of the supra-aortic trunks [10]:

- **Type G-1:** ARSA present as the last aortic arch branch, with normal origins for the right common carotid, left common carotid, and left subclavian arteries;
- **Type CG-1:** Similar to G-1, but with the left vertebral artery originating directly from the aortic arch;
- **Type H-1:** ARSA as the last branch, associated with a bicarotid trunk (common origin for both common carotid arteries);
- **Type N-1:** Mirror image of G-1, with a right-sided aortic arch and an aberrant left subclavian artery.

Variations in the ARSA origin from the arch (superior-posterior vs. inferior-posterior) and significant variability in the luminal diameter of the ARSA have also been reported, with some cases exhibiting unusually narrow lumens [11].

In 1994, Kieffer et al. proposed a more clinically oriented classification system for ARSA, aimed at guiding surgical strategy in symptomatic patients. Summarizing their experience with surgical interventions in thirty-three adult patients with ARSA, the authors described four types of pathology [12].

- **Kieffer type I:** non-stenotic and non-aneurysmal ARSA causing exclusively compressive symptoms;
- **Kieffer type II:** presence of stenosis or occlusion of non-aneurysmal ARSA;
- **Kieffer type III:** aneurysm of the origin of ARSA (Kommerell diverticulum) without aortic involvement;
- **Kieffer type IV:** Kommerell diverticulum associated with aneurysmal dilatation of the aorta.

Associated anomalies

ARSA frequently coexists with other anatomical variations or anomalies, which can have significant clinical implications.

Kommerell diverticulum. This is an aneurysmal dilatation or outpouching at the origin of the ARSA from the descending aorta [1, 4]. The presence of a Kommerell diverticulum is clinically significant, as it may increase the likelihood and severity of compressive symptoms (dysphagia, dyspnea) and carries a risk of aneurysmal degeneration, dissection, or rupture, particularly if the diameter exceeds certain thresholds (often cited as 3-5 cm) [10].

Non-recurrent inferior laryngeal nerve. A strong association exists between ARSA and a non-recurrent course of

the right inferior laryngeal nerve [1, 13, 14]. Reports suggest this occurs in up to 87% of ARSA cases. Awareness of this association is critical during thyroid, parathyroid, esophageal, or neck surgeries to prevent nerve injury [15, 16].

Bicarotid trunk. This anomaly involves a common origin for the right and left common carotid arteries directly from the aortic arch [1, 13]. It is reported to coexist with ARSA in a significant minority of cases (19.2% in one review) [13]. The presence of a bicarotid trunk alongside ARSA may increase the likelihood of dysphagia, possibly by limiting the anterior displacement of the trachea and esophagus, thus exacerbating the posterior compression by the ARSA.

***Chromosomal abnormalities and syndromes*.** There is a well-established increased frequency of ARSA in individuals with certain chromosomal abnormalities, particularly Trisomy 21 (Down syndrome) and Trisomy 18 (Edwards syndrome) [17, 18]. Prevalence in Down syndrome has been reported as high as 26-35%.

ARSA is consistently cited as the most common congenital anomaly of the aortic arch [1, 2]. Reported prevalence figures in the general population typically range from 0.5% to 2.0% [4-7], although some sources cite wider ranges up to 4.4% [19, 20]. Specific studies provide context to these figures:

***CTA studies*.** Large retrospective analyses of adult chest CTA scans consistently report prevalence figures around 0.5%. For instance, one study found ARSA in 32 out of 6833 patients (0.47%), and another identified 17 cases in 3460 patients (0.49%) [21].

***Fetal ultrasound studies*.** Prenatal screening studies report prevalence rates often around 1% or slightly higher. One study detected ARSA in 20 of 1913 second-trimester fetuses (1.04%), while another found an incidence of 0.6% in 3266 fetal sonographies. Feasibility of ultrasound detection is high, estimated at 85-95% depending on trimester and factors like operator experience and maternal habitus.

***Specific populations*.** Studies focusing on patients with known arteriopathies found a prevalence of 2.4%. As mentioned, prevalence is significantly higher in populations with Down syndrome or 22q11.2 deletion [6].

The observed variation in prevalence likely reflects differences in study methodology, population characteristics, and the sensitivity of the detection method used. CTA in adults provides a reliable estimate for that demographic, likely around 0.5%. Fetal ultrasound identifies cases early, potentially including those that remain asymptomatic, with a rate of around 1%. Cadaveric studies provide anatomical confirmation, though results may vary depending on the studied population. The increasing use and sensitivity of imaging modalities, particularly prenatal ultrasound, may lead to more frequent identification of ARSA [15], raising important questions regarding the natural history and clinical significance of incidentally or prenatally detected cases. Several studies suggest a female predominance for ARSA, with reported female-to-male ratios ranging up to 2:1 or 3:1 in some series [22].

Traditional open surgical interventions for symptomatic ARSA. Open surgical repair is the traditional approach for treating symptomatic ARSA and remains indicated for specific patient groups. The primary indication is the presence of significant symptoms, particularly dysphagia that is severe, persistent, progressive, unresponsive to conservative management, or associated with significant weight loss or nutritional compromise [23]. Other compressive symptoms, like severe dyspnea or cough, may also warrant intervention.

Anatomical factors are also crucial indications for open repair. The presence of aneurysmal dilatation of the ARSA itself often necessitates surgical intervention, even in asymptomatic patients, due to the risk of rupture, dissection, or thromboembolism [16]. While the exact size threshold for prophylactic repair of asymptomatic Kommerell diverticulum remains debated, diameters exceeding 3 cm or 5.0-5.5 cm are often cited as indications, particularly in low-risk patients.

The presence of concomitant thoracic aortic aneurysm or dissection involving the ARSA origin also typically requires open surgical management. Symptomatic occlusive disease of the ARSA, leading to arm claudication or ischemia, is another indication for intervention, usually involving revascularization. Open repair may also be preferred over endovascular options when significant esophageal or tracheal compression is the primary symptom, as endovascular exclusion alone may not relieve the mass effect [24].

Surgical approaches. Several open surgical approaches have been described, each with advantages and disadvantages, largely dictated by the specific anatomy and the goals of the operation.

Thoracotomy

Left Posterolateral Thoracotomy: Historically considered the standard approach, especially by Gross, who first described surgical correction [13]. It provides excellent exposure of the ARSA origin from the descending aorta, facilitating secure ligation or division, particularly when a Kommerell diverticulum is present or the origin is dilated [25]. However, performing revascularization (reimplantation or bypass) from this approach can be technically challenging due to the distance to suitable inflow sources, like the ascending aorta or right carotid artery.

Right Thoracotomy: Offers better access for anastomosing the distal ARSA to the right common carotid artery or ascending aorta, either directly or with an interposition graft [4]. It may provide less optimal exposure of the ARSA origin, potentially increasing the risk of leaving a residual stump if ligation is not performed flush with the aorta, which could lead to persistent symptoms or thrombosis. This approach is preferred by some for pediatric patients due to better overall exposure in smaller chests. It is also the typical approach for patients with a right-sided aortic arch and an aberrant left subclavian artery [25]. Thoracotomy is generally more invasive than cervical approaches, involving muscle division, potential rib spreading, and longer recovery times [22, 24].

***Median sternotomy*.** This approach provides excellent access to the ascending aorta and brachiocephalic vessels, making it suitable for reimplanting the ARSA onto the ascending aorta or performing bypasses from it. However,

exposure of the posteriorly located ARSA origin on the descending aorta for ligation or division can be difficult from this anterior approach.

Supraclavicular (cervical) approach. This is the least invasive of the open approaches, avoiding entry into the thoracic cavity [10, 22]. It provides good access to the distal ARSA in the neck for transposition or bypass to the right common carotid artery. It is generally considered suitable for uncomplicated, non-aneurysmal ARSA. However, this less traumatic approach is not suitable for patients with aneurysmal dilatation of the ARSA and those with dysphagia lusoria (as in our case), because the origin of the anomalous subclavian artery can be securely suture-ligated only via thoracotomy.

Hybrid and endovascular management. Development and implementation of endovascular techniques has offered new possibilities for the treatment of adult patients with symptomatic ARSA, avoiding potential morbidity associated with thoracotomy. Since revascularization of the right upper limb using carotid-to-subclavian bypass or subclavian-to-carotid transposition can be performed through a less traumatic supraclavicular approach, the endovascular part of the intervention is primarily aimed at occluding the origin of the ARSA. For this purpose, the majority of authors describe the off-label use of the Zenith (Cook Medical, Inc.) iliac occlusion device or an Amplatzer vascular plug (Abbott, USA), positioned at the origin or retroesophageal portion of the aberrant artery [26, 27]. Despite the relative simplicity and efficacy of this intervention, there is a considerable risk of at least two potential complications. First, in patients with large-diameter ARSA or the presence of Kommerell diverticulum, there exists the possibility of occlusion device migration. To prevent this problem, a 50% oversize in diameter of the device is usually recommended [26, 28]. However, positioning of a large occlusion device in the retroesophageal portion of the artery could result in persisting or worsening dysphagia, or even in the formation of an esophageal-subclavian fistula with fatal consequences [29].

Implantation of the thoracic aortic stent-graft (as an isolated endovascular technique or in combination with the use of vascular plugs) was proposed to prevent above-mentioned complications. This approach is indispensable in patients with large Kommerell diverticulum and concomitant aneurysmal disease of the aorta – ARSA type Kieffer IV. It should be mentioned that an adequate landing zone for aortic endograft implantation is required to prevent device migration and endoleaks. In a significant proportion of cases, a simultaneous left-sided carotid-subclavian bypass may be needed to prevent acute deterioration of the posterior cerebral circulation [30].

Conclusions

The presented clinical case and literature review demonstrate that symptomatic ARSA represents a rare cause of dysphagia in adult patients, creating significant challenges in diagnosis and management. Dysphagia lusoria should be included in the list of possible diagnoses in patients with an unusual clinical presentation of aero-digestive symptoms. Open surgical or hybrid treatment is indicated in cases with severe symptoms and, due to a lack of strong evidence, must be personalized based on individual anatomical characteristics, physician experience, and patient preferences.

Competing interests

None declared.

Authors' contributions

IB, SD, AP, and SG performed data collection and interpretation. DC, IM, AP, and SG performed a search of the bibliographic references and drafted the manuscript. All authors critically reviewed the manuscript and approved the final version of the article.

Ethics approval

Not needed for this study.

Patient consent

Obtained.

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Provenance and peer review

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CASE STUDY

Psoriasis vulgaris and B-cell non-Hodgkin lymphoma: a complex case with rare cephalic localization

Mihaela Rotari[1*], Mircea Beţiu[1], Sanda Buruiană[2], Valeria Pînzaru[3], Valeriu David[4], Lilian Şaptefraţi[5]

[1]Department of Dermatovenerology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chişinău, Republic of Moldova
[2]Discipline of Hematology, Department of Internal Medicine, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chişinău, Republic of Moldova
[3]Department of Oncology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chişinău, Republic of Moldova
[4]Laboratory of Morphology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chişinău, Republic of Moldova
[5]Department of Histology, Cytology and Embryology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chişinău, Republic of Moldova

ABSTRACT

Introduction. Psoriasis is a chronic immune-mediated inflammatory condition and is considered a potential risk factor for the development of hematologic malignancies, particularly in the context of immunosuppressive therapy and T-cell dysfunction. B-cell non-Hodgkin lymphomas are neoplasms of the lymphatic system with variable clinical manifestations, most commonly presenting with peripheral lymphadenopathy. Primary localization in the soft tissues of the head, with bone invasion, is rare.

Case presentation. We report a rare case of cephalic aggressive NHL Not Otherwise Specified (NOS) in a 63-year-old patient with a history of psoriasis vulgaris and Clear cell carcinoma (T1N0M0, treated in 2021 at the Oncology Institute in Chişinău), who presented with a painless right temporo-parietal mass. MRI revealed a 48×19×50 mm lesion in the temporal soft tissues with extension into the frontal bone. Surgical biopsy and immunohistochemistry (CD20+, CD79a+, CD45+, BCL6-) confirmed the diagnosis of B-cell NHL NOS. In 2022, the patient received 8 induction cycles of immunochemotherapy followed by maintenance therapy with Rituximab. PET/CT evaluation showed a Deauville score of 3, indicating a partial favorable response. Associated comorbidities (psoriasis, type 2 diabetes mellitus, hypertension) required multidisciplinary monitoring.

Conclusions. This case illustrates an unusual cranial localization of aggressive B-cell lymphoma NOS and highlights the potential link between psoriasis and lymphoproliferative risk, as previously suggested in the medical literature.

Keywords: non-Hodgkin lymphoma, psoriasis vulgaris, cranial localization, bone invasion, rituximab.

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***Corresponding author: Mihaela Rotari**, MD, PhD fellow
Department of Dermatovenerology
Nicolae Testemiţanu State University of Medicine and Pharmacy
165 Ştefan cel Mare şi Sfânt blvd, Chişinău, Republic of Moldova, MD2004
e-mail: m.garbatovschi1@gmail.com

Authors' ORCID IDs
Mihaela Rotari – https://orcid.org/0009-0002-6833-1845
Mircea Beţiu – https://orcid.org/0000-0003-0824-3480
Sanda Buruiană – https://orcid.org/0000-0003-2341-0099
Valeria Pînzaru – https://orcid.org/0009-0000-8834-7743
Valeriu David – https://orcid.org/0000-0001-9799-7369
Lilian Şaptefraţi – https://orcid.org/0000-0003-2779-718X

Key messages

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

The association between psoriasis vulgaris and the development of B-cell non-Hodgkin lymphoma (NHL) is rarely documented, especially in cases with cranial localization and bone invasion. Current literature provides limited data on atypical clinical presentations of NHL in patients with chronic inflammatory diseases.

The research hypothesis

Chronic inflammation in psoriasis, combined with potential immunosuppression induced by systemic therapy, may contribute to the development of lymphoproliferative malignancies with unusual clinical manifestations.

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

This manuscript documents a rare case of B-cell non-Hodgkin lymphoma with temporoparietal localization and frontal bone invasion in a patient with psoriasis vulgaris and an oncologic history. It emphasizes the importance of differential diagnosis and multidisciplinary collaboration in evaluating subcutaneous cranial masses in patients with chronic inflammatory conditions.

Introduction

Psoriasis is a chronic inflammatory disease with a global prevalence ranging between 0.5% and 11.4% [1], characterized by T-cell dysfunction and persistent release of proinflammatory cytokines [2]. Beyond cutaneous and joint manifestations, the disease is associated with systemic comorbidities such as metabolic and cardiovascular disorders, and a moderately increased risk of lymphoproliferative malignancies, particularly lymphomas [3].

The etiology of non-Hodgkin lymphomas (NHL) remains incompletely understood. However, both severe immunosuppression and chronic immune stimulation are recognized as key risk factors. Immune homeostasis imbalance and chronic inflammation, together with immunosuppressive treatments (methotrexate, cyclosporine, anti-TNF agents), appear to contribute to lymphoma development in patients with psoriasis, especially in moderate-to-severe forms [3, 4].

A comprehensive 2020 meta-analysis including over 2 million patients reported a relative risk of 1.56 for lymphomas in general [5], while a 2024 North American study demonstrated a 1.5–1.8-fold increase in NHL risk in patients with mild to severe psoriasis [6].

Recent clinical case reports have also shown potential associations: Alali et al. (2021) described a case of Hodgkin's lymphoma in a patient with psoriasis vulgaris under immunosuppressive therapy [7], and Scott et al. (2023) reported psoriasis–CTCL coexistence in 5.2% of analyzed cases [8]. Nevertheless, B-cell NHLs with cranial soft tissue localization and bone invasion remain exceptional.

In this context, we present a rare case of B-cell NHL with cranial involvement and bone extension in a patient with psoriasis vulgaris and an oncologic history. Its documentation offers valuable insights into the role of chronic inflammation in lymphoproliferative disorders and underlines the importance of an interdisciplinary clinical approach.

Case presentation

A 63-year-old male patient with a history of plaque-type psoriasis vulgaris and previous oncologic pathology (Clear cell renal carcinoma, T1N0M0, treated in 2021) presented for evaluation of a progressively enlarging, painless right temporo-parietal swelling, which had evolved over several months.

His psoriasis began in adulthood, around the age of 40, and followed a chronic, therapy-refractory course, with no complete remissions reported. Initial management included topical corticosteroids, but persistent lesions led to the initiation of systemic therapy with methotrexate. On dermatologic examination, the patient displayed extensive erythematous plaques with adherent white scaling and well-defined borders, located predominantly on the trunk, flanks, and lower limbs. Some lesions were confluent and lichenified, reflecting long-standing inflammation and insufficient treatment response (Figure 1a–d). Although the patient reported intermittent joint pain, standard radiographs revealed no significant articular changes suggestive of psoriatic arthritis.

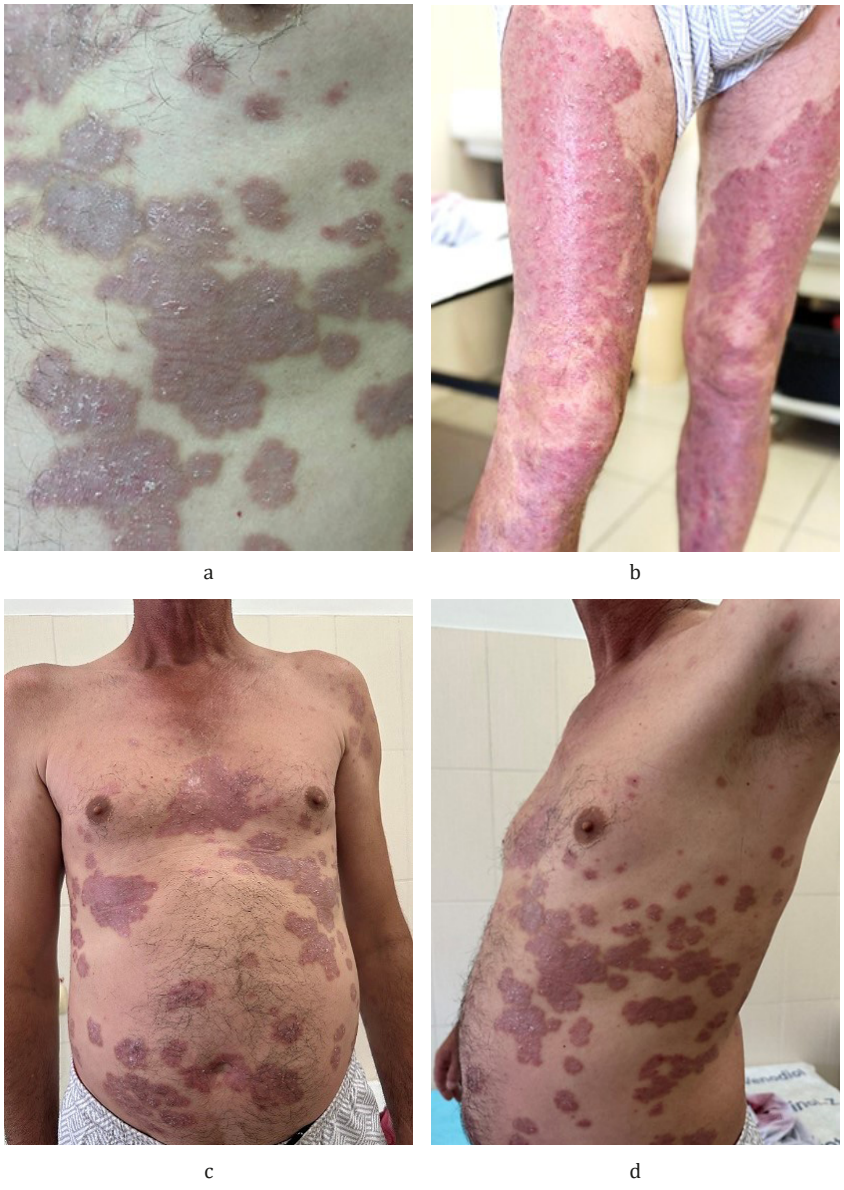
In February 2021, a left renal tumor was surgically treated and histologically confirmed as clear cell carcinoma. In March 2021, the patient noted a painless subcutaneous nodular lesion in the right temporo-parietal area, initially interpreted as inflammatory or rheumatologic in origin, and received symptomatic treatment. Associated general symptoms included asthenia and xerostomia. In March 2022, cerebral MRI revealed a tumor in the right temporal soft tissues (48×19×50 mm), anterosuperior to the parotid gland, showing a gadolinium-enhancing lesion (~37 mm) in the parasagittal frontal bone on the right – suspicious for secondary bone invasion.

In April 2022, a biopsy of the tumor fragment was performed. Hematoxylin–eosin staining revealed a diffuse malignant lymphoid proliferation with areas of crush artifact. Immunohistochemical (IHC) analysis demonstrated diffuse positivity for CD20 and CD79a, consistent with mature B-cell phenotype, while CD45 confirmed lymphoid origin. CD3 expression was restricted to scattered reactive T-cells. The neoplastic cells were negative for BCL6, pancytokeratin (pCK), CD15, CD30, and CD68, findings that excluded a germinal-center phenotype, epithelial origin, classical Hodgkin lymphoma, or a histiocytic process. The overall immunophenotype supported the diagnosis of B-cell NHL NOS (WHO/ICD-O code 9590/3) .The disease was staged as IVA, with bone involvement and enlarged parotid lymph nodes on the right side.

Induction immunochemotherapy was initiated with 8 cycles of R-CHOP, consisting of rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone. PET/CT evaluation in June 2023 revealed a Deauville score of 3, indicating a partial favorable response. The patient was transitioned to maintenance biological therapy with rituximab monotherapy every 2 months, which was well tolerated and led to overall clinical improvement.

Discussion

B-cell NHL is a heterogeneous lymphoproliferative neoplasm with variable clinical presentations, ranging from indolent forms to aggressive variants with rapid systemic



a b c d

Figure 1a–d. Clinical appearance of chronic plaque-type psoriasis in a 63-year-old male patient.

Multiple erythematous plaques with well-demarcated borders and thick, adherent silvery-white scales are observed on the anterior trunk (a, c), extensively on the lower limb (b), and left flank (d). Some lesions are confluent and show signs of chronicity, including lichenification and post-inflammatory hyperpigmentation. The patient reported persistent lesions despite topical corticosteroids and systemic methotrexate therapy.

progression. It most commonly presents with peripheral lymphadenopathy and constitutional symptoms, while primary involvement of the soft tissues of the head with bone invasion is rarely documented in the literature [9]. In our case, the initial presentation was a subcutaneous, painless, slowly progressive right temporo-parietal mass, later confirmed by imaging and histology as aggressive B-cell lymphoma NOS with frontal bone invasion.

In the present case, the diagnostic process was particularly challenging due to the patient's complex clinical background. The history of clear cell renal carcinoma raised an initial concern for metastatic disease, especially given the osteotropic behavior of renal cell carcinoma. However, the immunophenotype and the absence of epithelial or histiocytic markers effectively excluded this possibility. Cutaneous T-cell lymphoma was also considered, particularly in the context of psoriasis vulgaris, which can mask or mimic early CTCL. CD3 positivity restricted to reactive T-cells and the absence of T-cell lineage markers ruled out CTCL. The lack of BCL6 expression further argued against a germinal-center B-cell phenotype and supported the NOS classification.

The differential diagnosis included: bone metastasis from clear cell renal carcinoma (diagnosed in 2021), which was not supported by the immunophenotype; cutaneous T-cell lymphoma (CTCL), particularly in the context of underlying psoriasis vulgaris, as early CTCL can be clinically and histologically misdiagnosed as psoriasis [8]; and chronic inflammatory or granulomatous pseudotumoral lesions, which were excluded morphologically and immunohistochemically.

Psoriasis vulgaris represents an additional layer of complexity. Chronic immune activation, persistent systemic inflammation, and long-standing T-cell dysregulation have been associated with a moderately increased risk of lymphoproliferative disorders. Recent meta-analyses and cohort studies report a relative risk of 1.5–1.8 for lymphoma in patients with mild to severe psoriasis [4–6]. Although most associations involve Hodgkin lymphoma or CTCL, B-cell lymphomas have also been documented, suggesting a broader impact of chronic immune stimulation on lymphomagenesis. This case underscores the need for diagnostic vigilance when evaluating new or atypical masses in patients with chronic inflammatory dermatoses. Early biopsy and multidisciplinary evaluation—including dermatology, oncology, hematology, and radiology—are essential to avoid diagnostic delay in complex cases such as this.

Conclusions

This case highlights a rare presentation of aggressive B-cell NHL NOS with cranial soft-tissue involvement and bone erosion, posing significant diagnostic challenges given the patient's long-standing psoriasis and prior oncologic history. Histopathology and immunohistochemistry were essential in distinguishing lymphoma from potential mimickers, including renal cell carcinoma metastasis and cutaneous T-cell lymphoma. The association with chronic psoriasis underscores the importance of considering lymphoproliferative disease in patients with persistent inflammatory dermatoses or atypical cutaneous lesions. This

report reinforces the need for multidisciplinary evaluation and timely biopsy in order to avoid diagnostic delay in similar complex clinical settings.

Competing interests

None declared.

Authors' contributions

MR conceived the study, participated in the clinical assessment and drafted the manuscript. MB contributed to dermatological evaluation and clinical monitoring of the patient. SB and PV were responsible for oncologic and hematologic management, including therapeutic decision-making and follow-up. DV performed the immunohistochemical analysis and interpretation. LS contributed to histopathological examination and diagnostic validation. All authors critically reviewed and approved the final version of the manuscript.

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Informed consent for publication

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CASE STUDY

Proteus syndrome in a young African woman: a clinically diagnosed ultra-rare mosaic overgrowth disorder

Chiril Voloc[1], Andrei Olaru[2], Irina Rusu[3], Ninel Revenco[4], Alexandru Voloc[4]

[1]Dental Clinic SRL "VIDENTY", Chișinău, Republic of Moldova

[2]Department of Orthopedics and Traumatology, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[3]Department of Tropical Infectious Diseases and Medical Parasitology *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

[4]Department of Pediatrics, *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

ABSTRACT

Proteus syndrome is an extremely rare congenital multisystem disorder characterized by highly variable clinical manifestations. Its exact prevalence remains unknown, with fewer than 200 cases reported in the medical literature worldwide.

Clinical case description. We report the case of an 18-year-old African woman diagnosed with Proteus syndrome, presenting with marked asymmetrical overgrowth affecting the upper limbs, trunk, and lower limbs. The distribution of the deformities resembled the appearance of a tree trunk with multiple stumps. Additionally, the patient exhibited soft tissue tumor-like formations at the left oral commissure and in the left retroauricular region.

Conclusion. The detailed phenotypic description of individuals with Proteus syndrome is essential due to the extreme rarity and clinical complexity of this disorder. Improved awareness and diagnostic acumen among healthcare professionals are critical for the early identification and appropriate multidisciplinary management of affected patients. Given the high risk of life-threatening complications, such as the development of malignant tumors and thromboembolic events, a structured and dynamic follow-up protocol is required. Moreover, the psychosocial dimension of the disease must not be overlooked. Many patients experience profound emotional distress, stigmatization, and moral suffering, which can significantly impact their quality of life. An integrated approach that combines medical surveillance with psychological and social support is imperative for optimizing long-term outcomes in Proteus syndrome.

Keywords: Proteus syndrome, asymmetry of the upper limbs, tumors, melanocytic nevi.

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[*]**Corresponding author**: **Alexandru Chiril Voloc**, MD, PhD, University Professor
Department of Pediatrics
Nicolae Testemițanu State University of Medicine and Pharmacy
165, Ștefan cel Mare si Sfânt blvd., Chișinău, Republic of Moldova, MD2004
e-mail: alexandru.voloc@usmf.md

Authors' ORCID IDs
Chiril Voloc – https://orcid.org/0000-0002-3428-9880
Andrei Olaru – https://orcid.org/0009-0008-8298-7238
Ninel Revenco – https://orcid.org/0000-0002-5229-7841
Irina Rusu – https://orcid.org/ 0000-0002-2759-3171
Alexandru Voloc – https://orcid.org/0000-0001-8882-3164

Key messages

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

Due to the extremely low number of cases–approximately 200 recorded worldwide–Proteus syndrome is poorly studied, and many aspects of its etiology, pathophysiological mechanisms, diagnostic criteria and treatment options remain obscure and unknown to this day.

The research hypothesis

Hypothetically, the hope of accurately diagnosing the syndrome and preventing multiple complications in all organs involved in the process could be placed on genetic investigations and genetic engineering.

The novelty added by the manuscript to the already published scientific literature.
The absence of such a publication in the country to date, the clinical mosaicism, the extremely low number of Proteus syndrome cases globally, and the lack of knowledge of this pathology by doctors will certainly be useful to all practitioners of different specialties in their daily work.

Introduction

Proteus syndrome (PS) is an extremely rare congenital multisystem disorder characterized by progressive, disproportionate, and asymmetric overgrowth of various tissues, including bone, skin, adipose tissue, and the central nervous system. The clinical presentation is highly variable, and no two individuals are affected in exactly the same way. To emphasize this phenotypic diversity, the term Proteus syndrome was proposed, referencing the ancient Greek sea god Proteus, who could alter his form at will.

Although the wide range of manifestations strongly indicated a genetic origin, the mode of inheritance remained unclear for decades. Early theories suggested a sporadic occurrence, but in 1993 it was hypothesized that somatic mosaicism could be the underlying mechanism. This hypothesis provided a plausible explanation for the segmental and patchy distribution of overgrowth seen in affected individuals, as well as the absence of vertical transmission.

A major breakthrough occurred in 2011, when Lindhurst et al. identified a mosaic activating mutation in the AKT serine/threonine kinase 1 (AKT1) gene (c.49G>A, p.E17K) in affected tissues of individuals with Proteus syndrome. This mutation leads to constitutive activation of the phosphoinositide 3-kinase (PI3K)/AKT1 signaling pathway, resulting in abnormal cell proliferation, survival, and growth. The identification of this postzygotic mutation confirmed the somatic mosaic nature of the disorder and provided a molecular basis for diagnostic confirmation in uncertain cases.

Due to its rarity, fewer than 200 cases have been reported worldwide, and the true prevalence of Proteus syndrome remains unknown. However, its clinical burden is profound, often involving life-threatening complications such as deep vein thrombosis, pulmonary embolism, and the development of benign or malignant tumors. In addition to the physical challenges, affected individuals frequently endure significant psychosocial suffering due to disfigurement and societal stigma.

Early recognition, multidisciplinary management, and regular follow-up are essential to mitigate medical risks and improve the quality of life for individuals living with this complex and enigmatic disorder.

Proteus syndrome (PS) is an ultrarare disease that manifests through combined damage to organs and systems [1-3]. The frequency of occurrence of PS is unknown, but is presumed to be $1:10^6$–10^7 people. About 200 cases of PS are described in the world literature [3-5]. In Russia, according to open sources of information, fewer than 10 patients have been reported [6-12]. This syndrome was first described by Michael Cohen and Patricia Hayden (M.M. Cohen, P.W. Hayden) in 1979 [13]. To emphasize the variety of phenotypic features, the name "Proteus syndrome" was proposed – in honor of the ancient Greek god, capable of taking any form. A wide range of clinical manifestations in patients with PS indicated a genetic nature of the disease, but the type of inheritance remained unclear for a long time. In 1993, it was hypothesized that the syndrome is based on somatic mosaicism [14]. With the development of molecular genetic diagnostic methods, a search began for the gene responsible for the development of PS. Thus, X. Zhou et al., in 2000, suggested the involvement of the phosphatase and tensin homolog (PTEN) gene in the pathogenesis of PS [15]. Currently, it is believed that Proteus syndrome is based on postzygotic pathological variants of the AKT1 gene (14q32.33) [2]. The AKT1 gene is involved not only in the development of PS but also in several types of solid tumors – breast cancer, endometrial cancer, thyroid cancer, lung cancer, and malignant neoplasms of the genitourinary tract, etc. [16], which makes it a prospective target for the development of targeted therapy.

Case report

In 2010, an 18-year-old woman presented herself to the surgical team of a provincial hospital in Angola with an extremely unusual and emotionally charged request: elective amputation of her right forearm below the elbow. Her motivation was not driven by physical pain, functional limitation, or medical necessity, but by profound social ostracism and psychological distress. The patient had been the target of intense stigma and discrimination within her community, where some individuals unjustly accused her of being a manifestation of malevolent spiritual forces. This case highlights not only the clinical complexity of rare congenital disorders but also the severe moral and emotional suffering that patients may endure due to cultural beliefs and social exclusion.

We convey our profound gratitude to Professor Michele Garabedian and his team at National Institute for Medical Research in France (Unité 621), Saint-Vincent-de-Paul Hospital, 75014 Paris, which also includes highly experienced geneticists, who were kind enough to remotely examine the images (only the images) provided and confirm the diagnosis of Proteus syndrome.

The diagnosis in question was also confirmed by the co-author of this article, A. Olaru, an experienced specialist in oncology-traumatology at the Oncological Institute in Chişinău.

Family and perinatal history

The patient was born to healthy parents: the mother was 29 and the father 32 years old at the time of conception.

The pregnancy was uneventful, aside from first-trimester hyperemesis gravidarum. She was delivered vaginally at term without complications. At birth, her weight was 3,500 grams, length 50 cm, and head circumference 33 cm. She had several siblings, all of whom were developmentally normal and healthy. There was no known family history of congenital anomalies or similar conditions.

This case highlights the tragic psychosocial consequences of congenital limb differences in certain cultural contexts and raises complex ethical considerations regarding bodily autonomy, stigma, and the role of medicine in addressing non-physical suffering.

Clinical findings

We observed marked asymmetry of the upper limbs, with significant elongation and disfigurement of the right forearm and hand. Two overlapping melanocytic nevi were noted at the left oral commissure, and a soft, mobile, non-tender retroauricular mass on the left side was identified, measuring approximately 7.0 × 5.5 cm and adherent to the auricle.

The right upper limb, particularly the arm and forearm, was longer by approximately 7.8 cm compared to the left, with evident vascular malformations along the forearm, digital hyperplasia, and deformation of the fingers. Soft tissue

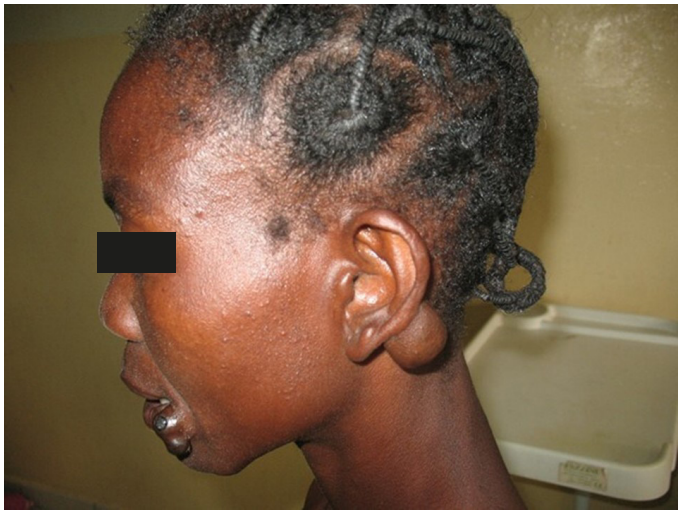

Fig. 1 Proteus syndrome

Note: *Two overlapping melanocytic nevi were noted at the left oral commissure, and a soft, mobile, non-tender retroauricular mass on the left side was identified, measuring approximately 7.0 × 5.5 cm and adherent to the auricle.*

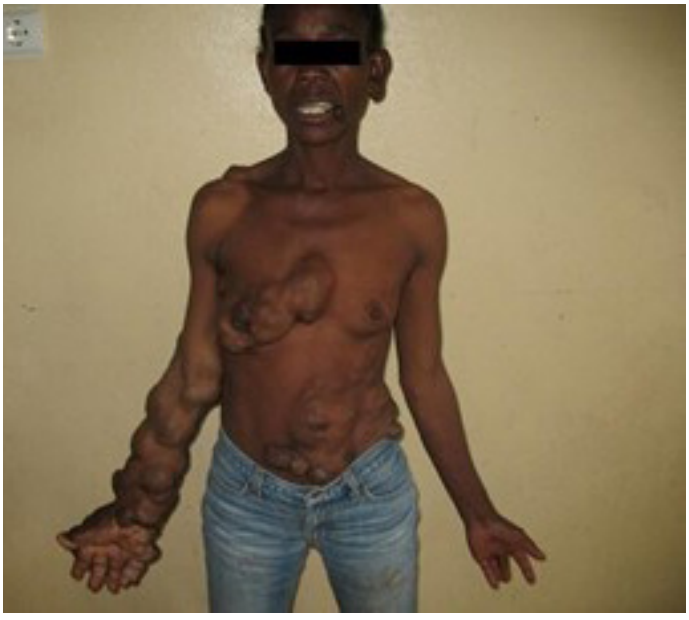

Fig. 2 Proteus syndrome

Note: *We observed marked asymmetry of the upper limbs, with significant elongation and disfigurement of the right forearm and hand. Soft tissue masses were also identified on the right side of the thorax, the anterior abdominal wall, and the left suprailiac region.*

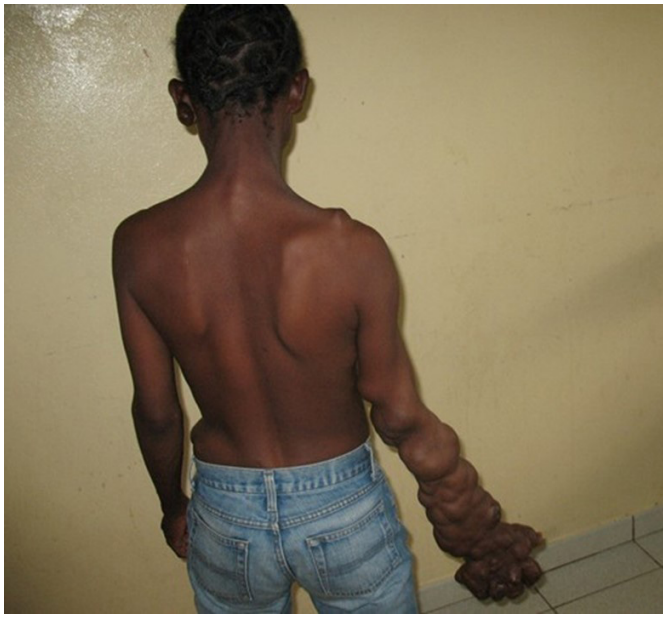

Fig. 3 Proteus syndrome

Note: *We observed marked asymmetry of the upper limbs, with significant elongation and disfigurement of the right forearm and hand.*

Fig. 4 Proteus syndrome

Note: *Significant elongation and disfigurement of the right forearm and hand.*

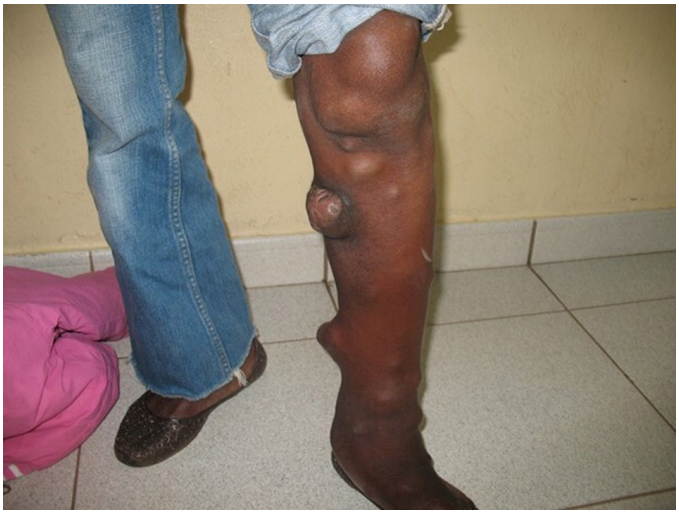

Fig. 5 Proteus syndrome

Note: *The left lower limb was slightly longer than the right by 2 cm, resulting in a tilted posture toward the right side, as visible in standing photographs. There was marked hypertrophy of the left calf (1.5 cm greater in circumference than the right), and the left foot exhibited soft tissue masses and hyperostoses on the surface of the calf, resembling the appearance of a tree trunk with multiple protruding branches.*

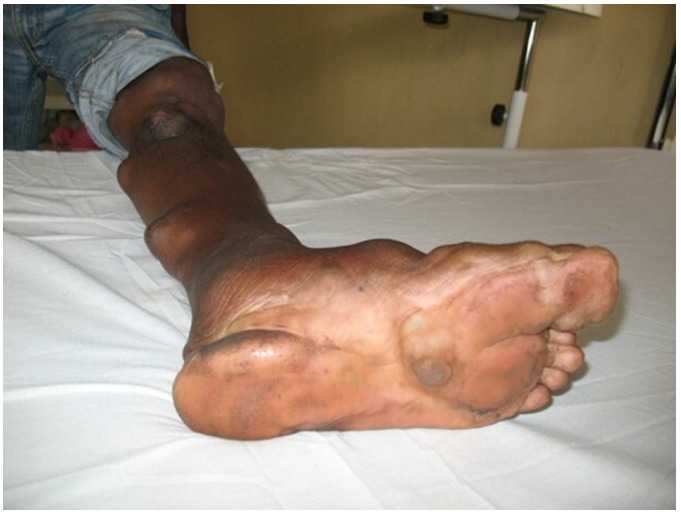

Fig. 6 Proteus syndrome

Note: *A pronounced plantar hyperkeratosis was also noted on the left foot.*

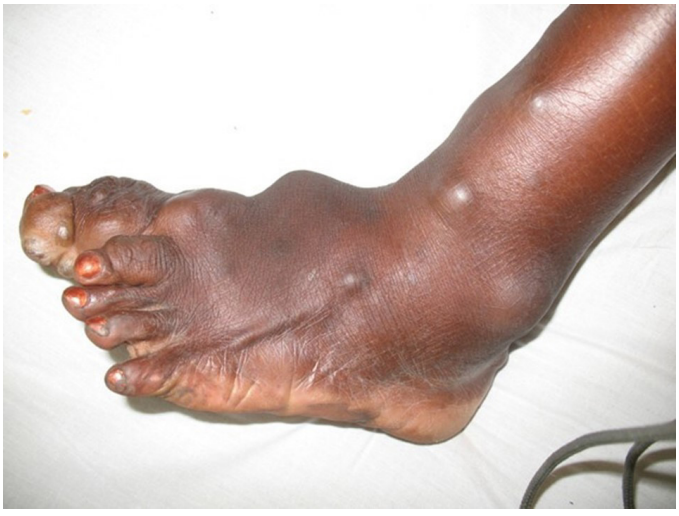

Fig. 7 Proteus syndrome

Note: *Digital hyperplasia and deformation of the fingers.*

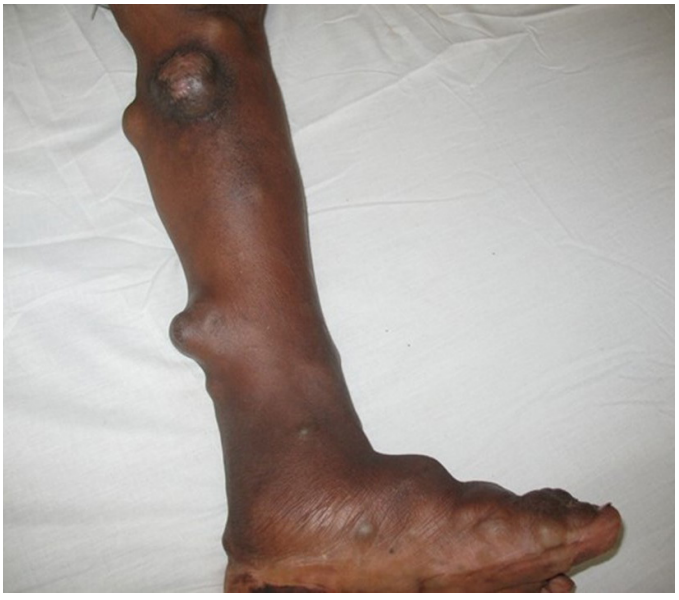

Fig. 8 Proteus syndrome

Note: *There was marked hypertrophy of the left calf (1.5 cm greater in circumference than the right), and the left foot exhibited soft tissue masses and hyperostoses on the surface of the calf, resembling the appearance of a tree trunk with multiple protruding branches.*

masses were also identified on the right side of the thorax, the anterior abdominal wall, and the left suprailiac region.

The left lower limb was slightly longer than the right by 2 cm, resulting in a tilted posture toward the right side, as visible in standing photographs. There was marked hypertrophy of the left calf (1.5 cm greater in circumference than the right), and the left foot exhibited soft tissue masses and hyperostoses on the surface of the calf, resembling the appearance of a tree trunk with multiple protruding branches. A pronounced plantar hyperkeratosis was also noted on the left foot.

In addition to her depressive symptoms, the patient complained of pain and paresthesia in the right arm and forearm, as well as in the left calf. She reported sensory disturbances in the affected limbs, periodic fronto-occipital headaches, dysmenorrhea with associated pain (algodysmenorrhea), and increased fatigability. Her intellectual development appeared normal, and her behavior was appropriate for her age.

No abnormalities were detected in other organ systems during the clinical examination. The diagnostic capacity of the hospital was extremely limited at the time, both in

terms of laboratory tests and imaging modalities. The patient could not specify when the noted changes began but stated that she became aware of the differences around the age of 4–5, when she was able to compare herself to her siblings and other children. She reported that the deformities became more prominent between the ages of 10 and 13, after which they appeared to stabilize.

We would like to emphasize from the very beginning that we did not have the opportunity or the necessary resources to carry out genetic investigations in such cases.

Discussion

Proteus syndrome is characterized by a polymorphic phenotype. Clinical findings change over time, making this syndrome difficult to diagnose. It is an extremely rare disease, and its natural history is not yet fully understood [17]. The most widely accepted etiological hypothesis for this disease is genetic and is believed to represent the existence of somatic mosaicism. The disease is lethal in the non-mosaic state, and cases are mainly sporadic. Three adults reported in the literature as affected by the disease subsequently gave birth to normal children [18].

In several cases, asymmetry and hemihypertrophy have been observed at birth, although these features typically become more pronounced during postnatal development. Excessive growth of bones and soft tissues usually progresses throughout childhood and adolescence but tends to plateau after puberty. Despite localized overgrowth, overall height and pubertal growth spurts in patients with Proteus syndrome are generally within normal limits [19-22]. In this case, the basic physical parameters appear to be normal (waist: 1m 58cm, body mass: 52 kg, BMI: 20, head circumference: 54 cm).

The main long-term complications of Proteus syndrome include premature death and the development of unusual tumors. Premature mortality is most commonly attributed to deep vein thrombosis, which may lead to pulmonary embolism, a potentially fatal complication that requires vigilant surveillance and prophylactic measures [19-24]. Attention is drawn to lipomas, which are histologically benign tumors but can show invasive intra-abdominal or intrathoracic behavior.

Special attention should be given to lipomas, which, although histologically benign, may exhibit aggressive behavior, particularly when located intra-abdominally or intrathoracically. These lesions can infiltrate surrounding tissues, making surgical management challenging and increasing the risk of recurrence [19]. Due to the difficulty in identifying this syndrome, diagnostic criteria were established at the first National Conference on Proteus Syndrome in 1998. To make a diagnosis, all of these criteria should be present: mosaic distribution of lesions, progressive course, and sporadic occurrence of the disease (i.e., not familial) [19].

Various clinical manifestations may arise throughout the progression of Proteus syndrome. Among the most common features are hemihypertrophy, cranial hyperostosis, cerebriform nevi, pigmented nevi, subcutaneous tumors, vascular malformations, abnormal adipose tissue distribution, central nervous system involvement, and a range of ophthalmologic abnormalities. Less frequent findings include craniofacial anomalies, distinctive facial phenotypes, internal tumors, splenomegaly, and thymic hypertrophy [18, 19].

Hemihypertrophy typically develops during childhood and progresses until late adolescence. It may be partial, complete, or mixed, often resulting in limb dysfunction and gait disturbances. Treatment options include epiphysiodesis, arthrodesis, limb shortening or lengthening procedures, and various methods aimed at reducing asymmetry. Cerebriform nevi are connective tissue lesions characterized by excessive collagen deposition. They most commonly occur on the plantar surface of the foot and the palms of the hands. While not mandatory for diagnosis, their presence is considered almost pathognomonic for Proteus syndrome when identified.

Subcutaneous tumors (lipomas, hemangiomas, and lymphangiomas) develop variably in any part of the body. They can grow to infiltrate local tissues, making surgical resection difficult. Treatment options include resection, dissection, and liposuction; however, results are often unsatisfactory due to recurrence and the formation of hypertrophic scars.

Management strategies include excision, surgical dissection, and liposuction. However, treatment outcomes are often suboptimal due to the high risk of recurrence and the development of hypertrophic scars [20, 23]. Although the histopathological appearance of these tumors is benign, they can exhibit aggressive behavior, depending on their location, especially if they are intrathoracic or intra-abdominal [19]. Both increases and reductions in adipose tissue may occur simultaneously in different regions of the same patient, reflecting a disrupted regulation of adipose tissue homeostasis. A distinct pattern is often observed in association with lipomas and abnormal subcutaneous fat accumulation, and ectopic fat deposits may also be found between muscle layers, further highlighting the complex and dysregulated nature of adipose tissue in Proteus syndrome [20]. Vascular malformations may have a single component (e.g., capillary, lymphatic, or venous) or may be combined (e.g., capillary and venous, or capillary, venous, and lymphatic). They grow proportionally with the patient, never involute, and may expand [19]. Less common tumors associated with the syndrome include ovarian cystadenomas, meningiomas, testicular tumors, and parotid gland adenomas [18].

The present case is of interest as an ultra-rare condition, not encountered in daily practice by many practitioners worldwide. Treatment is largely palliative, and the complications that arise along the way shorten patients' lives. Moreover, the spectrum of clinical manifestations often overlaps with other genodermatoses, which complicates timely diagnosis. Based on the analysis of the literature, in most cases the diagnosis of PS was made based on clinical manifestations, as in our case, without performing the necessary genetic testing.

Management of these patients should be carried out by a multidisciplinary team, involving specialists in genetics, or-

thopedics, dermatology, neurology, radiology, and psychosocial care. Comprehensive clinical evaluations should be performed to identify the major manifestations of the syndrome, given its complex and heterogeneous presentation.

Long-term follow-up is essential due to the polymorphic nature of the disease, with the potential for progressive changes over time. Special caution is warranted when performing surgical procedures or during periods of prolonged immobilization, due to the increased risk of deep vein thrombosis and subsequent pulmonary embolism [18-24].

Surgical interventions in Proteus syndrome mainly involve the resection of large, highly vascularized tumors. Both conventional and ultrasound-assisted liposuction techniques have been tried, with the aim of reducing the volume of adipose tumors while preserving the surrounding vascular structures. However, the methods have proven insufficient, resulting in limited aesthetic and functional improvement.

In this case, the patient encountered significant difficulties and suffering due to isolation from major medical centers and the lack of access to specialized high-performance healthcare. Being located hundreds of kilometers from the capital, performing genetic testing, ultrasound examinations, and comprehensive biochemical laboratory analyzes was not feasible, although such evaluations are recommended by some authors [1]. Given the possible complications of this condition, patients with PS require lifelong surveillance and continuous risk prevention strategies, including [1]:

- Annual examinations by a dermatologist, surgeon, orthopedist, pulmonologist, ophthalmologist, gynecologist/urologist, and oncologist (more frequently if clinically indicated);
- Assessment of the blood coagulation system every six months;
- Annual ultrasound examination of the veins of the lower extremities, particularly after the age of 25;
- Annual pelvic ultrasound examination;
- Annual chest X-ray.

Currently, the use of targeted therapy in the treatment of PS is not supported by clinical guidelines, but it may be considered in selected cases. This underscores the importance of timely diagnosis and molecular (DNA) testing [1].

NGS sequencing using a gene panel targeting the PI3K/AKT/mTOR signaling pathway, as emphasized by some authors from the Russian Federation [1], enables the differential diagnosis of phenotypically similar disorders and help determine the appropriate therapeutic strategy [1].

Consultation, ongoing supervision, and moral support from a qualified psychologist are extremely important and necessary for such patients. Without appropriate psychological support, individuals may develop severe depression, potentially leading to life-threatening consequences.

Conclusions

The description of the phenotypic characteristics of patients with Proteus syndrome is particularly important given the extreme rarity of this condition worldwide. It is necessary to increase the awareness and professionalism of clinicians regarding this disease in order to develop a dynamic follow-up plan, taking into account life-threatening complications (malignant tumors and risk of thromboembolism) and the moral suffering, sometimes unbearable, that patients encounter throughout their lives.

Today, the diagnosis of mosaic genodermatoses, including Proteus syndrome, is routinely performed in specialized laboratories. Molecular testing enables the detection of pathogenic variants in the AKT1 gene, even when present at low variant allele frequencies, thereby significantly increasing the rate of genetic confirmation. DNA-based diagnosis has become a critical tool not only for confirming clinical suspicion but also for guiding targeted therapies and conducting differential diagnosis with other disorders within the spectrum of segmental overgrowth syndromes.

Competing interests

None declared.

Authors' contributions

ACV, NR, IR, AO, and CAV conceived the study. ACV, CAV, AO, and NR participated in the study design. ACV and IR helped to outline and draft the manuscript. ACV, CAV, and IR participated in the search and compilation of bibliographic sources. All authors critically reviewed the work and approved the final version of the manuscript.

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ANNIVERSARY

Professor Valeriu Revenco at 70 – a life dedicated to excellence in cardiology

In the cold days of January, when the Soroca Fortress seems to have stood guard for centuries at the country's border, we celebrate a Man of rare spiritual nobility who, for a lifetime, has watched over the health of the heart of his fellow men – Valeriu Revenco, University Professor, Habilitated Doctor of Medical Sciences. Born on January 16, 1956, in the city of Soroca, Professor Revenco is, for many generations of doctors and patients in the Republic of Moldova, a professional and human beacon, an important name in local medicine, and truly the right person in the right place.

His roots, deeply planted in the fertile northern soil within a family of diligent householders from Cureșnița, shaped his love for work, discipline, and modesty from an early age, moral values that define him. After graduating from high school in his hometown, he chose to study at the State Institute of Medicine in Chișinău (today USMF *Nicolae Testemitanu*), from which he graduated with honors in 1979. Subsequently, he consolidated his professional training in cardiology at the All-Union Scientific Center in Moscow. Professor Valeriu Revenco's academic path is marked by notable successes: the defense of his doctoral thesis in medical sciences (1984) and obtaining the title of Habilitated Doctor in Medicine (1992), steps that confirmed his vocation as a researcher and outlined the beginning of a fruitful stage in training new generations of doctors.

In 1984, he began his teaching career at USMF *Nicolae Testemițanu* following a continuously ascending academic itinerary: from university assistant (1984–1992) to university professor (since 1993) and Head of the Cardiology Department (from 1998 to the present). Parallel to his teaching activity, he also held administrative positions: Vice-Rector for Clinical Activity and Residency, Dean of the Faculty of Residency and Clinical Fellowship, and served as a member of the University's governing bodies. The respect and trust he enjoys in the academic community are due to his exemplary responsibility, organizational skills, and tireless commitment to improving the professional training of new generations of doctors.

Valeriu Revenco has coordinated and participated in numerous national and international scientific projects, serving as a mentor for young researchers. His contribution to the field of cardiology, as a professor and coordinator of scientific projects, is confirmed by multiple publications, numbering over three hundred: articles in national and international journals, monographs, textbooks, guidelines, and methodological recommendations.

Colleagues, residents, and trainees admire and appreciate his professional competence and that "innate gift" of seeing the potential in people, guiding his disciples with great care toward professional fulfillment. Through his honest and demanding, yet benevolent approach, Professor Valeriu Revenco remains a benchmark for young specialists – a model of scientific rigor, professionalism, and devotion to patients. Numerous generations of specialists have perfected their professional training under his guidance, becoming scientists and professionals of real value to the country.

Beyond the lecture hall and the medical office, Professor Valeriu Revenco is known as a man who cherishes beauty – literature, history, art – passions that have always accompanied him and shaped his reflective discourse. He is an admirer of Eminescu's work and of great cultural values, and in his vision, medicine transcends the boundaries of a simple profession, becoming a noble vocation rooted in the love for people.

Beyond his academic track record and the prestige of the positions held, Professor Valeriu Revenco is, in essence, a Man of rare simplicity and decency: a son of peasants who, through assiduous work, became a university professor; he is a devoted husband, a loving father, and a caring grandfather who finds peace and joy in his grandchildren's smiles. Modesty, humanity, and generosity are the virtues he has kept intact, regardless of the high responsibilities he has assumed throughout his life.

On his 70th anniversary, the messages addressed to him are full of gratitude: former disciples thank him for the "school of life", while colleagues consider the chance to work together a privilege. The multiple appreciations confirm that Professor Valeriu Revenco remains a standard of professionalism, intelligence, nobility, honor, humanity, modesty, and generosity.

At this anniversary milestone, we wish Professor Valeriu Revenco health and many years with a strong heart and the same passion for the people he helps and trains. May he delight us with beautiful new achievements in the coming years, continue to research, write, instruct, and guide new generations, but above all, may he continue to save hearts and soothe suffering.

Happy Birthday, Esteemed Professor! *Vivat, Crescat, Floreat!*

With great respect and appreciation,

L. Grib, PhD, University Professor,
Head of the Internal Medicine Department.
Nicolae Testemițanu State University of Medicine and Pharmacy

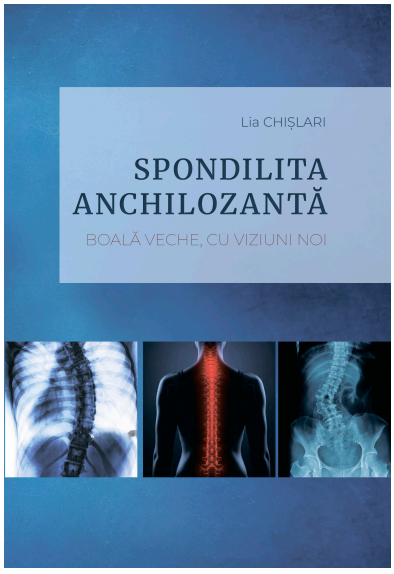
MONOGRAPH REVIEW

"Ankylosing spondylitis – an old disease with new perspectives"

Author: Lia Chişlari, MD, PhD, associate professor
Discipline of Rheumatology and Nephrology, Department of Internal Medicine,
Nicolae Testemiţanu State University of Medicine and Pharmacy, Republic of Moldova

Monograph details: Chişlari L. Spondilita anchilozantă – boală veche, cu viziuni noi [Ankylosing spondylitis – an old disease with new perspectives]. Chişinău; 2025. 180 p. ISBN 978-9975-173-85-8. Romanian.

Ankylosing spondylitis (AS) represents the prototype of seronegative spondyloarthritis, characterized by chronic inflammation, structural damage of the axial skeleton, and significant long-term functional impairment. In this context, the monograph *"Ankylosing spondylitis – an old disease with new perspectives"*, authored by Lia Chişlari, MD, PhD, associate professor, constitutes a comprehensive and timely scientific work that addresses both classical concepts and modern advances in the understanding and management of this disease.



The monograph is developed under the auspices of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy and has been favorably evaluated by academic scientific bodies, confirming its scholarly quality. It is addressed to a wide audience, including rheumatologists, internists, family physicians, and specialists involved in spinal disorders, serving as both a theoretical reference and a practical guide.

A major strength of this work is the integration of original research conducted by the author. The monograph includes well-designed clinical and translational studies, particularly focusing on the role of the intestinal microbiome in ankylosing spondylitis. The author presents original data obtained from a cohort of patients with AS compared to healthy controls, demonstrating significant differences in gut microbiota composition and structure. These findings are analyzed using appropriate statistical methods and are interpreted in the context of current international literature, supporting the hypothesis that microbiota alterations may play a role in disease pathogenesis and may represent potential biomarkers for early diagnosis.

In addition to microbiome research, the monograph discusses clinically relevant pathogenetic associations, including infectious triggers and their relationship with specific clinical phenotypes of spondyloarthritis. The author critically evaluates these associations, avoiding speculative conclusions and maintaining a balanced, evidence-based perspective. This analytical approach reflects scientific maturity and methodological rigor.

From a clinical standpoint, the monograph offers a thorough and up-to-date overview of diagnostic strategies, including clinical assessment, classification criteria (ASAS), imaging techniques, and disease activity indices. Therapeutic management is presented in accordance with current international recommendations, covering non-pharmacological interventions, non-steroidal anti-inflammatory drugs, and advanced biological therapies. The emphasis on individualized treatment strategies and long-term disease monitoring enhances the practical value of the work.

The didactic quality of the monograph is also noteworthy. The content is logically structured, progressing from fundamental concepts to advanced pathogenetic mechanisms and therapeutic approaches. The inclusion of illustrative figures, tables, and clinical algorithms significantly improves clarity and usability, making the monograph suitable for medical education at both undergraduate and postgraduate levels.

In conclusion, *"Ankylosing spondylitis – an old disease with new perspectives"* is a scientifically solid, original, and clinically relevant monograph. It successfully combines comprehensive literature synthesis with original research and practical clinical guidance. The work represents a valuable contribution to Romanian-language medical literature and serves as an important reference for specialists, trainees, and educators in rheumatology and related fields.

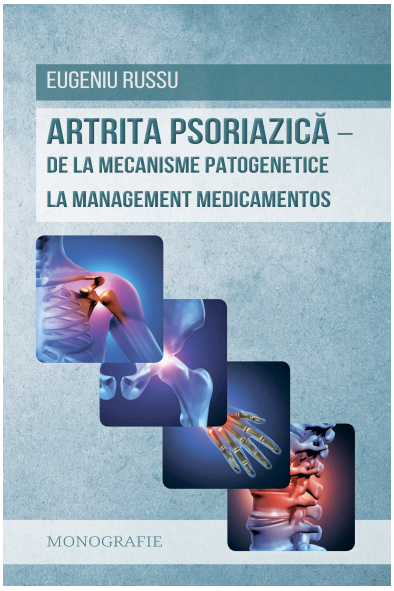
Ninel Revenco, MD, PhD, Dr. Hab., university professor
Head of Department of Pediatrics
Nicolae Testemiţanu State University of Medicine and Pharmacy, Republic of Moldova

MONOGRAPH REVIEW

"Psoriatic arthritis – from pathogenetic mechanisms to pharmacological management"

Author: Eugeniu Russu, MD, PhD, associate professor
Discipline of Rheumatology and Nephrology, Department of Internal Medicine,
Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova

Monograph details: Russu E. Artrita psoriazică – de la mecanisme patogenetice la management medicamentos [Psoriatic arthritis – from pathogenetic mechanisms to pharmacological management]. Chișinău; 2025. 224 p. ISBN 978-9975-173-86-5. Romanian.



Psoriatic arthritis (PsA) is a complex, chronic inflammatory disease with heterogeneous clinical manifestations and significant functional, social, and economic impact. In this context, the monograph *"Psoriatic arthritis – from pathogenetic mechanisms to pharmacological management"* authored by Eugeniu Russu, MD, PhD, associate professor, represents a timely and valuable contribution to the medical literature, particularly within the Romanian-language academic space.

The monograph provides a comprehensive and well-structured overview of psoriatic arthritis, integrating current international scientific evidence with original clinical and immunological research conducted on patient cohorts from the Republic of Moldova. The author succeeds in combining fundamental concepts of immunopathogenesis with clinical realities, offering a coherent and up-to-date synthesis of PsA as a systemic inflammatory disease rather than a purely articular condition.

A major strength of this work lies in its original research component. The author presents detailed analyses of immunological disturbances observed in patients with PsA, highlighting significant alterations in cellular and humoral immunity, particularly in patients with severe clinical phenotypes. These findings contribute novel regional data and enrich existing international knowledge regarding disease heterogeneity and immune dysregulation in psoriatic arthritis.

Equally important is the exploration of genetic susceptibility factors. The monograph discusses the association of various HLA alleles with PsA in the studied population and emphasizes correlations between specific genetic profiles and distinct clinical forms of the disease. These observations underline the multifactorial nature of PsA and support the concept of personalized risk stratification and prognosis assessment.

The interdisciplinary approach adopted throughout the monograph is particularly noteworthy. Concepts from clinical rheumatology, immunology, genetics, and molecular biology are integrated into a unified pathogenetic model, facilitating a deeper understanding of disease mechanisms. This approach reflects contemporary trends in translational medicine and supports the development of individualized therapeutic strategies.

From a practical perspective, the monograph has substantial clinical applicability. The author presents current diagnostic algorithms, emphasizes the importance of early detection in patients with cutaneous psoriasis, and reviews modern imaging techniques used for diagnosis and monitoring. The therapeutic chapters are aligned with the most recent international guidelines (ACR/NPF and EULAR), covering conventional synthetic DMARDs, biological therapies, and targeted synthetic agents, as well as treatment optimization and tapering strategies.

The work also addresses prognosis, comorbidities, and patient education, reinforcing the importance of a patient-centered, multidisciplinary management approach. The clear and accessible academic style makes the monograph suitable not only for specialists but also for residents, doctoral students, and clinicians involved in the care of patients with psoriatic disease.

In conclusion, *"Psoriatic arthritis – from pathogenetic mechanisms to pharmacological management"* is a scien-

tifically rigorous, original, and clinically relevant monograph. It fills an important gap in Romanian-language medical literature and represents a valuable reference for rheumatologists, dermatologists, and other healthcare professionals. The author demonstrates academic maturity, critical thinking, and a strong capacity to integrate research findings into clinical practice. This monograph fully meets the standards of a modern scientific work and deserves wide dissemination within the medical and academic community.

Ninel Revenco, MD, PhD, Dr. Hab., university professor
Head of Department of Pediatrics
Nicolae Testemiţanu State University of Medicine
and Pharmacy, Republic of Moldova

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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.
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