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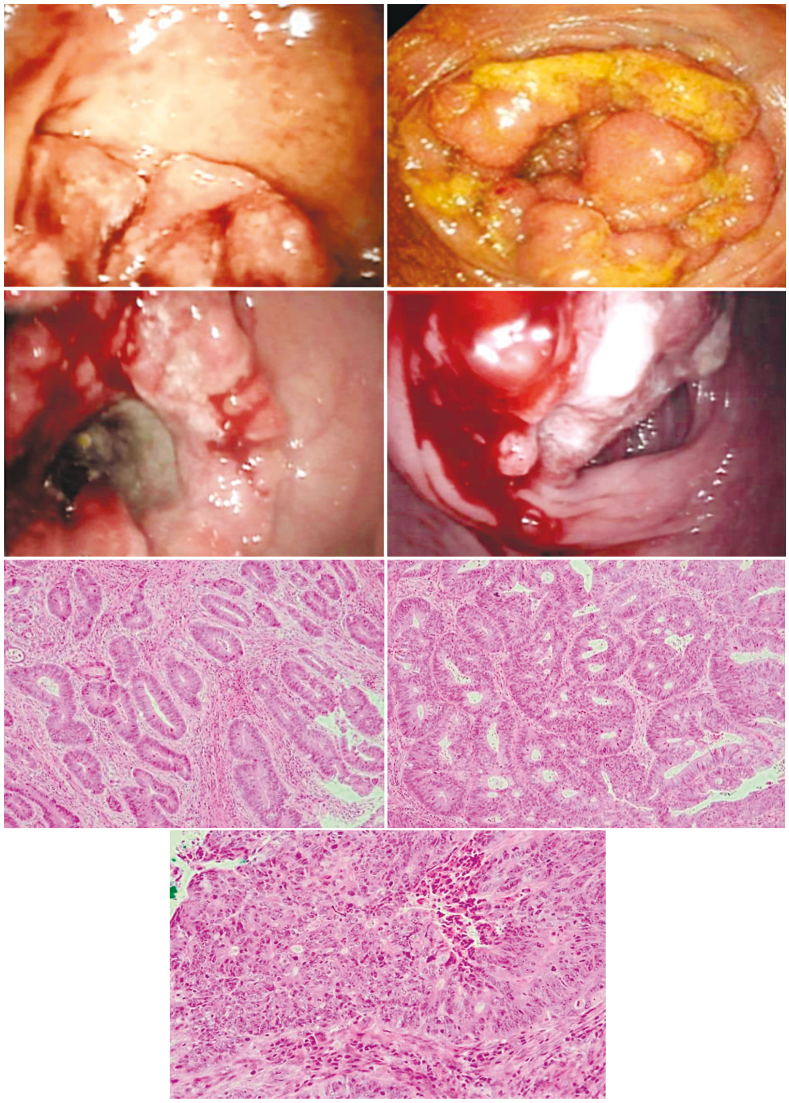
CONTENT HIGHLIGHTS:

Alexandr Ursu, Andrei Dolghii, Mihaela Cozma,
Eugen Melnic, Gheorghe Rojnoveanu

Morphological and histopathological characteristics of primary colon neoformationssuitable for staining of formalin-fixed, paraffin-embedded tissue



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Doze: Doza recomandată este de o capsulă pe zi, de preferință dimineața, la aceeași oră în fiecare zi. Doza zilnică maximă este de o capsulă.Dacă apare hipotensiune arterială la începutul tratamentului, pacientul trebuie așezat în decubit dorsal, tratamentul cu Ekvapress trebuie întrerupt și este necesar sfatul medicului. Un răspuns hipotensiv tranzitoriu nu este, de obicei, o contraindicație pentru doze ulterioare, deși reducerea dozei trebuie luată în considerare. Dacă devine necesară ajustarea dozei, trebuie avută în vedere titrarea dozei cu componentele individuale. *Doze omise:*Dacă pacientul omite o doză, doza omisă trebuie sărită și pacientul trebuie să ia următoarea doză la ora obișnuită. Nu este recomandat să luați o doză dublă pentru a compensa doza uitată.*Vârstnici (peste 65 de ani):*Pacienții vârstnici trebuie tratați cu prudență.Nivelul creatininei plasmatice trebuie monitorizat la pacienții geriatrici, în funcție de vârstă, greutate corporală și sex.În cadrul studiilor clinice, nu au fost observate modificări legate de vârstă ale profilurilor de eficacitate sau siguranță ale amlodipinei sau lisinoprilului. *Pacienți cu insuficiență renală:*Monitorizarea funcției renale, a concentrațiilor plasmatice de potasiu și sodiu trebuie continuată în timpul tratamentului cu Ekvapress. În cazul deteriorării funcției renale, Ekvapress trebuie să fie retras și înlocuit cu terapia cu doze individuale de componente active ajustate corespunzător. *Pacienți cu insuficiență hepatică:* Eliminarea amlodipinei poate fi prelungită la pacienții cu insuficiență hepatică. Nu au fost stabilite recomandări precise privind dozele pentru astfel de cazuri și, prin urmare, Ekvapress trebuie administrat cu precauție la pacienții cu insuficiență hepatică. *Copii și adolescenți (sub18 ani):*Nu au fost stabilite siguranța și eficacitatea administrării Ekvapress la copii și adolescenți cu vârsta sub 18 ani. Mod de administrare: Pentru administrare orală, indiferent de mese. **Contraindicații:** Hipersensibilitate la amlodipină sau la oricare alt derivat de dihidropiridină.Hipersensibilitate la lisinopril sau la orice alt inhibitor al enzimei de conversie a angiotensinei (ECA).Hipersensibilitate la indapamidă, alți derivați de sulfonamide. Hipersensibilitate la oricare dintre excipienți.Hipotensiunea arterială severă (TA sistolică mai mică de 90 mmHg).Antecedente de angioedem, inclusiv antecedente de angioedem asociate cu terapia anterioară cu ECA. Angioedem ereditar sau idiopatic.Insuficiență renală severă (clearance al creatininei <30 mL/min).Encefalopatie hepatică sau tulburări severe ale funcției hepatice.Hipokaliemie.Obstrucția tractului de scurgere al ventriculului stâng (inclusiv stenoză aortică de grad înalt, cardiomiopatie obstructivă hipertrofică), stenoză mitrală semnificativă hemodinamic.Insuficiență cardiacă instabilă hemodinamic după infarctul miocardic.Șocul (inclusiv șocul cardiogen). Angină instabilă (cu excepția anginei Prinzmetal).Utilizarea concomitentă cu produse care conțin aliskiren este contraindicată la pacienții cu diabet zaharat sau insuficiență renală ușoară sau severă (rata de filtrare glomerulară mai mică de 60 mL/min/1,73 m² suprafață corporală). Utilizarea concomitentă cu antagoniști ai receptorilor de angiotensină II (ARAII) la pacienții cu nefropatie diabetică. Utilizarea concomitentă cu inhibitori neutri ai endopeptidazei (de exemplu, produse care conțin sacubitril) datorită riscului ridicat de angioedem. Sarcina și alăptarea. **Atenționări și precauții speciale pentru utilizare:** La utilizarea Ekvapress trebuie luate în considerare toate avertismentele referitoare la monocomponentele individuale detaliate mai jos. Legat de lisinopril: *Hipotensiune arterială simptomatică:* La pacienții cu insuficiență cardiacă cronică cu TA normală sau scăzută, administrarea de lisinopril poate fi asociată cu scăderea TA; de obicei nu poate deveni un motiv pentru retragerea tratamentului. Dacă hipotensiunea arterială devine simptomatică, pot fi necesare reducerea dozei sau întreruperea tratamentului cu lisinopril. Trebuie controlat efectul dozei inițiale de lisinopril asupra tensiunii arteriale. *Disfuncție renală:* La pacienții cu infarct miocardic acut și disfuncție renală semnificativă (nivelul creatininei serice >177 micromol/L și/sau proteinurie >500 mg/zi) lisinoprilul nu trebuie utilizat. Dacă disfuncția renală apare în timpul tratamentului cu lisinopril (clearance-ul creatininei seric > 265 micromol/L sau se dublează comparativ cu valorile de dinainte de tratament), trebuie de luat în considerare întreruperea tratamentului cu lisinopril. *Hipersensibilitate/angioedem:* Pacienții cu antecedente de angioedem, fără legătură cu tratamentul anterior cu inhibitori ai ECA, pot prezenta un risc mai mare de angioedem în timpul tratamentului cu inhibitori ai ECA. *Reacții anafilactice asociate desensibilizării cu himenoptera:* Aceasta poate fi evitată prin retragerea temporară a inhibitorilor ECA înainte de fiecare desensibilizare. *Hemodializă:* În cazul acestor pacienți trebuie luată în considerare efectuarea dializei cu alt tip de membrane sau tratamentul cu alte clase de medicamente antihipertensive. *Tuse:*Administrarea inhibitorilor ECA poate fi asociată cu tusea.Tuse uscată pe termen lung dispare de obicei după retragerea inhibitorului ECA. *Chirurgie/anestezie generală:*Pacienții care iau inhibitori ai ECA trebuie să își informeze chirurgul/anestezistul înainte de intervenția chirurgicală. *Potasiul seric:* Dacă administrarea concomitentă de lisinopril și aceste medicamente este esențială, acestea trebuie utilizate cu prudență, cu control regulat al nivelului de potasiu al serului. *Blocarea dublă a sistemului renină-angiotensină-aldosteron (SRAA):* Administrarea concomitentă de inhibitori ai ECA cu medicamente care conțin aliskiren este contraindicată la pacienții cu diabet zaharat și/sau insuficiență renală moderată sau severă (RFG mai mică de 60 mL/min/1.73 m2 de suprafață corporală) și nu este recomandată la alți pacienți. Inhibitorii ECA și blocanții receptorilor de angiotensină II sunt contraindicați la pacienții cu nefropatie diabetică și nu sunt recomandați la alți pacienți. *Neutropenie/Agranulocitoză/Trombocitopenie/Anemie:*Ekvapress trebuie utilizat cu prudență extremă la pacienții cu boală vasculară de colagen, terapie imunosupresoare, tratament cu alopurinol sau procainamidă sau o combinație a acestor factori de complicare, mai ales dacă există afectare preexistentă a funcției renale. *Stenoza valvei aortice și mitrale/cardiomiopatia hipertrofică:* lisinoprilul trebuie utilizat cu precauție la pacienții cu stenoză a valvei mitrale și obstrucție a ejecției din ventriculul stâng. *Insuficiență hepatică:* Pacienții care administrează lisinopril și care dezvoltă icter sau creșteri marcate ale enzimelor hepatice trebuie să întrerupă tratamentul cu Ekvapress. Legat de amlodipină: Siguranța și eficacitatea amlodipinei în criza hipertensivă nu au fost stabilite. *Probleme gingivale:*Este necesară igiena dentară și supravegherea unui igienist dentar. *Vârstnici:* pacienții din acest grup necesită o monitorizare atentă. *Retragerea:* se recomandă încetarea amlodipinei prin reducerea progresivă a dozei. *Insuficiență cardiacă. Fertilitate.* Legat de indapamidă: *Tulburări ale funcției hepatice. Fotosensibilitate. Echilibrul hidro-electrolitic. Diureticele și funcția renală. Revărsat coroidian/Miopie acută/Glaucom secundar cu unghi închis. Sportivi.* **Reacții adverse:** Rezumatul profilului de siguranță: Cele mai frecvente reacții adverse, raportate în timpul monoterapiei cu Amlodipină, Indapamidă și Lisinopril au fost: amețeli, cefalee, somnolență, tulburări de vedere, tinitus, palpitații, înroșire, scăderea tensiunii arteriale (și efecte asociate hipotensiunii), tuse, dispnee, tulburări gastrointestinale (dureri abdominale, constipație, diaree, greață, dispepsie, vărsături), erupții cutanate maculopapulare, crampe musculare, umflarea gleznelor, astenie, edem și oboseală. Următoarele reacții adverse la medicamente (RAM) au fost raportate în timpul tratamentului cu amlodipină, indapamidă și lisinopril, în mod independent. Foarte frecvente (≥1/10): edem. Frecvente (≥1/100 până la <1/10): amețeală, cefalee, somnolență, tulburări ale vederii (inclusiv diplopie), palpitații, efecte ortostatice, bufeuri, hipotensiune, dispnee, tuse, dureri abdominale, grețuri, dispepsie, alterarea obiceiului intestinal, diaree, constipație, vărsături, reacții de hipersensibilitate, erupții cutanate maculopapulare, crampe musculare, edem periferic (glezne și picioare), disfuncție renală, oboseală, astenie. **Data și numărul autorizației de punere pe piață:** Nr.29235; Nr. 29234; Nr.29233 din 25.08.2023. **Statutul legal:** cu prescripție medicală. **Data revizuirii textului:** August 2023.
Acest material publicitar este destinat persoanelor calificate să prescrie, să distribuie și/sau să elibereze medicamente. Pentru informații complete vă rugăm să consultați rezumatul caracteristicilor produsului. Informații detaliate privind acest medicament sunt disponibile pe site-ul Agenției Medicamentului și Dispozitivelor Medicale (AMDM) http://nomenclator.amdm.gov.md/. Pentru mesaje de siguranță și informații medicale: e-mail: drugsafety.md@gedeonrichter.com sau tel.: (022) 20-21-90.

1. Williams, Mancia et al., J Hypertens 2018; 36:1953-2041 and Eur Heart J 2018; 39: 3021-3104 ; 2. RCP Ekvapress caps eliber modif 25.08.2023;

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RESEARCH ARTICLE

The interrelationship of clinical and paraclinical parameters depending on disease severity in children with hemophilia

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ABSTRACT

Introduction. Hemophilia is a genetic disorder characterized by impaired blood coagulation, leading to increased bleeding risk. The severity of hemophilia varies significantly among individuals, influenced by genetic factors, family inheritance patterns, and the occurrence of complications such as hemarthrosis. Understanding these interrelationships is crucial for developing tailored management strategies for affected children. The purpose of this article is to explore the correlations between clinical severity and various factors, including modes of inheritance, hemarthrosis incidence, types of genetic mutations, and inhibitor presence in pediatric patients with hemophilia. By elucidating these relationships, the study aims to contribute to improved diagnostic and therapeutic approaches in this population.

Material and methods. This retrospective analysis included 90 pediatric patients diagnosed with hemophilia. Clinical data regarding disease severity, inheritance patterns, hemarthrosis incidents, genetic mutation types, and inhibitor levels were collected and analyzed statistically to identify significant associations.

Results. The analysis revealed a strong correlation between familial inheritance patterns and disease severity, with moderate forms predominating in known inheritance cases. Hemarthrosis was most prevalent in severe cases, particularly affecting the knee and elbow joints. The study also found significant associations between genetic mutations, especially missense mutations, and the severity of hemophilia. Furthermore, elevated inhibitor levels were exclusively observed in severe forms of the disease.

Conclusions. The findings highlight the intricate relationships between clinical characteristics and hemophilia severity, emphasizing the necessity for individualized treatment strategies. Understanding these dynamics can facilitate better management of hemophilia in pediatric patients, ultimately improving their quality of life.

Keywords: hemophilia, pediatric patients, disease severity, genetic mutations, hemarthrosis, inhibitors, personalized treatment.

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Key messages

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

The interaction between clinical and paraclinical parameters and its variation with hemophilia severity in pediatric patients is not fully understood. Limited data exist on the predictive value of specific parameter combinations for disease progression and treatment response, particularly in children. Additionally, the impact of individualized treatment models on these relationships remains unclear, hindering the optimization of pediatric hemophilia management.

The research hypothesis

Specific paraclinical markers may strongly correlate with clinical severity, offering predictive insights into disease progression and treatment outcomes. Identifying these markers can guide severity-based, individualized treatment strategies to improve pediatric hemophilia care.

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

The manuscript explores the unique interplay between clinical and paraclinical parameters in pediatric hemophilia, focusing on disease severity and providing region-specific insights from Moldova. It highlights how combined markers can guide individualized treatment strategies, improving precision in managing the condition.

Introduction

Hemophilia is a hereditary bleeding disorder characterized by the deficiency of specific coagulation factors, leading to a predisposition to bleeding episodes. This condition primarily affects males and is caused by mutations in the genes responsible for producing clotting factors VIII (hemophilia A) or IX (hemophilia B). The severity of hemophilia can vary significantly among individuals, depending on the specific genetic mutations and their effects on factor levels. Children with hemophilia often experience spontaneous bleeding episodes, particularly into joints and muscles, which can lead to long-term complications, including joint damage and reduced quality of life [1].

Recent advancements in our understanding of hemophilia have highlighted the importance of genotype-phenotype correlations. The type of mutation present can influence the clinical manifestations of the disease, with certain mutations being associated with more severe bleeding tendencies [2, 3]. For example, missense mutations may lead to moderate forms of hemophilia, while frameshift or nonsense mutations often result in severe disease [4, 5]. Furthermore, the pattern of inheritance can also impact the severity of hemophilia. Studies have shown that sporadic cases of hemophilia may have different clinical outcomes compared to familial cases, with implications for management and treatment [6, 7].

Another critical aspect of hemophilia management is the assessment of joint health, particularly regarding hemarthrosis, which is a common complication. Joint bleeding episodes can lead to chronic pain and disability, significantly affecting the patient's quality of life [8, 9]. Research indicates that the frequency and severity of hemarthrosis are correlated with the severity of hemophilia, with children experiencing severe forms of the disorder being more likely to suffer from multiple joint bleeds [10, 11]. Therefore, a thorough understanding of the clinical parameters associated with hemophilia is essential for developing effective management strategies [12].

Material and methods

The study was conducted within the Department of Pediatrics of *Nicolae Testemițanu* State University of Medicine and Pharmacy, at the Pediatric Hematology Clinic of Mother and Child Institute. It involved 90 children aged 0–18 years from urban and rural areas. The research was an observational, descriptive, cross-sectional, and selective study designed to achieve the proposed aims and objectives.

Research stages

Participant selection: The research group included 90 children diagnosed with hemophilia (type A or B), aged 0–18 years, citizens of the Republic of Moldova, with informed consent signed by parents or guardians. Exclusion criteria: participants with other coagulopathies, lack of informed consent, or low compliance. Participants underwent a comprehensive examination, which included: Clinical evaluation (petechiae, purpura, hematomas, bleeding); Laboratory tests: coagulation panel (aPTT (activated Partial Thromboplastin Time), PT (Prothrombin Time), fibrinogen), hemoglobin, red blood cells, ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), bilirubin levels, factors VIII and IX; Identification of genetic mutations in FVIII and FIX genes in a subgroup of 39 children; Determination of inhibitor titers using the national protocol for diagnosing inhibitor hemophilia.

Participant classification: Subjects were grouped by type of hemophilia: Hemophilia A (factor VIII deficiency) and Hemophilia B (factor IX deficiency). Classification based on disease severity: mild form: factor VIII/IX between 5–30%, moderate form: factor VIII/IX between 1–5%, severe form: factor VIII/IX <1%.

Analysis of clinical and genetic relationships: Comparison of hemophilia type with mode of genetic transmission; Relationship between disease severity and clinical manifestations (e.g., hemarthrosis); Phenotype-genotype analysis.

Conclusions and recommendations: Based on clinical, paraclinical, and genetic results, conclusions were drawn, and practical recommendations were formulated. Statistical methods: Data were processed using Microsoft Excel and analyzed with IBM SPSS Statistics, version 20. Statistical methods included: ANOVA (Analysis of Variance): For comparing the means of multiple groups; Chi-square test (X2): For differentiating qualitative variables; validated using the Fisher Exact test where necessary; Kruskal-Wallis test: For comparing independent groups in cases of non-homogeneous variances. Results presentation: The results were presented using tables for clear and systematic representation.

Results

1. Interrelationship between disease severity and mode of transmission

In our analysis regarding the association between famil-

ial transmission type and disease severity, we observed the following relevant aspects:

- Concerning the severity criterion, we found that the most frequently encountered familial transmission type was associated with moderate forms of the disease, present in 45.45% of cases (15 cases). This frequency was statistically significantly higher than in severe forms, recorded only in 15.91% of cases (7 children), and mild forms, where no cases were identified.
- In severe forms, the familial transmission type was most often unknown, occurring in 54.55% of cases (24 children), while in mild forms, this proportion was 76.92% (10 children). However, in moderate forms, the frequency of cases where the transmission type was unknown was significantly lower, at just 3.03% (X^2=32.216, df=4 (degrees of freedom), p<0.001).
- The sporadic familial transmission type was most frequently associated with cases of moderate severity, present in 51.52% of these cases (17 children). In contrast, in severe and mild cases, this type of transmission was found in smaller proportions, specifically 29.55% (13 children) and 23.08% (3 children), respectively.

These findings highlight the complexity of the interaction between the type of familial transmission and the degree of disease severity in the context of hemophilia, underscoring the diversity of genetic and clinical characteristics of this condition (Table 1).

Table 1. Results of the interrelationship between transmission type and disease severity

		Severity							
		Mild		Moderate		Severe		Total	
		Absolut	%	Absolut	%	Absolut	%	Absolute	%	
Transmission type	Familial	0	0.00*	15	45.45*	7	15.91*	22	24.44
	Unknown	10	76.92*	1	3.03*	24	54.55*	35	38.89
	Sporadic	3	23.08	17	51.52	13	29.55	33	36.67
	Total	13	100.00	33	100.00	44	100.00	90	100.00

Note: *The abbreviations used in the table are as follows: "Familial" refers to cases of hemophilia transmitted through genetic inheritance, "Unknown" indicates cases with an unknown family history, and "Sporadic" represents cases of hemophilia occurring in isolation. The values are presented as both absolute numbers and percentages (%). The statistical analysis was performed using the Chi-square (χ^2) test to compare the distribution of frequencies between the transmission types and disease severity. A significance level of* $p < 0.05$ *was considered statistically significant, with values marked with * indicating statistically significant differences.*

2. Interrelationship between disease severity and hemarthrosis incidence

We analyzed the association between hemarthrosis, the most common complication in hemophilia, and disease severity and identified several significant findings:

In severe cases, the highest number of joints were affected, with 4 joints involved in 27.27% of cases (12 children), 5 joints in 18.18% (8 children), and similarly for cases involving 2 and 3 joints. The involvement of 6 joints was observed in 13.64% (6 children). Only 4.55% (2 children) had involvement of a single joint.

The elbow joint was more frequently affected in severe cases, with 54.55% (24 children), where both joints were involved in 31.82% (14 children) and only one in 22.73% (10 children). In moderate cases, the elbow joint was affected in 39.39% (13 children), with both joints involved in 36.36% (12 children) and only one in 3.03% (1 child). In mild cases, the elbow joint was rarely affected, at only 23.08% (3 children), and was recorded only in a single joint. In severe cases, the involvement of only one elbow was recorded in 22.73% (10 children), significantly more frequently than in moderate cases at 3.03% (1 child) (X^2=11.81, df=4, p=0.019).

Another joint affected was the knee. Similarly, it was more frequently affected in severe cases, at 88.64% (39 children), with both joints involved in 72.73% (32 children) and only one in 15.91% (7 children). In moderate cases, the knee joint was affected in 93.94% (31 children), with both joints involved in 84.85% (28 children) and only one in 9.09% (3 children). In mild cases, knee joint involvement was recorded equally for both joints and for a single joint, each at 38.46% (5 children). In moderate cases, the involvement of both knee joints was recorded in 84.85% (28 children), significantly more frequently than in mild cases at 38.46% (5 children) (X^2=10.07, df=4, p=0.039).

Another affected joint was the ankle. Similarly, it was more frequently affected in severe cases, at 72.73% (32 children), with both joints involved in 43.18% (19 children) and only one in 29.55% (13 children). In moderate cases, ankle joint involvement was observed in 66.66% (22 children), with equal involvement of both or just one joint at 33.33% (11 children). In mild cases, the ankle joint was the least affected at 92.31%, which significantly differed from the moderate form at 33.33% and the severe form at 27.27% (X^2=7, df=2, p=0.030).

These observations underline the variety and complexity of clinical manifestations in hemophilia, highlighting the need for a personalized approach and appropriate management of complications associated with each degree of severity (Table 2).

Table 2. Results of the interrelationship between hemarthrosis and disease severity

		Severity							
		Mild		Moderate		Severe		Total	
		Nr.	%	Nr.	%	Nr	%	Nr.	%	
The number of affected joints	0	2	15.38	1	3.03	0	0.00	3	3.33
	1	5	38.46	0	0.00	2	4.55	7	7.78
	2	6	46.15	7	21.21	8	18.18	21	23.33
	3	0	0.00	8	24.24	8	18.18	16	17.78
	4	0	0.00	10	30.30	12	27.27	22	24.44
	5	0	0.00	3	9.09	8	18.18	11	12.22
	6	0	0.00	4	12.12	6	13.64	10	11.11
	Total	13	100.00	33	100.00	44	100.00	90	100.00
Elbow	0	10	76.92	20	60.61	20	45.45	50	55.56
	1	3	23.08	1	3.03*	10	22.73*	14	15.56
	2	0	0.00	12	36.36	14	31.82	26	28.89
	Total	13	100.00	33	100.00	44	100.00	90	100.00
Knee	0	3	23.08	2	6.06	5	11.36	10	11.11
	1	5	38.46	3	9.09	7	15.91	15	16.67
	2	5	38.46*	28	84.85*	32	72.73	65	72.22
	Total	13	100.00	33	100.00	44	100.00	90	100.00
Ankle	0	12	92.31*	11	33.33*	12	27.27*	35	38.89
	1	1	7.69	11	33.33	13	29.55	25	27.78
	2	0	0.00	11	33.33	19	43.18	30	33.33
	Total	13	100.00	33	100.00	44	100.00	90	100.00

Note: *The abbreviations used in the table are as follows: "Nr." refers to the number of cases, and "Severity" represents the severity levels of hemophilia: Mild, Moderate, and Severe. The values are presented as both absolute numbers and percentages (%). The statistical analysis was performed using the Chi-square (χ^2) test to compare the distribution of affected joints between different severity levels of hemophilia. A significance level of* p *< 0.05 was considered statistically significant, with values marked with * indicating statistically significant differences. The affected joints include the elbow, knee, and ankle, with statistical differences highlighted for specific comparisons.*

3. Association of disease interrelationship with type of genetic mutation

We analyzed the types of genetic mutations recorded with disease severity, describing the genotype association with disease severity.

In mild forms of hemophilia, the most common mutation was Missense, at 7.69% (16 children). The Inv Intr 22 mutation was recorded in 13.33% (12 children) and was found only in moderate forms at 15.15% (5 children) and in severe forms at 15.91% (7 children). Another mutation type encountered was Frameshift in 7.78% (7 children), with a rate of 12.12% (4 children) in moderate forms and 6.82% (3 children) in severe forms. The least common mutation was Nonsense, found in 3.33% (3 children), of which 4.55% (2 children) were in the severe form and 3.03% (1 child) in the moderate form.

A statistically significant difference at the level of $p<0.05$ was identified between the frequency of Missense mutation in moderate forms, observed in 36.36% of cases (12 children), and in severe forms, found in 6.82% of cases (3 children) ($X^2=24.015$, df=10, p=0.008).

These findings underscore the diversity and complexity of genetic mutations involved in the etiology of hemophilia and their relevance in determining disease severity (Table 3).

Table 3. Results of the phenotype-genotype interrelationship

		Severity							
		Mild		Moderate		Severe		Total	
	Absolute	%	Absolute	%	Absolute	%	Absolute	%	
Mutation	Frame-shift	0	0.00	4	12.12	3	6.82	7	7.78
	Inv Intr 22	0	0.00	5	15.15	7	15.91	12	13.33
	Missens	1	7.69	12	36.36*	3	6.82*	16	17.78
	Negative	0	0.00	1	3.03	0	0.00	1	1.11
	Nonsens	0	0.00	1	3.03	2	4.55	3	3.33
	Mutation was not assesed	12	92.31*	10	30.30*	29	65.91*	51	56.67
	Total	13	100.00	33	100.00	44	100.00	90	100.00

Note: *The abbreviations used in the table are as follows: "Mutation" refers to the specific genetic mutations identified, including frameshift, Inv Intr 22, missense, negative, and nonsense mutations. The values are presented as both absolute numbers and percentages (%). The statistical analysis was performed using the Chi-square (χ^2) test to compare the distribution of mutations across different severity levels of hemophilia. A significance level of* p *< 0.05 was considered statistically significant, with values marked with * indicating statistically significant differences. «Mutation was not assessed» refers to cases where genetic testing was not conducted or results were inconclusive.*

4. Association of Disease Interrelationship with Inhibitor Titer

In our analysis of the incidence of inhibitors and disease severity in hemophilia, we observed a significant finding:

All 4 recorded cases with elevated inhibitor titers, according to both measurements, were exclusively identified within the severe forms of the disease, representing 9.09% of these cases.

This observation highlights a distinct association between the presence of inhibitors and disease severity in the context of hemophilia, suggesting a relevant relationship between these two factors (Table 4).

Table 4. Results of the Interrelationship between Disease Severity and Inhibitor Titer

		Severity							
		Mild		Moderate		Severe		Total	
		Nr	%	Nr	%	Nr	%	Nr	%	
Was not performed		2	15.38	28	84.85	19	43.18	49	54.44
Inhibitor titer determination - Stage I	Present	0	0.00	0	0.00	4	9.09	4	4.44
	Absent	11	84.62	5	15.15	21	47.73	37	41.11
	Total	13	100.00	33	100.00	44	100.00	90	100.00
Inhibitor titer determination - stage II	Present	0	0.00	0	0.00	4	16.00	4	9.76
	Absent	11	100.00	5	100.00	21	84.00	37	90.24
	Total	11	100.00	5	100.00	25	100.00	41	100.00

Note*: The abbreviations used in the table are as follows: "Nr." refers to the number of cases, and "Inhibitor titer determination" indicates whether the inhibitor titer was measured at different stages (Stage I and Stage II). The values are presented as both absolute numbers and percentages (%). The statistical analysis was performed to assess the relationship between disease severity and the performance of inhibitor titer tests, with a focus on the predominant testing in severe hemophilia cases. A significance level of* p *< 0.05 was considered statistically significant. Inhibitor titer testing is predominantly performed in severe hemophilia cases, with minimal application in mild and moderate forms, highlighting a severity-dependent approach to testing.*

Discussions

The management of hemophilia, particularly in pediatric patients, continues to evolve with advancements in both understanding the pathophysiology of the disease and improving treatment protocols. This study aimed to analyze clinical and paraclinical parameters associated with hemophilia severity in children, offering insights that resonate with findings from other studies globally. Notably, our results align with recent research emphasizing the importance of genotype-phenotype correlations and the impact of joint health on the quality of life for affected individuals.

Genotype-phenotype correlations

The significance of genetic mutations in determining clinical outcomes in hemophilia has been underscored in numerous studies. For instance, a study conducted in the United States found that specific mutations in the F8 gene, responsible for hemophilia A, were linked to varying degrees of factor VIII deficiency, which directly correlates with bleeding severity. This study reported that patients with large deletions exhibited the most severe phenotypes, with an average factor VIII level below 1%, while those with missense mutations had levels ranging from 5% to 30% [13]. Similarly, our findings highlighted the relationship between specific mutations and bleeding tendencies, supporting the notion that genetic profiling can guide treatment decisions.

Incidence of joint bleeding

Joint health is a critical concern in pediatric hemophilia, as repeated bleeding episodes can lead to severe complications such as hemophilic arthropathy. Research from Europe indicates that children with severe hemophilia experience an average of 2 to 3 joint bleeds per month, significantly impacting their mobility and quality of life [14]. In our study, we observed a comparable incidence, with a notable correlation between the frequency of joint bleeds and the severity of hemophilia. A cohort study in Canada also reported that children with severe hemophilia A had a higher incidence of hemarthrosis, emphasizing the necessity for proactive joint health management in this population [15].

Treatment modalities and outcomes

The treatment landscape for hemophilia has changed dramatically over the past two decades, particularly with the advent of recombinant factor therapies and gene therapy. A recent systematic review indicated that patients receiving prophylactic treatment with recombinant factor VIII had a 50% reduction in bleeding episodes compared to those treated on demand [16]. Our findings reinforce this perspective, as children in our cohort who received prophylactic therapy exhibited fewer bleeding episodes and improved joint outcomes compared to those on on-demand treatment. Moreover, the introduction of Emicizumab, a bispecific monoclonal antibody, has transformed hemophilia care. Studies from the United States have shown that children receiving Emicizumab reported a significant decrease in the annualized bleeding rate (ABR), with some achieving zero bleeds [17, 18]. Our analysis indicated that children treated with Emicizumab also experienced improved quality of life metrics, mirroring the positive outcomes reported internationally.

Statistical significance and global perspectives

It is essential to consider the statistical significance of findings in the context of international studies. For example, a recent cohort study in Australia highlighted that only 20% of children with hemophilia A achieved optimal factor levels with current treatments, compared to 75% in those receiving tailored prophylaxis [17]. Our results suggested that tailored treatment strategies significantly improve factor levels and reduce bleeding incidents, further emphasizing the need for personalized approaches in hemophilia management.

Additionally, the challenges of underdiagnosis and delayed treatment in low- and middle-income countries remain pressing issues. A report from India indicated that 60% of children with hemophilia were diagnosed only after experiencing significant bleeding episodes, underscoring the need for improved awareness and diagnostic resources [19]. Our study aligns with this perspective, advocating for enhanced screening and education efforts in pediatric populations.

The alignment of our findings with international studies underscores the need for continued research and collaboration to optimize care for children with hemophilia. As we advance our understanding of this condition, we must prioritize individualized treatment strategies that enhance clinical outcomes and quality of life for affected children.

Conclusions

The study highlights the significant interrelationships between clinical and paraclinical parameters in children with hemophilia, shedding light on the complexity of this disorder. Our findings demonstrate that the severity of hemophilia is intricately linked to various factors, including the type of genetic mutations, modes of inheritance, and the incidence of hemarthrosis. Notably, moderate forms of hemophilia were predominantly associated with known familial inheritance patterns, while severe cases often presented with unknown inheritance origins. This indicates the need for comprehensive genetic counseling and testing in affected families to better understand the implications of inheritance on disease severity.

Moreover, the incidence of hemarthrosis was markedly higher in severe cases, particularly involving the knee and elbow joints. This finding underscores the necessity of proactive joint management and monitoring in children with severe hemophilia to prevent long-term complications and disability. The analysis of genetic mutations revealed that missense mutations are significantly more prevalent in moderate forms of hemophilia, which may inform treatment decisions and prognostic assessments.

Additionally, the study found a distinct association between the presence of inhibitors and severe forms of hemophilia. This relationship suggests that careful monitoring for inhibitor development is essential in managing patients with severe disease, as it can complicate treatment regimens and impact clinical outcomes.

Competing interests

None declared.

Authors' contributions

VT and DA had a crucial role in the collection and analysis of empirical data, laying the foundations for the central argumentation of the paper. Their meticulous work allowed them not only to interpret the data in a new and innovative way, but also to integrate it into the wider context of specialist research. GE, DA on the other hand, focused on building the theoretical framework, exploring, and synthesizing the existing specialized literature. All authors have read and approved the final version of the manuscript.

Ethical statement and patient consent

No approval was required for this study.

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References

1. Srivastava A, Santagostino E, Dougall A, et al. Guidelines for the management of hemophilia, 3rd edition. Haemophilia. 2020;26(Suppl 6):1-158. doi: 10.1111/hae.14046.
2. Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. Lancet. 2016;388(10040):187-197. doi: 10.1016/S0140-6736(15)01123-X.
3. Blanchette VS, Key NS, Ljung LR, et al. Definitions in hemophilia: communication from the SSC of the ISTH. J Thromb Haemost. 2014;12(11):1935-1939. doi: 10.1111/jth.12672.
4. Vyas S, Enockson C, Hernandez L, Valentino LA. Towards personalizing haemophilia care: using the Haemophilia Severity Score to assess 178 patients in a single institution. Haemophilia. 2014 Jan;20(1):9-14. doi: 10.1111/hae.12227.
5. Sanchez NA, Brissia L. Gene therapy for hemophilia: current status and future perspectives. GSC Adv Res Rev. 2023;15(03):295-303. doi: 10.30574/gscarr.2023.15.3.0192.
6. Franchini M, Mannucci PM. Past, present and future of hemophilia: a narrative review. Orphanet J Rare Dis. 2012;7:24. doi: 10.1186/1750-1172-7-24.
7. Konkle BA, Huston H, Nakaya Fletcher S. Hemophilia A. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle: University of Washington; 1993 [cited 2024 Jul 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1404/.
8. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med. 2007;357(6):535-544. doi: 10.1056/NEJMoa067659.
9. Poonnoose P, Keshava S, Gibikote S, Feldman BM. Outcome assessment and limitations. Haemophilia. 2012 Jul;18 Suppl 4:125-30. doi: 10.1111/j.1365-2516.2012.02837.x.
10. Berntorp E, Shapiro A. Modern haemophilia care. Lancet. 2012;379(9824):1447-1456. doi: 10.1016/S0140-6736(11)61139-2.
11. Mahlangu J, Powell JS, Ragni MV, et al. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. Blood. 2014;123(3):317-325. doi: 10.1182/blood-2013-10-529974.
12. Coppola A, Morfini M, Cimino E, et al. Current and evolving features in the clinical management of haemophilia. Blood Transfus. 2014 Apr;12(Suppl 3):s554-s562. doi: 10.2450/2014.0043-14s.
13. Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear follow-up of AAV5-hFVIII gene therapy for hemophilia A. N Engl J Med. 2020;382(1):29-40. doi: 10.1056/NEJMoa1908490.
14. Poonnoose PM, Hilliard P, Doria AS, et al. Correlating clinical and radiological assessment of joints in haemophilia: results of a cross sectional study. Haemophilia. 2016 Nov;22(6):925-933. doi: 10.1111/hae.13023.
15. Powell JS, Pasi KJ, Ragni MV, et al. Phase 3 study of recombinant factor IX Fc fusion protein in hemophilia B. N Engl J Med. 2013 Dec 12;369(24):2313-2323. doi: 10.1056/NEJMoa1305074.
16. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays in the testing of factor VIII activity. J Thromb Haemost. 2016;14(2):248-261. doi: 10.1111/jth.13215.
17. Shima M, Hanabusa H, Taki M, et al. Factor VIII-mimetic function of humanized bispecific antibody in hemophilia A. N Engl J Med. 2016;374(21):2044-2053. doi: 10.1056/NEJMoa1511769.
18. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Haemophilia. 2013;19(1):e1-e47. doi: 10.1111/j.1365-2516.2012.02909.x.
19. Vagrecha A, Stanco J, Ulus D, Acharya S. Real-world experience using emicizumab prophylaxis for hemophilia A. Blood. 2020;136(Suppl 1):36. doi: 10.1182/blood-2020-139568.

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RESEARCH ARTICLE

The pathogenetic intersection between axial spondylitis and inflammatory bowel diseases: prevalence, risk factors, and clinical implications

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ABSTRACT

Introduction. Axial spondylitis is a chronic inflammatory disease primarily affecting the axial skeleton but can also involve peripheral joints. Axial spondylitis is often associated with extra-articular manifestations, such as inflammatory bowel diseases, emphasizing the need for rigorous monitoring and personalized therapeutic approaches. The interactions between axial spondylitis and inflammatory bowel diseases fall under the concept of "immune-mediated inflammatory diseases", sharing common pathogenetic mechanisms. This study analyzes the prevalence and characteristics of inflammatory bowel diseases in patients with axial spondylitis.

Objective. The objective of this study was to describe the baseline characteristics of patients with axial spondylitis, evaluate the prevalence of inflammatory bowel diseases in this population, and identify correlations between the two conditions, contributing to a better understanding of their pathogenetic and clinical interactions.

Material and methods. This prospective observational study included 257 axial spondylitis patients followed over two years. Patients were selected according to ASAS criteria for axial spondylitis and clinical guidelines for inflammatory bowel diseases. Analyses included clinical evaluations, laboratory tests, and imaging studies. Data were processed using SPSS v22.0. Continuous variables were expressed as mean ± standard deviation or median and interquartile range, and categorical variables as percentages. Correlations were assessed using Spearman's coefficient, with results considered significant at $p<0.05$.

Results. Among the 257 patients included (168 men and 89 women, mean age 48.2 ± 13.1 years), 13.2% were recently diagnosed with axial spondylitis. Of these, 5.1% had inflammatory bowel diseases, distributed as follows: Crohn's disease (3.1%), ulcerative colitis (1.2%), and indeterminate colitis (0.8%). In 53.8% of cases, the diagnosis of inflammatory bowel diseases preceded axial spondylitis. Multivariate analysis identified the absence of a family history of axial spondylitis as a significant risk factor for inflammatory bowel diseases (OR = 3.4; $p = 0.025$). The prevalence of inflammatory bowel diseases increased with axial spondylitis duration, reaching 6.5% in patients with disease progression over eight years.

Conclusions. The study highlights a high prevalence of inflammatory bowel diseases in axial spondylitis patients, indicating the need for rigorous clinical monitoring. The absence of a family history of axial spondylitis was identified as a major risk factor for inflammatory bowel diseases. These findings emphasize the importance of a multidisciplinary clinical approach, including active screening for inflammatory bowel diseases and collaboration between rheumatologists and gastroenterologists, to improve patient prognosis and quality of life.

Keywords: axial spondylitis, inflammatory bowel disease, prevalent, risk factors.

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Key messages

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

The exact pathogenic mechanisms linking axial spondylitis and inflammatory bowel disease, particularly their shared cytokine path-

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ways and environmental triggers, remain incompletely understood. Additionally, the role of genetic predisposition and the impact of early inflammatory bowel disease management on long-term outcomes in axial spondylitis patients warrant further investigation.

The research hypothesis

Inflammatory bowel disease is more prevalent in patients with ankylosing spondylitis than in the general population, with the absence of a family history of axial spondylitis being a significant predictive factor.

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

The manuscript adds novelty by highlighting the significant prevalence of inflammatory bowel disease in patients with axial spondylitis, emphasizing the temporal relationship between the diagnoses, with inflammatory bowel disease often preceding axial spondylitis. Additionally, it identifies the absence of a family history of axial spondylitis as a novel risk factor for inflammatory bowel disease, offering new insights into their shared pathogenesis and clinical management.

Introduction

Axial spondylitis (AxS) is part of a group of chronic inflammatory rheumatic diseases that predominantly affect the axial skeleton but may also involve peripheral joints at various stages of disease progression [1, 2]. The prevalence of these conditions is estimated to be approximately 1.5–2% of the general population [1, 3]. AxS is often associated with extra-articular manifestations, such as inflammatory bowel diseases (IBD), along with cardiovascular and renal complications, underscoring the need for rigorous monitoring and personalized therapeutic approaches [2, 4].

From a pathogenetic perspective, AxS is characterized by cytokine dysregulation, similar to that observed in other inflammatory diseases, including IBD [1, 4-6]. While IBD can appear as an associated manifestation of AxS, it may also evolve independently, indicating a complex pathogenetic relationship between the two conditions. These connections fall under the umbrella term "immune-mediated inflammatory diseases" (IMID), reflecting their shared immune mechanisms. Genetic and environmental factors play a central role in the onset and progression of these diseases [3, 5, 7].

Identifying associations between AxS and IBD is crucial for optimizing patient treatment and monitoring. Our prospective observational study, conducted over two years, investigated three cohorts of IMID patients (AxS, IBD, and other associated conditions) to evaluate the prevalence of these conditions.

The aim of this study was to describe the baseline characteristics of patients with AxS and IBD, evaluate the prevalence of IBD within the AxS cohort, and identify potential correlations between these two conditions. This work offers valuable insights into the relationship between these two pathological entities.

Material and methods

The study was designed as prospective observational research with two independent cohorts of patients: one with AxS and the other with IBD. Patients were selected based on their primary diagnosis established at the time of study inclusion. The study was conducted at the clinical base of the Rheumatology and Nephrology Discipline of the Internal Medicine Department at the *Nicolae Testemițanu* University of Medicine and Pharmacy, involving two main clinical specialties: rheumatology and gastroenterology.

Patient enrollment was carried out according to specific inclusion and exclusion criteria. Inclusion criteria: patients aged ≥18 years; diagnosed with AxS (including ankylosing spondylitis, enteropathic arthritis, or undifferentiated spondyloarthritis) or IBD; without other autoimmune inflammatory diseases that could interfere with the primary diagnosis; written informed consent for study participation. Exclusion criteria: patients with multiple IMID diagnoses managed by different specialists, preventing the establishment of a clear primary diagnosis; lack of continuous monitoring by the same specialist during the study.

Patients were recruited from the rheumatology and gastroenterology outpatient clinics of the participating centers and were followed up for two years by the same specialist who enrolled them in the study. Monitoring included clinical evaluations, laboratory tests, and relevant imaging investigations.

Cohort distribution: the AxS cohort comprised patients diagnosed with axial spondylitis or other forms of spondyloarthritis according to ASAS criteria. The IBD cohort included patients with diagnoses of Crohn's disease or ulcerative colitis established per current clinical guidelines. Data were centralized to evaluate interactions between AxS and IBD. Demographic (Table 1) and clinical data were collected for each cohort, ensuring group comparability in terms of age, sex, and disease duration.

The data were processed using SPSS v22.0 statistical software. Continuous variables were expressed as mean ± standard deviation (M ± SD) or median and interquartile range (IQR), while categorical variables were expressed as

percentages. For cohort comparisons, Student's t-test or Mann-Whitney test was used for continuous variables, and Chi-square or Fisher's exact test for categorical variables. Correlations were evaluated using Spearman's coefficient. Results were considered significant at $p<0.05$.

Table 1. Regional distribution of AxS patients in the Republic of Moldova

Region	AxS patients (n = 257)	Percentage (%)
Southern Districts	115	44.7%
- Cahul	53	20.6%
- Taraclia	34	13.2%
- Ciadâr-Lunga	28	10.9%
Northern Districts	51	19.8%
- Edineț	21	8.2%
- Drochia	18	7.0%
- Dondușeni	12	4.7%
Other Regions	91	35.4%

***Note**: The data presented in the table were analyzed using descriptive statistical methods. The absolute number of patients (n) and corresponding percentages (%) were calculated for each region relative to the total AxS cohort (n = 257). The percentages reflect the proportional representation of patients from different geographic districts. Abbreviations:AxS – axial spondylitis.*

This cohort structure and rigorous analytical approach enable a detailed investigation of the relationships between AxS and IBD, contributing to a deeper understanding of these immune-mediated inflammatory diseases. The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (No.5 from 03.03.2020).

Results

Baseline characteristics of patients. The study included 257 patients with AxS, of whom 168 (65.4%) were male and 89 (34.6%) were female. The mean age was 48.2 ± 13.1 years. Only 34 patients (13.2%) were newly diagnosed, while the majority (86.8%) had a known diagnosis of AxS. The median disease duration was 9.1 years (IQR 25–75: 3.5–16.8).

The baseline diagnoses for AxS were as follows: ankylosing spondylitis: 70.1% (n = 180); undifferentiated spondylarthritis: 25.5% (n = 66); enteropathic arthritis: 4.4% (n = 11). 11.0% of patients exhibited extra-articular manifestations associated with AxS (other than IBD), including: conjunctivitis: 5.5% (n = 14); cystitis: 2.5% (n = 6); nail hyperkeratosis: 3.0% (n = 8). Less common manifestations (<1%) included pulmonary fibrosis, aortic insufficiency, and renal amyloidosis.

The family history of AxS was reported in 18.7% (n = 48) of patients. The most frequent comorbidities were depression – 7.8% (n = 20); anemia – 5.1% (n = 13); cardiovascular risk factors – obesity – 21.2% (n = 54); smoking – 27.0% (n = 69); arterial hypertension – 22.8% (n = 58); hypercholesterolemia – 15.7% (n = 40); diabetes mellitus – 5.9% (n = 15).

Frequency of Extra-Articular Immune-Mediated Inflammatory Diseases (IMID). In the cohort of patients with AxS, 112 patients exhibited at least one of these extra-articular manifestations, with a prevalence of 43.6% (95% CI: 37.6–49.6). The most frequent extra-articular manifestation was inflammatory bowel disease (IBD), diagnosed in 13 patients, corresponding to a prevalence of 5.1% (95% CI: 2.8–8.6). Among these, the distribution was as follows: Crohn's disease: 8 patients (3.1%, 95% CI: 1.3–6.1); ulcerative colitis: 3 patients (1.2%, 95% CI: 0.3–3.4); indeterminate colitis: 2 patients (0.8%, 95% CI: 0.1–2.8).

In 7 cases (2.7%, 95% CI: 1.1–5.6), patients exhibited two concurrent inflammatory diseases in addition to AxS.

The prevalence of extra-articular inflammatory manifestations by primary diagnoses is detailed in Table 2. Among patients with axial spondylitis, inflammatory bowel disease was the most frequent extra-articular manifestation, with a prevalence of 3.9% (95% CI: 1.7–7.5). Additionally, all patients with enteropathic arthritis exhibited a form of IBD (100%).

Table 2. Prevalence of most common IBD among different AxS type

Category	Total (n = 257)	Ankylosing spondylitis (n = 180)	Enteropathic arthritis (n = 11)	Undifferentiated AxS (n = 66)
At least one, n (%)	112 (43.6%)	47 (26.1%)	11 (100%)	18 (27.3%)
IBD (any), n (%)	13 (5.1%)	7 (3.9%)	11 (100%)	2 (3.0%)
Crohn's disease, n (%)	8 (3.1%)	4 (2.2%)	7 (63.6%)	1 (1.5%)
Ulcerative colitis, n (%)	3 (1.2%)	1 (0.6%)	3 (27.3%)	0 (0.0%)
Indeterminate colitis, n (%)	2 (0.8%)	2 (1.1%)	1 (9.1%)	0 (0.0%)

***Note**: Patients with other diagnoses were excluded. Data are presented as numbers (n) and percentages (%). Statistical tests used include frequency analysis and Chi-square tests for categorical data. Abbreviations: IBD – inflammatory bowel disease; AxS – axial spondylitis.*

These data underscore the importance of monitoring inflammatory bowel disease in AxS patients, considering its frequency, especially among those with enteropathic arthritis.

Diagnosis chronology and prevalence of inflammatory bowel disease in axial spondylitis. According to the reviewed data, the diagnosis chronology for IBD and AxS is shown in Table 3. In 50% of IBD cases, the diagnosis preceded AxS, with a median of 10 years prior. In another 40% of cases, IBD was diagnosed after AxS, with a median of 9.5 years later. This temporal relationship suggests that IBD may precede or coexist with AxS, emphasizing the need for rigorous monitoring of AxS patients for early detection of IBD.

Table 3. Temporal relationship between AxS and IBD diagnosis

Temporal sequence relative to diagnosis	Before, n (%)	Same year, n (%)	After, n (%)
IBD (n = 13)	7 (53.8%)	1 (7.7%)	5 (38.5%)
Median, years (IQR 25–75)	-10.0 years		9.5 years

***Note**: Data are presented as numbers (n) and percentages (%) or median and interquartile range (IQR). Statistical tests applied include descriptive analysis of temporal distribution. Abbreviations: IBD – inflammatory bowel disease; AxS – axial spondylitis.*

IBD prevalence in different patient subgroups. The study demonstrated that the overall prevalence of IBD in the cohort of AxS patients was 5.1%. Subgroup analysis revealed the following (Table 4):

- The prevalence of IBD was similar between men (5.4%) and women (5.6%), with no statistically significant differences.
- No significant differences were observed between patients with previously known or newly diagnosed AxS regarding IBD prevalence.
- Patients with a family history of AxS had a lower prevalence of IBD (2.4%) compared to those without a family history (7.8%, p = 0.048).
- The prevalence of IBD increased with AxS disease duration: 3.9% in patients with a duration of less than 4 years, 5.1% between 4 and 8 years, and 6.5% in those with a duration of over 8 years.

Table 4. Prevalence of IBD by AxS patient subgroups

Category	IBD prevalence (%)	p
Sex		
Men (n = 168)	5.4%	0.834
Women (n = 89)	5.6%	
AxS diagnosis		
Known (n = 224)	5.8%	0.257
Recent (n = 33)	2.7%	
Family history of AxS		
Yes (n = 48)	2.4%	0.048
No (n = 209)	7.8%	
AxS disease duration		
<4 years (n = 89)	3.9%	0.223
4–8 years (n = 61)	5.1%	
>8 years (n = 107)	6.5%	

***Note**: Data are presented as percentages (%) and include statistical comparisons using Fisher's exact tests (p values). Abbreviations: IBD – inflammatory bowel disease; AxS – axial spondylitis.*

These findings highlight the importance of monitoring inflammatory bowel disease in AxS patients, particularly in those with a longer disease duration and no family history, indicating a potential interaction between these inflammatory conditions.

Multivariable analysis: correlation of variables with the presence of IBD in AxS. To assess variables associated with the presence of IBD in patients with AxS, a multivariable analysis was conducted (Table 5). The analysis included the following variables: age, sex, diagnosis (new or known), family history of AxS, disease duration (<4 years, 4–8 years, ≥8 years), and the presence of other extra-articular manifestations associated with AxS.

The results of the multivariable model demonstrated that IBD prevalence was significantly influenced by the absence of a family history of AxS (OR = 3.4; p = 0.025), suggesting a reduced genetic predisposition for these associated inflammatory conditions.

Beyond family history, other extra-articular manifestations associated with AxS (such as conjunctivitis, balanitis, or nail hyperkeratosis) contributed significantly to the prevalence of IBD. These findings underscore the importance of rigorous monitoring for early detection of IBD, particularly in AxS patients without a family history or typical extra-articular manifestations.

Table 5. Multivariable analysis of factors associated with the presence of IBD

Variable	OR (95% CI)	p
IBD		
Absence of a family history of AxS	3.4 (1.0–15.5)	0.025
Age (per year increase)	Not relevant	Not calculated
Sex (female vs. male)	Not relevant	Not calculated
Disease duration (<4 years vs. ≥8 years)	Not relevant	Not calculated

***Note**: Data are presented as odds ratios (OR) with 95% confidence intervals (CI). Statistical significance (p values) was determined using multivariable logistic regression analysis. Abbreviations: IBD – inflammatory bowel disease; AxS – axial spondylitis.*

These observations complement the previously presented data, confirming that IBD may occur independently of AxS family history and that disease duration does not significantly influence its prevalence. As such, screening for IBD should be included as part of the standard monitoring protocol for AxS patients, regardless of the time since diagnosis.

Discussion

The results of this study demonstrate that a significant proportion of patients with AxS also present with other inflammatory conditions, among which IBD was identified at a prevalence higher than expected in the general population. In our cohort, ankylosing spondylitis was the most frequent form of AxS (65.4%) [2, 5, 8, 9], followed by undifferentiated spondylarthritis (28.8%) and enteropathic arthritis (5.8%) [3-5, 8-10]. This distribution aligns with findings from other studies conducted in hospital-based rheumatology consultations, suggesting that this cohort reflects the profile of patients seen in routine clinical practice [3, 7, 11].

The prevalence of IBD (6.2%) observed in this study is significantly higher than that expected in the general population, estimated at approximately 0.2–0.3%. The most common form was Crohn's disease (3.9%) [1-3], followed by ulcerative colitis (1.9%) [2, 6, 9] and indeterminate colitis (0.4%) [3, 6, 12]. Notably, IBD diagnosis preceded AxS diagnosis in approximately 50% of cases, with a median lead time of 10 years, suggesting a potential pathogenic link between these conditions. In another 40% of cases, IBD was diagnosed after AxS, underscoring the need for monitoring gastrointestinal symptoms in these patients [3-7].

Multivariable analysis revealed that the absence of a family history of AxS is a significant risk factor for developing IBD (OR = 3.4; p = 0.025) [5, 8, 13]. Other variables, such as sex or AxS disease duration, did not significantly influence this model. This suggests that IBD can emerge as a manifestation independent of the genetic mechanisms associated with AxS. Additionally, the presence of other extra-articular manifestations, such as conjunctivitis or nail hyperkeratosis, was associated with an increased prevalence of IBD, indicating a more severe clinical expression of AxS in these patients [2-5, 14, 15].

This study has several important limitations. The data were collected through patient interviews and medical record reviews without incorporating additional diagnostic investigations for active IBD detection. Consequently, some

cases might have been overlooked, leading to an underestimation of the true prevalence [3-5]. Furthermore, patients were recruited from a hospital setting, which might limit the generalizability of these findings to the broader AxS population, as hospital-treated patients tend to have more severe or complex disease forms. Additionally, the predominantly consecutive rather than random inclusion of patients introduces a potential selection bias.

Conclusions

Our study highlights a high prevalence of inflammatory bowel disease in patients with AxS and its association with other extra-articular manifestations. These findings underscore the necessity of an integrated clinical approach for AxS patients, including the monitoring of gastrointestinal symptoms and multidisciplinary collaboration with gastroenterologists. Early identification and management of IBD may contribute to improving patient quality of life and optimizing therapeutic outcomes.

Competing interests

None declared.

Patient consent

Obtained.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (No.5 from 03.03.2020).

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References

1. Karreman MC, Luime JJ, Hazes JMW, Weel AEAM. The prevalence and incidence of axial and peripheral spondyloarthritis in inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis. 2017 May 1;11(5):631-42. doi: 10.1093/ecco-jcc/jjw199.
2. Ossum AM, Palm Ø, Cvancarova M, Bernklev T, Jahnsen J, Moum B, et al. The impact of spondyloarthritis and joint symptoms on health-related quality of life and fatigue in IBD patients. Results from a population-based inception cohort (20-year follow-up in the Ibsen study). Inflamm Bowel Dis. 2020;26(1):114-24. doi: 10.1093/ibd/izz105.
3. Ossum AM, Palm Ø, Cvancarova M, Solberg IC, Vatn M, Moum B, et al. Peripheral arthritis in patients with long-term inflammatory bowel disease. Results from 20 years of follow-up in the IBSEN study. Scand J Gastroenterol. 2018 Nov 2;53(10-11):1250-6. doi: 10.1080/00365521.2018.1518482.
4. Luchetti MM, Benfaremo D, Ciccia F, Bolognini L, Ciferri M, Farinelli A, et al. Adalimumab efficacy in enteropathic spondyloarthritis: a 12-mo observational multidisciplinary study. World J Gastroenterol. 2017. Oct 21;23(39):7139-49. doi: 10.3748/wjg.v23.i39.7139.
5. Kopylov U, Starr M, Watts C, Dionne S, Girardin M, Seidman EG. Detection of crohn disease in patients with spondyloarthropathy: the SpACE Capsule Study. J Rheumatol. 2018 Apr;45(4):498-505. doi: 10.3899/jrheum.161216.
6. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018 Feb;11(1):1-10. doi: 10.1007/s12328-017-0813-5.
7. Gill T, Asquith M, Brooks SR, Rosenbaum JT, Colbert RA. Effects of HLA-B27 on gut microbiota in experimental spondyloarthritis implicate an ecological model of dysbiosis. Arthritis Rheumatol. 2018;70(4):555-65. doi: 10.1002/art.40405.
8. Asquith M, Sternes PR, Costello ME, Karstens L, Diamond S, Martin TM, et al. HLA alleles associated with risk of ankylosing spondylitis and rheumatoid arthritis influence the gut microbiome. Arthritis Rheumatol. 2019;71(10):1642-50. doi: 10.1002/art.40917.
9. Angelidou I, Chrysanthopoulou A, Mitsios A, Arelaki S, Arampatzioglou A, Kambas K, et al. REDD1/autophagy pathway is associated with neutrophil-driven IL-1β inflammatory response in active ulcerative colitis. J Immunol. 2018 Jun 15;200(12):3950-61. doi: 10.4049/jimmunol.1701643.
10. Di Carlo M, Luchetti MM, Benfaremo D, Di Donato E, Mosca P, Maltoni S, et al. The DETection of Arthritis in Inflammatory boweL diseases (DETAIL) questionnaire: development and preliminary testing of a new tool to screen patients with inflammatory bowel disease for the presence of spondyloarthritis. Clin Rheumatol. 2018;37(4):1037-44. doi: 10.1007/s10067-017-3937-6.
11. Variola A, Zanolin ME, Cipriano G, Macchioni P, Martinis F, Pasetti A, et al. The IBIS-Q [IBd Identification of Spondyloarthritis Questionnaire]: a novel tool to detect both axial and peripheral arthritis in inflammatory bowel disease patients. J Crohn's Colitis. 2020;14(12):1680-6. doi: 10.1093/ecco-jcc/jjaa096.
12. Felice C, Leccese P, Scudeller L, Lubrano E, Cantini F, Castiglione F, et al. Red flags for appropriate referral to the gastroenterologist and the rheumatologist of patients with inflammatory bowel disease and spondyloarthritis. Clin Exp Immunol. 2019 Apr 9;196(1):123-38. doi: 10.1111/cei.13246.
13. Østgård RD, Deleuran BW, Dam MY, Hansen IT, Jurik AG, Glerup H. Faecal calprotectin detects subclinical bowel inflammation and may predict treatment response in spondyloarthritis. Scand J Rheumatol. 2018 Jan 2;47(1):48-55. doi: 10.1080/03009742.2017.1299216.
14. Fauny M, D'Amico F, Bonovas S, Netter P, Danese S, Loeuille D, et al. Faecal calprotectin for the diagnosis of bowel inflammation in patients with rheumatological diseases: a systematic review. J Crohns Colitis. 2020 Jun 19;14(5):688-93. doi: 10.1093/ecco-jcc/jjz205.
15. Ward MM, Deodhar A, Gensler LS, Dubreuil M, Yu D, Khan MA, et al. 2019 update of the American College of Rheumatology / Spondylitis Association of America / Spondyloarthritis Research and Treatment Network recommendations for the treatment of ankylosing spondylitis and nonradiographic axial spondyloarthritis. Arthritis Care Res (Hoboken). 2019;71(10):1285-99. doi: 10.1002/acr.24025.

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RESEARCH ARTICLE

Immunogenetic profiling of HLA antigens in psoriatic arthritis: insights into clinical variability

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ABSTRACT

Introduction. Psoriatic arthritis (PsA) is a complex autoimmune disease with genetic and immunological components influencing its pathogenesis. HLA antigens are critical in determining genetic predisposition and clinical variability. This study aims to explore HLA antigen diversity in PsA patients and its relationship to clinical variants.

Material and methods. A cohort of 103 PsA patients, diagnosed according to CASPAR (2006) criteria, was studied. Patients were received treatment in rheumatology departments from 2005–2024. Two groups were formed: 76 patients with PsA and cutaneous psoriasis (Group I) and 27 without cutaneous manifestations (Group II). Each group was further subdivided into clinical variants: axial, oligoarticular, polyarticular, distal interphalangeal, and mutilans.

Results. Significant correlations were identified between HLA antigens and PsA severity. Aggressive HLA antigens, including HLA-B27, B8, and B62, were associated with severe disease forms and high DAPSA scores (≥50), while protective antigens like HLA-A2 and A3 correlated with reduced activity (DAPSA <20). Group I exhibited HLA-B27/B62 and HLA-B27/A3 combinations linked to mixed articular and cutaneous involvement, whereas Group II had distinct profiles (e.g., HLA-B27/B62, HLA-B27/B11). Factorial analysis highlighted the immunogenetic variability between clinical subtypes, emphasizing HLA antigens' predictive and therapeutic relevance.

Conclusions. HLA antigens significantly influence PsA severity and clinical diversity. Integrating genetic profiling into clinical practice offers promising opportunities for improving diagnostic precision, therapeutic outcomes, and patient quality of life.

Keywords: psoriatic arthritis, HLA, clinical variant.

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Key messages

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

The specific associations between HLA antigens and the clinical variants of psoriatic arthritis (PsA), particularly in patients with and without cutaneous psoriasis, remain insufficiently understood. Additionally, the role of these antigens in influencing disease activity and progression, as measured by DAPSA, needs further clarification to enhance personalized treatment.

The research hypothesis

Specific HLA antigens are significantly associated with distinct clinical variants of PsA, influencing disease severity, activity (measured by DAPSA), and the presence or absence of cutaneous psoriasis.

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

The manuscript highlights the unique immunogenetic profiles of

HLA antigens in PsA, demonstrating their distinct associations with clinical variants and disease activity. It provides novel insights into the differences between PsA with and without cutaneous psoriasis, emphasizing the potential for HLA antigen profiling to guide personalized treatment strategies.

Introduction

Psoriatic arthritis (PsA) is a complex condition, with its pathogenesis involving both genetic and immunological factors. HLA antigens play a crucial role in determining genetic predisposition and influencing the severity and clinical variability of the disease [1]. Studies have shown that certain HLA alleles, such as HLA-B27, B8, B11, and B62, are associated with severe forms of PsA, while others, like HLA-A2, A3, and A5, may have a protective effect [2, 3].

Despite progress in identifying the role of these antigens, their specific relationship with various clinical variants of the disease remains incompletely understood. Investigating HLA antigens in patients with PsA in the context of clinical diversity can provide valuable insights for early diagnosis, risk stratification, and personalized treatment [1, 4, 5]. In the Republic of Moldova, such studies are rare but essential for gaining a deeper understanding of the genetic impact on this pathology and for tailoring therapeutic interventions to the specific needs of the local population.

The aim of the study is to evaluate the diversity of HLA antigens in patients with psoriatic arthritis, depending on the clinical variants of the disease.

Material and methods

To achieve the study's aim and objectives, a cohort of 103 patients diagnosed with PsA according to the CASPAR criteria (2006) was selected. The patients received treatment in the rheumatology and arthrology departments of *Timofei Moşneaga* Republican Clinical Hospital and *Holy Trinity* Municipal Clinical Hospital in Chişinău during the period 2005–2024. Inclusion criteria: confirmed diagnosis of PsA according to CASPAR criteria (2006); availability of relevant clinical and laboratory data; absence of other inflammatory rheumatic diseases (such as rheumatoid arthritis or systemic lupus erythematosus); informed consent obtained from patients for participation in the study. The study was approved by the Research Ethics Committee of *Nicolae Testemiţanu* State University of Medicine and Pharmacy (No.21 from 21.12.2019).

To investigate the relationships between the clinical-evolutionary variants of PsA and genetic factors, participants were divided into two groups. Group I: 76 patients with PsA associated with cutaneous psoriasis; group II: 27 patients with PsA without cutaneous manifestations.

To minimize selection bias, patients were allocated into comparable groups based on age, sex, disease duration, and arthritis severity. The demographic and clinical variables distribution showed no statistically significant differences ($p > 0.05$). The mean age of patients in Groups I and II was comparable (48.7±10.5 vs. 46.9±11.2, $p > 0.05$), as was the mean PsA duration (7.3±3.1 vs. 7.1±2.7 years, $p > 0.05$). Disease severity was evaluated using the DAPSA (Disease Activity in Psoriatic Arthritis) score, and patients were stratified into categories of low (<20), moderate (20–50), and high activity (>50).

Patients were further subdivided into five subgroups based on the clinical variant of the disease:

- Group I: oligoarticular – 13 patients; polyarticular – 23 patients; distal interphalangeal – 11 patients; axial – 21 patients; mutilans – 8 patients.
- Group II: oligoarticular – 5 patients; distal interphalangeal – 4 patients; polyarticular –: 9 patients; axial – 5 patients; mutilans – 4 patients.

HLA antigen determination was performed using standard molecular typing techniques via PCR-SSP (polymerase chain reaction with sequence-specific primers). Imaging investigations included joint ultrasound and MRI to identify structural changes.

This structured grouping and the application of rigorous investigative methods enable a detailed analysis of the relationship between HLA antigens and clinical variants of PsA, providing valuable insights for early diagnosis and risk stratification.

The statistical analysis was performed using Microsoft Excel, Statistica 9.0, and EpiInfo version 5. Descriptive statistics were applied, with mean±standard deviation (M±SD) for normally distributed variables and median (Me) with interquartile range (25%; 75%) for non-normal distributions. The Student's T-test was used for parametric data, and the Mann-Whitney U test for non-parametric data. Non-parametric correlations were assessed using Spearman's method. The significance of differences in HLA antigens and disease risk measures was determined using the X^2 test, Fisher's exact test, and odds ratio (OR), with results considered significant at $p<0.05$.

Results

Factorial analysis revealed significant correlations between HLA antigens identified in patients with PsA and cutaneous psoriasis ($p = 0.00043$), except for HLA-B32 and HLA-B62 antigens ($p>0.05$). The antigens HLA-B27/A2 (CI+95% = 217.7), HLA-B27 (CI+95% = 187.5), HLA-B27/A3 (CI+95% = 176.8), and HLA-A2/A3 (CI+95% = 140.5) were common to both subgroups, leading to similar osteoarticular impairments without influencing the distinct evolution of the disease (Figure 1).

In the group of patients with PsA associated with cutaneous psoriasis, HLA-A2/A5 (CI+95% = 193.5), HLA-B62/B8 (CI+95% = 211.3), HLA-B27/B8 (CI+95% = 207.1), and HLA-B27/32 (CI+95% = 188.2) antigens were associated with pronounced systemic involvement. Conversely, in patients without cutaneous psoriasis, HLA-B27/62 (CI+95% =

223.6) and HLA-B27/11 (CI+95% = 215.6) antigens showed a high prevalence, reflecting a distinct immunological profile.

These results confirm the presence of specific genetic HLA characteristics in PsA, with variations among clinical subgroups. They underscore the importance of these antigens in determining the disease's severity and systemic manifestations.

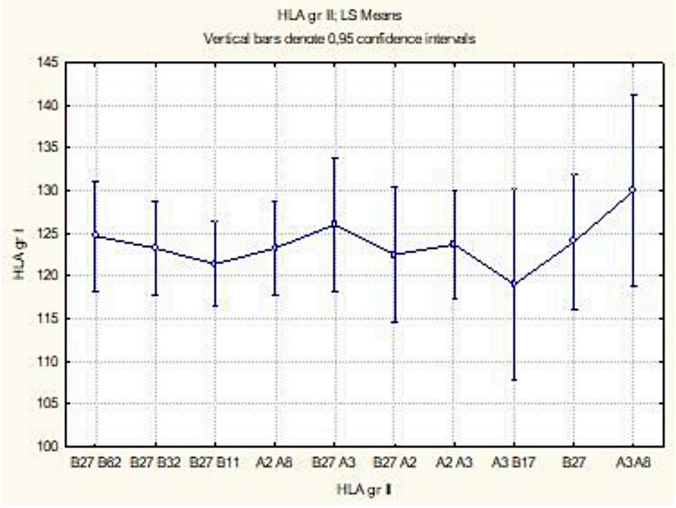

Fig. 1 Factorial correlation of HLA antigens among patient groups with PsA associated with cutaneous psoriasis (Group I) and without cutaneous psoriasis (Group II), p > 0.05.

***Note**: HLA – human leukocyte antigen; PsA – psoriatic arthritis*

The associative analysis using ANOVA revealed significant correlations between the clinical variants of PsA and HLA antigen sets in patients with cutaneous psoriasis (p<0.01).

For the axial variant, HLA-B27, HLA-B27/A2, and HLA-B27/A3 antigens were predominant, reflecting their involvement in inflammatory mechanisms of the spine and sacroiliac joints. In the distal interphalangeal variant, HLA-A2/A3, HLA-A2/A5, and HLA-A2/A8 antigens were associated with relatively benign involvement of small joints. The oligoarticular variant was correlated with HLA-B27/A3, HLA-A2/A5, and HLA-A3/A8, indicating a slower and less aggressive progression.

The polyarticular variant showed an association with HLA-B27/B8, HLA-B27/B62, and HLA-B27/B11, which were frequently observed in patients with systemic involvement and rapid progression. The most severe form, the mutilans variant, was dominantly associated with HLA-B27/B8, indicating a genetic risk for extensive joint destruction.

These findings underscore the immunogenetic role of HLA antigens in the clinical variability of PsA, offering perspectives for personalized diagnostic and therapeutic strategies. The relationships are detailed in Figure 2, which highlights the antigen-specific differences for each clinical subtype.

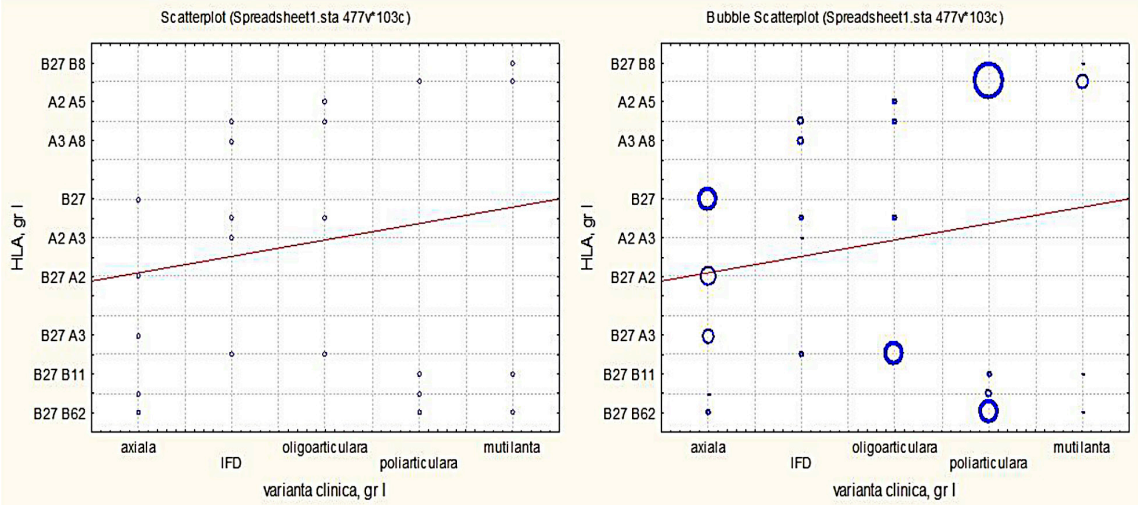

Fig. 2 The associative correlation of HLA antigens with clinical variants of PsA in patients with PsA associated with cutaneous psoriasis, p < 0.01.

***Note**: HLA – human leukocyte antigen; PsA – psoriatic arthritis*

The analysis of HLA antigens in the group of patients with PsA without cutaneous psoriasis revealed a general pattern similar to that observed in the group with cutaneous psoriasis, but with significant differences. The HLA-B27 antigen showed a uniform distribution across all clinical variants, unlike its predominant association with the axial form in the group with skin involvement. This suggests a more generalized genetic role of HLA-B27 in the pathogenesis of PsA without cutaneous manifestations.

Furthermore, an increased similarity was observed between the distal interphalangeal and oligoarticular variants, both associated with HLA-B27/A3 and HLA-A3/A8 alleles (for the distal interphalangeal form) and HLA-B27/A3 and HLA-A2/A3 (for the oligoarticular form). This antigenic

overlap indicates common pathogenic mechanisms, despite differing clinical manifestations between these forms.

Figure 3 illustrates the distribution of HLA antigens, highlighting the relatively reduced specificity of HLA-B27 for the axial form and the antigenic similarity between the distal interphalangeal and oligoarticular forms. These observations suggest that the distribution of HLA antigens influences the clinical characteristics of PsA depending on the presence or absence of cutaneous psoriasis.

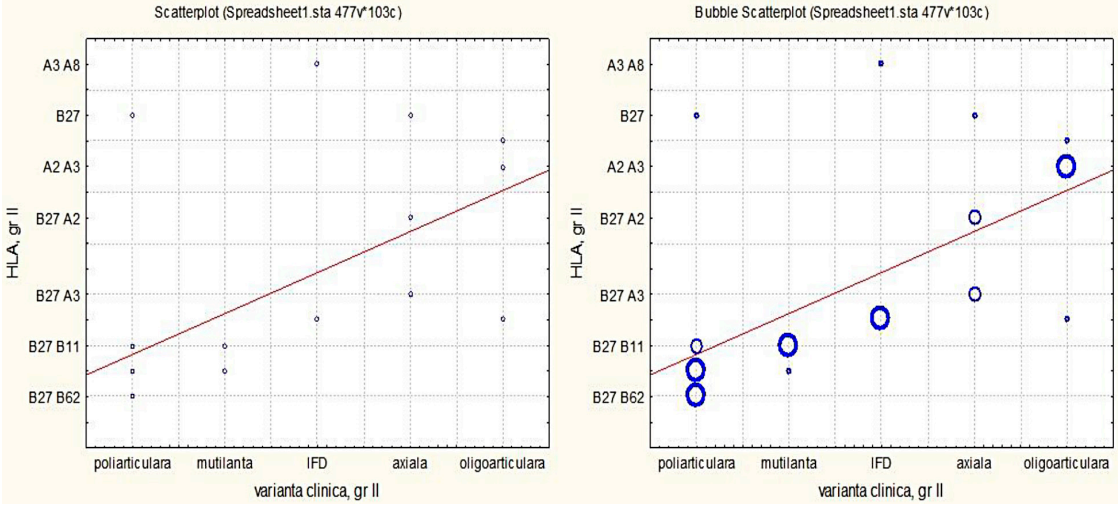

Fig. 3 Associative correlation of HLA antigens with clinical variants of PsA in patients with PsA without cutaneous psoriasis, $p < 0.01$.

***Note**: HLA – human leukocyte antigen; PsA – psoriatic arthritis*

The association of HLA antigens between the polyarticular and mutilans variants of PsA suggests a direct evolutionary link between these forms. In both studied groups, HLA-B27/B8, HLA-B27/B62, and HLA-B27/B11 were strongly correlated with these clinical variants, supporting the hypothesis that the mutilans form represents a final stage of aggressive polyarthritis. These findings align with the literature, which identifies these antigens as predictive markers of disease severity and progression.

For the distal interphalangeal, oligoarticular, and axial forms, although antigenic similarities exist, specific associations for each variant were identified. Antigens such as HLA-A2, HLA-A3, HLA-A5, HLA-A8, and HLA-B27, along with various combinations among them, were frequently observed, highlighting the differentiated role of genetic components in clinical manifestations.

The data in Table 1 illustrate the specificity of HLA antigens for each clinical variant of PsA, emphasizing the potential of HLA profiling in risk stratification and personalized treatment. Particularly for polyarticular and mutilans forms, early identification of these genetic associations can guide aggressive therapeutic interventions, preventing progression to severe disabilities.

The study revealed significant correlations between HLA antigens and disease severity, as assessed by the DAPSA score (Table 2). In patients with PsA associated with cutaneous psoriasis, HLA-B27/B8, HLA-B27/B62, and HLA-B27/B11 antigens were linked to high DAPSA scores (≥50), indicating severe and progressive clinical forms. Conversely, HLA-A2/A5 and HLA-A3/B17 antigens were associated with moderate DAPSA scores (30-40), suggesting less aggressive forms. These findings confirm the impact of HLA antigens on disease activity and highlight their utility in risk stratification and guiding personalized treatment strategies.

Table 1. Associations of HLA antigens with clinical variants of PsA in patients with PsA associated with cutaneous psoriasis (group I) and without cutaneous psoriasis (group II)

Clinical variant	General group	Group I	Group II
Axial	HLA-B27 HLA-B27/A2 HLA-B27/A3	HLA-B27 HLA-B27/A2 HLA-B27/A3	HLA-B27/A2 HLA-B27/A3
Distal interphalangeal	HLA-A2/A3 HLA-A2/A5 HLA-A2/A8 HLA-B27/A3 HLA-A3/A8	HLA-A2/A3 HLA-A2/A5 HLA-A2/A8	HLA-B27/A3 HLA-A3/A8
Oligoarticular	HLA-B27/A3 HLA-A2/A5 HLA-A3/A8 HLA-A2/A3	HLA-B27/A3 HLA-A2/A5 HLA-A3/A8	HLA-B27/A3 HLA-A2/A3
Polyarticular	HLA-B27/B8 HLA-B27/B62 HLA-B27B11	HLA-B27/B8 HLA-B27/B62 HLA-B27B11	HLA-B27/B62 HLA-B27/B11
Mutilans	HLA-B27/B8 HLA-B27/B11	HLA-B27/B8	HLA-B27/B11

Note: HLA – human leukocyte antigen; PsA – psoriatic arthritis

The research revealed significant correlations between HLA antigen variants and the clinical activity of PsA, as measured by the DAPSA score. The isolated HLA-B27 antigen was associated with DAPSA scores ranging from 25 to

35 (p<0.0001), reflecting moderate disease activity. However, when HLA-B27 was combined with antigens such as HLA-B32, HLA-B62, HLA-B11, and HLA-B8, it resulted in DAPSA scores of ≥50, indicating severe forms of the disease with pronounced articular inflammation. Conversely, combinations of HLA-B27 with HLA-A2 or HLA-A3 correlated with DAPSA scores below 20 (p<0.05), suggesting a protective effect.

Table 2. Factorial correlation of HLA antigens in patients with PsA associated with cutaneous psoriasis (group I) and without psoriasis (group II) with DAPSA scores

	DAPSA ≥25 and <35	DAPSA ≥35
Group I	HLA-B27/B32; HLA-A3/A8	HLA-B27/B62; HLA-B27/B11; HLA-B27/B8; HLA-B62/B8
Group II	HLA-B27; HLA-A3/A8	HLA-B27/B62; HLA-B27/B32; HLA-B27/B11

***Note**: HLA – human leukocyte antigen; PsA – psoriatic arthritis; DAPSA – disease activity index for psoriatic arthritis*

In patients with PsA without cutaneous psoriasis, the results were similar. However, the influence of HLA-B27 was more variable across clinical forms, and the protective effects of combinations with HLA-A2 and HLA-A3 were less pronounced. These findings underscore the critical role of HLA antigens in determining disease severity and influencing evolutionary risk, providing a foundation for personalized patient evaluation.

The study also highlighted significant correlations between other HLA antigens and PsA activity, measured by the DAPSA score. Antigens from other categories were associated with DAPSA scores between 20 and 30 (p<0.0001), reflecting moderate disease activity. HLA-B27 stood out due to its association with high DAPSA scores, emphasizing its role in amplifying inflammation and disease severity. In contrast, HLA-A2 and HLA-A3 demonstrated a significant protective effect (p<0.01), contributing to reduced clinical activity.

HLA-A2 was identified as the most protective antigen (CI+95% = 302.8), followed by HLA-A5 (CI+95% = 243.6), HLA-A3 (CI+95% = 214.7), and HLA-A8 (CI+95% = 126.1). These findings highlight the importance of HLA antigen profiling in assessing risk and disease progression, offering a basis for personalized therapeutic interventions.

Discussions

PsA is a complex autoimmune disease influenced by genetic predisposition and environmental factors, which significantly contribute to its clinical variability [1, 5, 6]. Our research, consistent with previous studies, has demonstrated that HLA antigens play a pivotal role in disease severity and clinical phenotypes, emphasizing their potential as biomarkers for risk assessment and personalized treatment strategies [7-9]. Aggressive HLA antigens, such as HLA-B27, B8, B11, B32, and B62, were frequently associated with severe forms of the disease characterized by intense inflammation and extensive systemic involvement. In contrast, protective antigens like HLA-A2, A3, A5, and A8 correlated with reduced inflammatory activity and minimal joint damage.

Our analyses revealed significant differences between patients with cutaneous psoriasis and those without skin manifestations. Among patients with cutaneous psoriasis, HLA-B27/B62 and HLA-B27/A3 combinations were frequently identified and linked to mixed articular and cutaneous involvement. On the other hand, patients without cutaneous psoriasis exhibited distinct combinations, such as HLA-B27/B62 and HLA-B27/B11, suggesting a specific genetic profile for this subgroup. Factorial analysis highlighted HLA-B27/B8 and HLA-B27/B62 as key markers for severe PsA forms with DAPSA scores ≥50, while combinations of HLA-B27 with HLA-A2 and HLA-A3 demonstrated protective effects, being associated with lower DAPSA scores (<20).

The distribution of HLA antigens across clinical subtypes provides valuable insights into the disease's pathogenesis. For instance, HLA-B27 antigens predominated in the axial variant, underscoring their role in vertebral and sacroiliac joint inflammation. Other clinical variants, such as the polyarticular and mutilating forms, were marked by the presence of HLA-B27/B8 and HLA-B27/B11, indicating an increased risk for aggressive and destructive progression. HLA-A2 and HLA-A3 antigens were associated with less severe forms, suggesting potential protective mechanisms [6, 9-11].

These findings underscore the importance of HLA antigens as predictive tools and guides for personalized treatment in PsA. Integrating these results into clinical practice could significantly enhance diagnosis, monitoring, and disease management, reducing both long-term complications and the therapeutic burden on patients [2, 5, 12-14]. Furthermore, the application of modern technologies, such as artificial intelligence and machine learning algorithms, may enable more precise risk stratification and efficient therapeutic interventions, paving the way for improved patient outcomes in PsA management [7, 15, 16].

Conclusions

The study highlights the role of HLA antigens in the pathogenesis and severity of PsA, demonstrating significant correlations between aggressive HLA antigens (HLA-B27, B8, B11, B32, B62) and severe disease forms, as well as protective antigens (HLA-A2, A3, A5, A8) and less aggressive forms. Antigenic differences between patients with and without cutaneous psoriasis suggest distinct immunogenetic mechanisms, reinforcing the need for genetic profiling for precise risk stratification.

The association of HLA antigens with disease activity, measured by the DAPSA score, validates the use of these markers for personalized treatments and early interventions. These findings emphasize the importance of integrating advanced technologies into clinical practice to enhance patient prognosis and quality of life.

Competing interests

None declared.

Patient consent

Obtained.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemiţanu* State University of Medicine and Pharmacy (No.21 from 21.12.2019).

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References

1. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. Lancet. 2021;397:1301-1315. doi: 10.1016/S0140-6736(20)32549-6.
2. Dand N, Mahil SK, Capon F, et al. Psoriasis and genetics. Acta Derm Venereol. 2020;100(3):adv00030. doi: 10.2340/00015555-3384.
3. Ogawa K, Okada Y. The current landscape of psoriasis genetics in 2020. J Dermatol Sci. 2020;99(1):2-8. doi: 10.1016/j.jdermsci.2020.05.008.
4. Lønnberg AS, Skov L, Skytthe A, et al. Heritability of psoriasis in a large twin sample. Br J Dermatol. 2013;169(2):412-416. doi: 10.1111/bjd.12375.
5. Singh S, Pradhan D, Puri P, et al. Genomic alterations driving psoriasis pathogenesis. Gene. 2019;683:61-71. doi: 10.1016/j.gene.2018.09.042.
6. Gudjónsson JE, Kárason A, Runarsdottir EH, et al. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients – an analysis of 1019 HLA-C- and HLA-B-typed patients. J Invest Dermatol. 2006;126(4):740-745. doi: 10.1038/sj.jid.5700118.
7. Mak RK, Hundhausen C, Nestle FO. Progress in understanding the immunopathogenesis of psoriasis. Actas Dermo-sifiliogr. 2009;100(Suppl 2):2-13. doi: 10.1016/s0001-7310(09)73372-1.
8. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009;361:496-509. doi: 10.1056/NEJMra0804595.
9. Gudjonsson JE, Karason A, Antonsdottir A, et al. Psoriasis patients who are homozygous for the HLA-Cw*0602 allele have a 2.5-fold increased risk of developing psoriasis compared with Cw6 heterozygotes. Br J Dermatol. 2003;148(2):233-235. doi: 10.1046/j.1365-2133.2003.05115.x.
10. Mallon E, Bunce M, Savoie H, et al. HLA-C and guttate psoriasis. Br J Dermatol. 2000;143(6):1177-1182. doi: 10.1046/j.1365-2133.2000.03885.x.
11. Szczerkowska-Dobosz A, Rebała K, Szczerkowska Z, Nedoszytko B. HLA-C locus alleles distribution in patients from northern Poland with psoriatic arthritis – preliminary report. Int J Immunogenet. 2005;32(6):389-391. doi: 10.1111/j.1744-313X.2005.00543.x.
12. Ho PY, Barton A, Worthington J, et al. HLA-Cw6 and HLA-DRB1*07 together are associated with less severe joint disease in psoriatic arthritis. Ann Rheum Dis. 2007;66(6):807-811. doi: 10.1136/ard.2006.064972.
13. Gudjónsson JE, Kárason A, Antonsdóttir AA, et al. HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. J Invest Dermatol. 2002;118(2):362-365. doi: 10.1046/j.0022-202x.2001.01656.x.
14. Jin Y, Zhang F, Yang S, et al. Combined effects of HLA-Cw6, body mass index and waist-hip ratio on psoriasis vulgaris in Chinese Han population. J Dermatol Sci. 2008;52(2):123-129. doi: 10.1016/j.jdermsci.2008.04.016.
15. Eder L, Abji F, Rosen CF, et al. The association of HLA-class I genes and the extent of atherosclerotic plaques in patients with psoriatic disease. J Rheumatol. 2016;43(10):1844-1851. doi: 10.3899/jrheum.151469.
16. Li K, Huang CC, Randazzo B, et al. HLA-C*06:02 allele and response to IL-12/23 inhibition: results from the ustekinumab phase 3 psoriasis program. J Invest Dermatol. 2016;136(12):2364-2371. doi: 10.1016/j.jid.2016.06.631.

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RESEARCH ARTICLE

The anatomical variability of the superficial circumflex iliac artery

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ABSTRACT

Introduction. The superficial circumflex iliac artery (SCIA) is a branch that originates either from the external iliac artery (EIA), or common femoral artery (CFA). Its anatomic variability is particularly relevant in plastic surgery, general surgery, and traumatology/orthopedics.

Material and methods. We retrospectively reviewed 2158 ultrasonographic images of the anterior thigh region from the Republican Medical Diagnostic Center, Functional Diagnosis Department, Chisinau, Republic of Moldova. Rigorous inclusion and exclusion criteria were applied. Additionally, 29 bibliographic sources were reviewed and discussed.

Results. The mean age of the patients was 63.4±10.68 years. In 387 cases (17.93%),SCIA originated from the CFA, while in 1771 cases (82.07%) it emerged from the EIA. SCIA originated from the CFA unilaterally in 194 cases (8.99%) on the left side and 142 cases (6.58%) on the right side, while bilateral origin from the CFA was observed in 51 cases (2.36%)

Discussion. The results in literature were suggestive for a higher prevalence of SCIA origin from the CFA with only one author suggesting the origin of this branch from the EIA, which aligns with our findings. This information is valuable for clinical applications, including hernia repairs, vascular and endovascular surgical interventions, nerve blocks, and skin grafts transplantation.

Conclusions. The most common origin of the SCIA was from the EIA. No significant sex differences were observed, but laterality showed notable variations. Age was analyzed as a factor.

Keywords: artery, anatomical variability, superficial circumflex iliac artery.

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Key messages

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

The exact trajectory and variation in number of the superficial circumflex iliac artery after its origin from either the external iliac, or femoral artery are not yet known.

The research hypothesis

The most common origin of the superficial circumflex iliac artery is from the common femoral artery, as reported in the literature.

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

Information on gender, laterality, and origin is unavailable for most arterial branches in the human body, this study aims to uncover these particularities for the population of the Republic of Moldova.

Introduction

The origin of the superficial circumflex iliac artery (SCIA) is from the external iliac artery (EIA) [1], or from the common femoral artery (CFA) [2]. Sometimes it may come as a branch of the superficial femoral artery (SFA), or profound femoral artery (PFA) [3, 4]. After originating, this artery will give 2 branches: superficial branch (SB), and profound branch (PB) [2], the first will be responsible for the antero-lateral abdominal wall blood supply, and the second will descend down to the sartorius muscle [2, 3]. It may have a variable diameter [1], and sometimes perforant branches [3].

A lack of knowledge in surgical anatomy along with the anatomical variability of certain vessels like the deep circumflex iliac artery (DCIA) may lead to severe surgical complications [5]. Skin flap reconstruction surgery is of great interest when it comes to the SCIA [6-8], and it is proven to be reliable in terms of blood supply [2]. Bone grafts for the surgical repair of the defects in the iliac bone and fibula can be regarded as a SCIA flap [9]. The anatomical variability of this artery should be considered in procedures that involve a femoral nerve (FN) block [10], and when predicting lymphedema patterns [11]. Surgical interventions in the groin and thigh regions have implications that regard the anatomical variability of this artery [12].

The anatomical variability of the neighboring structures like the FN, and DCIA may complicate the accurate identification of the SCIA [13, 14]. This necessitates advanced knowledge for the precise determination of these structures.

Vascular compression syndromes like May-Thurner syndrome [15] may co-interest the SCIA in an unexpected way because the arterial circulation in this region may be highly dependent on the vessels like CFA, EIA, and their branches that could in turn lead to the pathological alterations in the ipsilateral lower extremity [16].

The access to the femoral arterial system in the thigh region is made through a transverse incision, or in the groin region with a vertical incision for vascular, and endovascular surgical interventions [17], thus making the SCIA vulnerable to potential lesions in both cases.

The goal of this study was to determine the anatomical variability of the SCIA depending on the origin, gender, and laterality.

Material and methods

Statistical considerations. We retrospectively reviewed 2158 Doppler ultrasonography images of the anterior thigh region from the Republican Medical Diagnostic Center, Functional Diagnosis Department, Chisinau, Republic of Moldova. These images were collected between 01.01.2020-31.12.2022. The randomized selection of the patients was not required, as all available cases were included in the study. The study population consisted of 885 females (41.01%) and 1,273 males (58.99%). We have calculated the mean age of the patients, the absolute, and relative values of the incidence in anatomical variability based on the origin, gender, and laterality.

Inclusion and exclusion criteria. We included only patients that underwent imaging investigations on both legs and excluded duplicate cases (i.e., patients who had multiple serial investigations over time). All included cases were eligible for visualizing the tissues, vessels, and some nerves that were located nearby. No data was available on the antero-lateral abdominal wall, or lower parts of the thigh.

Equipment. The „GE Healthcare Vscan Air™" ultrasonografic device was used in order to perform these investigations. A single sensory device was of interest, operating in Doppler mode. No electronic applications were used, all the investigations were described manually.

Literature review. We have reviewed the available literature from PubMed, Google Scholar, HINARI, EMBASE, Elsevier, and Research Gate in order to find the most suitable research papers and have completed a comparative review in order to emphasize the significance of our findings. Overall, 28 literary sources were selected. Additionally, we have reviewed a book. A total of 29 bibliographic sources were referenced in this study.

Results

The mean age of the patients was 63.4±10.68 years. In 387 cases (17.93%), the SCIA originated from the CFA, while in 1771 cases (82.07%), it emerged from the EIA. Among the cases withorigin from the CFA,163 (7.55%) were female and 224 (10.38%) were male. For those with origin from the EIA were 722 (33.46%) were female, and 1049 (48.61%) were male. Thus, the most common origin of the SCIA was from the EIA.

Taking into consideration the gender, the SCIA originated from the CFA in 42.12% of female cases and in 57.88% of male cases. For the EIA origin, 40.77% of cases were female, and49.23% were male. Comparing these distributions, we conclude that there are no significant gender differences for the anatomical variability of the origin for this artery.

Based on laterality, the SCIA originated from the CFA in males as follows: 118 (5.5%) cases unilaterally on the left side, 82 (3.78%) cases unilaterally on the right side, and 24 (1.1%) cases bilaterally. In females, the origin from the CFA was observed in 76 (3.52%) cases unilaterally on the left side, 60 (2.78%) cases unilaterally on the right side, and 27 (1.25%) cases bilaterally. In total, there were 194 (8.99%) cases unilaterally on the left side, 142 (6.58%) cases unilaterally on the right side, and 51 (2.36%) cases bilaterally. The most common incidence of origin from the CFA was unilaterally on the left side.

Proportionally, males, the SCIA originated from the CFA unilaterally on the left side in 30.49% of cases, unilaterally on the right side 21.19% of cases on, and bilaterally in 6.2% of cases. In females the corresponding proportions were 19.64% on the left side, 15.50% on the right side, and 6.98% bilaterally. When considering proportions within each gender separately, in males 52.68% of cases were unilateral on the left side, 36.61% were unilateral on the right side, and 10.71% were bilaterally. In females 46.63% were unilateral on the left side, 36.81% were unilateral on the right side, and 16.56% were bilateral. The bilateral origin of the SCIA

from the CFA is more common in the females compared to males, while the most frequent pattern in both genders was a unilateral origin on the left side.

Age was not quantified as a factor that may influence the anatomical variability of SCIA. There were no variations that regarded supranumerary arteries, or trunks from which these arteries may emerge. Arterio-venous fistulas along with many other anomalies were considered as a potential bias factor but we have not identified any in our study. Atherosclerosis, thrombosis, thromboembolism, arterial dysplasia, and calcifications were impending factors in some cases. The SCIA had a diameter that may be affected by these conditions and yet remain visible in the Doppler ultrasonography sections.

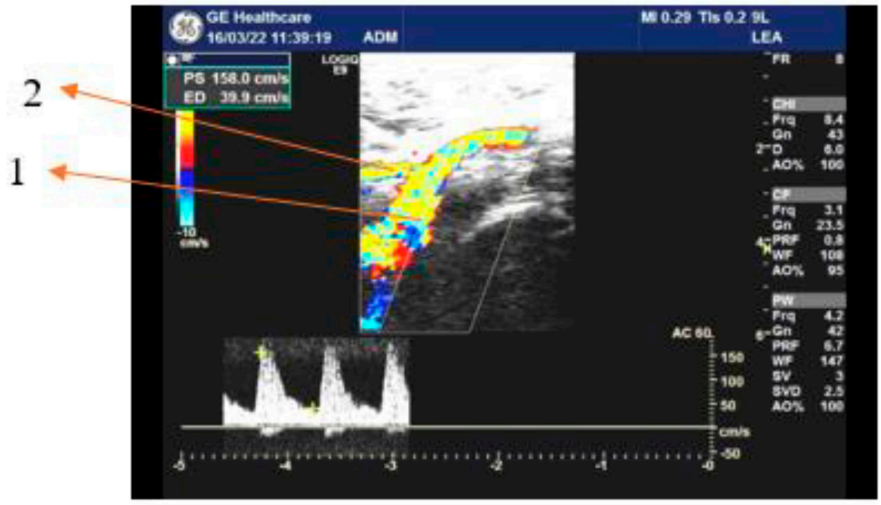

Fig. 1 Left upper thigh anterior Doppler ultrasonography

1 – common femoral artery; 2 – superficial circumflex iliac artery

Figure 1 shows the ultrasonographic image of a 72-year-old patient with the SCIA originating from the CFA. The emergence was directly from the main arterial trunk without a common origin with another artery, or a second artery of the same kind. The SCIA on the right upper thigh of the same patient had an identical origin. No pathological conditions were detected in this patient.

Discussion

According to Gandolfi *et al.* (2020), and Song *et al.* (2020) the most frequent origin of the SCIA was from the CFA [3, 18]. In our study the most common origin was from the EIA, just as in the reports of Kovanov V. V. (1974) [1].

There were no significant gender differences in the proportionality of this artery's origin while the laterality may play a key role, the bilateral origin of the SCIA from the CFA is more common in females, and in both genders the left unilateral type is more frequent rather than the right unilateral, or bilateral. No previous studies or literature sources were available form comparison with these findings.

This information is of great interest during potential groin, and thigh hernia repairs (direct, and indirect inguinal, or femoral) [12], femoral artery incisions [17], or for flap reconstruction surgical interventions that may involve bone grafting [9].

A study conducted in Denmark [19] has proven that a laparoscopic approach reduces the incidence of recurrences in femoral hernias, and also there is a higher mortality in the emergency femoral hernia repairs [20], thus an origin of the SCIA from the EIA may pose a risk for these interventions, especially due to the fact that it is highly prevalent (82.07%) compared to the origin from the CFA (17.93%).

Flap reconstruction surgeries are essential for revascularizing traumatized regions, and portions of the skin affected by burns [21, 22]. Our findings may be of great interest when identifying the origin of the SCIA in order to apply a ligature, although the SCIA is not involved in the collateral circulation of its anatomical region [23] while the DCIA is involved in this compensatory mechanism [24].

The vertical incision may cause increased morbidity due to an increased incidence of SCIA lesion, the same was stated in a study made by Caiati *et al.* in 2000 where the oblique incision is proposed for the purpose of reducing this burden [25] while the transverse incision is not proven to reduce the morbidity compared to the vertical one [17].

Other implications like truncal blocks [10], and the prediction of post-surgical complications is also of great interest in this anatomical variability [12], thus this study may provide useful data for further assessment of these patients because the identification of the FN, or DCIA can become even more difficult after we have reported these anatomical variations [13, 14].

Vascular compression syndromes may be of interest in this context, because they may induce alternations in the SCIA via chronic ischemia, and excessive continuous collateral circulation for this particular anatomical region [15, 16].

The higher prevalence of bilateral SCIA origin from the CFA in females may be of particular interest in the May-Thurner syndrome, which predominantly affects women, especially in their second or third decade as a result of multifactorial risk exposure [16, 26]. This syndrome is also treated using endovascular interventions that require further knowledge of the groin, and thigh regional anatomy [27].

There were no literature reports that regarded the aneurysms, or pseudoaneurysms of the SCIA but there were some that were located in the DCIA during diagnostic maneuvers, both iatrogenic, and idiopathic [28, 29], thus we may hypothesize that SCIA could be affected as well due to their similar morphological macroscopic, and microscopic aspect.

We were unable to measure the internal diameter of the SCIA. The trajectory of both superficial and deep branches was not assessed. The visualization of the perforasomes was not feasible. We could not have compared the topography of this artery in relation to other branches in the same anatomical region.

Conclusions

In our study the most common origin of the superficial circumflex iliac artery was from the external iliac artery (EIA). There was no significant difference between genders in the overall origin of the SCIA. However, a bilateral origin from the common femoral artery (CFA) was more frequently observed in females, while the most common pattern in both genders was a unilateral origin on the left side. Age was not assessed as a factor that determined the anatomical variability.

Competing interest

None declared.

Contribution of authors

DC designed the study, collected the data and analyzed it, VN revised the results critically and evaluated their applicability, SV revised the manuscript critically, SC revised the manuscript critically.

Ethics approval

No approval was required for this study.

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Bibliography

1. Kovanov VV, Anikina TI. Khirurgicheskaia anatomiia arterii cheloveka [Surgical anatomy of human arteries]. Moscow: Meditsina; 1974. 359 p. Russian.
2. Zubler C, Haberthür D, Hlushchuk R, Djonov V, Constantinescu M, Olariu R. The anatomical reliability of the superficial circumflex iliac artery perforator (SCIP) flap. Ann Anat. 2021;234:1-9. doi: 10.1016/j.aanat.2020.151624.
3. Gandolfi S, Postel F, Auquit-Auckbur I, Boissière F, Pelissier P, Casoli V, Duparc F. Vascularization of the superficial circumflex iliac perforator flap (SCIP flap): an anatomical study. Surg Radiol Anat. 2020;42(4):473-481. doi: 10.1007/s00276-019-02402-9.
4. Yoshimatsu H, Steinbacher J, Meng S, Hamscha U, Weninger W, Tinhofer I, Harima M, Fuse Y, Yamamoto T, Tzou C. Superficial circumflex iliac artery perforator flap: an anatomical study of the correlation of the superficial and the deep branches of the artery and evaluation of perfusion from the deep branch to the sartorius muscle and the iliac bone. Plast Reconstr Surg. 2019;143(2):589-602. doi: 10.1097/PRS.0000000000005282.
5. Singh B, Gupta V, Gupta S. Pseudoaneurysm: a complication of laparoscopic inguinal hernia repair. Int J Surg Case Rep. 2019;54:39-41. doi: 10.1016/j.ijscr.2018.11.045.
6. Messa CA 4th, Carney MJ 3rd, Tantillo K, Othman S, Moores C, Mirzabeigi MN, Weissler JM, Cook T, Kovach SJ. Characteristics of the superficial circumflex iliac artery perforator flap in a western population and a practice approach for free flap reconstruction. J Reconstr Microsurg. 2021;37(6):486-491. doi: 10.1055/s-0040-1719051.
7. Hong JP. The superficial circumflex iliac artery perforator flap in lower extremity reconstruction. Clin Plast Surg. 2021;48(2):225-233. doi: 10.1016/j.cps.2020.12.005.
8. Berner JE, Nikkhah D, Zhao J, Prousskaia E, Teo TC. The Versatility of the superficial circumflex iliac artery perforator flap: a single surgeon's 16-year experience for limb reconstruction and a systematic review. J Reconstr Microsurg. 2020;36(2):93-103. doi: 10.1055/s-0039-1695051.
9. Yoshimatsu H, Iida T, Yamamoto T, Hayashi A. Superficial circumflex iliac artery-based iliac bone flap transfer for reconstruction of bony defects. J Reconstr Microsurg. 2018;34(9):719-728. doi: 10.1055/s-0038-1651489.
10. Ogami K, Murata H, Sakai A, Sato S, Saiki K, Okamoto K, Manabe Y, Hara T, Tsurumoto T. Deep and superficial circumflex iliac arteries and their relationship to the ultrasound-guided femoral nerve block procedure: a cadaver study. Clin Anat. 2017;30(3):413-420. doi: 10.1002/ca.22852.
11. Campos JL, Suominen S, Pons G, Al-Sakkaf AM, Lusetti IL, Sirota M, Vela FJ, Pires L, Sánchez-Margallo FM, Abellán E, Masiá J. Lymphatic patterns in the superficial circumflex iliac artery perforator flap. J Reconstr Microsurg. 2024 Jul 9. doi: 10.1055/a-2340-9629. Epub ahead of print.
12. Brunetti B, Camilloni C, Putti A, Petrucci V, Pazzaglia M, Papalia R, Persichetti P. Use of superficial circumflex iliac artery perforator flap for soft tissues and lymphatic genital reconstruction after hidradenitis suppurativa resection. J Plast Reconstr Aesthet Surg. 2024;88:122-124. doi: 10.1016/j.bjps.2023.10.140.
13. Park JA, Lee SH, Koh KS, Song WC. Femoral nerve split with variant iliacus muscle: a potential source of femoral nerve entrapment. Surg Radiol Anat. 2020;42(10):1255-1257. doi: 10.1007/s00276-020-02502-x.
14. Sarna K, Amuti T, Butt F, Kamau M, Muriithi A. Variations of the anatomy and bony landmarks of deep circumflex iliac artery in a select Kenyan population. Craniomaxillofac Trauma Reconstr. 2020;13(4):300-304. doi: 10.1177/1943387520958333.
15. Farina R, Foti PV, Pennisi I, Vasile T, Clemenza M, Rosa G, Crimi L, Catalano M, Vacirca F, Basile A. Vascular com-

pression syndromes: a pictorial review. Ultrasonography. 2022;41(3):444-461. doi: 10.14366/usg.21233.
16. Poyyamoli S, Mehta P, Cherian M, Anand RR, Patil SB, Kalva S, Salazar G. May-Thurner syndrome. Cardiovasc Diagn Ther. 2021;11(5):1104-1111. doi: 10.21037/cdt.2020.03.07.
17. Canteras M, Baptista-Silva JC, do Carmo Novaes F, Cacione DG. Transverse versus vertical groin incision for femoral artery approach. Cochrane Database Syst Rev. 2020;4(4):1-32. doi: 10.1002/14651858.CD013153.pub2.
18. Song C, Bhogesha S, Chu S, Song C. Review of the superficial circumflex iliac artery perforator flap: recommendations to the approach of a groin perforator flap. Australas J Plast Surg. 2020;3(2):20-29. doi: 10.34239/ajops.v3n2.193.
19. Andresen K, Bisgaard T, Kehlet H, Wara P, Rosenberg J. Reoperation rates for laparoscopic vs open repair of femoral hernias in Denmark: a nationwide analysis. JAMA Surg. 2014;149(8):853-7. doi: 10.1001/jamasurg.2014.177.
20. Dahlstrand U, Wollert S, Nordin P, Sandblom G, Gunnarsson U. Emergency femoral hernia repair: a study based on a national register. Ann Surg. 2009;249(4):672-6. doi: 10.1097/SLA.0b013e31819ed943.
21. Hussain T, Khan FH, Rahman OU, Beg MSA. Superficial circumflex iliac artery free flap for coverage of hand injuries. Cureus. 2022;14(11):e31520. doi: 10.7759/cureus.31520.
22. Prasetyo AT, Hasibuan LY, Arsyad M. Pedicled abdominal skin flap technique for fingers salvaging and reconstruction in a complex palmar hand burn injury: a case report. Int J Surg Case Rep. 2024;114:109199. doi: 10.1016/j.ijscr.2023.109199.
23. Akinwande O, Ahmad A, Ahmad S, Coldwell D. Review of pelvic collateral pathways in aorto-iliac occlusive disease: demonstration by CT angiography. Acta Radiol. 2015;56(4):419-427. doi: 10.1177/0284185114528172.
24. Hardman RL, Lopera JE, Cardan RA, Trimmer CK, Josephs SC. Common and rare collateral pathways in aortoiliac occlusive disease: a pictorial essay. Am J Roentgenol. 2011;197(3):519-524. doi: 10.2214/AJR.10.5896.
25. Caiati JM, Kaplan D, Gitlitz D, Hollier LH, Marin ML. The value of the oblique groin incision for femoral artery access during endovascular procedures. Ann Vasc Surg. 2000;14(3):248-253. doi: 10.1007/s100169910042.
26. Mousa AY, AbuRahma AF. May-Thurner syndrome: update and review. Ann Vasc Surg. 2013;27(7):984-995. doi: 10.1016/j.avsg.2013.05.001.
27. Ibrahim W, Al Safran Z, Hasan H, Zeid WA. Endovascular management of may-thurner syndrome. Ann Vasc Dis. 2012;5(2):217-221. doi: 10.3400/avd.cr.12.00007.
28. Satija B, Kumar S, Duggal RK, Kohli S. Deep circumflex iliac artery pseudoaneurysm as a complication of paracentesis. J Clin Imaging Sci. 2012;2:1-4. doi: 10.4103/2156-7514.94022.
29. Miyagi K, Mulchandani M, Marks DJB, Mohamed M. Aneurysm of the deep circumflex iliac artery: a rare cause of rectus sheath haematoma. Eur J Vasc Endovasc Surg Extra. 2010;19(2):e19-e21. doi: 10.1016/j.ejvsextra.2009.11.002.

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RESEARCH ARTICLE

Morphological and histopathological characteristics of primary colon neoformations

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ABSTRACT

Introduction. Globally, colorectal cancer (CRC) has become one of the top three causes of death from neoplasms. CRC represents a heterogeneous group of tumors, manifested both by clinical signs and the pathogenesis of its development. Most colorectal carcinomas develop from preexisting adenomas. The aim of this article is to assess the histopathological aspects and variants of primary colon neoformations in correlation with their location and morphological characteristics.

Material and methods. Prospective clinical study based on the analysis of treatment outcomes in 255 patients with colonic and rectal neoformations, treated in the *Nicolae Anestiadi* Surgery Department No.1, Institute of Emergency Medicine, between 2018 and 2022. The mean age was 61.3±1.05 years. There were 145 (56.9%) men and 110 (43.1%) women, with a male to female ratio of 1.31:1. The following qualitative nominal variables were analyzed: location, number, dimensions, tumor appearance, histopathological type, and degree of tumor extension.

Results. Through imaging, colonoscopy, and intraoperative methods, 255 patients with colonic neoformations were identified. Upon analyzing these subjects, 77 (30.2%) patients were diagnosed with malignant neoplasm of the colon or rectum with various histological types, and 178 (69.8%) patients were diagnosed with precursor lesions of malignancy.

Conclusions. The detailed analysis of the morphopathological characteristics of the tumor formations, in addition to confirming the malignancy, provides important information for establishing the therapeutic attitude.

Keywords: colorectal cancer, polyps, dysplasia, colonic neoformation, morphology.

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Key messages

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

It is vital for endoscopists to identify high-risk polyps during colonoscopy to prevent colorectal cancer. This necessitates a more comprehensive description and histopathological analysis of these precursor lesions throughout the entire colon.

The research hypothesis

Since the incidence of carcinoma of the colon is on the rise and burgeoning evidence supports a polyp-cancer sequence, a vigorous program for endoscopic detection and excision of colorectal polyps will favorably influence the management of this disease.

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

This article has demonstrated that the detailed analysis of the morphopathological characteristics of tumor formations, in addition to confirming malignancy, provides important information for establishing therapeutic management.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies, being the second most common type of cancer in women and the third most common in men. Although many advances have been made in oncological and surgical treatment, and through the implementation of screening programs, CRC still remains first among malignant causes of death [1]. This condition is slowly progressive, developing over time. Most cases of CRC begin at the level of polyps in the epithelium of the colon and rectum, but there are other predisposing causes, such as inflammatory bowel diseases and certain hereditary genetic changes [2]. Approximately 80% of CRC cases have colorectal adenomatous polyps as a precursor [3], demonstrating the enormous benefit brought by screening patients for the detection and early removal of primary colonic neoformations (PCN). The terms" primary neoformations" and "incipient neoplasia" of the colon were formulated in 1983 by the Japanese Society for the Study of Colorectal Cancer as tumors of the colon that are limited to the mucosa and submucosa, without the presence of secondary lesions, local, or distant metastases *(Japanese Research Society for Cancer of the Colon and Rectum, 1983*) [4]. CRC occurs through the malignant and uncontrolled transformation of the cells of the lining mucosal epithelium of the rectum and colon. Most colorectal carcinomas develop from preexisting adenomas. This concept of carcinogenesis is called *the adenoma–carcinoma sequence*. Progression from adenoma to colorectal carcinoma occurs through successive genetic abnormalities and mutations, involving oncogenes and tumor suppressor genes [5, 6]. The evolution from normal colonic epithelium to adenoma and carcinoma extends over several years, providing a sufficiently long period during which, through the use of diagnostic methods, precancerous lesions can be detected [7, 8].

The aim of this article is to evaluate the histopathological aspects and variants of primary colon neoplasms in correlation with their location and morphological characteristics.

Material and methods

The research presents a prospective clinical study based on the analysis of the treatment results of 255 patients with colonic and rectal neoformations, treated in the *Nicolae Anestiadi* Surgery Department No.1, Institute of Emergency Medicine, during the period 2018–2022. The study was approved by the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, No. 52 dated 16.03.2018 and No. 7 dated 18.05.2022. The representative sample was calculated in the EpiInfo Program 7.2.2.6, "Stat Calc-Sample Size and Power" section, based on the following parameters: a confidence interval for 95% significance of the results, statistical power of 80%, the presence of colonic neoplasms in the population being 27% [Bray F. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries], and effect design = 4 (symptoms, laboratory paraclinical data, endoscopic examinations, and histological type of the formation). The calculated value is 216, with adjustment for the non-response rate, estimated at 10%.

The mean age was 61.3 ± 1.05 years. There were 145 (56.9%) men and 110 (43.1%) women, with a male to female ratio of 1.31:1. To conduct the study, the demographic data of the patients, operative protocols, endoscopic investigation protocols, histopathological reports, and multidisciplinary clinical, paraclinical, operative, and histopathological evaluations were collected and processed within the departments of Surgery, Endoscopy, and Pathological Anatomy. Regarding the morphopathological characteristics of colonic neoformations, the following qualitative nominal variables were analyzed: location, number, size, tumor appearance, histopathological type, and degree of tumor extension.

For the research, three groups were created based on the morphological results and the method of case management, with 58 respondents per group, in compliance with the study's inclusion and exclusion criteria:

- Group I – patients with colorectal polyps
- Group II – patients with colorectal cancer in early stages (stages I-II)
- Group III – patients with colorectal cancer in advanced stages (stages III-IV).

To ensure greater accuracy, a series of inclusion and exclusion criteria were applied, thereby refining the study and focusing on a specific representative group.

Inclusion criteria for the research group:

- Patients with colonic polypoid formations examined colonoscopically
- Individuals over 18 years of age
- Patients who provided informed consent to participate in the study
- Patients whose mental state allows participation in the study.

Exclusion criteria were:

- Patients hospitalized in emergency settings without endoscopic examination
- Patients with malignancies in other locations or a history of surgery for any type of cancer
- Patients with previously treated colorectal cancer (surgery, chemotherapy, or radiotherapy)
- Patients with immunological disorders
- Patients who declined participation in the study.

Statistical results were generated and processed using the R Studio program. The following descriptive statistics were estimated for numerical variables: minimum value, maximum value, mean value with standard deviation, and median value with interquartile range. For all statistical tests applied in the study, the significance threshold (p) was set at 0.05, complemented by 95% confidence intervals for relative frequencies.

Results

Through imaging, colonoscopy, and intraoperative methods, 255 patients with colonic neoformations were identified. Among these, 77 (30.2%) patients were diagnosed with malignant neoplasms of the colon or rectum, exhibiting various histological types, while 178 (69.8%) patients were diagnosed with precursor lesions of malignancy. The distribution of colonic neoformations by location is presented in Table 1.

Table 1. Distribution of colorectal neoplasms and premalignant lesions by location

Location	Right colon (abs, %)	Left colon (abs, %)	Rectum (abs, %)	Total (abs, %)
Premalignant lesions	52 (20.4%)	78 (30.6%)	48 (18.8%)	178 (69.8%)
Neoplasm	35 (13.7%)	26 (10.2%)	16 (6.3%)	77 (30.2%)
Total	87 (24.1%)	104 (40.8%)	64 (25.1%)	255 (100%)

Note: abs - absolute value in percentage

It was found that there are no significant differences in lesion distribution between patients with colorectal neoplasms and those with premalignant lesions ($p > 0.05$). The left colon has the highest proportion of premalignant lesions (30.6%), followed by the right colon (20.4%) and the rectum (18.8%). Overall, premalignant lesions are relatively evenly distributed among the three locations but predominate in the left colon, suggesting that this site may be more prone to such lesions. Neoplasms are more common in the right colon (13.7%) but have a lower incidence in the left colon (10.2%) and rectum (6.3%).

Regarding the endoscopic aspect, colorectal neoplasm presented in the following forms: vegetative formations accounted for 54.6% (n=42) of the 77 patients, vegetative-ulcerative tumor formations were observed in 24.6% (n=19), infiltrative formations with areas of ulceration were described endoscopically in 10.4% (n=8), while the classic infiltrative tumor aspect was detected in 8 patients (10.4%), as summarized in Figure 1.

Figure 2 shows endoscopic images that reflect the appearance of different forms of colorectal tumors, all of which require histopathological confirmation. The ulcerated type, the most common, appears as a circular mass with a raised, irregular, and externalized border. It is frequently large, occupying an extensive portion of the colonic circumference. The polypoid form presents as a large mass protruding into the lumen. In 10% of cases, it has a mucinous structure characteristic of colloid carcinoma. The annular or stenotic form occupies the entire colonic lumen, with variable extension along the longitudinal axis of the colon.

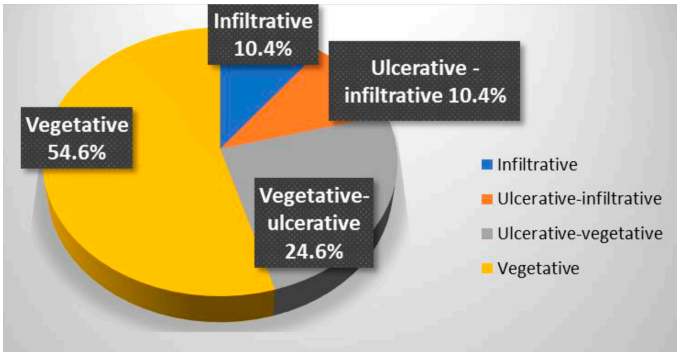

Fig. 1 Endoscopic appearance of colonic neoplasia in patients included in the study

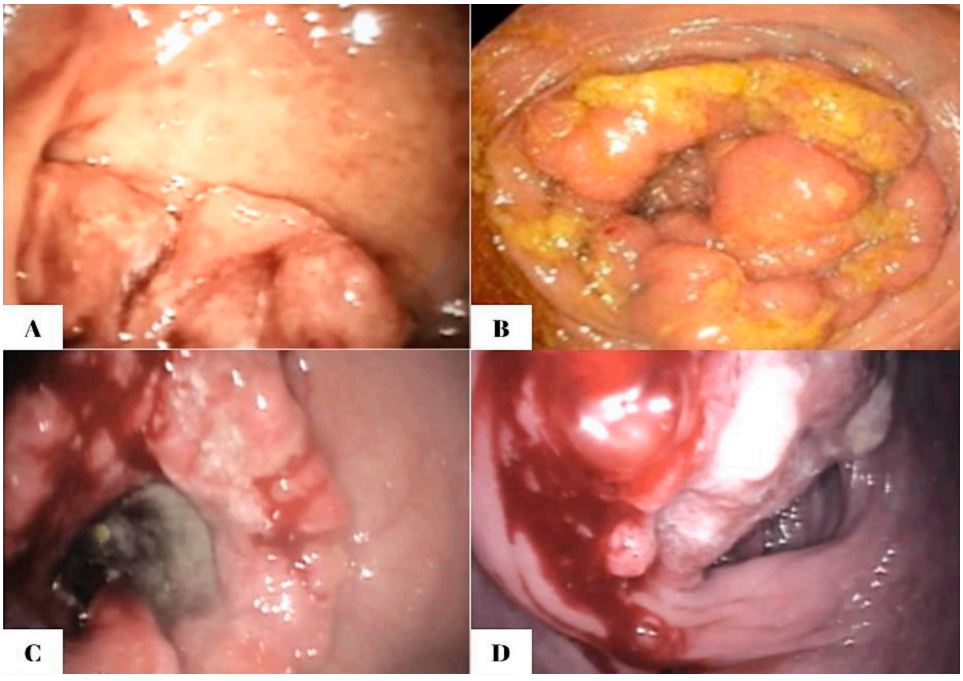

Fig. 2. Endoscopic images of colorectal tumors

A – vegetative formation; B – circumferential stenosing vegetative formation, impassable with the colonoscope; C – ulcer-infiltrative formation; D – vegetative formation covered by areas of necrosis.

In the present research, microscopic histological examination revealed that various variants of adenocarcinoma clearly dominate (100% of all identified tumors). Among them, conventional adenocarcinoma has the highest incidence, accounting for 87.1%. Table 2 presents the histological typology of colonic neoplasia.

Table 2. Histological typology of colonic neoplasia in CRC patients included in the study

Histological typology of the tumor	Cases (abs, %)
Conventional adenocarcinoma	67 (87.1%)
Mucinous adenocarcinoma	6 (7.8%)
Solid trabecular adenocarcinoma	4 (5.1%)
Total	77 (100%)

Note: abs - absolute value in percentage

The present study concluded that, out of the total 77 patients with colorectal neoplasia, 17 (22.1%) had a high degree of differentiation (G1), 51 (66.2%) had a moderate degree of differentiation (G2), and 9 (11.7%) had a low degree of differentiation (G3). Figure 3 illustrates the different morphopathological forms of colorectal neoplasia observed in the patients included in the study.

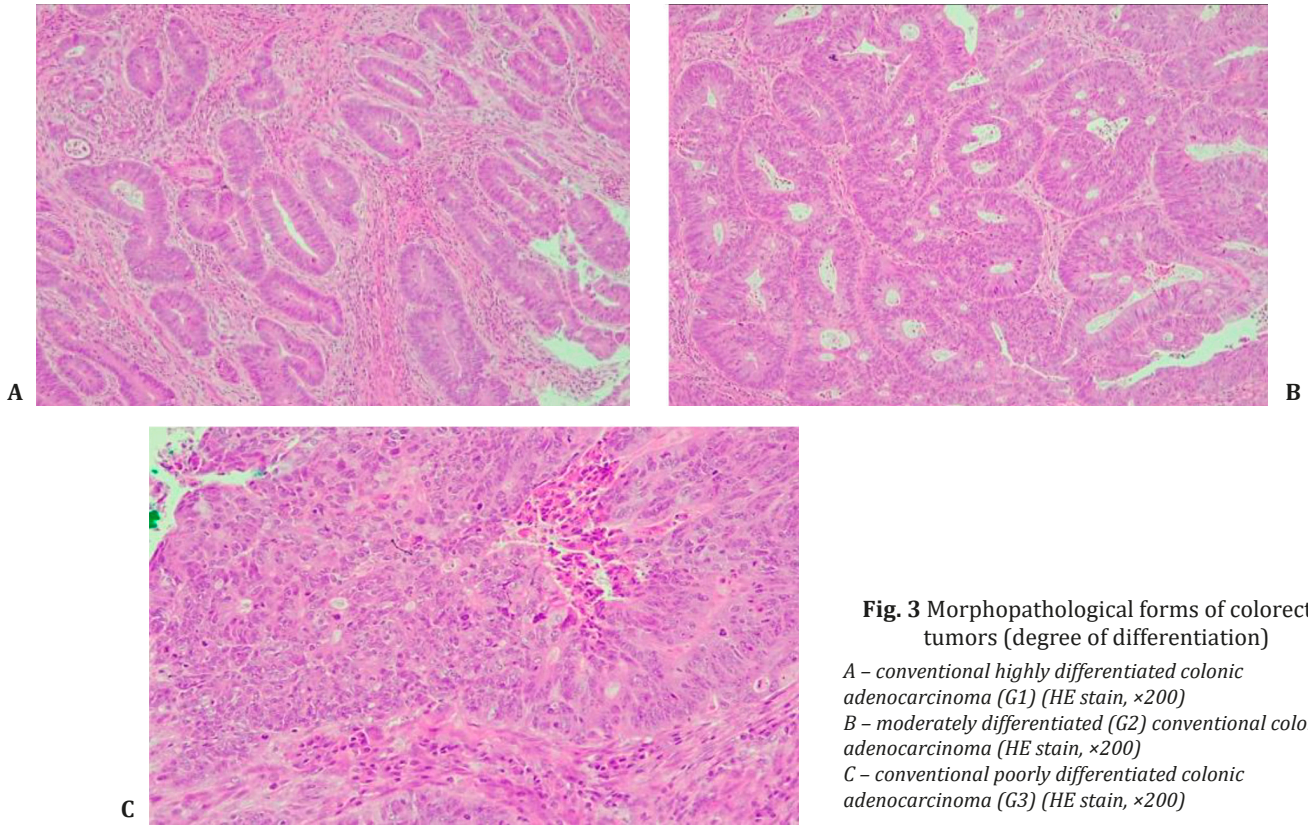

Fig. 3 Morphopathological forms of colorectal tumors (degree of differentiation)

A – conventional highly differentiated colonic adenocarcinoma (G1) (HE stain, ×200)
B – moderately differentiated (G2) conventional colonic adenocarcinoma (HE stain, ×200)
C – conventional poorly differentiated colonic adenocarcinoma (G3) (HE stain, ×200)

For patients with colorectal neoplasm, investigating the correlation between the macroscopic aspect of the tumor formation visualized endoscopically and the degree of differentiation described histologically, it was found that there is no statistically significant relationship ($p > 0.05$), which is above the threshold accepted for demonstrating a significant statistical correlation. It is known that most colorectal adenocarcinomas develop at the site of precursor lesions, such as adenomas and dysplasia. Residual adenoma is a phenomenon commonly found in colorectal adenocarcinomas. Typical adenomas are subclassified into tubular, tubulovillous, and villous types based on their architectural and histological features. Tubular adenomas consist of dysplastic glands that resemble cryptic intestinal glands and contain less than 25% villous component. Villous adenomas are composed of more than 75% villous components, which appear as fibrovascular rods covered by dysplastic epithelium. Tubulovillous adenomas represent intermediate lesions with a villous component ranging from 25% to 75%.

Premalignant lesions are described in terms of number, shape, size, location, histological type, and degree of malignancy. In terms of shape, the polyps were sessile, semipediculated or pedicled, round-oval, or polylobate (Figure 4).

Numerically, polyps can be solitary, multiple, or present in very large numbers, which can reach up to thousands (as in familial adenomatous polyposis, an autosomal dominant hereditary pathology). These can be detected by chance in a family member or transmitted through a genetic abnormality across multiple generations.

In the present research, regarding the number of polyps, solitary polyps were detected in 166 (93.2%) patients, two polyps in 10 (5.6%) patients, and three and more polyps in 2 (1.1%) patients.

In terms of size, polyps were categorized as follows: under 5 mm (very small polyps), between 5 and 10 mm (small polyps), and over 10 mm (large polyps) [9]. Very small polyps predominated in 86 (48.3%) cases, small polyps in 61 (34.3%), and large polyps represented 17.4% (n = 31).

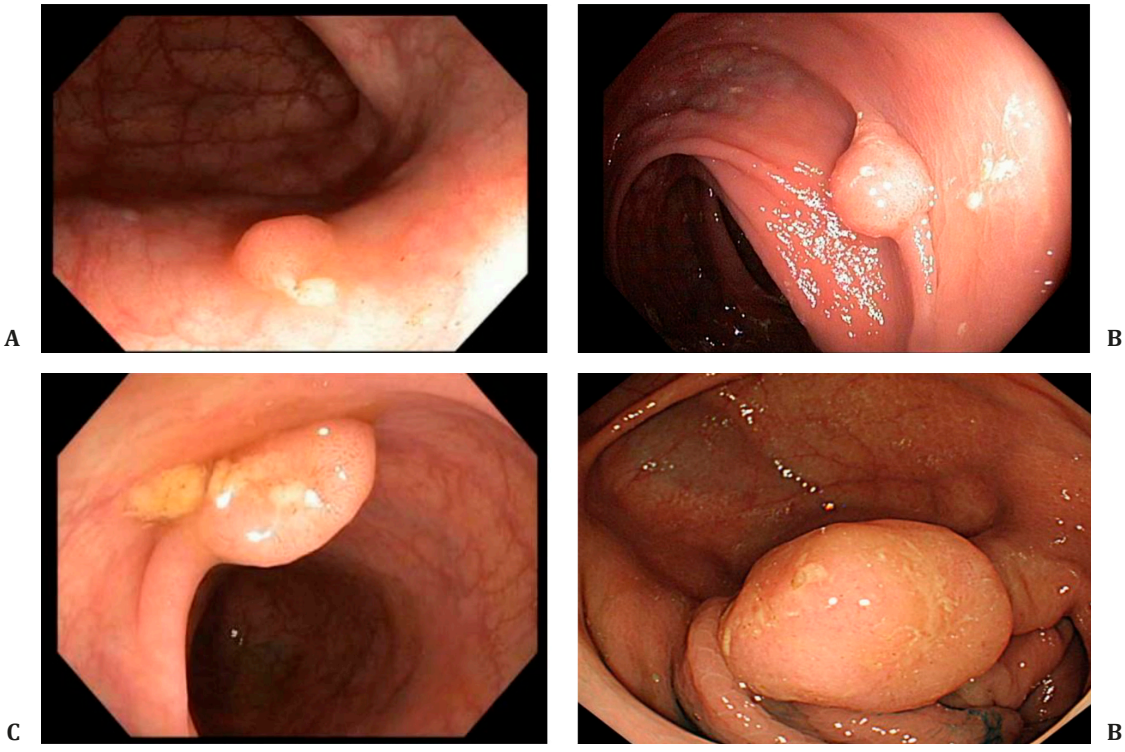

Fig. 4 Endoscopic appearance of polyps

A – sessile polyp; B – semipediculated polyp; C – pedunculated polyp; D – voluminous villous polyp

Regarding the histological type, the majority were neoplastic, with tubuloadenomatous polyps predominating (162, 91%), followed by hyperplastic polyps (12, 6.75%) and tubulovillous polyps (4, 2.25%) (Figure 5).

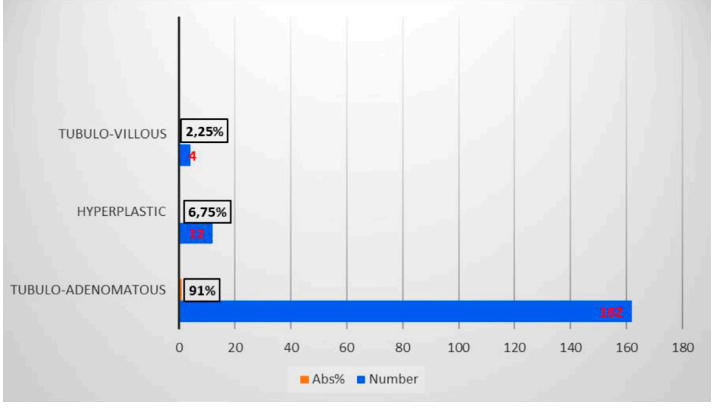

Fig. 5 Histological types of premalignant colonic lesions in patients included in the study

Note: abs - absolute value in percentage

The distribution of polyps varied statistically significantly (p = 0.0001) in relation to polyp size and histology. The most frequent, tubuloadenomatous (n = 86), were of very small size, while tubulovillous polyps (n = 4) were of large size, and hyperplastic polyps (n = 12) were equally distributed between small and large sizes (Table 3).

Table 3. Associative distribution of polyps in relation to size and histological type

Histological type		Size			Total
		very small	small	large	
tubuloadenomatous	frequency % of histology	86 (48.3%)*	55 (30.9%)	21 (11.8%)*	162 (91%)
tubulovillous	frequency % of histology	-	-	4(2.2%)	4 (2.2%)
hyperplastic	frequency % of histology	-	6 (3.4%)	6 (3.4%)	12 (6.8%)
	Total/size	86 (48.3%)	61 (34.3%)	31 (17.4%)	178 (100%)

Note: p < 0.05*, which allows us to conclude that very small sizes are much more frequent than large sizes

The results indicate that the distribution of case sizes is not uniform, and there is a significant difference between very small, small, and large sizes. Thus, p < 0.05 suggests that very small sizes are much more frequent than large sizes.

Dysplasia refers to a pathological adaptive cellular alteration, which involves changing in volume, shape, and organization within a tissue. This includes the variation in the size and shape of the cells, an increase in the volume of the nuclei (which become irregular and hyperchromic) disturbances in the stratification, disproportion between layers, dedifferentiation, depolarization of cells, and a disordered arrangement of cells within the epithelium. Depending on

the severity of these changes, dysplasia can be classified as mild (simple), moderate, or severe. The World Health Organization (WHO) updated its classification system in 2019 to use a two-grade system: low-grade dysplasia (encompassing mild and moderate dysplasia) and high-grade dysplasia.

According to the degree of dysplasia, the premalignant lesions were described as follows: 106 (59.5%) with mild dysplasia, 48 (26.8%) with moderate dysplasia, and 24 (13.5%) with severe dysplasia. When grouped according to the WHO classification (2019), the premalignant lesions exhibited low-grade dysplasia in 154 (86.5%) patients and high-grade dysplasia in 24 (13.5%). Figure 6 shows different morphopathological forms of premalignant lesions from the series of patients included in the study.

The distribution of polyps varied statistically significantly (p=0.0001) in relation to the size and dysplasia of the premalignant lesions. The smallest size was recorded in polyps with low-grade dysplasia (n=153), while large sizes were more commonly found in those with high-grade dysplasia (n=23). This demonstrates a direct relationship between size and dysplasia, with 74.2% of polyps with high dysplasia being classified as large (Figure 7).

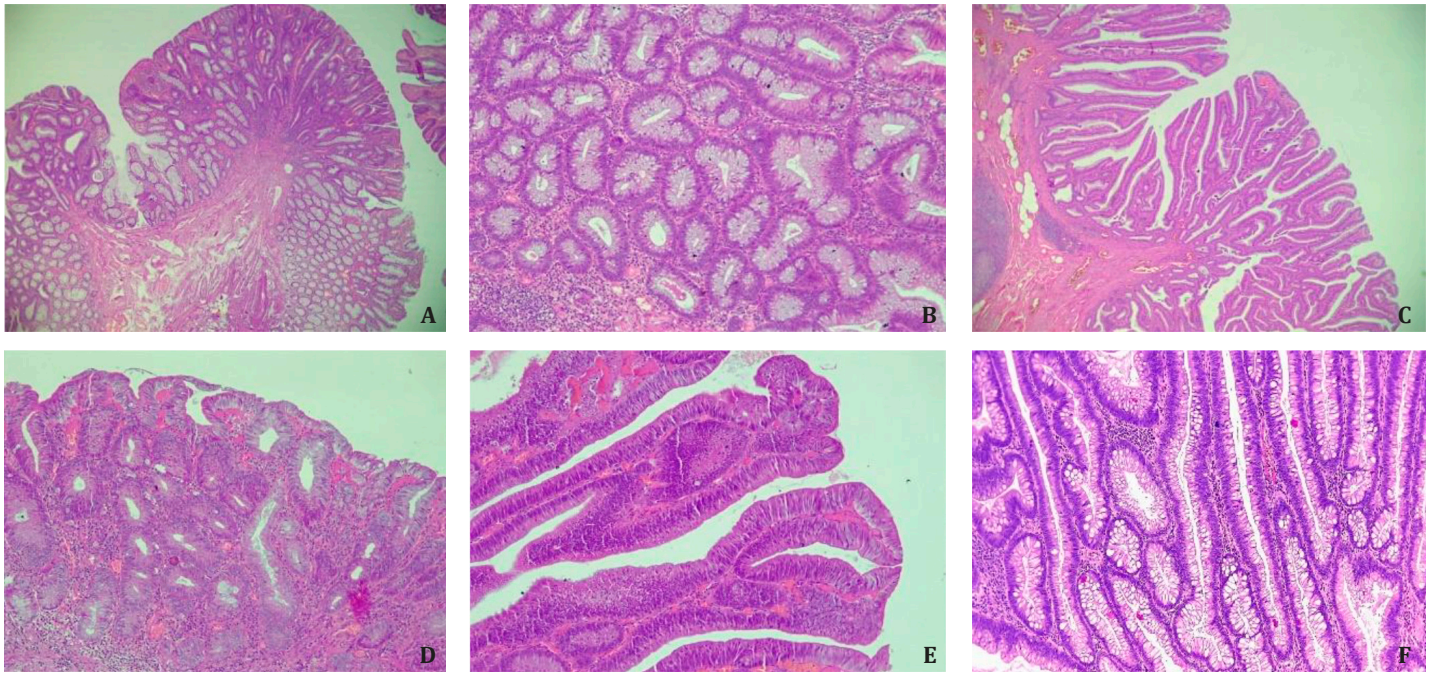

Fig. 6 Morphological subtypes of conventional adenomas according to the WHO classification (2019)

A, B – tubular adenoma with moderate epithelial dysplasia (A - HE stain, ×100; B - HE stain ×200); C – villous adenoma with moderate epithelial dysplasia (HE stain, x100); D – tubular adenoma with high epithelial dysplasia (HE stain, x200); E – tubulovillous adenoma with high epithelial dysplasia (HE stain, x200); F – tubulovillous adenoma with low epithelial dysplasia (HE stain, x200)

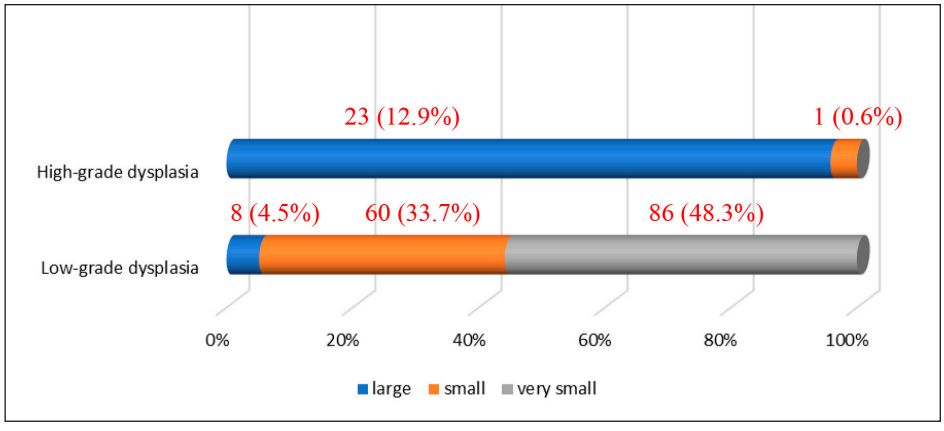

Fig. 7 Distribution of polyps in relation to size and their degree of dysplasia in the patients included in the study

Note: size of the polyps – large, small, very small

Discussions

As cell proliferation exceeds degradation, accumulations of cells appear, forming small protrusions in the intestinal lumen that represent benign tumors (polyps). Adenomatous polyps are defined as lesions containing epithelial neoplasia. According to their histological structure, adenomas can be of three types. The most common are tubular adenomas, which are formed predominantly (more than 80%) from a complex network of branched adenomatous glands. The second type is represented by villous adenomas, composed predominantly (>75%) of adenomatous glands that extend linearly from the surface to the center of the polyp, creating a digitiform appearance. The third type of adenomatous polyp is represented by tubulovillous lesions, which are a combination of the other two histological types (25-75%) [10]. Colorectal adenomas are, by definition, considered dysplastic, as they all contain foci of dysplasia. At their level, the glandular epithelium exhibits abnormalities in cellular differentiation and renewal, leading to hypercellularity in the colonic crypts, with cells containing mucin in variable amounts. Based on the extent of cytological and architectural alterations, two types of dysplasia are described: mild and severe [11, 12].

Currently, the architecture of the colonic mucosa is also considered in determining the form of neoplastic growth. According to the Paris classification, colonic neoplastic lesions are categorized into the following types:

- type Ip – protruding, pedunculated
- type Is – protruding, sessile, broad-based
- type IIa – superficial, flat, and elevated
- type IIb – completely flat
- type IIc – superficially depressed
- type III – excavated/ulcerated [13].

Kudo classifies adenomas into protruding (polypoid lesions), flat-elevated, and flat (Figure 8). Flat-elevated lesions are further divided into flat-elevated and flat-elevated with central depression. The flat type of lesion is subdivided into flat and depressed. In this classification, the flat type corresponds to the initial description of flat adenomas. within 2001, Kudo and colleagues morphologically analyzed approximately 20,000 colonoscopic lesions and determined that polypoid and non-polypoid lesions accounted for 55% and 45% of cases, respectively. Non-polypoid lesions are present in the proximal and distal regions of the colon in equal proportion, whereas polypoid lesions are more frequent in the distal colon [14, 15].

Type	Pit Pattern	Definition	Usual histopathological findings
I		Round pits	Normal
II		Asteroid or papillary pits	Hyperplastic
IIIs		Small tubular or roundish pits	Intramucosal adenocarcinoma (28.3%) Adenoma (73%) (depressed lesion)
IIIL		Large tubular or roundish pits	Adenoma (86.7%) (protruded lesion)
IV		Branch-like or gyrus-like pits	Adenoma (59.7%) (Almost tubulo-villous adenoma) Intramucosal adenocarcinoma (37.2%)
V		Non-structural pits	Submucosal adenocarcinoma (62.5%)

Fig. 8 Kudo classification of colonic neoplastic lesions [14, 15]

Note: Pit pattern classification of colorectal neoplasia (Kudo *et al.*). *I* – Round pit (normal pit), *II* – asteroid pit, III_S – tubular or round pit (smaller than the normal pit, i.e., type I), III_L – tubular or round pit (larger than the normal pit, i.e., type I), *IV* – dendritic or gyrus-like pit, *V* – amorphous, nonstructured pit.

World Health Organization (WHO) in 2010 classified serrated polyps into non-dysplastic polyps, which include hyperplastic polyps and sessile serrated polyps, and dysplastic polyps, which include sessile serrated polyps with dysplasia and traditional serrated adenomas [16, 17].

The prognostic significance of histopathological grading has been recognized in numerous studies over time. In 1949, Dukes found a correlation between colonic tumor

grading and lymph node metastasis, indicating a worse prognosis. Poorly differentiated adenocarcinomas are associated with lymph node metastases in more than 50% of cases. In contrast, moderately and highly differentiated tumors have a lower rate of lymph node metastasis. The degree of tumor differentiation also has prognostic significance, as it correlates with local invasion of the intestinal wall and adjacent organs, as well as with venous and lymphatic invasion. An obvious relationship between survival rate and histopathological grading of colorectal neoplasia has been demonstrated. The higher the grading–meaning the more undifferentiated the tumor–the more aggressive its progression and the lower the chances of long-term survival. According to Morson (2001), the five-year survival rates for highly differentiated, moderately differentiated, and undifferentiated forms are 80%, 60%, and 25%, respectively [18, 19].

However, there are challenges in interpreting studies due to the use of various older grading systems. Additionally, classifying tumors according to the current staging system, which includes 4 grades–from G1 (well-differentiated) to G4 (undifferentiated, anaplastic) – can be complex. There may also be staging errors, as most invasive tumors contain focal areas of undifferentiated cells at the site of invasion. However, these areas are not necessarily representative of the entire tumor's grade [20, 21].

The various histopathological types of primary colonic neoplasms may allow for the classification of patients into different prognostic groups. The histopathological aspect has negative prognostic significance that has been proven only for certain histological subtypes of adenocarcinomas: colloid (mucinous) carcinoma, "signet ring" cell carcinoma, small cell carcinoma, and squamous carcinoma. Despite advances in surgery and adjuvant therapy for colonic neoplasms in recent years, the average 5-year overall survival of patients with curative resections for CRC remains only 62%, and local recurrences occur in more than 90% of cases where therapy has failed [22]. The prognosis of CRC depends on a multitude of factors that can be grouped into categories: clinical factors, tumor stage factors, histopathological factors, and biological (molecular) factors [23]. Undoubtedly, the most important factors for survival prognosis are the degree of tumor invasion into the intestinal wall. Obviously, the more advanced the stage of neoplasia, the shorter the life expectancy. The TNM classification for CRC is based on the observation that the size of the tumor correlates directly with local invasion, and thus, implicitly with the prognosis. No significant relationship between neoplasm size or diameter and 5- or 10-year survival has been demonstrated. Moreover, some studies suggest that large-sized NPCs may have a better prognosis. A possible explanation for this could be that the size of the tumor is often amplified by peritumoral inflammatory phenomena of the body's defense mechanisms, while in reality, the actual tumor volume is smaller.

The macroscopic anatomopathological form is a parameter in formulating the prognosis, as the appearance of the neoplasia reflects its biological nature. Patients with exophytic or polypoid tumors seem to have a better prognosis compared to those with ulcerative or infiltrative tumors. Grinnell, who classifies tumors into protruding (exophytic, vegetative), intermediate, and infiltrative types, found as early as 1939 that 83% of patients with vegetative neoplasms survive five years, while the percentage drops to only 38% for those with infiltrative tumors or 48% for those with intermediate tumors. The explanation for these differences lies in the lower percentage of intestinal wall penetration in vegetative tumors compared to ulcerative ones (24% *vs* 39%), the lower frequency of nodal and/or hematogenous metastasis in vegetative tumors (about 24% *vs* 31 % for infiltrative tumors), and the fact that, in general, vegetative tumors are more limited in depth within the intestinal wall compared to ulcerative-infiltrative ones [24, 25]. There are divergent opinions on how the location of CRC in various segments of the colon influences survival. One study suggests that the location of CRC in the subperitoneal space on the rectum decreases the 5-year survival rate compared to intraperitoneal locations. Tumors located in the right hemicolon tend to have a less favorable prognosis than those in the left hemicolon, with a survival rate 2-14% higher at 3 years post-resection for neoplasms in the left hemicolon compared to those on the right. This difference is probably explained by the more technically challenged dissection of the central lymphatic stations at the level of the origin of the superior mesenteric artery [26]. The histopathological aspect of the primary neoplasm has negative prognostic significance proven only in the case of certain histological subtypes of adenocarcinomas: colloid (mucinous) carcinoma, "signet ring " cell carcinoma, small cell carcinoma, and squamous carcinoma. These adenocarcinomas are aggressive tumors, usually discovered at a more advanced stage, with extensive pericolonic invasion. They present an increased incidence of lymph node metastases and have a high degree of malignancy, leading to lower long-term survival rates [27]. The effects of the main histopathological prognostic factors in CRC and NPC are shown in Table 4.

Research on the molecular biology of CRC, along with increasingly detailed genomic studies, raises hope for better control of this pathology. The only chance to improve patient survival is through early diagnosis, appropriate management at each stage by a multidisciplinary team, and active, systematic surveillance of all patients. Treatment must be differentiated by stage and tailored to each patient, who may develop a unique form of neoplasia. Stage 0 malignant polyps (TisN0M0) less than 2 cm, with submucosal infiltration, endoscopically resected, with non-infiltrating margins, well-differentiated cancer *in situ*, or severe dysplasia, which usually have a low chance of lymph node infiltration, can be followed up colonoscopically. If the pedunculated polyps are over 2 cm or the microscopic malignant tissue is poorly differentiated, or if the invasion exceeds the submucosa or there is suspicion of damage to a single lymph node, segmental colectomy with lymphadenectomy is required after polypectomy. In cases where endoscopic resection margins

are positive, immediate colectomy is mandatory [28]. In stages I and II (any T, N0, M0), where the neoplasia is considered localized, surgery is the standard treatment. For T3 tumors, a D3 lymphadenectomy is required, involving complete dissection of all regional, paracolic, intermediate, and central lymph nodes (a minimum of 12 lymph nodes should be removed and examined). In stage III, where lymph node invasion is demonstrated (any TN1-3M0), if there is a risk of local or distant recurrence, sectoral colectomy with D3 lymphadenectomy is necessary. This procedure is important for correct stagingand should be followed by adjuvant chemotherapy [29].

Table 4. Histopathological prognostic factors in CRC and NPC [20-27]

Histopathological prognostic factors	Effect on prognosis
TNM stage (documented histopathologically)	increasing the stage significantly decreases the prognosis
The degree of intestinal wall invasion	the depth of invasion negatively affects the prognosis
The degree of differentiation	well-differentiated tumors have a better prognosis than poorly- or undifferentiated ones
Local inflammatory and immunological reaction	favorable prognosis
Primary tumor morphology	polypoid/exophytic tumors have a better prognosis than ulcerative/ infiltrative ones
The size of the primary tumor	no effect in most studies
Tumor location in the colon	better prognosis for colon tumors compared to rectal tumors * better prognosis for left hemicolon locations compared to right hemicolon *
Presence of lymphovascular invasion (LVI) and perineural invasion (PNI)	favorable prognosis in their absence
Status of surgical resection margins	favorable for negative resection margins
Status of lymph nodes (total number identified, number of positive lymph nodes)	favorable for the lack of metastases in the lymph nodes

***Note*: * -** prognostic significance for which the conducted studies are contradictory

Active surveillance of patients who have undergone surgery for CRC, as well as those at an increased risk of developing this neoplasia (e.g., patients who have undergone polypectomies for stage 0 cancer), is essential. The most effective way to detect recurrences and, especially, metastases in a timely manner is the systematic follow-up of patients with CRC in stages I-III. The most comprehensive surveillance protocol is that recommended by the Japanese Colorectal Cancer Society (2019), which recommends:

- clinical examination and determination of CEA (Carcinoembryonic Antigen) every 3 months during the first 3 years and every 6 months up to 5 years.
- abdominal ultrasound and chest X-ray every 6 months for up to 5 years.
- thoraco-abdominal computed tomography and colonoscopy annually for up to 5 years [30].

The surgeon, as a member of the multidisciplinary team managing colorectal neoplasia, plays an important role in diagnosis, treatment, and follow-up.

Conclusions

A detailed analysis of the morphopathological characteristics of tumor formations, beyond confirming malignancy, provides important information for determining therapeutic management. Classification into a risk group that assigns the patient a specific prognosis can also contribute to a better understanding of tumor genesis, allowing for the stratification of patients for individualized treatment–either more aggressive or less aggressive–based on the prognosis. Further extensive studies are required to analyze the correlations between histopathological form, treatment, and survival rate.

Competing interests

None declared.

Authors' contributions

AU – conceptualization, investigation, methodology, writing – original draft, writing – review and editing, visualization, project administration, data curation, resources. GR – investigation, project administration, validation, supervision, data curation. AD – data acquisition. MC, EM – analysis and interpretation of data. All authors critically reviewed the work and approved the final version of the manuscript.

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Patient consent

Obtained.

Ethics approval

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi: 10.3322/caac.21492.
2. Bujanda L, Cosme A, Gil I, Arenas-Mirave I. Malignant colorectal polyps. World J Gastroenterol. 2010;16(25):3103-3111. doi: 10.3748/wjg.v16.i25.3103.
3. Târcoveanu E. Cancerul de colon – o problemă de sănătate publică [Colon cancer – a public health problem]. [J Surg] (Iași). 2007;3(4):34-41. Romanian.
4. Japanese Research Society for Cancer of the Colon and Rectum. General rules for clinical and pathological studies on cancer of the colon, rectum and anus. Jpn J Surg.1983;13(6):557-573. doi: 10.1007/BF02469505.
5. Nikolaou S, Qiu S, Fiorentino F, Rasheed S, Tekkis P, Kontovounisios C. Systematic review of blood diagnostic mark-

ers in colorectal cancer. Tech Coloproctol. 2018;22(7):481-498. doi: 10.1007/s10151-018-1820-3.
6. Meng C, Yin X, Liu J, Tang K, Tang H, Liao J. TIMP-1 is a novel serum biomarker for the diagnosis of colorectal cancer: a meta-analysis. PLoS One. 2018;13(11):e0207039. doi: 10.1371/journal.pone.0207039.
7. Ahlquist D, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, et al. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. Gastroenterology. 2012;142(2):248-56. doi: 10.1053/j.gastro.2011.10.031.
8. Pisano M, Zorcolo L, Merli C, et al. 2017 WSES guidelines on colon and rectal cancer emergencies: obstruction and perforation. World J Emerg Surg. 2018;13:36. doi: 10.1186/s13017-018-0192-3.
9. Kumar V, Abbas AK, Aster J, editors. Robbins and Cotran pathological basis of disease. Philadelphia: Elsevier/Saunders; 2015. 1391 p.
10. Itzkowitz S, Potack J. Colonic polyps and polyposis syndromes. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management. 9th ed. Philadelphia: Saunders/Elsevier; 2010. p. 31-44.
11. Robert M. The malignant colon polyp: diagnosis and therapeutic recommendations. Clin Gastroenterol Hepatol. 2007;5(6):662-667. doi: 10.1016/j.cgh.2007.04.001.
12. Sweetser S, Smyrk T, Sugumar A. Serrated polyps: critical precursors to colorectal cancer. Expert Rev Gastroenterol Hepatol. 2011;5(5):627-635. doi: 10.1586/egh.11.67.
13. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon. Gastrointest Endosc. 2003;58(6 Suppl):S3-43. doi: 10.1016/s0016-5107(03)02159-x.
14. Kudo S, Kashida H, Tamura T, et al. Colonoscopic diagnosis and management of nonpolypoid early colorectal cancer. Word J Surg. 2000;24(9):1081-1090. doi: 10.1007/s002680010154.
15. Rembacken B, Fujii T, Cairns A, et al. Flat and depressed colonic neoplasms: a prospective study of 1000 colonoscopies in the UK. Lancet. 2000;355(9211):1211-1214. doi: 10.1016/s0140-6736(00)02086-9.
16. Snover D, Ahnen D, Burt R, et al. Serrated polyps of the colon and rectum and serrated polyposis. In: Nagtegaal ID, Arends MJ, Odze RD, Lam AK. WHO Classification of Tumours of the Digestive System. Lyon: IARC Press; 2010. p. 160-165.
17. Sobin LH, Gospodarowicz MK, Wittekind C, editors; Union for International Cancer Control UICC. TNM Classification of Malignant Tumors. 7th ed. Hoboken: John Wiley & Sons; 2009. p. 100-105.
18. Morino M, Allaix ME, Caldart M, et al. Risk factors for recurrence after transanal endoscopic microsurgery for rectal malignant neoplasm. Surg Endosc. 2011;25(11):3683-3690. doi: 10.1007/s00464-011-1777-z.
19. Friel C, Cromwell JW, Marra C, et al. Salvage radical surgery after failed local excision for early rectal cancer. Dis Colon Rectum. 2002;45(7):875-879. doi: 10.1007/s10350-004-6320-z.
20. Day LW, Velayos F. Colorectal cancer screening and surveillance in the elderly: updates and controversies. Gut Liver. 2015;9(2):143-151. doi: 10.5009/gnl14302.
21. Lu J, Lin GL, Qiu HZ, et al. Comparison of transanal endoscopic microsurgery and total mesorectal excision in the treatment of T1 rectal cancer: a meta-analysis. PLoS One. 2015;10(10):14-27. doi: 10.1371/journal.pone.0141427.
22. Zauber AG, Winawer S, O'Brien J. Colonoscopic polypectomy and long-term prevention of colorectal cancer deaths. N Engl J Med. 2012;366(8):687-696. doi: 10.1056/NEJMoa1100370.
23. Niv Y, Half N, Moshkowitz M, et al. Surveillance of patients after colonoscopic polypectomy and curative resection of colorectal cancer. Harefuah. 2010;149(10):670-673.
24. Mills SE, editor. Histology for pathologists. Philadelphia: Wolters Kluwer; 2012. p. 316-322.
25. Grigorescu M, Irimie A, Beuran M, editors. Tratat de oncologie digestivă [Treatise on digestive oncology]. Vol. 3. Bucharest: Editura Academiei Române; 2015. p. 205-227. Romanian.
26. Bărbulescu M. Factorii prognostici ai cancerului colorectal [Prognostic factors of colorectal cancer]. [J Surg] (Iași). 2007;3(2):117-129. Romanian.
27. Osorio H, Finol HJ, Gonzalez LR, et al. Ultrastructure of colorectal adenocarcinoma and peritumoral tissue in untreated patients. Ultrastruct Pathol. 2018;42(2):81-90. doi: 10.1080/01913123.2017.1422064.
28. Voloyiannis T, Snyder M, Bailey R, Pidala M. Management of the difficult colon polyp referred for resection. Dis Colon Rectum. 2008;51(3):292-295. doi: 10.1007/s10350-007-9175-2.
29. Tholoor S, Tsagkournis O, Basford P, Bhandari P. Managing difficult polyps: techniques and pitfalls. Ann Gastroenterol. 2013;26(2):114-121.
30. Hashiguchi Y, Muro K, Saito Y, et al. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. Int J Clin Oncol. 2020;25(1):1-42. doi: 10.1007/s10147-019-01485-z.

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RESEARCH ARTICLE

Knowledge, attitudes, and behaviors of the population of Chisinau municipality regarding antibiotic use

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ABSTRACT

Introduction. The irrational use of antibiotics is one of the main factors contributing to the accelerated development of antimicrobial resistance worldwide. Despite the regulations established to control the procurement of antimicrobial drugs, the rate of self-prescribing and self-treatment with antimicrobials remains very high in most countries. To plan further measures to combat this resistance, a thorough understanding of the current reasons behind this behavior is required. The aim of the study is to explore the general public's knowledge, attitudes, and behavior regarding antibiotic consumption.

Material and methods. A cross-sectional study was conducted using a questionnaire developed by the World Health Organization to assess the general public's knowledge, attitudes, and behavior regarding the use of antimicrobial preparations. The questionnaire was administered in 10 randomly selected locations in the Chisinau municipality, including bus and trolleybus stops, shopping centers, universities, and medical institutions.

Results. The survey included a sample of 572 participants with an average age of 36 years. The majority were female (60.0%). The overall level of awareness of the benefits and harms of antibiotics was 63.8%, and appropriate antibiotic use practices were demonstrated by 68.3% of respondents. These practices include purchasing antibiotics with a prescription (60.0%) and taking antibiotics until completing the course of treatment (77.0%).

Conclusions. Misconceptions and counterintuitive antimicrobial use practices undoubtedly complicate efforts to combat AMR and can lead to unintended consequences. Developing educational measures and promoting the responsible use of antibiotics are crucial priorities in combating antibiotic resistance and ensuring effective treatment outcomes.

Keywords: antibiotics, antimicrobial resistance, antibiotic use, awareness.

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Key messages

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

The cause of irrational antimicrobial consumption differ across age groups and social classes, and identifying and analyzing these reasons is essential to determine where and how to address the problem of inappropriate antimicrobial use.

The research hypothesis

The degree to which a population complies with corresponding safety measures and regulations regarding antimicrobial use is undoubtedly influenced by people's knowledge, attitudes, and practices (KAP) concerning antimicrobial resistance.

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

This study highlights the main factors related to the population's level of awareness about antibiotics that influence individuals' treatment decisions for various diseases. Understanding these factors is essential to implementing effective measures tailored to specific population groups to combat antimicrobial resistance.

Introduction

The discovery of antimicrobial drugs undoubtedly marked a turning point for humanity, changing the course of medicine and offering a new hope for life [1, 2]. Scientists believe this important event in medical history added a decade to human life expectancy [1]. It is known that the human body has its own defense mechanisms against infection, and even if some symptoms occur, a strong immune system can respond effectively and quickly to bacterial infections. However, in certain circumstances, the human body needs assistance to combat infection. In the case of bacterial infections, antibiotics provide that help [2, 3].

Over time, antimicrobial therapy has become increasingly used in the treatment of both confirmed and suspected bacterial infections, as well as for infection prophylaxis in surgical wards, intensive care units, and even pediatric wards [3, 4]. Due to the widespread use of antibiotics, appropriate prescribing has become a key topic among international researchers [2]. International studies on antibiotic prescription practices in hospitalized children indicate that antibiotics are being misused [3]. As a result of antibiotic overuse, many microorganisms have developed resistance through various defense mechanisms, particularly Gram-negative bacteria, which pose a major challenge worldwide [2, 5].

Antimicrobial resistance is associated with ineffective therapies, increased mortality, prolonged hospitalization, and additional costs for both patients and countries [2]. Given the current uncontrolled spread of resistant bacteria, it is imperative to identify the causes of this phenomenon. Numerous international studies have found that a major determinant in the development of resistance is the irrational and uncontrolled consumption of antibiotics within the population [3, 6]. Irrational antibiotic use includes prescribing antibiotics in incorrect doses, self-medication, and the treatment of viral or non-bacterial diseases [3]. It has been estimated that many factors such as politics, economics, doctors' knowledge and experience, diagnostic uncertainty, and pharmaceutical marketing contribute to the irrational use of antibiotics [7].

International research has estimated that patients' high expectations of the therapeutic effects of antibiotics influence their behavior and attitudes towards the use of antimicrobial preparations. Thus, measures to combat the spread of resistant micro-organisms should focus on increasing public awareness of how antibiotics are used, prescribed, and dispensed [4].

The aim of the present study is to assess the general public's knowledge, attitudes, and behavior regarding antibiotic use and to identify the factors associated with irrational antibiotic use.

Material and methods

A cross-sectional study was conducted to assess the general public's knowledge, attitudes, and behavior regarding the use of antimicrobial preparations in the treatment of infectious diseases. As of January 1, 2021, the population of the Republic of Moldova aged 18 to 74 was 2,619,950. With a sample size of 572 persons, the margin of error at a 95% confidence interval was no more than 4.3%. The sample size was calculated using the following formula:

$$n' = \frac{n}{1 + \frac{z^2 \, x \, p(1-p)}{N \, \varepsilon^2}} \quad (1)$$

where: **z** is the z-score, equal to 1.96 for a 95% confidence interval

ε is the margin of error

N is the population size

p is the population proportion (around 60%).

Inclusion criteria for the study population. Adults aged 18 to 74 years were included in the study. Individuals under 18 and over 74 years of age were excluded, as these groups rarely make independent decisions regarding their own health. Informed consent was obtained from each respondent at the beginning of the interview. Participants were selected using a random sampling approach.

Training for research team (interviewers). The research team underwent training in an online session conducted via the Zoom platform by three researchers from the University of Copenhagen. This training covered key aspects of qualitative research methodology, an introduction to qualitative research, and practical sessions. The team consisted of 7 interviewers who were assigned to the selected sites to conduct interviews and collect data between November 8 and November 17, 2022. The team was formed by order number 250-d, issued on November 8, 2022, by the National Agency for Public Health of the Republic of Moldova.

The study utilized a questionnaire developed and validated by the World Health Organization as its primary instrument. This opinion questionnaire featured closed-ended questions, which were administered by interviewers during face-to-face interviews. The survey included dichotomous (Yes/No), multiple choice, and hierarchical questions to comprehensively analyze the knowledge, attitudes, and behaviors of the interviewed population. Data collection was conducted using Android-based tablets, with the questionnaire implemented through KoBoToolbox, a free and open-source suite of tools for mobile data collection.

To conduct this survey, 10 locations in the Chisinau municipality, typically characterized by a higher flow of people,

were randomly selected. These locations included bus and trolleybus stops, shopping centers, universities, and medical facilities (Table 1).

Table 1. Locations selected for the survey

Trolleybus and bus stops	Shopping centers	Medical facilities	Universities
«Construction College»	Gemenii Shopping Center, 136 Stefan cel Mare şi Sfânt Boulevard	«Toma Ciorba» Clinical Hospital of Infectious Diseases, 163 Stefan cel Mare si Sfant Boulevard	Nicolae Testemiţanu State University of Medicine and Pharmacy, 165 Stefan cel Mare Boulevard
«Grand National Assembly Square»	Grand Hall Shopping Center, 2/4 Constantin Negruzzi Boulevard	Oncological Institute, 30 Nicolae Testemiţanu Street	
«House of Press»	UNIC Shopping Center, 8 Stefan cel Mare **şi** Sfant Boulevard	Institute of Cardiology, 29/1 Nicolae Testemiţanu Street	

The collected data was analyzed using Microsoft Excel (Microsoft, Redmond, Washington) tools. Relative statistical parameters were calculated, specifically the ratio, using the formula:

$$(2) \quad R = \frac{a\ part\ of\ the\ phenomenon}{the\ overall\ phenomenon} \text{ x } 100\%$$

Using this formula and raw data, the demographic parameters of the respondents were determined, including: (1) distribution by gender; (2) distribution by age groups; (3) distribution by socio-professional groups. The same principle was applied to analyze the responses to the questionnaire.

The study was confirmed as exempt from review by the WHO Ethics Review Committee (Protocol Number ERC.0003790).

Results

According to the interview location, 25.0% of respondents were interviewed while waiting at bus or trolleybus stops, 39.0% near selected shopping centers, 12.0% in the vicinity of universities, and 24.0% near the mentioned medical institutions.

Females accounted for 60.0% of the respondents, while males comprised 40.0%.

The individuals included in the study were almost evenly distributed across age groups: 21.0% of respondents were aged 18-24 years, 34.0% were in the 25-39 years age group, 25.0% were in the 40-54 years group, and 20.0% were aged 55 years or older.

Respondents were categorized into socio-professional groups based on their primary activity. The largest group consisted of employees performing physical labor (23.0%), followed by students (22.0%), office workers (17.0%), and retired persons (15.0%).

Of all the individuals included in the study, 49.0% reported having taken antibiotics in tablet, powder or syrup form within the past 12 months. In 60.0% of cases, these antibiotics were obtained with a prescription, 21.0% were administered by a healthcare professional, 10.0% were taken from leftover medication from previous treatments, and 7.0% were taken without prescription.

The reasons for using these antimicrobial preparations varied (Fig. 1).

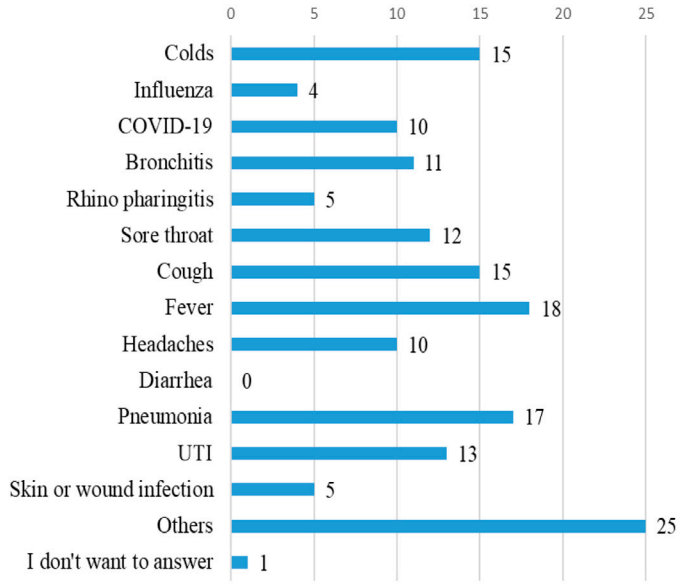

Fig. 1. Reason provided by respondents for frequent antibiotic use, %

Note: UTI – urinary tract infection

Most people used antibiotics to manage a fever (18.0%), while 17.0% took antibiotics to treat pneumonia. Additionally, 15.0% used antibiotics for colds and coughs, 13.0% for urinary tract infections, 12.0% for sore throat, 11.0% for bronchitis, and 10.0% for headaches.

In response to the question "Did you have any tests–such as blood tests, urine analysis, or pharyngeal exudate–to determine the cause of the illness before or at the same time as starting antibiotics?", 63.0% answered "Yes," and 33.0% answered "No".

To assess the level of knowledge regarding the use of antimicrobials, respondents were asked several general questions about antimicrobial preparations. The statement "Antibiotics kill viruses and are effective against colds" was considered true by 43.0% of respondents and false by 44.0%. Meanwhile, 66.0% agreed with the statement "Using antibiotics when not needed makes them ineffective", and 56.0% identified diarrhea as one of the common side effects of antibiotics.

An interesting pattern emerged when responses to the statement "Antibiotics kill viruses" were disaggregated by age group (Fig. 2).

The rate of correct response was higher in age groups under 40 years, which is an important milestone in assessing population knowledge and identifying target groups for public health interventions. Representing more than half of the respondents, an average of 55.64% chose the correct answer to the "trap" question in the survey.

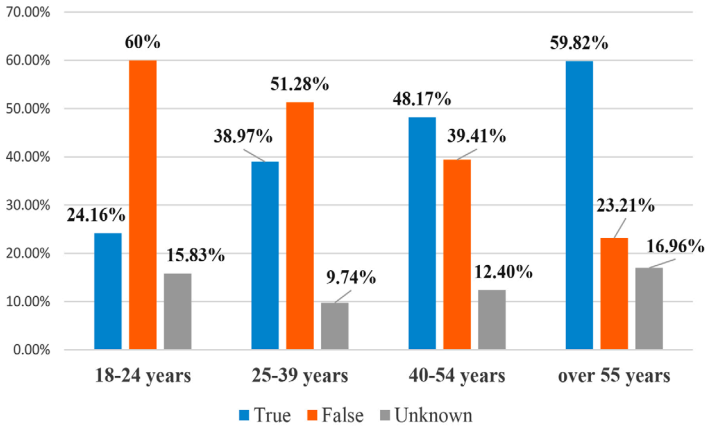

Fig. 2. Disaggregation of responses to the statement "Antibiotics kill viruses" by age group, %.

Note: *The figure illustrates the distribution of "True," "False," and "Don't know" answers (blue, orange, and grey columns) among respondents, categorized into age groups: 18-24 years, 25-39 years, 40-54 years, and over 55 years.*

The majority of individuals included in the study (77.0%) believed that antibiotics should be taken until the treatment was completed, while 16.0% were of the opinion that the antimicrobial treatment should last only until the person felt well.

Of the individuals who were advised not to take antibiotics unnecessarily in the past 12 months, 51.0% received this advice from a doctor, 47.0% from the news or other TV programs, 29.0% from online sources, and 19.0% from family members or friends. For 64.0% of respondents, this information was sufficient to change their perspective on antimicrobials. Specifically, they reported: consulting a doctor when they believe they need antibiotic treatment (95.0%); stopping self-medication with antibiotics (49.0%); and not keeping leftover antibiotics for future infections (21.0%).

When asked which sub-topics they would like more information about, most respondents indicated an interest in learning how to prescribe antibiotics (35.2%), followed by antimicrobial resistance (20.5%) and the administration or use of antibiotics (20.0%). In 84.0% of cases, respondents preferred to receive this information from a healthcare worker, while 22.0% favored an official health website, 9.0% mentioned TV, and 8.0% cited relatives and friends.

Among those who had COVID-19, 33.0% had not taken antibiotics to treat the infection, 16.0% had taken antibiotics prescribed by a doctor, and 3.0% had taken antibiotics without a prescription. Slightly more than a third of respondents (32%) reported not having had COVID-19.

To address antimicrobial resistance, 14.0% believe that preventive and control measures should be taken at the individual level, 12.0% at the national level, 15.0% at the global level, and 39.0% think actions should be implemented at all levels.

Discussion

Population knowledge on how to use antimicrobial preparations varies by country/territory, culture, socioeconomic status, education, age, and personality [8].

The main objective of this study was to identify the main factors associated with antimicrobial misuse in order to recommend the development of targeted measures to prevent and control antibiotic resistance [8].

Due to the growing levels of resistance, in 2017, the World Health Organization implemented the first antibiotic release restrictions. Since then, most European countries have developed and approved several regulations in this area [9, 10]. However, the results of the current study, recent studies conducted in Russia, and numerous similar studies in other countries show that dispensing antibiotics in pharmacies without a prescription remains a common practice, for known reasons–primarily the commercial interests of pharmacists or their compassion for the patient's condition [9, 11, 12].

In this context, it is very important to note that such reasons cannot justify a 'fair' policy of marketing antibacterial preparations in contravention of the regulations set by government authorities. International researchers have also reported the results of quantitative studies, which demonstrate the common practice of over-the-counter dispensing of antibiotics in pharmacies [9, 13, 14].

The results of the study showed a moderate level of awareness (63.8%) of antibiotic use, higher than the levels reported by El Zowalaty and Al-Shawi et al. (48.0% and 48.4% respectively) [15, 16].

In this study, 81.0% of the respondents indicated that they had taken antibiotics on prescription, while 18.0% had not been prescribed by a doctor. Among the participants in the study conducted by El Zowalaty, 62.0% took antibiotics for treatment according to a doctor's prescription, and 48.0% took them without a prescription [16].

As in the study by Alnasser et al., the majority of respondents believed that antimicrobial preparations or prescriptions should not be kept for later use and should not be passed on to another person for treatment [17].

The majority of study participants indicated that antibiotics should be discontinued after completing the full course of treatment prescribed by the doctor (77.0%), while 16.0% believed treatment should be stopped when feeling better. This contrasts with 56.8% of participants in the study by Alkhalifah et al., who frequently stopped antibiotics when symptoms improved [18]. Results from other local studies conducted in Riyadh, Saudi Arabia, show that between 48.0% and 67.0% of respondents stopped antibiotics after feeling better [16].

Importantly, a significant proportion of people in the survey have misconceptions about the need for antibiotics. Thus, 43.0% of respondents believe that antibiotics kill viruses, and 48.0% agree that they are effective against colds. These results align with those obtained in the study by Dopelt et al. in 2023, conducted with a group of students at Ashkelon Academic College in Israel, the majority of whom believed that antibiotics could be used to cure a cold, fever, sore throat, and viral infections [19]. Similar results have also been obtained in other research highlighting the problem of a lack of knowledge about antibiotic use [20, 21].

Moreover, these people believe that they should receive antibiotics with their doctor's prescription whenever they

fall ill or use those left over from previous treatment. Such misconceptions are also reflected in the findings of studies by Sambakunsi and Marzan et al. in Malawi and Bangladesh, where many respondents practiced self-medication, including with antibiotics [22, 23].

A significant proportion of participants in a study conducted in Russia believe that antibiotics are generally easy to obtain at any time and can be self-prescribed based on symptoms previously experienced by the person themselves, family members, or friends. In these situations, self-treatment with antibiotics recommended by close individuals is practiced without any medical education background [9]. Similar self-medication practices have been demonstrated in other studies in north-west Russia [12].

In these cases, associated with the irrational use of antibiotics, individuals were unaware of the potential adverse reactions to antimicrobial preparations or the risk of developing bacterial resistance to them.

Another important finding based on the study results was the positive correlation between employment status and appropriate antibiotic use. Thus, the socio-professional status of the interviewee serves as a determinant of antibiotic use. Such observations were also made by Napolitano et al. in a study from Italy, in which unemployed respondents were less informed about antibiotics [24]. Several international studies have shown the relationship between awareness of antibiotic use and antibiotic resistance with the level of education and the presence of children [25], and in a study in Saudi Arabia, appropriate antibiotic use practices were associated with marital status and the presence of children (80% of married participants), explained by more information about antibiotics received during pediatric patients' treatment [18].

To combat antimicrobial resistance, 14.0% believe that measures to prevent and control this phenomenon should be taken at the individual level, 12.0% at the national level, 15.0% at the global level, and 39.0% believe that actions should be taken at all levels.

It is gratifying that most of the respondents are aware of the impact of AMR on the health of the population and believe that prevention and control measures to combat this phenomenon should be taken at the individual, national, and global levels. Insignificant differences in this respect were found in the study conducted by Rachina et al. in Russia, where less than 10.0% of respondents regarded AMR as a global problem that affects the individual not only at the moment but also as a problem for the future [9]. These results are also consistent with other studies in Germany [26] and Australia [27].

Among the limitations of this study, we mention the cross-sectional study design, which does not allow us to determine causality but only to highlight associations between variables. Also, the survey conducted in urban settings cannot speak to the behavior of rural people or the behavioral patterns of healthcare personnel and policymakers who interact with the general public through awareness-raising activities. Given that it was conducted in one step and in a very short period, our survey was not able to capture internal migration or seasonal changes that affect the epidemiologic environment.

Conclusions

Despite all the regulations regarding the use of antibiotics, common practices of self-diagnosis and self-treatment with antibiotics persist, along with attempts to purchase antimicrobial preparations in pharmacies without a prescription. Moreover, treatment advice often comes from friends or family members who lack any medical education.

The general population has insufficient knowledge about antibiotics and misconceptions about the impact of their irrational use and the role of individual users in the development of bacterial resistance. A lack of clarity about the nature of infections continues to contribute to the inappropriate use of antibiotics – treating different viral or bacterial diseases without prior investigation – and, consequently, to the development of resistance in microorganisms.

Addressing these misconceptions through educational interventions and promoting the responsible use of antibiotics remains a priority in combating antibiotic resistance and ensuring optimal treatment outcomes.

Insufficient knowledge among study participants about the effects and appropriate practices of antibiotic use highlights the need to strengthen awareness-raising measures, as well as to promote effective collaboration between family doctors and pharmacists to ensure the rational use of antibiotics.

The findings of the study emphasize the need for interventions at both the individual and community level through healthcare policies and public education programs. These findings provide insight into the population's understanding of the phenomenon of resistance, serving as a benchmark for designing interventions aimed at increasing knowledge and awareness of the problem.

Competing interests

None declared.

Authors' contributions

Conception and design of the work - MA, IOS, MP, VC, EB, OB. Drafting the article - MA, IOS. Reviewing the article for important intellectual content - MA, IOS, OB. All authors critically reviewed the work and approved the final version of the manuscript.

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Ethics approval

Favorable opinion of the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, No. 1, dated September 27, 2022.

References

1. Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: the most critical pathogens. Pathogens. 2021;10(10):1310. doi: 10.3390/pathogens10101310.
2. World Health Organization. 10 threats to global health in 2018 [Internet]. Medium.com; c2018 [cited 2024 Jun 16].

Available from: https://medium.com/@who/10-threats-to-global-health-in-2018-232daf0bbef3
3. Al-Taani GM, Karasneh RA, Al-Azzam S, et al. Knowledge, attitude, and behavior about antimicrobial use and resistance among medical, nursing and pharmacy students in Jordan: a cross sectional study. Antibiotics (Basel). 2022;11(11):1559. doi: 10.3390/antibiotics11111559.
4. Shahpawee NS, Chaw LL, Muharram SH, et al. University students' antibiotic use and knowledge of antimicrobial resistance: what are the common myths? Antibiotics. 2020;9(6):349. https://doi.org/10.3390/antibiotics9060349.
5. Van Hecke O, Butler CC, Wang K, Tonkin-Crine S. Parents' perceptions of antibiotic use and antibiotic resistance (PAUSE): a qualitative interview study. J Antimicrob Chemother. 2019;74(6):1741-1747. doi: 10.1093/jac/dkz091.
6. Duan L, Liu C, Wang D, et al. The vicious cycle of the public's irrational use of antibiotics for upper respiratory tract infections: a mixed methods systematic review. Front Public Health. 2022;10:985188. doi: 10.3389/fpubh.2022.985188.
7. Bassetti M, Giacobbe DR. A look at the clinical, economic, and societal impact of antimicrobial resistance in 2020. Expert Opin Pharmacother. 2020;21(17):2067-2071. doi: 10.1080/14656566.2020.1802427.
8. Baddal B, Lajunen TJ, Sullman MJM. Knowledge, attitudes and behaviours regarding antibiotics use among Cypriot university students: a multi-disciplinary survey. BMC Med Educ. 2022;22(1):847. doi: 10.1186/s12909-022-03853-2.
9. Rachina S, Zakharenkova P, Kozlov R, et al. The antibiotic knowledge, attitudes and behaviours of patients purchasing antibiotics with prescription in Russia: a qualitative, comparative analysis. JAC Antimicrob Resist. 2024;6(2):41. doi: 10.1093/jacamr/dlae041.
10. Ndaki PM, Mushi MF, Mwanga JR, et al. Dispensing antibiotics without prescription at community pharmacies and accredited drug dispensing outlets in Tanzania: a cross-sectional study. Antibiotics (Basel). 2021;10(8):1025. doi: 10.3390/antibiotics10081025.
11. Rachina S, Kozlov R, Kurkova A, et al. Antimicrobial dispensing practice in community pharmacies in Russia during the COVID-19 pandemic. Antibiotics. 2022;11(5):586. https://doi.org/10.3390/antibiotics11050586.
12. Cantarero-Arevalo L, Nørgaard LS, Sporrong SK, et al. A qualitative analysis of the culture of antibiotic use for upper respiratory tract infections among patients in Northwest Russia. Front Pharmacol. 2022;13:800695. doi: 10.3389/fphar.2022.800695.
13. Zaidi SF, Alotaibi R, Nagro A, et al. Knowledge and attitude towards antibiotic usage: a questionnaire-based survey among pre-professional students at King Saud bin Abdulaziz University for Health Sciences on Jeddah Campus, Saudi Arabia. Pharmacy (Basel). 2020;8(1):5. doi: 10.3390/pharmacy8010005.
14. Gunasekera YD, Kinnison T, Kottawatta SA, Silva-Fletcher A, Kalupahana RS. Misconceptions of antibiotics as a potential explanation for their misuse. A survey of the general public in a rural and urban community in Sri Lanka. Antibiotics (Basel). 2022;11(4):454. doi: 10.3390/antibiotics11040454.
15. Miyano S, Htoon TT, Nozaki I, Pe EH, Tin HH. Public knowledge, practices, and awareness of antibiotics and antibiotic resistance in Myanmar: the first national mobile phone panel survey. PLoS One. 2022;17(8):e0273380. doi:10.1371/journal.pone.0273380.
16. El Zowalaty ME, Belkina T, Bahashwan SA, et al. Knowledge, awareness, and attitudes toward antibiotic use and antimicrobial resistance among Saudi population. Int J Clin Pharm. 2016;38(5):1261-1268. doi: 10.1007/s11096-016-0362-x.
17. Alnasser AHA, Al-Tawfiq JA, Ahmed HAA, et al. Public knowledge, attitude and practice towards antibiotics use and antimicrobial resistance in Saudi Arabia: a web-based cross-sectional survey. J Public Health Res. 2021;10(4):2276. doi:10.4081/jphr.2021.2276.
18. Alkhalifah HM, Alkhalifah KM, Alharthi AF, Elzahrany YR, Aljuhani MA. Knowledge, attitude and practices towards antibiotic use among patients attending Al Wazarat health center. J Family Med Prim Care. 2022;11(4):1299-1307. doi: 10.4103/jfmpc.jfmpc_1431_21.
19. Dopelt K, Amar A, Yonatan N, Davidovitch N. Knowledge, attitudes, and practices regarding antibiotic use and resistance: a cross-sectional study among students in Israel. Antibiotics. 2023;12(6):1028. https://doi.org/10.3390/antibiotics12061028.
20. Sakeena MHF, Bennett AA, Jamshed S, et al. Investigating knowledge regarding antibiotics and antimicrobial resistance among pharmacy students in Sri Lankan universities. BMC Infect Dis. 2018;18(1):209. doi: 10.1186/s12879-018-3107-8.
21. Sakr S, Ghaddar A, Hamam B, Sheet I. Antibiotic use and resistance: an unprecedented assessment of university students' knowledge, attitude and practices (KAP) in Lebanon. BMC Public Health. 2020;20(1):535. https://doi.org/10.1186/s12889-020-08676-8.
22. Sambakunsi CS, Småbrekke L, Varga CA, Solomon V, Mponda JS. Knowledge, attitudes and practices related to self-medication with antimicrobials in Lilongwe, Malawi. Malawi Med J. 2019;31(4):225-232. doi: 10.4314/mmj.v31i4.2.
23. Marzan M, Islam DZ, Lugova H, Krishnapillai A, Haque M, Islam S. Knowledge, attitudes, and practices of antimicrobial uses and resistance among public university students in Bangladesh. Infect Drug Resist. 2021;14:519-533. doi: 10.2147/IDR.S289964.
24. Napolitano F, Izzo MT, Di Giuseppe G, Angelillo IF. Public knowledge, attitudes, and experience regarding the use of antibiotics in Italy. PLoS One. 2013;8(12):e84177. doi: 10.1371/journal.pone.0084177.
25. Guo H, Hildon ZJ, Chow A. "Antibiotics are for everyone, our past and our future generations, right? If antibiotics are dead, we will be in big trouble": building on community values for public engagement on appropriate use of antibiotics in Singapore. Front Public Health. 2022;10:1001282. doi: 10.3389/fpubh.2022.1001282.
26. Schneider S, Salm F, Schröder C, Ludwig N, Hanke R, Gastmeier P. Antibiotikaeinnahme und Resistenzentwicklung–Wissen, Erfahrungen und Einnahmeverhalten innerhalb der deutschen Allgemeinbevölkerung [Antibiotic intake and resistance development - Knowledge, experience and behavior among the German general population]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2016;59(9):1162-1170. https://doi.org/10.1007/s00103-016-2417-5. German.
27. McClelland JW, Norris JM, Dominey-Howes D, Govendir M. Knowledge and perceptions of Australian postgraduate veterinary students prior to formal education of antimicrobial use and antimicrobial resistance. One Health. 2021;14:100366. doi: 10.1016/j.onehlt.2021.100366.

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RESEARCH ARTICLE

Testing and validation of the questionnaire for evaluating the chemical composition of bottled water in relation to public health

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ABSTRACT

Introduction. In the context of increasing global consumption of bottled water, assessing its chemical composition and impact on public health becomes essential. Although perceived as a safe alternative, bottled water exhibits variability in its chemical composition, and advanced filtration methods can remove essential minerals. Consumers opt for bottled water due to its taste and convenience, yet awareness of associated risks remains limited. This study proposes the development and validation of a questionnaire to evaluate public perceptions of bottled water quality and its health impact, providing a valuable tool for public education and regulatory policies.

Material and methods. The questionnaire was developed to analyze consumer attitudes and behaviors regarding bottled water. Structured into four sections (socio-demographic data, consumption habits, perceptions of quality, and health impact), it underwent multiple validation stages. A panel of experts assessed the relevance of the questions, and a pilot study was conducted with a sample of 32 adults (aged 24-62) to evaluate validity and internal consistency using the Cronbach's alpha coefficient. Final validation was based on the feedback collected and statistical analysis performed using SPSS Statistics 27.

Results. S-CVI/Ave and S-CVI/UA are indicators of questionnaire content validity, calculated based on item validity scores and the percentage of agreement among evaluators. The S-CVI/Ave and S-CVI/UA values exceeded the minimum standard of 0.80, while the I-CVI index ranged between 0.83 and 1.00, demonstrating excellent item validity. Following respondent feedback, 18 questions were revised, and 6 were removed, resulting in a second version with 61 items. The validity sample comprised 84.4% women and 15.6% men, aged 24 to 62 years. Most respondents considered the questions clear and easy to understand, although suggestions were made to improve clarity and avoid redundancy. Internal consistency was confirmed through the Cronbach's alpha coefficient, which was acceptable for most domains, except one, where the coefficient was below 0.70 but was retained due to the validity of the questions.

Conclusions. The questionnaire for assessing bottled water consumption was successfully validated, demonstrating content validity and internal consistency. Face validity ensured the clarity of the questions.

Keywords: bottled water, water mineralization, questionnaire, validation, pre-testing.

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Key messages

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

The impact of the chemical composition of bottled water, particularly its mineralization indices, on public health remains insufficiently explored. Moreover, there is no validated tool designed to assess this relationship comprehensively.

The research hypothesis

The questionnaire developed to evaluate the population's perception

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of bottled water quality, particularly its chemical composition, is a valid and reliable tool for collecting relevant data to analyze the relationship between consumer perceptions and public health impact.

The novelty added by manuscript to the already published scientific literature

The study presents the development and validation of a novel questionnaire for assessing the chemical composition of bottled water and its potential health impacts, marking a significant contribution to scientific literature. This tool is particularly innovative for the Republic of Moldova, as it represents the first standardized method tailored to evaluate the relationship between water quality and public health in the region.

Introduction

In the context of growing concerns about the impact of bottled water on public health, assessing its chemical composition has become essential to ensuring safe consumption. Bottled water is often perceived as a safer alternative to tap water; however, the variability in its chemical composition, including the presence of potentially hazardous substances, can affect the long-term health of the population [1]. Although numerous studies have focused on the physicochemical analysis of bottled water, tools for evaluating consumer perceptions and raising awareness about the associated risks remain limited.

The increasing consumption of bottled water can be attributed to greater public awareness of its perceived health benefits. This trend initially emerged in Western Europe, followed by the United States and Asian countries. Global bottled water consumption reached 329.33 billion liters in 2015 [2]. The technological process of bottled water production includes reverse osmosis, nanofiltration, or ultrafiltration, wherein water passes through membranes measuring 0.0001, 0.001, and 0.01 μm, respectively, before being packaged. However, these filtration methods often remove essential minerals along with impurities. The World Health Organization has emphasized that consumers primarily choose bottled water for its taste and convenience, but safety and potential health benefits should be fundamental priorities [3]. As bottled water becomes increasingly popular, the Codex Alimentarius Commission of the WHO (2011) established an international framework for regulating bottled water.

Recent studies have shown that in blind taste tests, consumers were unable to distinguish between tap water and bottled water. Nevertheless, more consumers choose bottled water over tap water [4]. Despite adverse reports regarding bottled water, much of the research focuses on exceeding limits rather than evaluating baseline thresholds. Establishing ideal global limits based solely on human health could lead to overestimation or underestimation, given regional variations in daily water consumption [5].

The aim of this study is to develop, test, and validate a questionnaire to assess the population's perception of bottled water quality, particularly regarding its chemical composition, and to establish the interrelationship between these perceptions and public health. The validation of the questionnaire will not only provide an effective data collection tool but also offer valuable insights into public education and regulatory policies for bottled water.

Material and methods

Stage 1. Development and design of the questionnaire and questionnaire validation

The questionnaire titled "Assessment of the chemical composition of bottled water and its impact on population health" was designed to be comprehensive, explicit, simple, and easy to understand.

The development process involved creating a detailed research tool capable of thoroughly investigating the attitudes, behaviors, and perceptions of the population regarding the consumption of bottled still water. The goal was to produce a well-structured material, organized into distinct sections, each targeting an essential aspect of the subject: from preferences and purchasing habits to perceptions of quality and health impacts.

The development process began with defining objectives, focusing on addressing key questions about bottled water consumption. These included identifying the factors influencing purchasing decisions, assessing consumers' awareness of the chemical composition and quality of bottled water, examining perceptions of product safety and sustainability, and evaluating the health impacts of consumption.

The questionnaire was structured with a clear organization, comprising four major components. The first part is dedicated to gathering general information about respondents, including socio-demographic characteristics. The second part examines consumption habits and brand preferences, as well as points of purchase. The third section explores perceptions of bottled water quality, trust in producers, and perceived risks. The final section addresses the connection between still water consumption and health, focusing on reported positive and negative influences as well as medical recommendations.

Specifically, a series of socio-demographic questions targeted biological gender, age, place of residence, profession, education level, family size, and average household income.

These questions focused on drinking water consumption and health conditions that might affect hydration and overall health, aiming to evaluate the respondent's profile. Detailed habits regarding the consumption of drinking water or beverages were recorded, requesting quantitative information about the volume in liters or the number of bottles consumed daily.

The questionnaire items were designed to allow the collection of both quantitative and qualitative data. The first version of the questionnaire was based on expert opinions and a bibliographic review focused on similar previously developed questionnaires. To minimize measurement errors, the questions were short, closed-ended, and crafted to be concise and easy to understand. Additionally, predefined response options were provided, drawing on previous studies and consultations with domain experts, offering detailed choices to better clarify the questionnaire's purpose.

To ensure the validity and relevance of the instrument, the questionnaire was tested on a sample of 32 respondents, allowing the identification and correction of any ambiguities or redundancies.

In July-August 2024, 32 adult individuals (5 men and 27 women) recruited from the general population of the Republic of Moldova, aged between 24 and 62 years, participated in the study. The questionnaire was applied online via the Google Forms platform, and the link was distributed through open social networks. The participants were informed about the study objectives and provided their consent to participate. The collected data were kept confidential, and the study was approved by the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, approval number 1, dated 09.10.2023.

Stage 2. Content validity

This phase aimed to ensure that the set of items accurately measures the expected construct of interest. Content validity assesses how well the items of a questionnaire reflect the concept or domain it aims to evaluate. It is a qualitative measure based on expert judgment. An independent panel of 4 experts participated in this initial stage, including 2 hygiene physicians, a specialist in social medicine and biostatistics, and a sociologist. Each expert received a copy of the first version of the questionnaire via email and was asked to assess the relevance and clarity of the items using two 4-point Likert scales. Content validity is a current and widely used method for assessing the quality of a questionnaire, especially in the early stages of development [6-8].

A content validity index (CVI) was calculated for each domain:

- S-CVI/Ave – scale level, using the average method; acceptable limit >0.80 [8].
- S-CVI/UA – scale level, using the universal agreement method; acceptable limit >0.80 [9].

Additionally, a CVI was calculated for each individual item (I-CVI; acceptable limit >0.83), along with the modified kappa concordance index (κ*):

- 0.75-1.00 – excellent
- 0.60-0.74 – good
- 0.40-0.59 – acceptable
- <0.40 – poor
- acceptable limit >0.60 [7, 10].

The experts were also given the opportunity to provide additional comments and suggestions for each item of the questionnaire. Following this stage, the second version of the questionnaire was developed. Researchers in the Republic of Moldova have extensive experience and have validated questionnaires both at the national level and through the evaluation of study feasibility and the Cronbach's alpha coefficient.

Stage 3. Apparent validity and internal consistency

A quantitative and qualitative study was conducted to analyze the items, aiming to assess their suitability for inclusion in the questionnaire through face validity and internal consistency. The questionnaire, distributed online, was completed by 32 adults, constituting a convenience sample with a wide age range and diverse preferences and practices regarding bottled water consumption.

Data were collected in July-August 2024. Participants completed the second version of the questionnaire and were provided with an open space to offer additional feedback regarding ease of completion, clarity, and suggestions for improvement. For face validity, frequencies were calculated, and open-ended narrative responses regarding opinions and/or instrument improvements were analyzed [11]. Internal consistency was determined using Cronbach's alpha coefficient for each dimension (acceptable limit >0.70) [12]. As a result of this stage, the third version of the questionnaire was developed.

Statistical analysis

The results were presented in terms of central tendency values, relative values, and absolute data. Additionally, Cronbach's alpha coefficient was used to determine internal consistency. Statistical analyses were performed using SPSS Statistics 27 (SPSS, Inc., Chicago, IL, USA).

Results

Stage 1. Development of the questionnaire and questionnaire validation

The first version of the questionnaire included 67 questions, structured into four parts: "General information", "Information about bottled drinking water consumption", "Public perception of bottled still water quality", and "Bottled still water and health". The main areas of interest were: "Information about bottled drinking water consumption", "Public perception of bottled still water quality", and "Bottled still water and health". The questions in the first domain had pre-established, clear, and concise response options. The questions in the second domain also had pre-established answers, with a minimum of 2 response options and a maximum of 20. For some questions (7 in total), multiple responses were allowed. The third domain of the questionnaire included questions with response options ranging from 2 to 15. Two questions allowed multiple responses. The maximum number of responses in the fourth section

was 7, with no multiple-response questions. Respondents were asked to freely express their opinions and knowledge regarding their perceptions of bottled still water, its consumption, water quality, and its impact on health.

Stage 2. Content validity

S-CVI/Ave represents the content validity index at the scale level, calculated as the average of the validity scores for all items, while S-CVI/UA indicates the percentage of items that received complete agreement between evaluators. S-CVI demonstrates content validity in terms of both relevance and clarity (Table 1). S-CVI/Ave ranged from 0.84 to 0.90 for the domains, exceeding the minimum standard of 0.80. S-CVI/UA ranged from 0.82 to 0.89 for the domains, surpassing the minimum standard of 0.80 for relevance and clarity in all domains.

The I-CVI values (Table 2) for the relevance criterion ranged from 0.83 to 1.00, and the κ* indices fell within the "excellent" category (κ* 0.75–1.00). Regarding clarity, the I-CVI values ranged from 0.83 to 1.00, and the κ* indices also fell within the "excellent" category (κ* 0.75–1.00).

Based on the evaluations and comments, modifications were made to 18 questions, including the removal of 6 questions. As a result, the second version of the questionnaire included 61 items.

Table 1. S-CVI/AVE and S-CVI/UA for the three domains of interest in the questionnaire

Domains of the questionnaire	S-CVI/Ave		S-CVI/UA	
	Relevance	Clarity	Relevance	Clarity
Information about bottled drinking water consumption	0.88	0.86	0.84	0.86
Public perception of bottled still water quality	0.86	0.84	0.82	0.82
Bottled still water and health	0.90	0.88	0.89	0.87

Note:
S-CVI/Ave – scale level, using the average method; acceptable limit >0.80;
S-CVI/UA – scale level, using the universal agreement method; acceptable limit >0.80.

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

Domains of the questionnaire	I-CVI [a] Relevance				K* for Relevance [b]				I-CVI [a] Clarity				K* for Clarity [b]			
	1.00	0.83	0.67	0.5	Excellent	Good	Acceptable	Poor	1.00	0.83	0.67	0.5	Excellent	Good	Acceptable	Poor
Information about bottled drinking water consumption	85.5	14.5	0	0	100	0	0	0	86.0	14.0	0	0	100	0	0	0
Public perception of bottled still water quality	84.3	15.7	0	0	100	0	0	0	83.2	16.8	0	0	100	0	0	0
Bottled still water and health	89.9	10.1	0	0	100	0	0	0	87.5	12.5	0	0	100	0	0	0

Note:
κ* represents the modified kappa concordance index;
[a] I-CVI indicates the content validity index at the item level;
[b] κ* 0,75-1,00: excellent, κ* 0,60-0,74: good, κ* 0,40-0,59: acceptable, and κ* <0,40: poor

Stage 3. Face validity and internal consistency

The structure of respondents who participated in the face validity phase was 84.4% women and 15.6% men, with ages ranging from 24 to 62 years (40.03±11.74). Of them, 84% were from urban areas, and 16% were from rural areas. Additionally, 96.9% had higher education, and 3.1% had specialized secondary education.

The questionnaire included three open-ended questions, to which respondents provided qualitative answers (Table 3).

Respondents were asked an open-ended question regarding the clarity and understanding of the questions in the questionnaire. Most respondents considered the questions clear and easy to understand, expressing themselves with phrases such as "Everything was clear", "All were clear", or "There are none", indicating the absence of significant issues related to content comprehension. Some participants noted that the questionnaire was lengthy, highlighting the risk that respondents might become bored and abandon it. Additionally, a few responses pointed out specific questions that were perceived as unclear or difficult to understand, such as questions 6, 10, 23, 36, 45, and 51. Furthermore, some responses highlighted the redundancy of certain questions, with one observation noting that some questions seemed to have the same meaning, while another remark pointed to a sequencing issue between questions 50 and 51, suggesting the need for conditional display logic.

Table 3. Results of the responses (n=32) to the three open-ended questions in the questionnaire during the apparent validity phase

	n (%)		
	Yes	Non	Excluded from analysis
1. Please indicate the question numbers of any unclear or difficult-to-understand questions in this questionnaire.	23 (71.9%)	5 (15.6%)	4 (12.5%)
2. Please indicate the question numbers of the clear questions.	24 (75.0%)	4 (12.5%)	4 (12.5%)
3. Please suggest ways to improve the unclear/difficult-to-understand questions in this questionnaire.	23 (71.9%)	5 (15.6%)	4 (12.5%)

Note: n (%) - relative values, and absolute data.

The analysis of responses regarding the questions considered clear in the questionnaire highlighted that most respondents found all the questions clear, using phrases such as "All," "Everything was clear," or "All are clear." One participant noted that some questions practically repeat, especially those referring to changes in health status after starting to consume bottled water. Additionally, question 57 was flagged as redundant in relation to question 60. One participant suggested adding filters to the questionnaire to tailor the questions to specific situations, stating: "Most are clear, but there should be some filters. For example, I consume 5 liters of water over 1.5-2 months...". Some respondents identified specific questions as clear, such as questions 35, 2, and 1, or provided general answers like "Basically all."

From the responses provided by the participants regarding ways to improve unclear or difficult-to-understand questions in the questionnaire, most considered the questions to be clear, offering answers such as "There are none", "They are quite clear", or "Everything is clear", suggesting that the questionnaire is, by and large, well-designed and accessible. However, some respondents provided valuable suggestions for improvements. A frequent observation was related to the repetitiveness of certain questions, such as those concerning changes in health status or questions about organoleptic qualities. For example, one participant mentioned that it was unclear whether the questions referred separately to characteristics such as transparency and taste or to all of them simultaneously. This issue was addressed by clarifying the wording and avoiding redundancy. Additionally, some responses emphasized the need for further explanations for technical or less familiar terms, so that the respondents could better understand the content of the questions. Furthermore, the introduction of filters or conditional logic in the questionnaire was recommended to adapt the questions to the specific situations of the participants, making the questionnaire more personalized and effective. Some participants also suggested adding the option "I don't know/I'm not sure" for all questions, which would allow for the collection of more authentic and less forced responses.

Some of the responses were ambiguous or incomplete, using expressions such as "I don't know", "I couldn't notify", or "-", which indicates either a lack of clear observations or difficulties in understanding the open-ended question posed. These responses were excluded from the analysis.

Internal consistency was demonstrated through the Cronbach's alpha coefficient parameters (Table 4). The Cronbach's alpha coefficient for the entire questionnaire was 0.717 (excluding the nine questions in the "general information" section), and for two domains, the coefficients were also higher than the minimum acceptable value of 0.70 (ranging between 0.702 and 0.815), with the exception of the "Information about bottled drinking water consumption" domain (Cronbach's alpha coefficient = 0.634). We decided to retain this domain because the questions related to it reflect subsequent domains, based on literature data and expert experience. Therefore, no changes were made to the questionnaire.

Table 4. Cronbach's alpha coefficient for each domain and for the entire questionnaire (internal consistency)

Domains of the questionnaire	Cronbach's alpha	
	value	n
Information about bottled drinking water consumption	0.634	21
Public perception of bottled still water quality	0.815	12
Bottled still water and health	0.702	19
The questionnaire in its entirety with all domains	0.717	52

Note: Cronbach's alpha (α) measures the internal consistency of a test, reflecting how well items in a set are related. Values above 0.70 are typically acceptable, while those over 0.90 suggest excellent reliability. Named after psychologist Lee Cronbach, it is widely used to assess test reliability.

Discussions

This study developed a questionnaire to assess the chemical composition of bottled drinking water in relation to public health. The questionnaire was developed through a detailed process that included stages of validity evaluation and content adjustment. Its validation was carried out using the S-CVI and I-CVI validity indices, which demonstrated excellent agreement regarding the relevance and clarity of the questions. Additionally, internal consistency was confirmed through Cronbach's alpha coefficient, with values exceeding the acceptable threshold for most of the domains investigated. Subsequent revisions allowed for the elimination of redundant questions and the improvement of item formulations, ensuring the relevance and accessibility of key topics: bottled drinking water consumption, perceptions of its quality, and health effects.

In particular, the most important result of the study was the creation of a questionnaire that can be used to assess public perception regarding the consumption of bottled drinking water, as well as its potential benefits and risks.

Our study highlights several key findings regarding the development and validation of the questionnaire used to evaluate perceptions and consumption of bottled drinking water. Its structure, the relevance of the questions, their clarity, as well as data on apparent validity and internal consistency, indicate the effectiveness of the research tool employed.

The questionnaire, structured into four domains–three of which are thematic and essential–was adapted based on suggestions provided by respondents and experts. The modifications resulted in a reduction in the number of questions from 67 to 61, while maintaining the relevance of the data collected. This process reflects best practices found in the literature, which emphasize the need for flexible and adaptable tools [13, 14].

The S-CVI/Ave and S-CVI/UA indicators, which exceeded the minimum standard of 0.80 for the three thematic domains of importance, confirm the quality of the questions in terms of both clarity and relevance. These results align with methodologies presented by other researchers, who have emphasized the importance of quantitative assessment of content validity [15]. Additionally, the I-CVI index and the modified kappa coefficient indicate excellent

agreement among evaluators, supporting the robustness of this process.

The respondent profile, predominantly consisting of individuals with higher education and from urban areas, ensured the gathering of diverse perspectives on the investigated topic. Their feedback revealed that the questions were generally well-formulated, although a few aspects were identified that required adjustments. The observations provided facilitated the optimization of the questionnaire, similar to the results presented in other significant studies [16, 17].

The Cronbach's alpha coefficient, with values above the minimum threshold of 0.70 for most domains, confirms the internal consistency of the questionnaire [18, 19]. The exception observed in one domain (0.634) did not, however, affect its use due to its importance within the overall framework of the study. This aspect is supported by similar analyses in the literature [20-22].

Among the identified limitations is the possibility that responses may be influenced by participants' subjectivity and the complexity of the questionnaire. To avoid situations that could jeopardize the survey process and data collection, the developed tool will be applied in a more diversified setting, including respondents from rural areas, for a more extensive validation.

Conclusions

The developed questionnaire for assessing bottled water consumption and public perception has proven to be a valid and effective tool. Content validity and internal consistency were confirmed, and the face validity process ensured the clarity of the questions.

Competing interests

None declared.

Authors' contributions

All authors contributed equally to the drafting and writing of the manuscript. The authors read and approved the final version of the manuscript.

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The study protocol was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Protocol No. 01 of 09.10.2023).

References

1. Talatala S. The effect of tap water perception on the consumption of bottled water. Public perception of tap water. 2008 [cited 2024 Dec 11]. Available from: https://nature.berkeley.edu/classes/es196/projects/2008final/Talatala_2008.pdf.
2. Qian N. Bottled water or tap water? A comparative study of drinking water choices on university campuses. Water. 2018;10(1):59. doi: 10.3390/w10010059.
3. World Health Organization. Guidelines for drinking-water quality. 4th ed. Geneva: WHO; 2011. 541 p.
4. Debbeler LJ, Gamp M, Blumenschein M, Keim D, Renner B. Polarized but illusory beliefs about tap and bottled water: a product- and consumer-oriented survey and blind tasting experiment. Sci Total Environ. 2018;643:1400-1410. doi: 10.1016/j.scitotenv.2018.06.190.
5. Manoj S, Kartheeshwari MR, Keerthan L, Elango L. Effects of low mineral content in bottled drinking water on human health and evaluation of optimal requirements. J Food Compos Anal. 2024;128:106052. doi: 10.1016/j.jfca.2024.106052.
6. Zamanzadeh V, Ghahramanian A, Rassouli M, Abbaszadeh A, Alavi-Majd H, Nikanfar AR. Design and implementation content validity study: development of an instrument for measuring Patient-Centered Communication. J Caring Sci. 2015;4(2):165-178. doi: 10.15171/jcs.2015.017.
7. Polit DF, Beck CT, Owen SV. Is the CVI an acceptable indicator of content validity? Appraisal and recommendations. Res Nurs Health. 2007;30(4):459-467. doi: 10.1002/nur.20199.
8. Davis LL. Instrument review: getting the most from a panel of experts. Appl Nurs Res. 1992;5(4):194-197. doi: 10.1016/S0897-1897(05)80008-4.
9. Waltz CF, Strickland OL, Lenz ER. Measurement in nursing and health research. 4th ed. New Yor: Springer; 2010. 492 p.
10. Wynd CA, Schmidt B, Schaefer MA. Two quantitative approaches for estimating content validity. West J Nurs Res. 2003;25(5):508-518. doi: 10.1177/0193945903252998.
11. Rattray J, Jones MC. Essential elements of questionnaire design and development. J Clin Nurs. 2007;16(2):234-243. doi: 10.1111/j.1365-2702.2006.01573.x.
12. Bland JM, Altman DG. Statistics notes: Cronbach's alpha. BMJ. 1997;314:572. doi: 10.1136/bmj.314.7080.572.
13. Krug K, Wensing M. Development and validation of questionnaires in health services research. In: Wensing M, Ullrich C, editors. Foundations of health services research: principles, methods, and topics. Cham: Springer; 2023. p. 139-152. doi:10.1007/978-3-031-29998-8_11.
14. Ajith V, Fishman R, Yosef E, Edris S, Ramesh R, Alookaran Suresh R, Pras A, Rahim V, Rajendran S, Yanko M, Amit A, Bhavani RR, Vinodini Ramesh M, Mamane H. An integrated methodology for assessment of drinking-water quality in low-income settings. Environ Dev. 2023;46:100862. doi: 10.1016/j.envdev.2023.100862.
15. Lynn MR. Determination and quantification of content validity. Nurs Res. 1986;35(6):382-385.
16. DeVon HA, Block ME, Moyle-Wright P, et al. A psychometric toolbox for testing validity and reliability. J Nurs Scholarsh. 2007;39(2):155-164. doi: 10.1111/j.1547-5069.2007.00161.x.
17. Boateng GO, Neilands TB, Frongillo EA, Melgar-Quiñonez HR, Young SL. Best practices for developing and validating scales for health, social, and behavioral research: a primer. Front Public Health. 2018;6:149. doi: 10.3389/fpubh.2018.00149.

18. Croitoru C, Ciobanu E, Ferdohleb A. Validarea chestionarului: Cunoştinţe, atitudini şi practici privind fenomenul de antibiorezistenţă la medici [Validation of questionnaire: Knowledge, attitudes and practices regarding the antibioresistance phenomenon to doctors]. Arta Medica (Chisinau). 2022;84(3):4-15. Romanian. doi: 10.5281/zenodo.7305448.
19. Bologan V, Mazur-Nicorici L, Spinei L, Cebanu S, Visnevschi A, Ciobanu E. Development and validation of the questionnaire for hygienic estimation of the impact of risk factors on morbidity due to osteoporosis. Mold J Health Sci. 2024;11(4):15-24. doi: 10.52645/MJHS.2024.4.03.
20. Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int J Med Educ. 2011;2:53-55. doi: 10.5116/ijme.4dfb.8dfd.
21. Malisova O, Bountziouka V, Panagiotakos DB, Zampelas A, Kapsokefalou M. The water balance questionnaire: design, reliability and validity of a questionnaire to evaluate water balance in the general population. Int J Food Sci Nutr. 2012;63(2):138-144. doi: 10.3109/09637486.2011.607799.
22. Ferdohleb A, Croitoru C, Ciobanu E, Tapu L, Spinei L. Questionnaire standardization knowledge, attitudes, and practices regarding the antimicrobial resistance of medical workers. In: Biomedical and health research: quality, excellence and performance, 19-21 October 2022, Chişinău, Republic of Moldova: Abstract book. Chişinău; 2022. p. 67.

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RESEARCH ARTICLE

The dosage and biological role of carotenoids and chlorophylls in species of the genus *Galium L.*

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ABSTRACT

Introduction. Carotenoids and chlorophylls are plant pigments that play a vital role in plant life, participating in the process of photosynthesis and in the synthesis of growth regulators, stress signalers and fungicide attack agents. Research from the last decades has shown that, widely used as nutrients, chlorophyll and carotenoid pigments have beneficial effects on the human body. They exert antioxidant, energizing, detoxifying, anti-inflammatory properties, and can be applied in the prevention and treatment of 21st century diseases (cancer, cardiovascular diseases, low immunity), which are related to numerous causal factors (excessive chemicalization of the food industry, progressive climate change and environmental pollution), which reflects on the quality of human health.

Material and methods. We aimed to determine the content of carotenoid and chlorophyll pigments in different plant organs (stems, leaves, flowers and aerial parts), collected during the flowering period from 2 species, *G. verum* and *G. aparine* from the spontaneous flora of the Republic of Moldova. Determination was carried out by spectrophotometric method with the Metertech UV/VIS SP 8001 spectrophotometer, used as an extractant ethyl alcohol at 60% and 95% concentration. The data were reported as mean values ± standard deviation (SD).

Results. Experimental data obtained on the comparative application of 2 concentrations of ethyl alcohol (60 and 95%) for the extraction of carotenoid and chlorophyll pigments show that 95% ethyl alcohol is the most efficient for all analyzed samples. The determination of carotenoid pigments in different organs of *G. verum* and *G. aparine* species indicates that the maximum carotenoid content is extracted from the aerial parts of *G. verum* sp. (52.66 mg/%) than those of *G. aparine* (27.38 mg/%); compared to the content of chlorophyll pigments, which is quantitatively predominant in *G. aparine* sp. (chlorophyll *a* – 2.85 and chlorophyll *b* – 4.33 mg/l) compared to *G. verum* sp. (chlorophyll *a* – 1.2 and chlorophyll *b* – 1.9 mg/l).

Conclusions. The results of spectrophotometric determination of carotenoids, chlorophylls *a* and *b* in different organs of *G. verum* and *G. aparine* sp. denote that the highest content of pigments is extracted with 95% ethyl alcohol in aerial parts in both species of the spontaneous flora of the Republic of Moldova.

Keywords: carotenoids, chlorophyll *a*, chlorophyll *b*, *Galium verum*, *Galium aparine*.

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Key messages

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

Determination of chlorophylls and carotenoids concentration in different organs of 2 species of the genus *Galium* (*G. verum* and *G. aparine*) collected from the spontaneous flora of the Republic of Moldova.

The research hypothesis

The species *Galium verum* and *Galium aparine* could serve as sources of chlorophylls and carotenoids.

The novelty added by manuscript to the already published scientific literature
Medicinal plant species with a high content of chlorophyll and carotenoid pigments can be used in the pharmaceutical industry for their antioxidant, energizing, detoxifying properties.

Introduction

The use of medicinal plants has a long history, dating back to ancient times, with its practice widespread in different regions around the world. Both the past and the present are marked by the fundamental importance of the use of plants in ethnomedicine [1]. Medicinal plants are used to treat a wide range of disorders, diseases and illnesses: dermal, cardiovascular, endocrine, gastrointestinal, genito-urinary, respiratory, musculoskeletal, hepatic, including cancer [2]. The therapeutic effects of medicinal plants are often attributed to secondary metabolites, but it should be noted that carotenoid and chlorophyll pigments also play a significant role in the prophylaxis and treatment of various diseases [1].

Carotenoids and chlorophylls are the most common pigments in nature, providing a wide range of colors (yellow, orange, red, purple, green) to plants. Carotenoids are tetraterpene pigments, predominantly composed of 8 isoprene units with a backbone of 40 carbon atoms. Carotenoids are divided into 2 groups: carotenes and xanthophylls. Carotenes, such as α, β-carotene, ψ-carotene (γ-carotene) and lycopene, are represented by hydrocarbons. About 50 types of carotenes are present in nature [3]. On the other hand, xanthophylls, such as β-cryptoxanthin, lutein, zeaxanthin, astaxanthin, fucoxanthin and peridinin, are carotenoids, which contain oxygen atoms in the form of hydroxy, carbonyl, aldehyde, carboxyl, epoxide and furanoxide groups in its molecules, which also confer pronounced antioxidant properties [4]. As of 2018, more than 800 types of xanthophylls have been reported in nature, some of which are present as fatty acid esters, glycosides, sulfates and protein complexes [3, 4].

Chlorophylls have a porphyrin ring at their base, to which a long carbon chain (phytol) is attached, and a magnesium ion in the center. The purpose of the porphyrin ring is to capture solar energy and the magnesium ion acts as an electron acceptor. There are several types of chlorophyll, such as chlorophyll *a*, *b*, *c*, *d* and chlorophyll *e* [1], with different colour shades, such as for chlorophyll *a* – bluish green, and chlorophyll *b* – yellowish green.

In the plant body, carotenoids and chlorophylls are essential compounds in providing two major physiological processes such as photosynthesis (being part of the light-harvesting complex) and protection against photo-oxidative damage in cells [4, 5].

Carotenoids and chlorophylls capture light energy in the blue-red spectral range (400-650 nm) and excite electrons in pigment molecules. After absorbing light energy, the excited electrons in chlorophyll are used to carry out the two phases of photosynthesis: light and dark. The first phase of photosynthesis is also known as light-dependent reactions, which take place in the thylakoid membrane of chloroplasts and is responsible for the production of ATP and NADPH. The second phase of photosynthesis is known as light-independent reactions, also called the Calvin cycle, which takes place in the stroma of chloroplasts. In this phase, ATP and NADPH produced in the light-dependent reactions are used to achieve the production of glucose and oxygen [1].

An important function of carotenoids is the absorption of excess energy from chlorophylls by triplet-triplet transfer and the release of excess energy by polyene vibration. Triplet-triplet transfer is an essential higher energy state in photoprotection. Reactive types of oxygen, such as singlet oxygen, hydroxyl radicals and superoxide anion radicals, are produced by oxygen and light during photosynthesis. Carotenoids with more than 11 conjugated double bonds show a marked ability to quench singlet oxygen [6].

Another major function of carotenoids is the synthesis of hormones (growth regulators), which are formed by their oxidative cleavage to form apocarotenoids. Apocarotenoids perform such vital functions as growth regulators, stress factor signalers and fungal attack agents. One of the best known phytohormones is abscisic acid, which regulates plant growth, seed dormancy, embryo maturation and germination, cell division and elongation, stomatal cell movement, floral growth and responses to biotic and abiotic stresses [3, 5]. Carotenoids are also present in non-photosynthesizing plant organs such as roots, flowers, fruit pericarp and seeds, acting as photoprotectants, antioxidants, color attractants and plant hormone precursors [7].

Approximately 50 types of carotenoids are found in the typical human diet, of which about 20 can be detected in blood (plasma or serum) following ingestion [8]. Carotenoids can be found in several human organs such as liver, adrenal glands, ovaries, skin, lungs, testes, prostate and blood serum. The distribution of carotenoids in human organs shows specificity. Lutein and zeaxanthin are found on the surface of skin and subcutaneous tissue in an esterified form and act as UV absorbers and singlet oxygen quenchers [9]. Xanthophylls, such as β-cryptoxanthin, lutein and zeaxanthin, are found in the brain [10]. In the eye, lutein and zeaxanthin are present as macular pigments [7]. Lycopene accumulates in the prostate [6].

The first report on the efficacy of dietary β-carotene in reducing human cancer rates [6] was followed by multiple preclinical and clinical studies demonstrating the role of intake of carotenoid-containing leafy green vegetables and fruits in reducing cancer risk. For example, the fruit of mandarin *Citrus unshiu,* cultivar 'Satuma' is characterized with high content of β-cryptoxanthin, and may be associated with reduced cancer risk. Consumption of lycopene has shown to improve health outcomes in prostate cancer pa-

tients. In addition, clinical studies have shown, that administration of natural carotenoid complex (mixture of α and β-carotene, lutein and lycopene) and α-tocopherol significantly suppresses hepatoma development in patients with hepatitis virus-induced cirrhosis. Carotenoids have also been reported to contribute to the prevention of cardiovascular disease, diabetes, obesity and several lifestyle-related diseases. In addition, carotenoids improve resistance, boost immunity and especially skin quality [4].

The bioactivity of chlorophylls is attributed to their ability to act as antioxidants, antimutagens and anticarcinogens. The unique chemical structure allows chlorophylls to scavenge harmful free radicals, mitigate DNA damage and modulate cellular processes involved in the development of disease. In addition, their hydrophobic side chains facilitate interactions with biological membranes, influencing cellular uptake and signalling pathways [1].

Species of the g. *Galium*, with a long history as medicinal plants in traditional medicine, have attracted the attention of researchers, who have scientifically demonstrated that their carotenoids and chlorophylls content provides biologically active substances with antioxidant, antiradiant and energizing properties [11-13]. Given these findings, our study aims to determine the content of carotenoid and chlorophyll pigments in different organs of *G. verum* and *G. aparine* from the wild flora of the Republic of Moldova.

Material and methods

Plant material. Different vegetal products (stems, leaves, flowers and aerial parts), collected during the flowering period from 2 species of *G. verum* and *G. aparine* from the spontaneous flora of the Republic of Moldova, were used for the study of pigments. The species were collected from the North of the Republic of Moldova: *G. verum* were collected from Brinzeni village, Edinet district (48°5′0″ North 27°10′28″ East), and *G. aparine* – from Taul village, Donduseni district (48°12′57″ North 27°40′22″ East). The collected material was dried and conditioned according to the technical normative requirements [14].

Preparation of plant extracts. The dried plant product was shredded using a grinder and the powder was sieved through a 0.5 mm pore sieve. Extracts were obtained by repeated extraction of the pulverized plant products with a mixture of ethanol:water at two concentrations (60 and 95%, w/w) for half an hour at each extraction step until the plant products were exhausted. Extraction was performed with a water bath coupled with a condenser. Extractive solutions obtained were concentrated at 40°C using a rotary evaporator – Laborota 4011.

***Chemical and reagents*.** Ethyl alcohol was used as the solvent to extract the carotenoids and chlorophylls at two concentrations: 60 and 95%.

Sphectrophotometric assay. The optical density of the extracts obtained was measured by Metertech UV/VIS SP 8001 spectrophotometer at different wavelengths.

For the determination of carotenoids, the optical density of the extract was determined at 448 nm, after which the absorbance was substituted in formula number 1 [15].

Formula for the determination of carotenoids:

$$x = \frac{\mathrm{A}*100*100*100*25*1000}{\mathrm{m}*2500*5*(100-\mathrm{w})*100} = \frac{\mathrm{A}*100*1000}{\mathrm{m}*5*(100-\mathrm{w})}, \quad (1)$$

where: A – absorbance; m – raw material weight; w – yield of plant product on drying (*G. verum* – 10.15; *G. aparine* – 11.12).

Chlorophyll optical density was determined at 664 nm for chlorophyll *a*, and at 649 nm for chlorophyll *b*. To determine the chlorophyll content, absorbance was substituted in formulas 2 and 3 for chlorophyll *a* and *b*, respectively.

Chl *a* = 13.36×A664−5.19×A649 (2),

Chl *b* = 27.43×A649−8.12×A664 (3).

Statistical analysis. All determinations were made in triplicate, and the results are reported as mean values ± standard deviation (SD) using Microsoft Excel 2021.

Results

The carotenoid contents of the analyzed alcohol extracts, calculated using formula 1, are presented numerically in Table 1 and diagrammatically in Figures 1 and 2.

Table 1. Carotenoid content (±SD) in different organs of *G. verum* and *G. aparine* species

Species, organ/ Analyzed extract	Carotenoid content (mg/%)							
	Organs of ***G. verum***				Organs of ***G. aparine***			
	stems	leaves	flowers	aerial parts	stems	leaves	flowers	aerial parts
Alcoholic extract (60%)	13.39 ± 0.07	20.03 ± 0.11	20.81 ± 0.1	38.03 ± 0.13	6.23 ± 0.09	15.91 ± 0.06	18.36 ± 0.07	20.92 ± 0.09
Alcoholic extract (95%)	23.85 ± 0.08	33.98 ± 0.11	35.1 ± 0.07	52.66 ± 0.09	7.23 ± 0.07	20.03 ± 0.06	24.26 ± 0.04	27.38 ± 0.11

Analysis of the obtained data (Table 1 and Fig. 1, 2) shows that 95% ethyl alcohol facilitates better extraction than 60% ethyl alcohol for all organs in both species of genus *Galium.* When comparing the results, we established that in extracts of organs of *G. verum* species, the content of carotenoids is obviously higher than in *G. aparine* (stems – 23.85 and 7.23; leaves – 33.98 and 20.03; flowers – 35.1 and 24.26; aerial parts – 52. 66 and 27.38).

The data in Table 1, Figure 1 and 2 indicate that the carotenoid content (mg/%) with 95% alcohol ranges from 23.85 for stems to 52.66 in aerial parts for *G. verum* and from 7.23 to 27.38 for *G. aparine*, respectively. Note that carotenoid content correlates with plant organ in the same consecutiveness for both species (decreasing): the highest values are for aerial parts, followed by flowers, leaves and the lowest are recorded for stems.

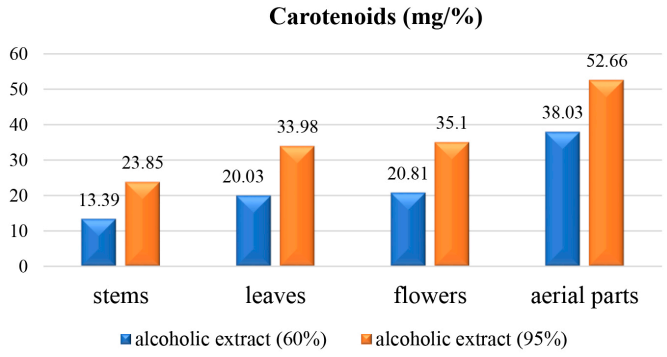

Fig. 1 Carotenoid content extracted with 60% and 95% ethyl alcohol from different organs of *G. verum* species

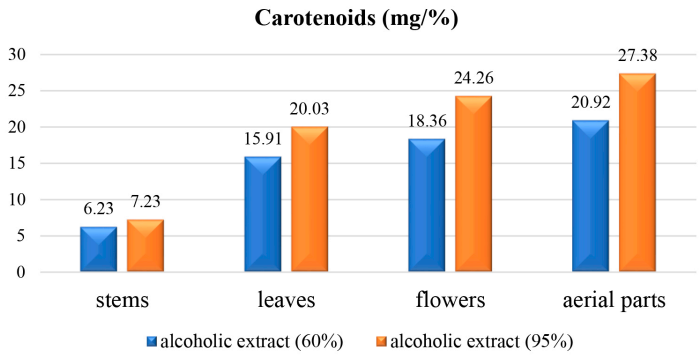

Fig. 2 Carotenoid content extracted with 60% and 95% ethyl alcohol from different organs of *G. aparine* species

The contents of chlorophylls *a* and *b* from the alcoholic extracts from vegetal products of *G. verum* and *G. aparine*, calculated according to formulas 2 and 3, expressed in mg/l, are given in Table 2.

Table 2. Content (±SD) of chlorophyll a and b pigments in the organs of *G. verum* and *G. aparine* species

Species	Chlorophyll content (mg/l)							
	Alcoholic extract 60%				Alcoholic extract 95%			
G. verum	stems	leaves	flowers	aerial parts	stems	leaves	flowers	aerial parts
Chl *a*	0.13 ± 0.09	0.23 ± 0.13	0.26 ± 0.18	0.33 ± 0.1	0.22 ± 0.12	1.09 ± 0.15	0.5 ± 0.05	1.2 ± 0.08
Chl *b*	0.26 ± 0.16	0.51 ± 0.15	0.65 ± 0.12	0.67 ± 0.08	0.23 ± 0.18	1.64 ± 0.16	0.74 ± 0.07	1.9 ± 0.05
	Alcoholic extract 60%				Alcoholic extract 95%			
G. aparine	stems	leaves	flowers	aerial parts	stems	leaves	flowers	aerial parts
Chl *a*	0.34 ± 0.02	0.41 ± 0.03	0.66 ± 0.016	1.1 ± 0.05	1.07 ± 0.04	2.28 ± 0.08	1.85 ± 0.11	2.85 ± 0.19
Chl *b*	0.48 ± 0.1	0.58 ± 0.18	0.89 ± 0.02	1.57 ± 0.13	1.46 ± 0.22	2.3 ± 0.19	1.87 ± 0.05	4.33 ± 0.14

Note: *Chl a – Chlorophyll a, Chl b – Chlorophyll b; SD – standard deviation*

The analysis of the chlorophyll content in the organs of *Galium* species shows a higher content (mg/l) of chlorophyll *a* and *b* in the aerial parts of *G. aparine* compared to *G. verum* in the 95% alcoholic extract (respectively chlorophyll *a*: aerial parts – 2.85 and 1.2; leaves – 2.28 and 1.09; flowers – 1.85 and 0.5; stems – 1.07 and 0.22 and chlorophyll *b*: aerial parts – 4.33 and 1.9; leaves – 2.3 and 1.64; flowers – 1.87 and 0.74 and stems – 1.46 and 0.23).

Discussion

This is the first study that analyzes and compares the content of chlorophyll and carotenoid pigments in the organs (stems, leaves, flowers and aerial parts) of 2 species of the genus *Galium* (*G. verum* and *G. aparine*) from the wild flora of the Republic of Moldova.

Previous studies [15] came to the same conclusion, that 95% ethyl alcohol facilitates better extraction of chlorophyll and carotenoid pigments compared to 60% ethyl alcohol.

Comparing the obtained results, we establish that in the alcoholic extracts from different organs of *G. verum,* the carotenoid content is obviously higher than in *G. aparine* with quantitative prevalence in aerial parts (aerial parts – 52.66 and 27.38; flowers – 35.1 and 24.26; leaves – 33.98 and 20.03; stems – 23.85 and 7.23). The comparative study of 6 species of genus *Galium* [12] similarly indicates higher carotenoid content in aerial parts of *G. verum* compared to *G. aparine* and 4 other studied species.

Carotenoids have a biologically determined role in energy transmission and singlet oxygen quenching chain, precursors of growth regulators [3, 4]. Additionally, numerous studies from different laboratories has been demonstrated the contribution of carotenoids in determining of antioxidant potential [7]. Recent studies identified the antimicrobial and antifungal activity of carotenoid-containing lipid complex in *Galium* species: *G. aparine* – the highest against strains of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus subtilis* and the lowest against *Escherichia coli*. *G. verum* exhibited strong sensitivity against *B. subtililis* and moderate activity against *Proteus vulgaris* [12]. The antifungal action denotes that the extract with carotenoids and chlorophylls from *G. aparine* has the highest sensitivity to *Candida albicans*, while *G. verum* was characterized with no significant antifungal effect.

Based on a comparison of the obtained results, we establish that the alcoholic extracts from the organs of *G. aparine* species are richer in chlorophylls *a* and *b* than those of *G. verum*, and the amount of chlorophyll *b* is higher than chlorophyll *a*, which has been mentioned in other studies [11].

Chlorophyll *a* is the primary bluish green pigment required for photosynthesis (it absorbs light with wavelength

430-662 nm from the orange-red and blue-violet regions of the electromagnetic spectrum, thus transferring energy to the reaction center and donating 2 excited electrons to the electron transport chain), and chlorophyll *b* – a yellowish green, accessory pigment, which is not always present in photosynthesizing organisms, but absorbs blue light (with peak absorption at around 453 nm) [1] and thus contributes to broadening the absorption spectrum of organisms. In this way, organisms can absorb more energy from the high-frequency blue light part of the spectrum [16]. The presence of chlorophyll *b* in cells demonstrates the adaptation of organisms to light-deficient conditions, helping them to convert a wider range of solar energy into the chemical one [17]. Thus, the higher values of chlorophyll *b* relative to *a* in all extracts analyzed in *Galium* sp., denote their high potential adaptability to light conditions to ensure an efficient photosynthetic process.

Besides the biological role of chlorophylls in the process of photosynthesis and the starting link for the biosynthesis of different chemical compounds [17], the therapeutic role of chlorophylls has been demonstrated through various studies, based on their ability to act as antioxidants, antimutagens and anticarcinogens, due to their unique chemical structure, which allows chlorophylls to scavenge harmful free radicals, attenuate DNA damage and modulate cellular processes involved in the development of diseases [1]. The antioxidant properties of *G. verum* were studied, on aerial parts extract, by 3 methods (DPPH, determination of OH radical scavenging activity and determination of hydrogen peroxide scavenging activity), the result of which shows that the extract with higher chlorophyll content has higher antioxidant capacity [11].

The chlorophyll *a* and *b* complex of *G. aparine* is characterized with half microbial inhibitory and bactericidal properties against *E. coli* strain, compared to Chlorophyllipt (the drug is a mixture of chlorophylls derived from eucalyptus leaves, which has bacteriostatic and bactericidal action, especially against staphylococci, including antibiotic-resistant strains, and has anti-inflammatory properties) but with low antifungal activity against *C. albicans* compared to Chlorophyllipt [12].

Conclusions

(1) Spectrophotometric determination of carotenoids (mg/%) in different vegetal products of *G. verum* and *G. aparine* species shows that the highest content of carotenoids is extracted with 95% ethyl alcohol in *G. verum*: aerial parts (52.66), flowers (35.1), leaves (33.98), stems (23.85), followed by carotenoids of *G. aparine.*

(2) Comparative analysis of chlorophyll *a* and *b* content (mg/l) in organs of the species presents their maximum concentration in aerial parts of both species (*G. verum* – chlorophyll *a* – 1.9 and chlorophyll *b* – 1.2 and *G. aparine* – 4.33 and 2.85, respectively) than in flowers, leaves and stems. In all analyzed extracts the content of chlorophyll *b* prevails numerically over chlorophyll *a*.

Competing interests

None declared.

Authors' contributions

AO collected the data, performed the experimental part; MCT coordinated experimental activity and approved the manuscript; TC interpreted the data and drafted the manuscript; All authors revised and approved the final version of the manuscript.

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References

1. Martins T, Novo Barros A, Rosa E, Antunes L. Enhancing health benefits through chlorophylls and chlorophyll-rich agro-food: a comprehensive review. Molecules. 2023;28(14):5344. https://doi.org/10.3390/molecules28145344.
2. Ravichandran S, Manju Bhargavi K, Rai A, et al. Medicinal plants for curing human diseases. Chin Med. 2023:6(1):570. doi: 10.18282/i-cm.v6i1.570.
3. Britton G, Liaaen-Jensen S, Pfander H. Introduction and guidelines on the use of the Handbook. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids. Basel: Birkhäuser; 2004. p. 1-33. https://doi.org/10.1007/978-3-0348-7836-4_1.
4. Maoka T. Recent progress in structural studies of carotenoids in animals and plants. Arch Biochem Biophys. 2009;483(2):191-195. doi: 10.1016/j.abb.2008.10.019.
5. Swapnil P, Meena M, Singh K, et al. Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. Curr Plant Biol. 2021;26:100203. doi: 10.1016/j.cpb.2021.100203.
6. Maoka T. Carotenoids as natural functional pigments. J Nat Med. 2020;74(1):1-16. doi: 10.1007/s11418-019-01364-x.
7. Britton G, Liaaen-Jensen S, Pfander H. Carotenoids. Vol. 4: Natural functions. Basel: Birkhäuser; 2008. 370 p. ISBN 978-3-7643-7498-3.
8. Khachik F, Beecher G, Goli M, Lusby W, Smith J. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. Anal Chem. 1992;64(18):2111-2122. doi: 10.1021/ac00042a016.
9. Wingerath T, Sies H, Stahl W. Xanthophyll esters in human skin. Arch Biochem Biophys. 1998;355(2):271-274. doi: 10.1006/abbi.1998.0734.
10. Craft N, Haitema T, Garnett K, Fitch K, Dorey C. Carotenoid, tocopherol, and retinal concentration in elderly human brain. J Nutr Health Aging. 2004;8(3):156-162.
11. Lakic N, Mimica-Dukic N, Isak J, Božin B. Antioxidant properties of Galium verum L. (Rubiaceae) extracts. Cent Eur J Biol. 2010;5(3):331-337. https://doi.org/10.2478/s11535-010-0022-4.

12. Ilyina T, Goryachaya O, Toryanik E. Antimicrobial activity of the genus Galium L. Pharmacogn Commun. 2016:6(1):42-47. doi: 10.5530/pc.2016.1.8.
13. Turcov D, Barna S. Antioxidants from Galium verum as ingredients for the design of new dermatocosmetic products. Plants. 2022;11(19):2454. https://doi.org/10.3390/plants11192454.
14. Nistreanu A. Farmacognozie [Pharmacognosy]. Chișinău: Tipografia Centrală; 2000. 672 p. Romanian.
15. Trineeva OV, Slivkin AI. Validatsiia metodiki opredeleniia karotinoidov v plodakh oblepikhi razlichnykh sposobov konservatsii [Validation of the methodology for the determination of carotenoids in sea buckthorn fruits of different preservation methods]. Vestnik VGU. Seriia: Khimiia. Biologiia. Farmatsiia [Proceedings of Voronezh State University. Series: Chemistry. Biology. Pharmacy]. 2016;(2):145-151. Russian.
16. Voitsekhovskaja OV, Tyutereva EV. Chlorophyll b in angiosperms: functions in photosynthesis, signaling and ontogenetic regulation. J Plant Physiol. 2015;189:51-64. https://doi.org/10.1016/j.jplph.2015.09.013.
17. Lakna P. Difference between chlorophyll A and B, definition, characteristics, role, comparison [Internet]. In: Pedia.com. 2017 Apr 11 [cited 2024 Jul 12]. Available from: https://pediaa.com/difference-between-chlorophyll-a-and-b/
18. Murariu A, Stratu A, Zamfirescu O. Ecophysiological research on some medicinal plants from Bălteni forest (Vaslui county). In: 4th Conference on Medicinal and Aromatic Plants of South-East European Countries; 2006, Iasi, Romania: Book of abstracts. Iasi: Alma Mater; 2006. p. 24.

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REVIEW ARTICLES

Gastric intestinal metaplasia and gastric epithelial dysplasia – precursor lesions of gastric cancer

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ABSTRACT

Introduction. Despite worldwide decreasing trends in the incidence of gastric cancer, the disease remains a significant global health burden, one of the leading causes of cancer death worldwide, and its prevention is a priority for the health system. Intestinal-type gastric carcinoma originates in dysplastic epithelium, which, in turn, develops in the environment of chronic atrophic gastritis and gastric intestinal metaplasia.

Material and methods. Narrative literature review. A bibliographic search was conducted in the databases PubMed, Hinari, SpringerLink, National Center for Biotechnology Information, and Medline. Articles published between 2000-2024 were selected based on the following keywords: "gastric intestinal metaplasia" and "gastric epithelial dysplasia", used in different combinations with the terms "epidemiology", "clinical picture", "risk factors", "classification", "diagnosis", and "management" to maximize the search yield. After processing the information from the databases according to the search criteria, 215 full articles were found. The final bibliography contains 34 relevant sources, considered representative of the materials published on the subject of this summary article.

Results. Gastric intestinal metaplasia represents the replacement of the gastric epithelium with two types of intestinal-type epithelium (enteric or colonic) as an adaptive response to chronic injury, while gastric epithelial dysplasia is defined as unequivocal neoplastic change of the gastric epithelium (intraepithelial neoplasia) without evidence of stromal invasion. Gastric intestinal metaplasia and gastric epithelial dysplasia are preneoplastic lesions of gastric cancer. The estimated annual risk of gastric adenocarcinoma in patients with gastric intestinal metaplasia is 0.13-0.25%, and in patients with gastric epithelial dysplasia it is 1.36%, depending on the extent and type of the lesion.

Conclusions. Despite the lack of a specific treatment for gastric intestinal metaplasia, the management strategy, according to current clinical guidelines, includes eradication of *Helicobacter pylori* infection, screening for early detection of gastric cancer, and control of other risk factors. Appropriate management of high-grade gastric epithelial dysplasia requires endoscopic resection due to its potential for progression to carcinoma and the possibility of coexisting carcinoma. For low-grade gastric epithelial dysplasia, which has a lower risk of malignant transformation, scientists recommend annual endoscopic surveillance with biopsy and histological examination.

Keywords: gastric intestinal metaplasia, gastric epithelial dysplasia, risk factors, serological examination, endoscopic examination, morphological examination, *Helicobacter pylori*.

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Key messages

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

An appealing approach to reducing the incidence of gastric adenocarcinoma is to identify high-risk individuals who may benefit from screening, prophylactic, and therapeutic measures to prevent the onset of gastric cancer.

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The research hypothesis
The analysis and synthesis of contemporary literature will enable a comprehensive characterization of patients with gastric intestinal metaplasia and gastric epithelial dysplasia to establish gastric cancer prevention strategies.

The novelty added by the manuscript to the already published scientific literature
The article summarizes the latest international publications on the epidemiology, clinical picture, risk factors, classification, diagnosis, and management of patients with gastric intestinal metaplasia and gastric epithelial dysplasia.

Introduction

Despite worldwide decreasing trends in the incidence of gastric cancer (GC), the disease remains a significant global health burden, one of the leading causes of cancer death worldwide, and its prevention is a priority for the health system [1].

The sequence leading to GC – the Correa cascade – can be schematically reduced to Helicobacter pylori infection (HP) – non-atrophic gastritis – chronic atrophic gastritis (CAG) – gastric intestinal metaplasia (GIM) – gastric dysplasia (GD) – neoplasia. Intestinal-type gastric carcinoma originates in dysplastic epithelium, which, in turn, develops in the environment of CAG and GIM. Prevention of HP infection and timely eradication (until the development of extensive atrophic changes) is the most effective strategy for preventing the development of precancerous gastric lesions (PGL) and the primary prophylaxis of GC. An attractive proposal to reduce the incidence of gastric adenocarcinoma is to identify high-risk individuals who may benefit from screening, prophylactic, and therapeutic measures to prevent the onset of malignancy. For this reason, the diagnosis and effective management of CAG and GIM are very important research topics for the prevention of GC [1-5].

Among PGL, GIM is a recognized precancerous lesion, defined as the replacement of gastric epithelium with intestinal-type epithelium. The reported prevalence of GIM in international databases of gastric biopsies varies widely – from 3.4% to 29.6% [6].

GIM is commonly diagnosed, but only a small proportion of these patients will eventually develop GC. Current clinical guidelines recommend screening for active HP infection in all patients with GIM. Eradication of HP infection reverses early histological changes in CAG patients and may slow the progression of GIM to GC [2].

In this context, the article aims to develop a narrative synthesis of contemporary studies to review current concepts regarding epidemiology, clinical picture, risk factors, classification, diagnosis, and management of patients with gastric intestinal metaplasia and gastric epithelial dysplasia to establish strategies for the prevention of gastric cancer.

Material and methods

To achieve the stated objective, an initial search of specialized scientific publications was conducted, identified through the Google Search engine and the databases PubMed, Hinari (Health Internet Work Access to Research Initiative), SpringerLink, National Center for Biotechnology Information, and Medline. The article selection criteria included contemporary data on the epidemiology, clinical picture, risk factors, classification, diagnosis, and management of patients with GIM and GD, using the following keywords: "gastric intestinal metaplasia" and "gastric epithelial dysplasia", combined in various ways with the terms "epidemiology", "clinical picture", "risk factors", "classification", "diagnosis", and "management" to maximize the search yield.

For the advanced selection of bibliographic sources, the following filters were applied: full text articles, articles in English, and articles published between 1990-2024. After a preliminary review of the titles, original articles, editorials, narrative synthesis articles, systematic reviews and meta-analyses were selected, containing relevant information and contemporary concepts regarding the epidemiology, clinical picture, risk factors (RF), classification, diagnosis, and management of patients with GIM and GD. Additionally, a search of the bibliographic reference lists of the identified sources was conducted to highlight additional relevant publications that were not found during the initial database search.

The information from the publications included in the bibliography was collected, classified, evaluated, and synthesized, highlighting the main aspects of the contemporary view on the epidemiology, clinical picture, RF, classification, diagnosis, and management of patients with GIM and GD.

To minimize the risk of systematic errors (bias) in the study, thorough searches were conducted in the databases to identify the maximum number of publications relevant to the study's purpose. Only studies that met the validity criteria were evaluated, and safe exclusion criteria were applied to the articles included in the study.

If necessary, additional sources of information were consulted to specify certain notions. Duplicate publications, articles that did not correspond to the purpose of the work, and those not accessible for full viewing were excluded from the list of publications generated by the search engine.

After processing the information identified by the Google Search engine and from the databases PubMed, Hinari, SpringerLink, National Center for Biotechnology Informa-

tion, and Medline according to the search criteria, 215 articles were found that address the topic of epidemiology, clinical picture, risk factors, classification, diagnosis, and management of patients with GIM and GD. After the primary analysis of the titles, 42 articles were considered possibly relevant for the given synthesis. Following repeated reviews of these sources, 34 publications relevant to the intended purpose were finally selected.

Results and discussions

The publications, the content of which did not reflect the topic addressed, although they were selected by the search program, as well as the articles that were not accessible for free viewing either through the HINARI database or in the medical scientific library of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, were subsequently excluded from the list.

Gastric intestinal metaplasia

GIM is defined as the replacement of gastric epithelium with two types of intestinal-type epithelium as an adaptive response to chronic injury, such as chronic inflammation due to HP infection [4, 5, 7-11].

Studies on the incidence of GIM are rare in the asymptomatic general population, as the diagnosis of GIM requires upper digestive endoscopy with biopsy and histological examination [3].

The prevalence of GIM is heterogeneous across different regions of the world, correlating with the endemicity of HP infection among other environmental factors and with the global incidence of GC. It increases significantly with age in both men and women [3, 8].

In previous studies, the prevalence of GIM generally ranged from 3.4% to 23.9% in patients undergoing upper digestive endoscopy with gastric biopsies and from 12% to 50% in high-risk patient groups [6, 10, 12-14]. The prevalence of GIM in large international databases of gastric biopsies also varied widely – from 3.4% to 29.6% [6]. According to the analysis of other studies, the prevalence of GIM varies from 7.1% to 42.5%, depending on the country and diagnostic methods [3].

More recent studies found GIM to be present in 25.3% of patients endoscopically evaluated for dyspepsia [15], in 2.5% of patients undergoing any upper digestive endoscopy, and in 4.8% of patients with gastric biopsies regardless of indication [6, 16-18]. The prevalence of GIM in HP-infected patients was 33.9% compared to 15.2% among HP-negative patients [15].

According to the results of a recent study published in 2020, among 223 patients with GIM, 194 (87%) had complete-type GIM, and 29 (13%) had incomplete-type GIM [5].

Risk factors. Male sex, age >50 years, and current HP infection are significant predictors of the presence of GIM [5, 8, 10].

Gene expression patterns found in different studies have provided new comparative information on CAG and GIM, which may play an important role in the development of GC [8]. An increased risk of GIM was found in subjects with the IL-8-251AT genotype (OR = 2.27; 95% CI: 1.25-4.14), in carriers of IL-8-251A alleles (OR = 2.07; 95% CI: 1.16-3.69) [19], and in subjects with the MIF-173GC genotype [20].

Several RFs have been associated with the development of GIM and GC, including HP infection and associated genomics, host genetic factors, environmental factors, rheumatological disorders, diet, and gut microbiota [3, 12]. However, for CAG, the most important RF is the virulence factor HP, while for GIM, environmental and host factors play a more significant role [3].

Intestinal metaplasia – pre-neoplastic step. GIM is a pre-malignant condition of the gastric mucosa, a precursor of GD, and an essential predisposing factor in the development of GC, associated with a more than 10-fold increase in the risk of GC [2, 5, 8, 16, 21-23].

The initial risk of GC among patients with GIM may be significantly higher depending on the anatomical extent, stage, severity, and histological subtype of metaplasia, as well as the presence of CAG and HP infection [10, 23]. The incomplete subtype of GIM is often detected around regions of GD or early gastric carcinoma and has an 8-fold higher risk of developing GC compared to the complete subtype [14, 22].

According to the results of a recent study, GIM was a significant RF for early GC (RR = 5.36; 95% CI: 1.51-19.0; p<0.01). Patients with OLGIM (Operative Link on Gastric Intestinal Metaplasia Assessment) in stages III-IV had the highest risk (RR = 20.7; 95% CI: 5.04-85.6; p<0.01) [23].

The cumulative rate of progression of GIM to GD at 3 and 5 years was 15% [16, 17, 18]. The estimated annual risk of GC in patients with GIM is 0.13–0.25% and depends on the extent and type of metaplasia [2, 4, 15, 24, 25]. The cumulative incidence rates of GC at 3, 5, and 10 years among patients with GIM were estimated to be 0.4%, 1.1%, and 1.6%, respectively [16-18, 26]. Incomplete GIM is an important FR for GC development [27]. Patients with incomplete GIM had a 3.33-fold higher risk of GC incidence compared to those with complete GIM [11].

According to the results of Japanese studies, the 5-year total cumulative incidence of GC is 1.9-10% in CAG patients and 5.3-9.8% in GIM patients [28].

GIM is considered an important stage along the continuum to GC and has an average latency period of approximately 6 years before progression to cancer, providing a window of opportunity for intervention. However, only a small proportion (0.25–2.5%) of patients with GIM ultimately progress to cancer [9].

In the process of carcinogenesis, GIM is considered an "irreversible point" that significantly increases the risk of GC. Therefore, elucidating the underlying mechanisms of GIM is of significant importance for the prevention and treatment of gastric mucosal carcinogenesis associated with HP infection [29]. Even in established GIM, HP eradication slows progression along the Correa cascade to GC [1, 9, 30].

Information on the RFs for the neoplastic progression of GIM is limited. Little is known about the molecular and genetic events that trigger GIM progression to adenocarcino-

ma. Smoking and a positive family history of GC in first-degree and/or second-degree relatives were associated with an increased but not statistically significant risk of GIM progression [31]. In addition to HP status, the two clinical factors that increase the risk of GIM progression to malignancy are age >50 years (RR = 8.8; 95% CI: 1.2-68.5) and a family history of gastric cancer in a first-degree relative (RR = 4.5; 95% CI: 1.3-15.5) [9].

The risk of GC is 4-11 times higher in patients with incomplete GIM compared to those with complete GIM [12]. Recent studies found no difference in progression to GC between extensive and limited GIM [13].

OLGIM is a validated GC risk assessment system that incorporates both the severity and topographical distribution of GIM. Patients with stage III or IV OLGIM have a significantly higher risk (20.8 times) of early gastric neoplasia compared to patients without GIM [10].

Classification. GIM is a highly heterogeneous lesion with multiple classification systems. One of the most commonly used classifications recognizes two types: complete (or enteric) GIM and incomplete (or colonic) GIM. Another classification (Jass and Filipe, 1981) currently in use recognizes 3 types of GIM: the complete "low risk" subtype (type I or small intestinal – characterized by the presence of mucin-producing goblet cells, Paneth cells, and columnar cells) and the incomplete "high-risk'' subtypes: type II (enterocolic, with mucin-producing goblet cells but lacking columnar and/or Paneth cells) and type III (colonic, with goblet cells containing irregular mucin vacuoles, and the absence of columnar and/or Paneth cells). However, the associations between the histological subtypes of GIM and the risk of GC are not universally accepted [2, 4, 11, 14, 15, 30].

Limited GIM was defined as involvement of only the distal stomach (antrum, pre-pylorus, or pylorus), while extensive GIM was defined as involvement of both the proximal and distal stomach or only the proximal stomach (body or fundus) [13].

In the specialized literature, a model of GIM known as SPEM (Spasmolytic polypeptide-expressing metaplasia) or pseudo-pyloric metaplasia, has been described. It represents the metaplastic replacement of oxyntic glands by mucin and is considered an alternative pathway to gastric neoplasia. This type of GIM represents a physiological healing response to acute injuries, but in cases of persistent injury and chronic inflammation, these reparative metaplastic lineages can evolve into pre-neoplastic (proliferative and self-renewing metaplastic and dysplastic) lesions, predisposing to GC development. Unlike intestinal metaplasia, SPEM develops in the gastric body and fornix and is strongly associated with HP infection and early GC [21, 24, 29, 30].

Diagnosis Three methods can be used to establish the diagnosis and extent of GIM: endoscopic evaluation, histological evaluation of biopsy specimens, and serological testing [30].

GIM can be diagnosed incidentally in patients with non-ulcer dyspepsia undergoing upper digestive endoscopy with random biopsies of normal-appearing gastric mucosa or targeted biopsies of subtle gastric mucosal abnormalities [6, 14].

Gross endoscopic features of GIM include dark-gray spots surrounded by pale or normal-colored gastric mucosa or irregular erythematous spots. Other endoscopic markers of GIM on narrow-band magnification endoscopy include the light blue crest (LBC), defined as a fine, light blue line on the crests of the villous and elongated epithelial *foveolae*, and opaque white matter (WOS - White Opaque Substance, lipid droplets). The latter has a distinctive appearance, which contributes to the endoscopic diagnosis of GIM with high specificity (100%) but limited sensitivity (50%) [4, 15, 25].

Several studies have suggested that endoscopy with magnification and chromoendoscopy identify GIM and GD lesions with high accuracy. The sensitivity and specificity of the endoscopic diagnosis of GIM, based on histological examination, were 24.0% and 91.9% for the antrum and 24.2% and 88.0% for the corpus [3].

Serum PGs have been used for the screening of CAG, GIM, and gastric adenocarcinoma for the past 3 decades due to their non-invasiveness and cost-effectiveness. In a prospective cohort study of 5,113 individuals in Japan, screening for GC with PG-I cutoff values <70 ng/mL and PGR < 3 showed a sensitivity of 84.6% and a specificity of 73.5% [3].

Management. To date, there are no unified clinical guidelines for the prevention of GC regarding the classification of high-risk groups that progress to GC. However, the prevention and treatment of CAG and GIM, considered precancerous lesions, could reduce the prevalence of GC [3].

Although GIM has no specific treatment, the management strategy includes the eradication of HP infection, screening for early detection of GC, and control of other RFs. Current clinical guidelines recommend that all patients with GIM be screened for active HP infection, as eradication of this infection reverses early histological changes in CAG patients and may slow the progression of GIM to GC [1, 2, 15, 16, 26, 30].

Despite the increased risk of GC among patients with GIM, there are no randomized controlled trials evaluating the benefits or harms of surveillance endoscopy in these patients. This has led to consensus-based recommendations for surveillance endoscopy in limited subgroups of patients at increased risk of developing GC [6]. Endoscopic surveillance every 3 years is recommended for patients with OLGA/OLGIM (Operative Link on Gastritis Assessment/Operative Link on Gastric Intestinal Metaplasia Assessment) stages III/IV [1, 4, 15, 16, 18, 30]. The purpose of GPL surveillance at defined intervals is to diagnose GC at an early stage and facilitate endoscopic or surgical resection with curative intent [4, 17, 22].

The results of studies suggest that GIM has a low probability of regression after HP eradication. GIM may be the "point of no return" if irreversible genetic damage occurs to gastric stem cells [24, 26], and these patients remain at risk of neoplastic progression regardless of HP infection status [17, 26]. Although substantial evidence supports the "point

of no return" concept, there is also evidence of regression with histological improvement in a subset of patients. These results indicate that GIM regression may be a long-term process, lasting many years after HP eradication [1, 17, 26, 30]. GIM does not always represent a "point of no return," as it can regress in some cases over an extended period (on average, 90 months), thus providing a mechanism for preventing intestinal-type GC through HP eradication [1, 30].

According to the results of a recent study published in 2020, among 50 patients with GIM who successfully eradicated HP, GIM disappeared in 62% of cases and persisted in 38% after a mean follow-up of 21 months [5].

According to the results of other long-term prospective studies, GIM is virtually irreversible at a more advanced stage, unless the lesion is minimal (e.g., focal and complete). These findings support the concept that the earlier HP is eliminated, the greater the benefits [27].

It is important to recognize the role of HP infection duration, among other factors, in the subsequent risk of GC [17]. No significant changes in GD were found, but there was a tendency toward greater regression and less progression among consistently HP-negative patients. A longer surveillance duration with an adequate sample size may help clarify the effect of HP eradication on GD [32].

Eradication of HP, compared with placebo, among individuals with or without GIM in the absence of gastric neoplasia was associated with a 32% reduction in the relative risk of GC incidence. Similarly, eradication of HP, compared to placebo, was associated with a 33% reduction in the relative risk of GC mortality [16]. Overall, HP testing and treatment in patients with confirmed HP infection (with or without GIM) demonstrated a protective effect against GC incidence and was associated with improved GC mortality compared to patients receiving placebo or non-antibiotic therapy [4, 17].

Dysplasia of the gastric epithelium

Definition. GD is defined as unequivocal neoplastic change of the gastric epithelium (intraepithelial neoplasia) without evidence of stromal (lamina propria) invasion [7, 21, 24]. GD is an advanced and direct precancerous lesion characterized by a combination of three basic morphologic abnormalities: (1) epithelial atypia (variation in size, shape, and orientation of epithelial cells) without deep invasion, (2) loss of native epithelial commitment, (3) disorganized glandular architecture, and (4) increased mitotic activity [7, 21].

Epidemiology. The endoscopic prevalence of GD ranges from 0.5% to 3.75% in Western countries and from 9% to 20% in areas with a high incidence of gastric adenocarcinoma [7, 15, 25, 33]. The prevalence of GD in patients with CAG, ulcers, or after gastrectomy ranges from 4% to 30%, and in patients with pernicious anemia, it can be as high as 40% [33].

GD – pre-neoplastic state. GD represents the penultimate stage of gastric carcinogenesis. Its clinical importance is determined by its close association with the risk of developing GC. Moderate to severe GD was associated with 40–100% of early GC and was detected in 5–80% of advanced adenocarcinomas, suggesting a direct role in cancer formation [33].

The results of several prospective longitudinal studies suggest that severe GD is a precursor of intestinal-type GC. In these studies, more than 30% of patients with moderate dysplasia and more than 70% of patients with severe dysplasia developed early or invasive carcinoma within a short or very short period of time [14].

The estimated risk of GC in patients with GD is 1.36% annually and 6% at 5 years [4, 25]. Both LGD (low-grade dysplasia) and HGD (high-grade dysplasia) have the potential to progress to carcinoma. The risk of progression to GC increases substantially and proportionally with the histological grade, ranging from LGD (4-18%) to HGD (up to 69%). It has been reported that approximately 15–30% of LGD progresses to HGD or adenocarcinoma [24].

A recent prospective study of a population of 9,740 subjects undergoing digestive endoscopic screening and followed dynamically for a median of 10 years identified cumulative incidence rates in patients with HGD, LGD, and CAG/GIM as 25%, 3.05%, and 1.58%, respectively. The rate of progression and risk of GC increased monotonically with each step in the Correa cascade [34].

Classification. There are several classifications of GD. According to the Vienna classification, dysplasia is currently divided into LGD and HGD [7, 21, 24, 30, 33]. LGD is characterized by minimal architectural disorder, mild to moderate cytological atypia, and mitotic activity, whereas HGD is marked by significant cytological atypia, strong mitotic activity, and complex glandular architecture [24].

The international Padua classification identifies five main categories for dysplastic lesions: 1) negative for GD, 2) indefinite for GD, 3) non-invasive neoplasia (divided into low-grade GD and high-grade GD), 4) suspicion for invasive carcinoma, and 5) invasive adenocarcinoma [7, 30, 33].

The term "dysplasia" is used synonymously with "intraepithelial neoplasia/dysplasia, non-invasive neoplasia/dysplasia" [4, 30].

Diagnostic. Detection of GD and early GC is difficult due to the lack of well-defined endoscopic criteria. Commonly described, but not exhaustive, features include color differences (more commonly red or pale), loss of vascularity, mild over- or under-elevation, nodularity, thickening, and abnormal convergence or flattening of the folds [15, 25].

Endoscopically, GD can present as a flat, depressed, or polypoid lesion, with the latter categorized as gastric (intestinal) and foveolar (adenomas) [21].

In a meta-analysis published in 2004, Dinis-Ribeiro and coauthors combined 42 studies, including 27 population studies (296,553 patients) and 15 studies of selected populations (4,385 patients), to assess the best cutoff for the diagnosis of dysplasia. A combination of PG-I ≤50 ng/mL and a PG-I/PG-II ratio ≤3.0 yilded the best results, with a sensitivity of 65%, a specificity of 74–85%, and a negative predictive value of >95% [1, 30].

Management. In general, there is no controversy regarding the appropriate management of defined HGD. Such le-

sions require endoscopic resection due to the potential for progression to carcinoma and the coexistence of carcinoma. If HGD is endoscopically indistinct, guidelines recommend immediate endoscopic reevaluation with extensive biopsy and surveillance at 6- to 12-month intervals. Given the lower risk of malignant transformation, some scholars recommend annual endoscopic surveillance with biopsy for LGD [1, 30]. Endoscopic surveillance is recommended every 6 months for high-grade GD and every 12 months for low-grade GD [1, 15, 30].

Conclusions

Gastric intestinal metaplasia represents the replacement of the gastric epithelium with two types of intestinal-type epithelium (enteric or colonic) as an adaptive response to chronic injury, while gastric epithelial dysplasia is defined as unequivocal neoplastic change of the gastric epithelium (intraepithelial neoplasia) without evidence of stromal invasion. Gastric intestinal metaplasia and gastric epithelial dysplasia are preneoplastic lesions of gastric cancer. The estimated annual risk of gastric adenocarcinoma in patients with gastric intestinal metaplasia is 0.13-0.25%, and in patients with dysplasia of the gastric epithelium, it is 1.36%, depending on the extent and type of the lesion. Advancing endoscopic technologies with high definition gastroscopes and improved imaging, medical training in endoscopy, risk stratification, and histological evaluation are essential for the diagnosis and management of precancerous gastric lesions. Despite the lack of specific treatment for gastric intestinal metaplasia, the management strategy according to current clinical guidelines includes eradication of Helicobacter pylori infection, screening for early detection of gastric cancer, and control of other risk factors. Adequate management of high-grade gastric epithelial dysplasia requires endoscopic resection because of the potential for progression to carcinoma and the coexistence of carcinoma. For low-grade gastric epithelial dysplasia, which has a lower risk of malignant transformation, scientists recommend annual endoscopic surveillance with biopsy and histological examination.

Competing interests

None declared.

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References

1. Pimentel-Nunes P, Libânio D, Marcos-Pinto R, Areia M, Leja M, Esposito G, et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. Endoscopy. 2019;51(4):365-388. doi: 10.1055/a-0859-1883.
2. Huang K, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan A, et al. Genomic and epigenomic profiling of high-risk intestinal metaplasia reveals molecular determinants of progression to gastric cancer. Cancer Cell. 2018;33(1):137-150.e5. doi: 10.1016/j.ccell.2017.11.018.
3. Park Y, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. J Cancer Prev. 2015;20(1):25-40. doi: 10.15430/JCP.2015.20.1.25.
4. Matysiak-Budnik T, editor. From premalignant lesions to early gastric cancer what is clinically relevant? Rouen: Le Grand Métier; 2021. 108 p.
5. Aumpan N, Vilaichone R, Nunanan P, Chonprasertsuk S, Siramolpiwat S, Bhanthumkomol P, et al. Predictors for development of complete and incomplete intestinal metaplasia (IM) associated with H. pylori infection: a large-scale study from low prevalence area of gastric cancer (IM-HP trial). PLoS One. 2020;15(10):e0239434. doi: 10.1371/journal.pone.0239434.
6. Altayar O, Davitkov P, Shah S, Gawron A, Morgan D, Turner K, et al. AGA technical review on gastric intestinal metaplasia - epidemiology and risk factors. Gastroenterology. 2020;158(3):732-744. doi: 10.1053/j.gastro.2019.12.002.
7. Yakirevich E, Resnick M. Pathology of gastric cancer and its precursor lesions. Gastroenterol Clin North Am. 2013;42(2):261-284. doi: 10.1016/j.gtc.2013.01.004.
8. Huang RJ, Choi AY, Truong CD, Yeh MM, Hwang JH. Diagnosis and management of gastric intestinal metaplasia: current status and future directions. Gut Liver. 2019;13(6):596-603. doi: 10.5009/gnl19181.
9. Tjandra D, Busuttil R, Boussioutas A. Gastric intestinal metaplasia: challenges and the opportunity for precision prevention. Cancers (Basel). 2023;15(15):3913. doi: 10.3390/cancers15153913.
10. Shah SC. Improving the endoscopic detection and management of gastric intestinal metaplasia through training: a practical guide. Gastroenterology. 2022;163(4):806-811. doi: 10.1053/j.gastro.2022.07.068.
11. Shah SC, Gawron AJ, Mustafa RA, Piazuelo MB. Histologic subtyping of gastric intestinal metaplasia: overview and considerations for clinical practice. Gastroenterology. 2020;158(3):745-750. doi: 10.1053/j.gastro.2019.12.004.
12. Jencks D, Adam J, Borum M, Koh J, Stephen S, Doman D. Overview of current concepts in gastric intestinal metaplasia and gastric cancer. Gastroenterol Hepatol (NY). 2018;14(2):92-101.
13. Laszkowska M, Truong H, Faye A, Kim J, Tan S, Lim F, et al. Prevalence of extensive and limited gastric intestinal metaplasia and progression to dysplasia and gastric cancer. Dig Dis Sci. 2022;67(8):3693-3701. doi: 10.1007/s10620-021-07276-9.
14. Kapadia CR. Gastric atrophy, metaplasia, and dysplasia: a clinical perspective. J Clin Gastroenterol. 2003;36(5 Suppl):S29-36. doi: 10.1097/00004836-200305001-00006.
15. Banks M, Graham D, Jansen M, Gotoda T, Coda S, di Pietro M, et al. British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric ad-

enocarcinoma. Gut. 2019;68(9):1545-1575. doi: 10.1136/gutjnl-2018-318126.
16. Gupta S, Li D, El Serag H, Davitkov P, Altayar O, Sultan S, et al. AGA clinical practice guidelines on management of gastric intestinal metaplasia. Gastroenterology. 2020;158(3):693-702. doi: 10.1053/j.gastro.2019.12.003.
17. Gawron A, Shah S, Altayar O, Davitkov P, Morgan D, Turner K, et al. AGA technical review on gastric intestinal metaplasia-natural history and clinical outcomes. Gastroenterology. 2020;158(3):705-731. doi: 10.1053/j.gastro.2019.12.001.
18. Shah S, Piazuelo M, Kuipers E, Li D. AGA Clinical Practice Update on the Diagnosis and Management of Atrophic Gastritis: Expert Review. Gastroenterology. 2021; 161(4): 1325-1332.e7. doi: 10.1053/j.gastro.2021.06.078.
19. Li Z, Wu Y, Sun Y, Liu L, Tian M, Feng G, et al. Inflammatory cytokine gene polymorphisms increase the risk of atrophic gastritis and intestinal metaplasia. World J Gastroenterol. 2010;16(14):1788-1794. doi: 10.3748/wjg.v16.i14.1788.
20. Karaman A, Aydin H, Geçkinli B, Binici D, Pirim I. Relation of chronic atrophic gastritis and intestinal metaplasia with Helicobacter pylori and tumor necrosis factor-α and macrophage GIMration inhibitory factor polymorphisms in a population of Easten Anatolia. Göztepe Tıp Dergisi. 2014;29(1):12-19. doi: 10.5222/J.GOZTEPETRH.2014.012.
21. Gullo I, Grillo F, Mastracci L, Vanoli A, Carneiro F, Saragoni L, et al. Precancerous lesions of the stomach, gastric cancer and hereditary gastric cancer syndromes. Pathologica. 2020;112(3):166-185. doi: 10.32074/1591-951X-166.
22. Lee J, Zhu F, Srivastava S, Tsao S, Khor C, Ho K, et al. Severity of gastric intestinal metaplasia predicts the risk of gastric cancer: a prospective multicentre cohort study (GCEP). Gut. 2022;71(5):854-863. doi: 10.1136/gutjnl-2021-324057.
23. Giroux V, Rustgi A. Metaplasia: tissue injury adaptation and a precursor to the dysplasia-cancer sequence. Nat Rev Cancer. 2017; 17(10): 594-604. doi: 10.1038/nrc.2017.68.
24. Businello G, Angerilli V, Parente P, Realdon S, Savarino E, Farinati F, et al. Molecular landscapes of gastric pre-neoplastic and pre-invasive lesions. Int J Mol Sci. 2021;22(18):9950. doi: 10.3390/ijms22189950.
25. White J, Banks M. Identifying the pre-malignant stomach: from guidelines to practice. Transl Gastroenterol Hepatol. 2022;7:8. doi: 10.21037/tgh.2020.03.03.
26. Tong Y, Wang H, Zhao Y, He X, Xu H, Li H et al. Diagnostic Value of Serum Pepsinogen Levels for Screening Gastric Cancer and Atrophic Gastritis in Asymptomatic Individuals: A Cross-Sectional Study. Front Oncol. 2021; 11: 652574. doi: 10.3389/fonc.2021.652574.
27. Piazuelo MB, Bravo LE, Mera RM, Camargo MC, Bravo JC, Delgado AG, et al. The Colombian chemoprevention trial: 20-year follow-up of a cohort of patients with gastric precancerous lesions. Gastroenterology. 2021;160(4):1106-1117.e3. doi: 10.1053/j.gastro.2020.11.017.
28. Young E, Philpott H, Singh R. Endoscopic diagnosis and treatment of gastric dysplasia and early cancer: current evidence and what the future may hold. World J Gastroenterol. 2021;27(31):5126-5151. doi: 10.3748/wjg.v27.i31.5126.
29. Chen H, Hu Y, Lu NH, Zhu Y. Caudal type homeoboxes as a driving force in Helicobacter pylori infection-induced gastric intestinal metaplasia. Gut Microbes. 2020;12(1):1-12. doi: 10.1080/19490976.2020.1809331.
30. Dinis-Ribeiro M, Areia M, de Vries A, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44(1):74-94. doi: 10.1055/s-0031-1291491.
31. Nieuwenburg S, Mommersteeg M, Eikenboom E, Yu B, den Hollander W, Holster I, et al. Factors associated with the progression of gastric intestinal metaplasia: a multicenter, prospective cohort study. Endosc Int Open. 2021;9(3):E297-E305. doi: 10.1055/a-1314-6626.
32. Mera R, Fontham E, Bravo L, Bravo J, Piazuelo M, Camargo M, et al. Long term follow up of patients treated for Helicobacter pylori infection. Gut. 2005;54(11):1536-1540. doi: 10.1136/gut.2005.072009.
33. Lauwers GY, Riddell RH. Gastric epithelial dysplasia. Gut. 1999;45(5):784-790. doi: 10.1136/gut.45.5.784.
34. Gu J, Xie S, Wang S, Xue L, Zhou J, Li M, et al. Surveillance of premalignant gastric cardia lesions: a population-based prospective cohort study in China. Int J Cancer. 2021;149(9):1639-1648. doi: 10.1002/ijc.33720.

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REVIEW ARTICLE

Artificial intelligence-based techniques for predicting outcomes in COVID-19 patients

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ABSTRACT

Introduction. Currently, extensive research has shown that almost all published prediction models are poorly studied and have significant limitations, leading to their predictive performance often being overestimated. Additionally, there is still no universally accepted scoring system, primarily due to the need for adaptation to heterogeneous patient samples (including patient numbers, clinical profiles, and risk factors) and/or ongoing differences in the organization of healthcare systems across various countries.

Materials and methods. This is a narrative literature review. A bibliographic search was conducted in *the PubMed, Hinari, SpringerLink, National Center for Biotechnology Information*, and *Medline* databases. Articles published between 2000 and 2024 were selected based on keyword combinations such as "artificial intelligence", "prediction model", "algorithm", "machine learning", and "COVID-19". Information on machine learning predictive models was selected and processed to identify characteristics that can be used to predict diagnosis, severity, length of hospital stay, ICU admission, treatment, vaccination, and mortality in COVID-19 patients. After processing the data according to the search criteria, 125 full-text articles were identified. The final bibliography includes 52 relevant sources, which were considered representative of the literature on this synthesis article topic.

Results. Artificial intelligence techniques are increasingly being used to predict outcomes in COVID-19 patients, particularly in estimating mortality among individuals infected with SARS-CoV-2, which can rapidly and effectively support clinical decision-making. According to the analysis of multiple studies, strong predictors of mortality in COVID-19 patients include advanced age, male gender, comorbidities, reduced levels of calcium, albumin, red blood cells, and oxygen saturation, as well as lymphopenia, elevated blood urea nitrogen, creatinine, lactate dehydrogenase, D-dimers, neutrophils, interleukin-6, procalcitonin, bilirubin, ferritin, aspartate aminotransferase, and troponin.

Conclusions. Artificial intelligence techniques provide potential advantages over conventional assessment methods. The information obtained from machine learning and deep learning algorithms, including easily accessible and interpretable data, can assist healthcare workers in making accurate decisions for the appropriate and timely care of COVID- 19 patients. This can improve patient outcomes, reduce the burden on healthcare systems, and ultimately decrease mortality rates.

Keywords: artificial intelligence, predictive model, algorithm, machine learning, deep learning, COVID-19.

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Key messages

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

Currently, many large-scale studies show that almost all prediction models based on artificial intelligence are not well-researched and have significant limitations, meaning their reported predictive performance is often overestimated.

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The research hypothesis

The contribution of machine learning techniques to medical practice is an emerging subject in the medical literature, with different opinions on their utility. Analyzing and reviewing relevant up-to-date articles will provide a detailed overview of predictive models based on *machine learning*, which use computer algorithms to assess patient health risks, manage clinical care, and predict mortality in COVID-19 patients, including those admitted to Intensive Care Units within the local healthcare system.

The novelty added by manuscript to the already published scientific literature

The article summarizes the latest international publications to identify a practical machine-based scoring system capable of predicting disease severity and risk of death, with the aim of reducing mortality rates.

Introduction

The rapid progression and worsening of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) highlight the need for early identification of high-risk patients, as well as the prompt and effective implementation of support measures to improve prognosis. Detecting patients at high risk of death can improve clinical outcomes through the rapid and individualized selection of effective treatment methods, resource optimization, and higher quality of healthcare delivery [1-6].

According to study results, *machine learning* and *deep learning* algorithms are highly effective in predicting mortality associated with coronavirus disease 2019 (COVID-19) [7, 8]. The indicators obtained were more effective compared to conventional statistical models (regression analysis, factor analysis, discriminant analysis) and traditional scoring systems (SOFA, SAPS-II, CURB-65, APACHE-II, APACHE-IV) [2, 3, 9-12].

Currently, extensive research shows that nearly all published prediction models are poorly studied and have significant limitations, leading to their predictive performance often being overestimated. Additionally, there is still no universally accepted scoring system, mainly due to the need for adaptation to heterogeneous patient samples (including patient numbers, clinical profiles, and risk factors) and/or ongoing differences in the organization of healthcare systems across various countries [13-16].

Artificial intelligence applications, especially machine learning and deep learning algorithms, have great potential to support healthcare professionals in decision-making, predicting complications and mortality in hospitalized patients, including those admitted to intensive care units (ICUs). Medical validation of predictive models should be performed by clinical experts, and the most effective models can be implemented in different hospitals and healthcare institutions [14, 17-19].

In this context, the **purpose** of this article is to present a synthesis of the most recent data regarding the effectiveness of *machine learning* algorithms and artificial intelligence in predicting mortality among COVID-19 patients.

Material and methods

To achieve the study purpose, an initial search was conducted for specialized scientific publications identified through Google Search and the *PubMed, Hinari (Health Internet Work Access to Research Initiative), SpringerLink, National Center for Biotechnology Information*, and *Medline* databases. The selection criteria for articles included state-of-the-art data on machine learning-based mortality prediction models using the following keywords: "artificial intelligence", "prediction model", "algorithm", "machine learning", "deep learning", and "COVID-19", which were used in various combinations to increase search efficiency.

For the advanced selection of bibliographic sources, the following filters were applied: full-text articles, articles in English, and articles published between 2000 and 2024. After a preliminary analysis of the titles, original articles, editorials, narrative reviews, systematic reviews, and meta-analyses containing relevant information and current concepts regarding the application of machine learning algorithms and artificial intelligence in the diagnosis, prediction of severity, and mortality risk in patients with COVID-19 were selected. Additionally, a search of the reference lists of the identified sources was conducted to identify additional relevant publications that were not found in the initial database search.

The information from the publications included in the bibliography was gathered, classified, evaluated, and synthesized, highlighting the main aspects of the contemporary perspective on the effectiveness of cutting-edge *machine learning* and *deep learning* algorithms, used either separately or in combination, in predicting mortality caused by SARS-CoV-2 infection.

To minimize the risk of systematic errors (bias) in the study, a thorough data search was conducted to identify the maximum number of relevant publications for the study's objectives. Only studies meeting reliability criteria were evaluated, while strict exclusion criteria were employed to remove articles from the present study. Moreover, both studies showing positive results and those not emphasizing the benefits of predictive models were analyzed.

If necessary, additional sources of information were consulted to clarify some concepts. Duplicate publications, articles that did not correspond to the purpose of the article, and those not available for full review were excluded from the list of publications generated by the search engine.

Results

After processing the information identified by the Google Search engine and from databases such as *PubMed, Hinari, SpringerLink, the National Center for Biotechnology Information*, and *Medline,* according to the established search criteria, a total of 125 articles addressing the application of machine learning algorithms and artificial intelligence in the diagnosis, prediction of severity, and mortality risk in COVID-19 patients were found. After an initial review of the titles, 59 articles were considered potentially relevant to this synthesis. Following a thorough review of these sources, 52 publications were ultimately selected as relevant to the stated objective. The final bibliography of the paper included these 52 articles, which were considered representative of the published materials on this synthesis topic.

Publications whose content did not reflect the considered topic, even though they were selected by the search program, as well as articles that were not accessible for free viewing through the *HINARI* database or available in the medical scientific library of the Nicolae Testemițanu State University of Medicine and Pharmacy, were subsequently excluded from the list.

The complexity of severe acute respiratory syndrome SARS-CoV-2 lies in the unpredictable clinical evolution of the disease, which develops rapidly, recording high morbidity and mortality rates. In this context, the identification and use of mortality prognostic models based on *machine learning* and *deep learning* algorithms become mandatory for the purposes of risk stratification, clinical management, and mortality prediction of COVID-19 patients, especially those hospitalized in intensive care units. Therefore, identifying a feasible mortality prediction score based on artificial intelligence techniques represents not only an urgent need for monitoring, predicting outcomes, and prognosis of the disease but also a key factor in reducing mortality rates [20-29].

Predictive models, also known as "prognostic models", "risk scores", or "prediction rules", are developed to assist healthcare providers in estimating the probability or risk of the presence of a condition or disease (diagnostic models) or the occurrence of an event in the future (prognostic models) for the purpose of informing and making appropriate decisions [4, 16, 30].

There are several studies that have shown that traditional risk scores generally underestimate mortality in patients with COVID-19 [31, 32]. Although multiple scores are currently used to predict mortality in patients with COVID-19, there is no universal score to date, and artificial intelligence has been used less than expected for this purpose. However, AI-based methods (*machine learning* and *deep learning*) are increasingly being used to study patient risk stratification in almost all areas of COVID-19 pandemic management. Numerous studies have been conducted to develop computerized predictive models for early disease prediction, diagnosis, severity assessment, progression, the need for ICU admission, and mortality risk evaluation in patients infected with SARS-CoV-2 [18, 25-27, 29, 33].

Firstly, feature classification (or selection or reduction) algorithm is used to pre-process the information to query new features, remove redundant values and unusable data, handle missing values, and select the most important features. SHAP *(SHaPley Additive exPlanations*) and LASSO (Least Absolute Shrinkage and Selection Operator) are most used for this purpose. After pre-processing the information, *machine learning* or *deep learning* algorithms are used to create a predictive model [18, 27, 34-36].

The latest generation of *machine learning* algorithms (*decision tree* – J48, *random forest* – RF, *artificial neural network* – ANN, *K-nearest neighbor* – k-NN, *multilayer perceptron* – MLP, *linear discriminant analysis* – LDA, *naive Bayes* – NB, *extreme gradient boosting* – XGBoost, *adaptive boosting* – AdaBoost, *support vector machine* – SVM, *logistic regression* – LR) and *deep learning* methods (*convolutional neural networks* – CNN, *feedforward neural network* – FNN, *long short-term memory* – LSTM, *autoregressive integrated moving average* – ARIMA, *partial least squares-discriminant analysis* – PLS-DA, *auto-encoder* – AE) are the most commonly used methods. Studies have investigated the effectiveness of these models both individually and in combination for predicting mortality caused by COVID-19 [18, 19, 26, 27, 37-39].

To improve the prognostic model for patients admitted to ICUs, a new strategy is needed that could be easily updated periodically and include the latest clinical data reflecting the local characteristics of each medical institution. Electronic medical records have provided the opportunity to extract a large amount of clinical information to improve the performance of prognostic models. The use of multiple artificial intelligence algorithms to select features with the highest mortality prediction values contributed to a significant increase in accuracy with an area under the ROC curve ≥90%, and the highest accuracy reaching an area under the ROC curve of 99.1-99.7% [8, 10, 19, 27, 34, 39, 40]. These methods are superior and more accurate than traditional scoring systems (SOFA, SAPS-II, SAPS III, APACHE-II, APACHE-III, APACHE-IV), which show moderate prediction accuracy (area under the ROC curve 0.73-0.96) [41-47].

AI techniques (*machine learning* and *deep learning*) have become valuable tools for supporting decision-making processes in healthcare, including diagnosis, monitoring, and predicting disease severity and mortality. These methods have demonstrated promising results across various medical applications, such as skin cancer classification, breast cancer detection, pneumonia classification, and predicting mortality from acute kidney injury [7, 14]. Emerging applications can provide higher prediction performance than classical statistical analysis by leveraging large-scale complex electronic health records and identifying the most reliable parameters, going beyond traditional statistical model-

ing. They can quickly evaluate large and complex databases with numerous variables to determine clinically significant risk levels for prognostic outcomes through intensive computational statistical modeling. In addition, machine and deep learning algorithms can identify hidden trends and unknown interactions between different variables that affect the outcome [18, 19, 27, 33, 34, 48].

AI techniques are increasingly being used to predict the outcomes of COVID-19 patients. In particular, the use of these algorithms to predict COVID-19 mortality is rapidly developing, which can quickly and effectively support clinical decision-making for COVID-19 patients at imminent risk of death [8, 10, 18, 36, 39, 48, 49].

AI applications, especially *machine learning* and *deep learning* algorithms, have great potential to support healthcare workers and professionals in decision-making, predicting complications and mortality rates in hospitalized patients. Medical validation should be performed by clinical experts, and these models can be implemented in various hospitals and healthcare settings [17].

However, despite the availability of several machine learning algorithms for predicting mortality in COVID-19 patients, their practical use is limited by factors such as the heterogeneity of patients' clinical profiles and risk factors, small sample sizes, and the lack of external validation of the prediction tools, which may reduce their applicability [14-16].

According to a review of multiple studies, the strongest predictors of mortality in COVID-19 patients, repeatedly reported, include advanced age, male gender, comorbidities (such as cardiovascular diseases, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, neurological disorders, chronic kidney disease, and cancer), low levels of calcium, albumin, red blood cells, and oxygen saturation, lymphocytopenia, and increased levels of blood urea nitrogen, creatinine, lactate dehydrogenase, C-reactive protein, D-dimers, respiratory rate, neutrophil count, interleukin-6, procalcitonin, bilirubin, ferritin, aspartate aminotransferase, and troponin [8, 18, 36].

In general, studies have highlighted the effectiveness of various *machine learning* models in predicting outcomes for COVID-19 patients, showing promising results in forecasting mortality and disease severity [8].

According to a study involving 235 hospitalized COVID-19 patients, the most important variables for predictive performance, in descending order, were lymphocytes, leukocytes, eosinophils, basophils, and hemoglobin. Among the six algorithms used, the SVM algorithm demonstrated the best predictive performance, with an ROC-AUC of 0.85, sensitivity of 0.68, specificity of 0.85, and an F1 score of 0.72. Thus, among patients with an estimated probability of 80-100% of having COVID-19, 82% were indeed infected, while only 12% of those with an estimated probability of 0-20% were diagnosed with the disease [21].

Aslam H. and Biswas S. predicted mortality in COVID-19 patients in real time using *machine learning* methods. The analysis evaluated well-known regression models (XGBoost, RF, and SVM) on datasets from the United States, India, Italy, and three continents - Asia, Europe, and North America. The dataset contained a total of 165,870 records, each with 67 parameters. The results demonstrated that these models are effective and can be used to predict mortality in COVID-19 patients [49].

Jamshidi E. et al. built a mortality prediction model based only on age, gender, and comorbidities (15 parameters) in 23,749 hospitalized and confirmed COVID-19 patients [25] according to the TRIPOD guidelines [50]. Six *machine learning* methods (LR, RF, ANN, k-NN, LDA, and NB) were evaluated. The RF mortality prediction algorithm had the highest efficiency: the area under the ROC curve was 0.79, sensitivity was 75%, and specificity was 70% [25].

The binary RF classifier, tested on 218 electronic medical records with 50 variables from ICU patients, achieved an accuracy of 80.28%, sensitivity of 81.82%, specificity of 79.43%, positive predictive value of 73.26%, negative predictive value of 84.85%, F1 score of 0.74, and an area under the ROC curve of 0.85. The reliable model for predicting ICU mortality identified lactate level as the most important factor, followed by temperature and the Glasgow Coma Scale [51].

A performance analysis of eight *machine learning* algorithms (J48, RF, k-NN, MLP, SVM, XGBoost, NB, and LR) for predicting mortality in COVID-19 patients used a dataset of 6,854 patients, including features such as CT severity score, demographics, risk factors, clinical symptoms, and lab results. The RF predictive model demonstrated the best results, with an accuracy of 97.2%, sensitivity of 100%, precision of 94.8%, specificity of 94.5%, F1 score of 97.3%, and an area under the ROC curve of 99.9%. This algorithm can quickly identify high-risk patients upon admission, potentially improving their survival chances. XGBoost, J48, k-NN, and MLP also showed good prognostic performance with ROC curve ≥93.9. Other *machine learning* algorithms (SVM, NB, and LR) also had acceptable performance, with the area under the ROC curve ranging from 81.2 to 88.9% [12].

Shi Y. et al. evaluated three *machine learning* algorithms (RF, PLS-DA, and SVM) for mortality prediction using a database of 4711 patients who were consecutively hospitalized in four hospitals. The analysis included only relatively accessible clinical parameters, including demographics, laboratory results, and clinical characteristics. The RF model, which evaluated 20 variables, showed the best performance with an area under the ROC curve of 0.859, with 5 significant predictors: mean arterial pressure, age, procalcitonin, blood urea nitrogen, and troponin. PLS-DA included 20 variables and had an area under the ROC curve of 0.775, with 5 significant predictors: procalcitonin, ferritin, C-reactive protein, D-dimers, and troponin. The SVM model analyzed 10 variables, with an area under the ROC curve of 0.828, and identified five key predictors: mean arterial pressure, age, aspartate aminotransferase, alanine aminotransferase, and C-reactive protein. Notably, nine variables (age, procalcitonin, ferritin, C-reactive protein, troponin, blood urea nitrogen, mean arterial pressure, aspartate aminotransferase,

and alanine aminotransferase) were common to all three models and were identified as the most critical risk factors for COVID-19 mortality [14].

RF was the most effective machine learning algorithm (area under the ROC curve: 88%) for predicting mortality in COVID-19 patients. The algorithm identified key predictors of in-hospital mortality, including age, severity of respiratory injury (PaO2/FiO2), cardiac damage biomarkers (troponin and BNP), inflammatory markers (interleukin-6 and procalcitonin), creatinine, urea, albumin, and red blood cell distribution [48].

The RF mortality prediction algorithm can reliably forecast mortality at the time of admission for patients infected with SARS-CoV-2 in the ICU, with an area under the ROC curve of 83%, sensitivity of 70%, and a specificity of 75%. The most significant prognostic factors included gender, age, blood urea nitrogen, creatinine levels, international normalized ratio, albumin, white blood cell count, segmented neutrophil count, lymphocyte count, hemoglobin, and a history of neurological, cardiovascular, and respiratory disorders [37].

In a large-scale study, 7 *machine learning* algorithms were tested on a cohort of 263,007 patients with 41 clinical and demographic parameters. XGBoost showed the best results in predicting COVID-19-related mortality, with 96% accuracy, 95% precision, an F1 score of 95%, and an area under the ROC curve of 96%. Older age, pneumonia, diabetes, obesity, cardiovascular diseases, and kidney diseases were statistically significant factors associated with COVID-19 mortality [17].

According to another study comparing the effectiveness of 4 *machine learning* techniques (RF, XGBoost, SVM, and LR), the XGBoost algorithm, based on initial clinical data (demographics and comorbidities), produced the most accurate prediction models. Through systematic design-based optimization, this algorithm performed better in predictive modeling applications involving structured data [52].

Zhao S. et al. tested 4 *machine learning* algorithms (LR, RF, XGBoost, and ANN) on a cohort of 12,393 ICU patients. Routine variables used included age, gender, physiological parameters, and use of vasoactive drugs during the first 24 hours of hospitalization. Among the tested models, the XGBoost algorithm showed the highest performance in predicting the risk of mortality within 24 hours, achieving an area under the ROC curve of 0.97 [47].

Another study evaluated 4 *machine learning* methods (RF, LR, XGBoost, and SVM) on a database containing 4120 records with 38 variables for each hospitalized COVID-19 patient across 5 hospitals in Tehran, Iran. The XGBoost model showed higher performance compared to other models (accuracy 70%, sensitivity 77%, specificity 69%, and AUC 0.857). For RF, LR, and SVM models, the AUC was 0.836, 0.818, and 0.794, respectively [28].

A study comparing two mortality prediction algorithms in 2,348 hospitalized COVID-19 patients using clinical and radiological information found similar results for both the SVM (*machine learning*) and FNN (*deep learning*) algorithms. The area under the ROC curve was 0.803 and 0.864, sensitivity 0.816 and 0.814, specificity 0.791 and 0.759, and accuracy 0.813 and 0.766, respectively [26].

Booth A. et al. used the SVM *machine learning* algorithm to predict mortality in patients with SARS-CoV2 based solely on a set of serum biomarkers. Using five readily available laboratory parameters (C-reactive protein, blood urea nitrogen, serum calcium, serum albumin, and lactic acid) from 398 patients, the model achieved a sensitivity of 91%, a specificity of 91%, and an area under the ROC curve of 0.93 [35].

Ali M. et al. evaluated 7 *machine learning* algorithms for predicting mortality in a cohort of 696 hospitalized COVID-19 patients. The scientists highlighted that the k-NN classifier performed best in predicting mortality compared to other machine learning algorithms, achieving an accuracy of 95.25%, a sensitivity of 95.30%, a precision of 92.7%, a specificity of 93.30%, an F1 score of 93.98%, and an area under the ROC curve of 96.90%. Male gender, intensive care unit admission, and alcohol consumption were the three most important predictors of COVID-19 mortality [8].

Pourhomayoun and Shakibi assessed the effectiveness of six machine learning algorithms (SVM, ANN, RF, J48, LR, and k-NN) for predicting mortality rates in COVID-19 patients. The study included a dataset of more than 2,670,000 patients infected with laboratory-confirmed SARS-CoV-2 from 146 countries. The original dataset contained 32 items for each patient, including symptoms, comorbidities, demographics, and physiological data. ANN achieved the highest performance in predicting the mortality of COVID-19 patients with an accuracy of 89.98%. This result is nearly equivalent to the k-NN (89.83%) and SVM (89.02%) algorithms, but slightly higher than RF (87.93%), LR (87.91%), and J48 (86.87%) [36].

Another prognostic model based on the SIMPLS (*Statistically inspired modification of partial least square*) algorithm, performed on a group of 250 hospitalized patients with COVID-19, assessed 18 clinical parameters and comorbidities, as well as 3 blood biochemical markers. The most significant predictors of in-hospital mortality were coronary artery disease, diabetes mellitus, altered mental status, age over 65 years, and dementia. C-reactive protein, prothrombin, and lactate dehydrogenase were the most important biochemical predictors of in-hospital mortality [24].

Some scientists believe that using more variables can improve the performance of mortality prediction models for patients with COVID-19 [28].

Conclusions

1. Artificial intelligence techniques, such as *machine learning* and *deep learning*, offer potential advantages over traditional scoring assessments, making them a valuable tool to support decision-making in healthcare, including diagnosis, monitoring, and predicting disease severity and mortality.
2. Information generated by *machine learning* and *deep learning* algorithms, which involve easily accessible and interpretable data, can help healthcare profes-

sionals make the right decisions to provide appropriate and timely care to COVID-19 patients, improve patient outcomes, reduce the burden on health systems, and ultimately reduce mortality.

3. Strong predictors of mortality in COVID-19 patients that have been repeatedly reported include older age, male gender, comorbidities, decreased calcium, albumin, and red blood cells, low oxygen saturation, lymphocytopenia, increased blood urea nitrogen, creatinine, lactate dehydrogenase, C-reactive protein, D-dimers, respiratory rate, neutrophil count, interleukin-6, procalcitonin, bilirubin, ferritin, aspartate aminotransferase, and troponin.
4. Implementing artificial intelligence algorithms in each specific healthcare service will be crucial to improving prediction efficiency, enhancing the quality of healthcare services, reducing the burden on healthcare workers, lowering overall patient care costs, and increasing their applicability in clinical practice.

Competing interests

None declared.

Authors' contributions

Substantial contributions to the concept and design of the work VM, CT, IG, SS, OA. Substantial contributions to the acquisition of data VM, CT, IG. Substantial contributions to the analysis and interpretation of data VM, CT, RB, SS, SC, OA. Drafting the article VM, CT, IG, OA. Critically reviewing the article for important intellectual content RB, SS, SC, OA. Final approval of the version to be published VM, CT, IG, RB, SS, SC, OA. Taking responsibility and being accountable for all aspects of the work VM, RB, SC, OA.

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References

1. Moon H, Kim K, Kang E, Yang H, Lee E. Prediction of COVID-19-related mortality and 30-day and 60-day survival probabilities using a nomogram. J Korean Med Sci. 2021;36(35):e248. doi: 10.3346/jkms.2021.36.e248.
2. Eldaboosy S, Almoosa Z, Saad M, Al Abdullah M, Farouk A, Awad A, et al. Comparison between physiological scores SIPF, and APACHE II as predictors of prognosis and mortality in hospitalized patients with COVID-19 pneumonia: a multicenter study, Saudi Arabia. Infect Drug Resist. 2022;15:7619-7630. doi: 10.2147/IDR.S395095.
3. Turda U, Deligöz O, Ekinci O. The effect of CRP/Albumin, Platelet/Lymphocyte, SOFA, and APACHE II in predicting mortality in Covid-19 patients in intensive care unit. J Cardiovasc Thorac Anaesth Intensive Care Soc (GKDA Derg). 2023;29(1):45-50. doi: 10.14744/GKDAD.2023.63383.
4. Barough S, Safavi-Naini S, Siavoshi F, Tamimi A, Ilkhani S, Akbari S, et al. Generalizable machine learning approach for COVID-19 mortality risk prediction using on-admission clinical and laboratory features. Sci Rep. 2023;13(1):2399. doi: 10.1038/s41598-023-28943-z.
5. Dessie Z, Zewotir T. Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. BMC Infect Dis. 2021;21(1):855. doi: 10.1186/s12879-021-06536-3.
6. Statsenko Y, Al Zahmi F, Habuza T, Gorkom K, Zaki N. Prediction of COVID-19 severity using laboratory findings on admission: informative values, thresholds, ML model performance. BMJ Open. 2021;11(2):e044500. doi: 10.1136/bmjopen-2020-044500.
7. Soundrapandiyan R, Manickam A, Akhloufi M, Murthy Y, Sundaram R, Thirugnanasambandam S. An efficient COVID-19 mortality risk prediction model using deep synthetic minority oversampling technique and convolution neural networks. Biomedinformatics. 2023;3(2):339-368. doi: 10.3390/biomedinformatics3020023.
8. Alie M, Negesse Y, Kindie K, Merawi D. Machine learning algorithms for predicting COVID-19 mortality in Ethiopia. BMC Public Health. 2024;24(1):1728. doi: 10.1186/s12889-024-19196-0.
9. Beigmohammadi M, Amoozadeh L, Rezaei Motlagh F, Rahimi M, Maghsoudloo M, Jafarnejad B, et al. Mortality predictive value of APACHE II and SOFA scores in COVID-19 patients in the Intensive Care Unit. Can Respir J. 2022;2022:5129314. doi: 10.1155/2022/5129314.
10. Genc A, Cekic D, Issever K, Genc F, Genc A, Toçoğlu A, et al. Can artificial intelligence predict COVID-19 mortality? Eur Rev Med Pharmacol Sci. 2023;27(20):9866-9871. doi: 10.26355/eurrev_202310_34163.
11. Mehryar H, Yarahmadi P, Anzali B. Mortality predictive value of APACHE II Scores in COVID-19 patients in the intensive care unit: a cross-sectional study. Ann Med Surg (Lond). 2023;85(6):2464-2468. doi: 10.1097/MS9.0000000000000641.
12. Zakariaee S, Naderi N, Ebrahimi M, Kazemi-Arpanahi H. Comparing machine learning algorithms to predict COVID-19 mortality using a dataset including chest computed tomography severity score data. Sci Rep. 2023;13(1):11343. doi: 10.1038/s41598-023-38133-6.
13. Wynants L, Van Calster B, Collins G, Riley R, Heinze G, Schuit E, et al. Prediction models for diagnosis and prognosis of Covid-19: systematic review and critical appraisal. BMJ. 2020;369:m1328. doi: 10.1136/bmj.m1328.
14. Shi Y, Qin Y, Zheng Z, Wang P, Liu J. Risk factor analysis and multiple predictive machine learning models for mortality in COVID-19: a multicenter and multi-ethnic cohort study. J Emerg Med. 2023;65(6):e584-e593. doi: 10.1016/j.jemermed.2023.06.012.
15. Shakibfar S, Zhao J, Li H, Nordeng H, Lupattelli A, Pavlovic M, et al. Machine learning-driven development of a disease risk score for COVID-19 hospitalization and mortality: a Swedish and Norwegian register-based study. Front Public Health. 2023;11:1258840. doi: 10.3389/fpubh.2023.1258840.
16. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRI-

POD Statement. BMC Medicine. 2015;13:1. https://doi.org/10.1186/s12916-014-0241-z.
17. Chadaga K, Prabhu S, Umakanth S, Bhat V, Sampathila N, Chadaga R, et al. COVID-19 mortality prediction among patients using epidemiological parameters: an ensemble machine learning approach. Eng Sci. 2021;16:221-233. https://dx.doi.org/10.30919/es8d579.
18. Alballa N, Al-Turaiki I. Machine learning approaches in COVID-19 diagnosis, mortality, and severity risk prediction: a review. Inform Med Unlocked. 2021;24:100564. doi: 10.1016/j.imu.2021.100564.
19. Lalmuanawma S, Hussain J, Chhakchhuak L. Applications of machine learning and artificial intelligence for Covid-19 (SARS-CoV-2) pandemic: a review. Chaos Solitons Fractals. 2020;139:110059. doi: 10.1016/j.chaos.2020.110059.
20. Kravchenko Y, Dakhno N, Leshchenko O, Tolstokorova A. Machine learning algorithms for predicting the results of COVID-19 coronavirus infection. In: Proceedings of the 7th International Conference "Information Technology and Interactions" (IT&I-2020). CEUR Workshop Proceedings; 2020 Dec 2-3; Kyiv, Ukraine. p. 371-381.
21. Moraes Batista AF, Miraglia JL, Rizzi Donato TH, Filho AD. COVID-19 diagnosis prediction in emergency care patients: a machine learning approach. In: medRxiv [Internet]. 2020 [cited 2024 Jul 18]. Available from: https://www.medrxiv.org/content/10.1101/2020.04.04.20052092v2
22. Lou X, Gao C, Wu L, Wu T, He L, Shen J, et al. Prediction of short-term progression of COVID-19 pneumonia based on chest CT artificial intelligence: during the Omicron epidemic. BMC Infect Dis. 2024;24(1):595. doi: 10.1186/s12879-024-09504-9.
23. Buvana M, Muthumayil K. Prediction of COVID-19 patient using supervised machine learning algorithm. Sains Malaysiana. 2021;50(8):2479-2497. http://doi.org/10.17576/jsm-2021-5008-28.
24. Banoei M, Dinparastisaleh R, Zadeh A, Mirsaeidi M. Machine-learning-based COVID-19 mortality prediction model and identification of patients at low and high risk of dying. Crit Care. 2021;25(1):328. doi: 10.1186/s13054-021-03749-5.
25. Jamshidi E, Asgary A, Tavakoli N, Zali A, Dastan F, Daaee A, et al. Symptom prediction and mortality risk calculation for COVID-19 using machine learning. Front Artif Intell. 2021;4:673527. doi: 10.3389/frai.2021.673527.
26. Verzellesi L, Botti A, Bertolini M, Trojani V, Carlini G, Nitrosi A, et al. Machine and deep learning algorithms for COVID-19 mortality prediction using clinical and radiomic features. Electronics. 2023;12(18):1-14. https://doi.org/10.3390/electronics12183878.
27. VanderZanden A, Amberbir A, Sayinzoga F, Huda F, Ntawukuriryayo J, Mathewos K, et al. Evidence of health system resilience in primary health care for preventing under-five mortality in Rwanda and Bangladesh: lessons from an implementation study during the Millennium development goal period and the early period of covid-19. J Glob Health. 2024;14:05023. doi: 10.7189/jogh.14.05023.
28. Emami H, Rabiei R, Sohrabei S, Atashi A. Predicting the mortality of patients with Covid-19: A machine learning approach. Health Sci Rep. 2023;6(4):e1162. doi: 10.1002/hsr2.1162.
29. Matta DM, Saraf MK. Prediction of COVID-19 using machine learning techniques. Karlskrona: Blekinge Institute of Technology; 2020. 52 p. [cited 2024 Jul 18]. Available from: https://www.diva-portal.org/smash/get/diva2:1454983/FULLTEXT02.pdf
30. Shamsabadi A, Mirzapour P, Heydari M, Mojdeganlou H, Karimi A, Pashaei Z, et al. Identifying techniques and models for COVID-19 prediction. J Iran Med Counc. 2022;6(2):207-228. https://doi.org/10.18502/jimc.v6i2.12230.
31. Yildrim S, Kirakli C. Accuracy of conventional disease severity scores in predicting COVID-19 ICU mortality: retrospective single-center study in Turkey. Ann Saudi Med. 2022;42(6):408-414. doi: 10.5144/0256-4947.2022.408.
32. Endo H, Ohbe H, Kumasawa J, Uchino S, Hashimoto S, Aoki Y, et al. Conventional risk prediction models fail to accurately predict mortality risk among patients with coronavirus disease 2019 in intensive care units: a difficult time to assess clinical severity and quality of care. J Intensive Care. 2021;9(1):42. doi: 10.1186/s40560-021-00557-5.
33. Satpathy S, Mangla M, Sharma N, Deshmukh H, Mohanty S. Predicting mortality rate and associated risks in COVID-19 patients. Spat Inf Res. 2021;29(4):455-464. https://doi.org/10.1007/s41324-021-00379-5.
34. Dwivedi A, Upadhyay A, Pal A. Mortality prediction of COVID-19 pandemic using artificial intelligence. In: 2nd International Conference on Advances in Computing, Communication Control and Networking (ICACCCN), 2020 Dec 18-19; Greater Noida, India. © *IEEE 2021.* p. 695-698. doi: 10.1109/ICACCCN51052.2020.9362740.
35. Booth A, Abels E, McCaffrey P. Development of a prognostic model for mortality in COVID-19 infection using machine learning. Mod Pathol. 2021;34(3):522-531. doi: 10.1038/s41379-020-00700-x.
36. Pourhomayoun M, Shakibi M. Predicting mortality risk in patients with COVID-19 using machine learning to help medical decision-making. Smart Health (Amst). 2021;20:100178. doi: 10.1016/j.smhl.2020.100178.
37. Jamshidi E, Asgary A, Tavakoli N, Zali A, Setareh S, Esmaily H, et al. Using machine learning to predict mortality for COVID-19 patients on day 0 in the ICU. Front Digit Health. 2022;3:681608. doi: 10.3389/fdgth.2021.681608.
38. Shakeel S, Kumar N, Madalli P, Srinivasaiah R, Swamy D. COVID-19 prediction models: a systematic literature review. Osong Public Health Res Perspect. 2021;12(4):215-229. doi: 10.24171/j.phrp.2021.0100.
39. Abegaz K, Etikan İ. Boosting the performance of artificial intelligence-driven models in predicting COVID-19 mortality in Ethiopia. Diagnostics (Basel). 2023;13(4):658. doi: 10.3390/diagnostics13040658.
40. Yildirim S, Sunecli O, Kirakli C. Mortality prediction with machine learning in COVID-19 patients in intensive care units: a retrospective and prospective longitudinal study. J Crit Intensive Care. 2024;15(1):30-36. doi: 10.14744/dcybd.2023.3691.

41. Locquet M, Diep A, Beaudart C, Dardenne N, Brabant C, Bruyère O, et al. A systematic review of prediction models to diagnose COVID-19 in adults admitted to healthcare centers. Arch Public Health. 2021;79(1):105. doi: 10.1186/s13690-021-00630-3.
42. Gao J, Zhu Y, Wang W, Wang Z, Dong G, Tang W, et al. A comprehensive benchmark for COVID-19 predictive modeling using electronic health records in intensive care. Patterns (N Y). 2024;5(4):100951. doi: 10.1016/j.patter.2024.100951.
43. Elgohary M, Ali A, El-Masry T, Faidah H, Bantun F, Elkholy A, et al. Development and validation of a predictive scoring system for in-hospital mortality in COVID-19 Egyptian patients: a retrospective study. Sci Rep. 2022;12(1):22352. doi: 10.1038/s41598-022-26471-w.
44. Bakin EA, Stanevich OV, Belash VA, Belash AA, Savateeveva GA, Bokinova VA, et al. A precise score for the regular monitoring of COVID-19 patients condition validated within the first two waves of the pandemic. In: medRxiv [Internet]. 2021 [cited 2024 Jul 18]. Available from: https://www.medrxiv.org/content/10.1101/2021.02.09.21249859v3.
45. Chen J, Liu B, Du H, Lin H, Chen C, Rao S, et al. Performance of CURB-65, PSI, and APACHE-II for predicting COVID-19 pneumonia severity and mortality. Eur J Inflam. 2021;19:1-12. https://doi.org/10.1177/20587392211027083.
46. Choi M, Kim D, Choi E, Jung Y, Choi Y, Cho J, et al. Mortality prediction of patients in intensive care units using machine learning algorithms based on electronic health records. Sci Rep. 2022;12(1):7180. doi: 10.1038/s41598-022-11226-4.
47. Zhao S, Tang G, Liu P, Wang Q, Li G, Ding Z. Improving mortality risk prediction with routine clinical data: a practical machine learning model based on eICU patients. Int J Gen Med. 2023;16:3151-3161. doi: 10.2147/IJGM.S391423.
48. Laino M, Generali E, Tommasini T, Angelotti G, Aghemo A, Desai A, et al. An individualized algorithm to predict mortality in COVID-19 pneumonia: a machine learning based study. Arch Med Sci. 2022;18(3):587-595. doi: 10.5114/aoms/144980.
49. Aslam H, Biswas S. Analysis of COVID-19 death cases using machine learning. SN Comput Sci. 2023;4(4):403. doi: 10.1007/s42979-023-01835-9.
50. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. BMJ. 2014;350:g7594. https://doi.org/10.1186/s12916-014-0241-z.
51. Caires Silveira E, Mattos Pretti S, Santos B, Santos Corrêa C, Madureira Silva L, Freire de Melo F. Prediction of hospital mortality in intensive care unit patients from clinical and laboratory data: a machine learning approach. World J Crit Care Med. 2022;11(5):317-329. doi: 10.5492/wjccm.v11.i5.317.
52. Yadaw A, Li Y, Bose S, Iyengar R, Bunyavanich S, Pandey G. Clinical features of COVID-19 mortality: development and validation of a clinical prediction model. Lancet Digit Health. 2020;2(10):e516-e525. doi: 10.1016/S2589-7500(20)30217-X.

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REVIEW ARTICLE

Current concepts in the management of calcaneal fractures

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ABSTRACT

Introduction. Despite advances in non-operative and surgical management, calcaneal fractures remain severe injuries with relatively poor clinical outcomes. These fractures predominantly affect young, active individuals and are often associated with long-term sequelae, permanent disability, a considerable reduction in quality of life, and a substantial economic impact due to work incapacity and rehabilitation needs.

Material and methods. This study is a narrative literature review. A bibliographic search was conducted using PubMed, Hinari, SpringerLink, National Center for Biotechnology Information, and Medline databases. Articles published from 1990 to 2024 were selected based on keyword combinations such as "calcaneal fracture," "comminuted calcaneal fracture," "orthopedic treatment", "surgical treatment," "minimally invasive treatment," "osteosynthesis," "locking plate," and "locked intramedullary nail." After processing information from these databases according to the search criteria, 225 full-text articles were identified. The final bibliography includes 56 relevant sources, which were considered representative of the materials published on the topic of this synthesis article.

Results. Surgical treatment using open reduction and internal fixation for displaced intra-articular calcaneal fractures was superior to non-surgical treatment in restoring Bohler's angle, achieving more stable calcaneal height and width, improving functional recovery, reducing the number of patients requiring orthopedic footwear, and enabling return to pre-injury activities, though it carries a high risk of complications. The minimally invasive approach via the sinus tarsi and the extended lateral L-shaped approach are equally effective for treating Sanders type II and III fractures in terms of restoring anatomical structures, radiological outcomes, and functional recovery. However, the sinus tarsi approach is effective in reducing wound complication rates (3.6–6.3% vs. 13.5–31.2%, respectively; $p < 0.05$), pain syndrome rates, time to surgery ($p < 0.0001$), surgery duration ($p < 0.05$), and hospital stay duration. Therefore, the minimal incision approach is a good alternative to the extended lateral L-shaped approach.

Conclusions. The current concept in managing calcaneal fractures involves developing an individualized treatment plan based on the patient's characteristics and functional requirements, comorbidities, fracture type, and associated injuries, as well as the surgeon's experience with the selected surgical technique.

Keywords: calcaneal fracture, comminuted calcaneal fracture, orthopedic treatment, surgical treatment, minimally invasive treatment, osteosynthesis, locking plate, locked intramedullary nail.

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Key messages

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

The inconsistent clinical outcomes and frequent complications associated with open reduction of calcaneal fractures have made it challenging to standardize the surgical management of these injuries. The optimal approach to managing displaced intra-articular calcaneal fractures, which are highly complex injuries, remains controversial, and there is currently no universal treatment protocol.

The research hypothesis

The analysis and synthesis of contemporary literature will allow for a comprehensive presentation of the current management of calcaneal fractures, determining the indications and contraindications for conservative treatment, surgical treatment, and minimally invasive treatment.

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

The article provides a synthesis of the latest international publications on the effectiveness of contemporary treatment methods for calcaneal fractures. The study results will contribute to refining the treatment protocol for initial management and optimizing fixation methods for calcaneal fractures by selecting appropriate types of fixators.

Introduction

Although new technologies and osteosynthesis materials have been developed, the treatment of calcaneal fractures (both non-surgical and open reduction with internal fixation) remains a controversial topic in the specialized literature due to the fracture's anatomical complexity, the fragile soft tissue surrounding the bone, and high complication rates. The literature presents contradictory findings regarding outcomes for calcaneal fractures and the potential superiority of one treatment option over another or over conservative treatment. Some reasons for this controversy include issues with different classification systems, varying indications for surgical treatment, and diverse evaluations for clinical and radiological outcomes [1-6].

Inconsistent clinical outcomes and frequent complications associated with open reduction have made it difficult to standardize the surgical management of these injuries. A universal treatment protocol has yet to be established. Even existing randomized controlled trials comparing operative and non-operative treatments over the past 25 years have failed to provide clarity. While many studies favor surgical treatment, others have found no difference between operative and non-operative management. The irregular bone anatomy, complex joint mechanics among the tarsal bones, and delicate soft tissue coverage make these fractures challenging. Systematic reviews and meta-analyses have not found conclusive evidence for the most effective treatment due to the use of diverse operative strategies and outcome measurements [5-17].

Given this context, the aim of this article is to provide a synthesis of the most recent data on the efficacy of modern treatment methods for patients with intra-articular calcaneal fractures.

Material and methods

To achieve the stated objective, an initial search of specialized scientific publications was conducted using Google Search and databases including PubMed, Hinari (Health Internet Work Access to Research Initiative), SpringerLink, National Center for Biotechnology Information, and Medline. The selection criteria for articles included contemporary data on treatment methods for patients with intra-articular calcaneal fractures, using keywords such as "calcaneal fracture," "comminuted calcaneal fracture," and "intra-articular calcaneal fracture" in various combinations with "orthopedic treatment," "surgical treatment," "minimally invasive treatment," "osteosynthesis," "locking plate," and "locked intramedullary nail" to maximize search yield.

For advanced source selection, the following filters were applied: full-text articles in English, published between 1990 and 2024. After a preliminary title analysis, original research articles, editorials, narrative reviews, systematic reviews, and meta-analyses containing relevant information and contemporary concepts on orthopedic, surgical, and minimally invasive treatment of intra-articular calcaneal fractures were selected. Additionally, reference lists from identified sources were searched to highlight further relevant publications not found during the initial database search.

The information from publications included in the bibliography was collected, classified, evaluated, and synthesized, highlighting key aspects of contemporary views on treatment methods for patients with intra-articular calcaneal fractures.

To minimize the risk of systematic errors (bias) in the study, we conducted thorough searches in databases to identify a maximum number of relevant publications for the study's purpose, assessed only studies meeting validity criteria, and applied reliable exclusion criteria for articles in the study.

Additional information sources were consulted as needed for clarification of certain concepts. Duplicate publications, articles that did not align with the study's purpose, and those not accessible for full viewing were excluded from the list of publications generated by the search engine.

Following the information processing through Google Search and databases such as PubMed, Hinari, SpringerLink, National Center of Biotechnology Information, and Medline, 225 articles addressing treatment methods for patients with intra-articular calcaneal fractures were identified based on the search criteria. After a primary title analysis, 62 articles were initially deemed potentially relevant for this synthesis. Upon further review, 56 publications were ultimately selected as relevant to the stated objective. These 56 articles were included in the final bibliography, representing the materials considered significant for the topic of this synthesis article.

Publications that did not address the topic, even if initially selected by the search program, and articles inaccessible for full viewing, either through the HINARI database or in the scientific medical library of the Nicolae Testemiţanu State University of Medicine and Pharmacy, were subsequently excluded from the list.

Results

The management of displaced intra-articular calcaneal fractures (DIACF) can be divided into four main categories: 1) non-operative management, 2) open reduction and internal fixation (ORIF), 3) minimally invasive reduction and fixation, and 4) primary subtalar arthrodesis (PSA) [18]. Therapeutic options include conservative non-surgical treatment, closed reduction with external fixation using Kirschner wires or an external fixator, closed reduction and internal fixation with screws or nails, closed reduction with calcaneoplasty, ORIF with K-wires, screws, or plates, arthroscopically assisted reduction with internal fixation, arthroscopic reduction with external fixation, and subtalar arthrodesis. Available fixation methods include external fixation, plate fixation, nails, Kirschner wires, and screws [1, 12, 15, 19-24].

A range of factors and their negative impacts on the healing process should be considered, as they inform strict indications for the optimal treatment strategy. These include patient factors (comorbidities, age, gender, functional needs, smoking, psychological disorders), limb and soft tissue injury characteristics (open fracture, bilateral fracture, severe edema, fracture blisters), and fracture features (Sanders classification, Bohler's angle, type of fracture—whether intra-articular or extra-articular) [18].

The most important imaging data for planning reconstructive surgery are: the number of posterior articular surface fragments (according to the Sanders classification, prognosis worsens with an increasing number of fragments, making surgical reconstruction of the posterior articular surface unfeasible in cases of excessive comminution with poor prognosis), the direction of fracture lines (the more medial, the harder it is to achieve reduction), the direction of displacement of the calcaneal tuberosity fragment (varus or valgus with rotational elements), the width and degree of collapse of the calcaneus (affecting footwear selection and the risk of peroneal tendon or malleolar impingement against a bulging lateral calcaneal wall), and involvement of the calcaneo-cuboid joint [25].

Orthopedic (non-surgical) treatment is indicated for extra-articular fractures without displacement or with displacement <2 mm from the anatomical position, fractures located in the anterior process affecting less than 25% of the calcaneo-cuboid joint, patients with comorbidities (e.g., diabetes, severe neurovascular insufficiency, peripheral vascular disease), severe local soft tissue conditions that preclude extensive approaches, or elderly patients unable to tolerate surgery [7, 16, 18, 26-30].

Conservative treatment for calcaneal fractures involves immobilizing the fracture area in a plaster splint (5–7 days), followed by a plaster cast or orthosis for the heel (60 days), use of nonsteroidal anti-inflammatory drugs, and physiotherapy procedures. For non-displaced calcaneal fractures, the healing time is approximately 10–12 weeks [7, 16, 18, 26-30].

Early joint mobilization is recommended, but without weight-bearing on the affected foot. After 10–12 weeks, the patient can begin full weight-bearing walking on the injured foot, but only after radiographic confirmation of bone consolidation [16]. Potential disadvantages of non-operative management compared to surgical treatment include the inability to achieve anatomic reduction of the posterior facet, insufficient functional recovery, higher rates of subtalar and calcaneo-cuboid arthritis, risk of malunion, and higher rates of subtalar arthrodesis (16% and 3%, respectively; $p < 0.0001$) [5, 7, 11, 18, 29, 31].

Modern surgical management of calcaneal fractures is based on the following steps:

1. **Surgical Approaches:**
   - **Medial approach** (for sustentaculum tali fractures)
   - **Extended lateral L-shaped approach (ELLA)** (for Sanders II, III, and IV intra-articular fractures) – This is the most used approach, suitable for most DIACF cases where access to the posterior facet, posterolateral and anterolateral fragments, and subtalar joint is necessary.
   - **AST** is a limited, customized lateral approach allowing access to the subtalar and calcaneo-cuboid joints.
2. **Combined approach (medial and lateral)** has been described for DIACF with wound complications [20, 23, 28, 31].
3. **Timing of surgery:**

Surgery is performed 10–14 days after the fracture occurs, allowing time for significant reduction or resolution of edema and blisters. Keeping the affected limb elevated and immobilized helps reduce the time needed for edema resolution. Surgery is considered safe when lateral skin folds appear [20]. However, in the case of an open fracture, immediate treatment is required due to the risk of osteitis [20, 30].

4. **Surgical objectives:**

The surgical goals include restoring heel height and width, reconstructing anatomy to approximate Bohler's and Gissane's angles, repairing and realigning the subtalar joint, restoring the functionality of the posterior mechanical axis, reconstructing the medial and lateral walls, and protecting tendons and neurovascular structures. There is a significant correlation between preoperative Bohler's angle and fracture severity, and postoperatively, Bohler's angle significantly correlates with functional recovery [4, 19-21, 32]. Restoration of the posterior facet is essential for achieving a normal gait, an earlier return to professional activities, and reducing the need for subtalar joint arthrodesis [33].

5. **Surgical technique**

The goal of surgical treatment is to restore the morphology of the calcaneus and joint congruence, and it includes:

- **Closed reduction and external (percutaneous) fixation with screws** – recommended for extra-articular fractures when the fractured fragments are large and for minimally displaced "tongue" type fractures.
- **Open reduction and internal fixation (ORIF) with screws or plates (conventional or locking)** – indicated for fractures that cannot be reduced by less invasive methods (displaced complex intra-articular

fractures, displaced "tongue" type fractures, Sanders type II and III fractures, fractures of the anterior process with over 25% involvement of the calcaneo-cuboid joint, displaced sustentaculum fractures, and cases with reduced Bohler's angle).

- **Minimally invasive osteosynthesis with or without arthroscopic reduction** [7, 8, 10, 12, 15, 16, 19, 21, 22, 34, 35].

Over the past two decades, surgical management of calcaneal fractures has increasingly become preferred over conservative management. With advances in surgical techniques and biomechanics, surgical treatment is now usually the first-line choice for calcaneal fractures [5]. The goals of modern surgical treatment in the literature are defined as follows: 1) reduction of the posterior articular facet, 2) restoration of the original height and width of the calcaneus, 3) reconstruction of Bohler's and Gissane's angles, 4) ensuring fibular tendon mobility, 5) re-establishment of the valgus position of the calcaneal tuberosity, 6) reduction of the calcaneo-cuboid joint, 7) early rehabilitation of the foot and ankle, and 8) minimization of soft tissue complications [21, 32, 36].

Thus, modern surgical treatment for fractures is designed to reduce bone fragments while achieving joint congruence and stable fixation, allowing for early mobilization [5, 30].

Surgical treatment is performed for patients with open fractures, displaced intra-articular fractures (Sanders types II, III, and IV), "tongue" type fractures that may compromise soft tissue, extensive fractures of the anterior process, fractures located at the posterior tuberosity, or calcaneal fractures with dislocations. An important rule is that calcaneal surgery should be done only after edema and blisters subside (indicated by the appearance of skin laxity—wrinkles), but the time from the fracture occurrence to surgery should not exceed 2–3 weeks to avoid fracture consolidation and development of fibrotic rigidity in the soft tissues surrounding the calcaneus. Additionally, the condition of the soft tissues is crucial [7, 16, 19, 21, 27, 28, 37].

Factors that may influence wound healing include the Sanders type of fracture, open fractures, smoking, comorbidities (especially diabetes and obesity), overcorrection of calcaneal height, early or delayed surgery, prolonged operating time, and wound closure technique [17, 32, 38]. Therefore, the decision to perform surgery is based on three main criteria: (1) displacement or comminution of the posterior facet; (2) the patient's age and surgical contraindications; (3) soft tissue trauma and associated conditions or polytrauma that can influence the timing of surgery and the choice of surgical technique (open reduction, external fixation, percutaneous technique, approach selection) [28, 30].

Since the 1990s, ORIF with a plate has been considered the "gold standard" for surgical treatment of DIACF, including comminuted fractures [23, 39-41]. For internal fixation, most authors use a single lateral plate of various shapes (locked or simple) that matches the anatomical features of the calcaneus and is secured with at least two screws, often in different planes, providing a more rigid fixation. These plates effectively resist rotation and axial loads to achieve stable fixation and are associated with low rates of loosening and fixation failure [26, 31, 39-41].

Locking plate systems were developed to enhance fracture fixation stability, allowing for early mobilization and rehabilitation. The introduction of locking plate technology in orthopedics has improved the biomechanical durability of bone and fracture fixation, representing a significant advancement in treating complex, periarticular, and osteopenic fractures [29, 30].

In recent years, the technology of locking plates has been evaluated, with researchers supporting that they provide stronger joint stability and fixation than non-locked plate constructs, particularly in comminuted fractures with a wide coverage area. They enable early weight-bearing on the affected limb without compromising fracture stability [27, 29, 40-42]. Although the advantages of locking plates in calcaneal fractures remain debated, multiple studies have demonstrated the superiority of locking plates over conventional constructs. Locking plates are also advantageous in treating DIACF in patients with osteoporotic bone [27, 29, 30, 40-42].

The locked intramedullary nail, a new minimally invasive technique, was developed to combine the benefits of a minimally invasive approach with stable percutaneous fixation. This system has demonstrated high primary stability, comparable to variable-angle locking plates, in fixing fractures in patients with Sanders type II and III DIACF [43, 44].

Among all methods, ORIF is considered the standard treatment and the best method for achieving anatomical joint reduction, restoring calcaneal morphology with a lower prevalence of post-traumatic arthritis. However, soft tissue complications are proportional to the extent of soft tissue trauma, which is why ORIF is not always feasible for every case and is typically performed when the soft tissue has recovered following fracture trauma [8-10, 12, 15, 45].

ORIF with a plate has shown acceptable radiological and functional outcomes: restoration of subtalar joint movement (67.8% of active movement and 80.7% of passive movement), attributed to rigid anatomical reduction and early postoperative rehabilitation [23, 40, 41]. The calcaneal locking plate provides a secure functional outcome by restoring the anatomical reconstruction of calcaneal height, width, Bohler's angle, and Gissane's angle, which leads to on early mobilization [39]. It has been shown that ORIF has higher complication and reoperation rates, yet yields better outcomes in terms of pain relief, walking restoration, time to return to work, and wearing regular footwear [19, 45]. All major randomized studies comparing non-operative treatment with surgical intervention via the extended lateral L-shaped approach (ELLA) found wound complication rates between 19% and 37% in the surgical group [14].

A meta-analysis published in 2012 examined 10 studies (6 randomized controlled trials and 4 controlled clinical studies) with a total of 891 patients, confirming that surgical

treatment of DIACF was superior to non-surgical treatment in restoring Bohler's angle, achieving more stable calcaneal height and width, better functional recovery, reducing the number of patients needing orthopedic footwear, and resuming pre-injury activities. Surgical management of DIACF was associated with a higher risk of complications (22.8% versus 16.2%, p=0.008), while function continued to deteriorate over long-term follow-up in conservative treatment. The authors concluded that surgery can effectively restore the anatomical structures of the calcaneus with better functional recovery, despite a high risk of complications [5, 46].

In a systematic review published in 2013, Veltman E. and co-authors evaluated 25 studies with a total of 1,730 calcaneal fractures and an average follow-up of 4.6 years. The studies reported functional, subjective, and radiographic outcomes following surgical or conservative treatment. The authors support surgical treatment of DIACF with ORIF as the method of choice [5, 47].

Wei N. and co-authors conducted a systematic literature review and meta-analysis published in 2017 that assessed the efficacy of surgical and non-operative management of DIACF. The analysis included 18 studies (8 randomized controlled trials and 10 controlled clinical studies) involving 1,467 patients. The authors concluded that surgical treatment of DIACF, compared with conservative treatment, may increase complication incidence but provides significantly better anatomic recovery, including restoration of Bohler's angle, calcaneal height, and width, and a higher likelihood of resuming previous professional activities [48]. Another similar and more recent systematic literature review and meta-analysis, published in 2022, included 13 studies, including 10 randomized controlled trials and 3 prospective clinical studies with 1,251 patients. The study found that the best available evidence at that time favored an advantage for operative treatment. Fewer footwear issues and a higher likelihood of returning to the desired activity level were observed in surgically treated patients [13].

The clinical significance of these findings is that surgical treatment should be recommended for DIACF, as it can better restore calcaneal anatomy, improve functional recovery, and increase the likelihood of returning to previous activity levels compared to conservative treatment [48]. A prospective, case-control, and prognostic study revealed that if severe post-traumatic subtalar arthritis does not develop, long-term functional outcomes (10–20 years) with mild pain, minimal changes in daily or professional activities, and normal footwear use are favorable results of ORIF via ELLA [49].

Therefore, ORIF can be performed through various approaches (lateral, medial, plantar, posterior, or combined), including minimally invasive options. ELLA has been the most popular technique, considered since the 1990s as the "gold standard" in treating DIACF. It offers wide and precise exposure of fracture fragments and the subtalar and calcaneo-cuboid joints, easier decompression of the lateral wall, and sufficient lateral space for plates of various shapes. With delicate soft tissue management in mind, particular attention is given to creating full-thickness flaps and using the "no-touch" technique. However, this method is associated with soft tissue complications (wound edge dehiscence or necrosis, superficial or deep infection, hematoma, sural nerve injury, peroneal tendon injuries or displacement, and subtalar arthritis), with complication rates ranging from 5.8% to 43%. Despite this, the ability of ORIF to directly visualize the posterior facet, the advantages of achieving better restoration, and the re-establishment of anatomical parameters (length, width, height, and calcaneal alignment) outweigh the increased complication risk.

Minimally invasive surgical treatment

Although ORIF for DIACF is favored by many authors, the increased risk of soft tissue complications makes this treatment method challenging, especially for patients who smoke or have diabetes. This may explain the growing use of minimally invasive techniques in managing intra-articular calcaneal fractures [3, 18, 24, 32].

Several minimally invasive surgical techniques have been developed for patients with calcaneal fractures to achieve good reductions, favorable clinical and radiographic outcomes, and lower complication rates, particularly for soft tissue complications. These techniques include percutaneous reduction and external fixation, arthroscopically assisted percutaneous reduction and fixation, surgery guided by a three-dimensional model or 3D printing of the fracture, calcaneoplasty, and open fixation via mini incisions. Minimally invasive techniques are more reliable when performed within the first 2 weeks after the fracture, as the fracture fragments are easier to manipulate during this period [8-11, 15, 22, 26, 31, 34, 37]. Some percutaneous techniques are used to realign the calcaneus with minimal tissue dissection and can be performed immediately after injury in cases of extensive soft tissue edema [34, 50].

The primary indications for using minimally invasive approaches in the surgical treatment of calcaneal fractures include fewer complex fractures, minimally fragmented posterior facet fractures, displaced Essex-Lopresti fractures, Sanders type II and III fractures in patients with multiple comorbidities, and those at high risk for significant postoperative wound complications [14, 24, 30, 50]. Recent prospective and retrospective studies, systematic reviews, and meta-analyses have shown promising results with minimally invasive techniques in terms of reducing preoperative time, surgery duration, hospital stay, and complication rates [8-11, 22, 26, 27, 34, 37, 45].

The sinus tarsi approach (STA) is used in minimally invasive reduction and percutaneous fixation of DIACF fragments. This technique, suitable for simple calcaneal fractures, allows a direct visualization of the subtalar joint surface and the exposure of the calcaneal joint by extending the incision if necessary. Additionally, locking plates can be introduced through STA to achieve firm fixation while minimizing soft tissue damage. Patients with diabetes, smokers, and those with comorbidities, who are at risk of wound healing complications, may benefit from minimally invasive techniques, especially STA. Furthermore, STA is the most

cost-effective treatment option for Sanders type II-III DIACF [1, 21, 22, 34, 36, 42, 45].

A comparison of two internal fixation techniques—cannulated screw fixation and plate with screws using STA showed no statistically significant difference in restoring, correcting, and maintaining Bohler and Gissane angles over time, radiographic parameters, or postoperative complication rates. Thus, both fixation methods are equally effective in restoring and maintaining Bohler and Gissane angles with a lower complication rate compared to ELLA. The implant costs and implant removal rate were significantly higher with the minimally invasive plate application [51].

A systematic literature review, three prospective, randomized controlled trials, and three retrospective studies were conducted to compare ORIF with minimally invasive reduction and percutaneous fixation for Sanders type II, III, or IV DIACF. No statistically significant difference was found between ORIF and percutaneous fixation groups regarding anatomical reduction, functional outcomes, or the need for revision surgery. The minimally invasive method was associated with the ability to perform the procedure immediately without waiting for soft tissue stabilization, significantly shorter surgery and hospital stay, lower rates of postoperative pain syndrome and wound complications, reduced subtalar joint stiffness, faster postoperative rehabilitation, better functional outcomes, and an earlier return to work [24, 34].

Researchers concluded that both techniques – percutaneous reduction and fixation and ORIF – can be considered for the surgical treatment of DIACF, as both are capable of restoring Bohler's angle and have relatively good long-term functional outcomes. Indications for each technique may vary among surgeons, and each has its own set of risk factors and complications; however, both have been shown acceptable reduction outcomes [24].

Four meta-analyses, published in 2017 and 2018, along with other prospective and retrospective cohort studies, suggest that the minimally invasive approach with STA and the extended lateral L-shaped approach (ELLA) are equally effective in treating Sanders type II and III fractures in terms of anatomical structure restoration, radiological outcomes, and functional recovery. However, STA has shown effectiveness in reducing wound complication rates (3.6–6.3% vs. 13.5–31.2%, respectively; $p < 0.05$), rates of pain syndrome, time to surgery ($p < 0.0001$), surgery duration ($p < 0.05$), and length of hospital stay [52-54]. Despite this, heterogeneity among studies and errors in including certain publications have raised doubts about the reliability of these conclusions [38].

A recent meta-analysis published in 2020, which included 17 randomized controlled trials and 10 retrospective studies with 2,179 patients and 2,274 DIACF cases monitored for an average of 22.41 months, evaluated the effectiveness of the minimally invasive incision approach and standard ELLA. Overall, results showed no statistically significant difference in Gissane's angle, calcaneal width and length, deep infection, or subtalar joint stiffness. When only randomized controlled trials were analyzed, there were no statistically significant differences between groups regarding Bohler's or Gissane's angles. A statistically significant difference was observed in wound complication rates, superficial wound infection, sural nerve injuries, AOFAS scores, time to surgery, surgery duration, calcaneal height, and postoperative Bohler's angle (when all studies were considered), all in favor of the minimally invasive incision approach. These results remained statistically significant when only randomized controlled trials were compared, except for Bohler's angle and AOFAS scores. This meta-analysis indicates that the minimally invasive incision approach is a good alternative to standard ELLA [33].

A more recent meta-analysis, published in 2021, found no differences in anatomical structure restoration, functional recovery, and clinical efficacy of DIACF treatment between STA and ELLA [55].

However, two retrospective cohort studies published in 2020 and 2021, along with three more recent meta-analyses from 2020 and 2021 that updated the literature and excluded inadequate studies previously included in meta-analyses, found that STA, compared to ELLA, is superior for treating calcaneal fractures due to improved anatomical reduction of the calcaneus, lower incidence of wound complications ($p < 0.001$), shorter preoperative time, reduced operative time ($p < 0.001$), shorter hospital stay ($p=0.002$), lower rates of secondary surgeries, and faster wound healing. There was no statistical difference in Bohler and Gissane angles, but better foot function scores according to AOFAS and Maryland scores which were significantly higher in the STA group ($p < 0.01$). Therefore, STA was identified as a superior alternative for DIACF treatment [38, 56].

Two recent systematic literature reviews and meta-analyses published in 2023 analyzed 59 studies with over 10,000 calcaneal fractures and 13 studies with 897 patients with calcaneal fractures. According to the results, most researchers agree on the superiority of surgical treatments for calcaneal fractures compared to conservative ones. Furthermore, minimally invasive access to the sinus tarsi showed better outcomes and lower complication rates than traditional ELLA, as it uses a smaller incision and involves less extensive tissue manipulation. In general, reduction and osteosynthesis with percutaneous and/or minimally invasive techniques offer better outcomes compared to open treatments, even when open reduction is contraindicated [12, 45].

Thus, minimally invasive techniques yield clinical and radiographic outcomes similar to ELLA but with lower wound complication rates for all DIACF types. There is no statistically significant difference between the two groups in terms of restoring anatomical structures, although some surgeons consider posterior facet restoration more challenging with the minimally invasive approach.

There is no universally applicable treatment method for all calcaneal fractures. The consensus among most researchers is as follows: 1) surgical treatment is superior to conservative treatment; 2) minimally invasive access via

STA provides better results and fewer complications than traditional ELLA; 3) reduction and osteosynthesis with percutaneous and/or minimally invasive techniques appear to offer better outcomes than open treatments. Therefore, the current concept in managing calcaneal fractures involves developing an individualized treatment plan based on the patient's characteristics and functional requirements, comorbidities, fracture type, associated injuries, and the surgeon's experience with the selected surgical technique. New technologies may improve calcaneal fracture management [5, 6, 12, 13, 45, 49].

Recent studies, systematic literature reviews, and meta-analyses have shown promising results with minimally invasive techniques in the treatment of Sanders type II, III, and IV DIACF. Although both surgical interventions are effective, the sinus tarsi approach (STA) in the treatment of calcaneal fractures has proven to be significantly safer and more effective, with similar functional and radiological outcomes but lower postoperative complication rates compared to ELLA. The advantages of this treatment method include a much shorter time from fracture to fixation (often 2–3 days), reduced intraoperative bleeding, higher mean AOFAS scores, lower postoperative complication rates, including wound complications, shorter operative time, much earlier active mobilization, shorter hospital stay and recovery periods, and a lower rate of secondary surgeries [15, 22, 32, 34, 36, 45, 51]. Therefore, the minimally invasive sinus tarsi approach is becoming the new "gold standard" in DIACF treatment [14, 37].

According to contemporary guidelines and the views of many researchers, patients with non-displaced Sanders type I and II calcaneal fractures should be treated conservatively. Sanders type II and III DIACF should be treated surgically. There remains debate regarding the treatment method for Sanders type IV fractures, which can be managed surgically or conservatively; however, this group of patients tends to have poor outcomes even after open reduction. Some patients with subtalar arthritis may require subtalar or triple arthrodesis (subtalar, talonavicular, and calcaneocuboid). Despite these general treatment principles, the treatment strategy should be individually tailored on a case-by-case basis, as various factors influence complication development in calcaneal fractures [35, 42].

Conclusions

(1) Over the past two decades, surgical management of calcaneal fractures has become increasingly preferred over conservative management. With advances in surgical techniques and biomechanics, surgical treatment is now the first-line choice for calcaneal fractures, aimed at reducing bone fragments, achieving joint congruence, and stable fixation with early mobilization.

(2) Surgical treatment through open reduction and internal fixation for displaced intra-articular calcaneal fractures has proven superior to non-surgical treatment in restoring Bohler's angle, achieving more stable calcaneal height and width, improving functional recovery, reducing the number of patients requiring orthopedic footwear, and facilitating the resumption of pre-injury activities, though there is a high risk of complications.

(3) The decision to perform surgery is based on three main criteria: displacement or comminution of the posterior facet; the patient's age and contraindications for surgery; and soft tissue trauma and associated injuries, which may influence the timing of surgery and the choice of surgical technique.

(4) The minimally invasive surgical method allows for immediate intervention without waiting for soft tissue consolidation, significantly shorter operative and hospitalization times, reduced rates of postoperative pain syndrome and wound complications, decreased subtalar joint stiffness, faster postoperative rehabilitation with better functional outcomes, and an earlier return to professional activity.

(5) The minimally invasive sinus tarsi approach and the extended lateral L-shaped approach are equally effective for treating Sanders type II and III fractures in terms of anatomical restoration, radiological outcomes, and functional recovery. However, the sinus tarsi approach is more effective in reducing wound complication rates (3.6–6.3% vs. 13.5–31.2%; $p < 0.05$) and rates of pain syndrome, reducing time to surgery ($p < 0.0001$), operative duration ($p < 0.05$), and hospital stay. Thus, the minimal incision approach is a good alternative to the extended lateral L-shaped approach.

(6) The current concept in managing calcaneal fractures involves developing an individualized treatment plan based on the patient's characteristics and functional requirements, comorbidities, fracture type, associated injuries, and the surgeon's experience with the selected surgical technique.

Competing interests

None declared.

Ethics approval

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References

1. Schepers T. Sinus tarsi approach with screws-only fixation for displaced intra-articular calcaneal fractures. Clin Podiatr Med Surg. 2019;36(2):211-224. doi: 10.1016/j.cpm.2018.10.004.
2. Ni M, Lv M, Sun W, Zhang Y, Mei J, Wong D, et al. Fracture mapping of complex intra-articular calcaneal fractures. Ann Transl Med. 2021;9(4):333. doi: 10.21037/atm-20-7824.
3. Wee AT, Wong YS. Percutaneous reduction and injection of Norian bone cement for the treatment of displaced intra-articular calcaneal fractures. Foot Ankle Spec. 2009;2(2):98-106. doi: 10.1177/1938640009334153.
4. Paul M, Peter R, Hoffmeyer P. Fractures of the calcaneum. A review of 70 patients. J Bone Joint Surg Br. 2004;86(8):1142-1145. doi: 10.1302/0301-620x.86b8.15219.
5. Buzzi R, Sermi N, Soviero F, Bianco S, Campanacci D. Dis-

placed intra-articular fractures of the calcaneus: ORIF through an extended lateral approach. Injury. 2019;50 Suppl 2:S2-S7. doi: 10.1016/j.injury.2019.01.037.
6. Shi G, Liu W, Shen Y, Cai X. 3D printing-assisted extended lateral approach for displaced intra-articular calcaneal fractures: a systematic review and meta-analysis. J Orthop Surg Res. 2021;16(1):682. doi: 10.1186/s13018-021-02832-5.
7. Allegra P, Rivera S, Desai S, Aiyer A, Kaplan J, Gross C. Intra-articular calcaneus fractures: current concepts review. Foot Ankle Orthop. 2020;5(3):2473011420927334. doi: 10.1177/2473011420927334.
8. Guerado E, Bertrand M, Cano J. Management of calcaneal fractures: what have we learnt over the years? Injury. 2012;43(10):1640-1650. doi: 10.1016/j.injury.2012.05.011.
9. Giannini S, Cadossi M, Mosca M, Tedesco G, Sambri A, Terrando S, et al. Minimally-invasive treatment of calcaneal fractures: a review of the literature and our experience. Injury. 2016;47 Suppl 4:S138-S146. doi: 10.1016/j.injury.2016.07.050.
10. Maskill JD, Bohay DR, Anderson JG. Calcaneus fractures: a review article. Foot Ankle Clin. 2005;10(3):463-489. doi: 10.1016/j.fcl.2005.03.002.
11. Kiewiet NJ, Sangeorzan BJ. Calcaneal fracture management: extensile lateral approach versus small incision technique. Foot Ankle Clin. 2017;22(1):77-91. doi: 10.1016/j.fcl.2016.09.013.
12. Leigheb M, Codori F, Samaila E, Mazzotti A, Villafañe J, Bosetti M, Ceccarini P, et al. Current concepts about calcaneal fracture management: a review of metanalysis and systematic reviews. Appl Sci. 2023;13(22):12311. https://doi.org/10.3390/app132212311.
13. Selim A, Ponugoti N, Chandrashekar S. Systematic review of operative vs nonoperative treatment of displaced intraarticular calcaneal fractures. Foot Ankle Orthop. 2022;7(2):24730114221101609. doi: 10.1177/24730114221101609.
14. Schepers T. Fixation by open reduction and internal fixation or primary arthrodesis of calcaneus fractures: indications and technique. Foot Ankle Clin. 2020;25(4):683-695. doi: 10.1016/j.fcl.2020.08.008.
15. Wei N, Zhou Y, Chang W, Zhang Y, Chen W. Displaced intra-articular calcaneal fractures: classification and treatment. Orthopedics. 2017;40(6):e921-e929. doi: 10.3928/01477447-20170907-02.
16. Gornea F. Ortopedie şi traumatologie [Orthopedics and traumatology]. Chişinău: Medicina; 2010. 588 p. Romanian.
17. Schindler C, Schirm A, Zdravkovic V, Potocnik P, Jost B, Toepfer A. Outcomes of intra-articular calcaneal fractures: surgical treatment of 114 consecutive cases at a maximum care trauma center. BMC Musculoskelet Disord. 2021;22(1):234. doi: 10.1186/s12891-021-04088-w.
18. Sharr PJ, Mangupli MM, Winson IG, Buckley RE. Current management options for displaced intra-articular calcaneal fractures: non-operative, ORIF, minimally invasive reduction and fixation or primary ORIF and subtalar arthrodesis. A contemporary review. Foot Ankle Surg. 2016;22(1):1-8. doi: 10.1016/j.fas.2015.10.003.
19. Box M, Domingue G, Werthmann N, Szatkowski J, Riehl J. Calcaneus fractures. In: Riehl J, Szatkowski J, editors. Current fracture care. London: Intechopen; 2024. doi: 10.5772/intechopen.114132.
20. Razik A, Harris M, Trompeter A. Calcaneal fractures: where are we now? Strategies Trauma Limb Reconstr. 2018;13(1):1-11. doi: 10.1007/s11751-017-0297-3.
21. Cottom JM, Douthett SM, McConnell KK. Intraoperative reduction techniques for surgical management of displaced intra-articular calcaneal fractures. Clin Podiatr Med Surg. 2019;36(2):269-277. doi: 10.1016/j.cpm.2018.10.008.
22. Hsu AR, Anderson RB, Cohen BE. Advances in surgical management of intra-articular calcaneus fractures. J Am Acad Orthop Surg. 2015;23(7):399-407. doi: 10.5435/JAAOS-D-14-00287.
23. Reddy M, Kiran R. Outcome of internal fixation of comminuted calcaneum fractures. IOSR J Dent Med Sci. 2016;15(8):29-35. doi: 10.9790/0853-1508102935.
24. Steelman K, Bolz N, Feria-Arias E, Meehan R. Evaluation of patient outcomes after operative treatment of intra-articular calcaneus fractures. SICOT J. 2021;7:65. doi: 10.1051/sicotj/2021065.
25. Grala P, Machyńska-Bućko Z, Kierzynka G. Radiographic imaging of calcaneal fractures – the surgeons view point. Pol J Radiol. 2007;72(2):88-91.
26. Rammelt S, Zwipp H. Fractures of the calcaneus: current treatment strategies. Acta Chir Orthop Traumatol Cech. 2014;81(3):177-196. doi: 10.55095/achot2014/020.
27. Herrera-Pérez M, Oller-Boix A, Valderrabano V, González-Casamayor S, Gutiérrez-Morales MJ, Guerra-Ferraz A, et al. Calcaneal fractures: controversies and consensus. Acta Ortop Mex. 2018;32(3):172-181.
28. Thermann H, Krettek C, Hüfner T, Schratt H, Albrecht K, Tscherne H. Management of calcaneal fractures in adults. Conservative versus operative treatment. Clin Orthop Relat Res. 1998;(353):107-124. doi: 10.1097/00003086-199808000-00013.
29. Shrestha R, Shrestha D, Kayastha S, Winker H. Displaced intra-articular calcaneal fractures: evaluation of clinical and radiological outcome following open reduction and internal fixation with locking branched calcaneal plate. Kathmandu Univ Med J. 2017;58(2):130-136.
30. Swanson SA, Clare MP, Sanders RW. Management of intra-articular fractures of the calcaneus. Foot Ankle Clin. 2008;13(4):659-678. doi: 10.1016/j.fcl.2008.09.006.
31. Rammelt S, Zwipp H. Calcaneus fractures: facts, controversies and recent developments. Injury. 2004;35(5):443-461. doi: 10.1016/j.injury.2003.10.006.
32. Gougoulias N, McBride D, Maffulli N. Outcomes of management of displaced intra-articular calcaneal fractures. Surgeon. 2021;19(5):e222-e229. doi: 10.1016/j.surge.2020.10.003.
33. Seat A, Seat C. Lateral extensile approach versus minimal incision approach for open reduction and internal fixation of displaced intra-articular calcaneal fractures: a me-

ta-analysis. J Foot Ankle Surg. 2020;59(2):356-366. doi: 10.1053/j.jfas.2019.08.007.
34. Park CH. Role of subtalar arthroscopy for displaced intra-articular calcaneal fractures. Clin Podiatr Med Surg. 2019;36(2):233-249. doi: 10.1016/j.cpm.2018.10.006.
35. Mladenović M, Milenkovic S, Stojiljković P, Krstic A. Sanders type III calcaneal fracture fixed with a locking angular plate: a case report. Acta Facultatis Medicae Naissensis. 2022;39(3):371-379. doi: 10.5937/afmnai39-33264.
36. Holmes GB. Treatment of displaced calcaneal fractures using a small sinus tarsi approach. Tech Foot Ankle Sur. 2005;4(1):35-41. doi: 10.1097/01.btf.0000156336.58175.e1.
37. Jiang G, Li J, Zhang X, Shu S, Ma Y, Zhang P, et al. Limb reconstruction system assisted reduction and internal fixation for intra-articular calcaneal fractures: a new application. Orthop Surg. 2023;15(10):2540-2548. doi: 10.1111/os.13828.
38. Peng C, Yuan B, Guo W, Li N, Tian H. Extensile lateral versus sinus tarsi approach for calcaneal fractures: a meta-analysis. Medicine (Baltimore). 2021;100(31):e26717. doi: 10.1097/MD.0000000000026717.
39. Jain S, Jain A, Kumar I. Outcome of open reduction and internal fixation of intraarticular calcaneal fracture fixed with locking calcaneal plate. Chin J Traumatol. 2013;16(6):355-360. https://doi.org/10.3760/cma.j.issn.1008-1275.2013.06.008
40. Fahmy M, Yasin E, Abdelmoneim M. Using calcaneal plates in fixation of comminuted posterior wall acetabular fractures with cranial or posterior extension: a prospective case series and novel technique. Eur J Orthop Surg Traumatol. 2024;34(7):3591-3597. doi: 10.1007/s00590-024-03939-1.
41. Pei X, Zheng Y, Zhou W, Wang G, Liu X. Calcaneal reticular plate with cannulated screws fixation for comminuted Hoffa fracture: a case report. Trauma Case Rep. 2022;40:100656. doi: 10.1016/j.tcr.2022.100656.
42. Yao H, Lu H, Zhao H, Lv L, Hou G. Open reduction assisted with an external fixator and internal fixation with calcaneal locking plate for intra-articular calcaneal fractures. Foot Ankle Int. 2017;38(10):1107-1114. doi: 10.1177/1071100717715908.
43. Amlang M, Zwipp H, Pompach M, Rammelt S. Interlocking nail fixation for the treatment of displaced intra-articular calcaneal fractures. JBJS Essent Surg Tech. 2017;7(4):e33. doi: 10.2106/JBJS.ST.17.00015.
44. Fascione F, Di Mauro M, Guelfi M, Malagelada F, Pantalone A, Salini V. Surgical treatment of displaced intraarticular calcaneal fractures by a minimally invasive technique using a locking nail: a preliminary study. Foot Ankle Surg. 2019;25(5):679-683. doi: 10.1016/j.fas.2018.08.004.
45. Bandyopadhyay A, Kumar S, Mandal P. Calcaneal fractures management, change of clinical practice in recent years from ELA to STA: a systematic review and meta-analysis. Indian J Orthop. 2023;57(6):800-817. doi: 10.1007/s43465-023-00871-0.
46. Jiang N, Lin Q, Diao X, Wu L, Yu B. Surgical versus nonsurgical treatment of displaced intra-articular calcaneal fracture: a meta-analysis of current evidence base. Int Orthop. 2012;36(8):1615-1622. doi: 10.1007/s00264-012-1563-0.
47. Veltman ES, Doornberg JN, Stufkens SA, Luitse JS, van den Bekerom MP. Long-term outcomes of 1,730 calcaneal fractures: systematic review of the literature. J Foot Ankle Surg. 2013;52(4):486-490. doi: 10.1053/j.jfas.2013.04.002.
48. Wei N, Yuwen P, Liu W, Zhu Y, Chang W, Feng C, et al. Operative versus nonoperative treatment of displaced intra-articular calcaneal fractures: a meta-analysis of current evidence base. Medicine (Baltimore). 2017;96(49):e9027. doi: 10.1097/MD.0000000000009027.
49. Sanders R, Vaupel Z, Erdogan M, Downes K. Operative treatment of displaced intraarticular calcaneal fractures: long-term (10-20 Years) results in 108 fractures using a prognostic CT classification. J Orthop Trauma. 2014;28(10):551-563. doi: 10.1097/BOT.0000000000000169.
50. Wireman G, Schultz A, Seligson D, Laco N. Medial percutaneous reduction of calcaneal fracture using a two pin ratcheting joint distractor: a case report. Foot Ankle Surg: Tech Rep Cases. 2023;3(4):100336. https://doi.org/10.1016/j.fastrc.2023.100336.
51. Pitts C, Almaguer A, Wilson J, Quade J, Johnson M. Radiographic and postoperative outcomes of plate versus screw constructs in open reduction and internal fixation of calcaneus fractures via the sinus tarsi. Foot Ankle Int. 2019;40(8):929-935. doi: 10.1177/1071100719848063.
52. Bai L, Hou Y, Lin G, Zhang X, Liu G, Yu B. Sinus tarsi approach (STA) versus extensile lateral approach (ELA) for treatment of closed displaced intra-articular calcaneal fractures (DIACF): a meta-analysis. Orthop Traumatol Surg Res. 2018;104(2):239-244. doi: 10.1016/j.otsr.2017.12.015.
53. Zhang F, Tian H, Li S, Liu B, Dong T, Zhu Y, et al. Meta-analysis of two surgical approaches for calcaneal fractures: sinus tarsi versus extensile lateral approach. ANZ J Surg. 2017;87(3):126-131. doi: 10.1111/ans.13869.
54. Nosewicz T, Dingemans S, Backes M, Luitse J, Goslings J, Schepers T. A systematic review and meta-analysis of the sinus tarsi and extended lateral approach in the operative treatment of displaced intra-articular calcaneal fractures. Foot Ankle Surg. 2019;25(5):580-588. doi: 10.1016/j.fas.2018.08.006.
55. Lv Y, Zhou Y, Li L, Yu Z, Wang Q, Sun Y, et al. Sinus tarsi approach versus the extended lateral approach for displaced intra-articular calcaneal fractures: a systematic review and meta-analysis. Arch Orthop Trauma Surg. 2021;141(10):1659-1667. doi: 10.1007/s00402-020-03554-9.
56. Ma D, Huang L, Liu B, Liu Z, Xu X, Liu J, et al. Efficacy of sinus tarsal approach compared with conventional L-shaped lateral approach in the treatment of calcaneal fractures: a meta-analysis. Front Surg. 2021;7:602053. doi: 10.3389/fsurg.2020.602053.

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CASE STUDY

Tooth extraction with immediate implantation and immediate loading

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ABSTRACT

Introduction. Immediate loading of dental implants is an evolving discipline requiring validation through clinical and statistical analyses. This study presents a case of immediate implantation and loading to evaluate predictability and outcomes. Immediate restoration reduces treatment time, promotes rapid aesthetic recovery, and addresses patient expectations for functional rehabilitation. Success in such cases relies heavily on maintaining primary stability and avoiding micromovements during osseointegration.

Materials and methods. A 47-year-old patient underwent extraction of teeth 11 and 13, followed by immediate post-extraction implantation. Implants were loaded with provisional restorations within 48 hours. Statistical analysis included torque measurements, Periotest values, and aesthetic evaluations. Comparative data were reviewed against existing literature to assess clinical significance.

Results. Primary stability was achieved with insertion torque of 50 Ncm and Periotest values of -5 and -6. Literature indicates success rates for immediate loading between 94-98%, and this case corroborated these findings with stable and aesthetic results. Surveys revealed 85% satisfaction with comfort and appearance, and the gingival profile remained stable post-treatment.

Conclusions. Immediate loading is a predictable and effective method when conditions for atraumatic extraction, implant stability, and soft tissue management are met. The study reinforces the viability of this approach in improving patient outcomes and minimizing recovery periods while maintaining aesthetics.

Keywords: immediate loading, dental implants, atraumatic extraction, provisional restoration, implant stability, gingival contour, primary stability, patient satisfaction.

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Key messages

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

Immediate loading of dental implants is gaining popularity due to reduced treatment time and improved patient comfort. However, the success and predictability of immediate loading protocols require further analysis, particularly in cases of compromised alveolar bone or soft tissue defects. This article aims to address the gap by providing detailed case study evidence supported by statistical support.

The research hypothesis

Immediate post-extraction implantation with immediate loading can yield predictable outcomes when appropriate conditions are met. This includes high primary stability, proper patient selection, and meticulous surgical protocols.

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

This study provides statistical evidence supporting the predictability of immediate loading under stringent clinical protocols. It emphasizes the importance of atraumatic techniques, proper implant positioning, and soft tissue preservation on both aesthetic and functional outcomes.

Introduction

Endoosseous dental implants are a reliable method for rehabilitating edentulous areas. The Branemark protocol, a standard insertion method, involves delayed loading after a 4–6-month osseointegration period [1]. High patient demands for faster and aesthetically superior outcomes have led to the development of immediate loading protocols.

Immediate loading offers faster aesthetic and functional results. According to studies, immediate loading prostheses have success rates ranging from 94% to 98%. Such outcomes depend heavily on patient-specific factors such as bone density, oral hygiene, and professional expertise [1-4].

The need for immediate aesthetic restoration is particularly critical in anterior zones. Research has shown that atraumatic extraction techniques preserve the alveolar ridge, minimizing bone resorption and supporting gingival contour stability. Proper implant positioning and achieving primary stability with torque values exceeding 35 Ncm are critical to avoiding micromovements that could jeopardize osseointegration [5, 6].

Additionally, the type of provisional restoration used plays a significant role in preserving the gingival architecture and ensuring occlusal harmony. Biomechanical studies emphasize the importance of limiting occlusal forces during the healing period to avoid complications. This study demonstrates the effectiveness of immediate post-extraction implantation and loading, supported by atraumatic extraction techniques and precise implant placement, ensuring both functional and aesthetic outcomes [5, 7-9].

Clinical case presentation

A 47-year-old patient presented with compromised teeth (11 and 13). Clinical examination revealed a mobile metal-ceramic bridge with vestibulo-oral mobility and sensitivity upon palpation and percussion. Radiographic analysis confirmed periapical pathology and significant bone loss, necessitating extraction (Fig. 1). Patient consent was obtained, and all procedures adhered to ethical guidelines.

Procedure

Atraumatic extraction of teeth 11 and 13 was performed to preserve the alveolar ridge. The post-extraction sockets were thoroughly curetted to remove pathological tissue and irrigated with antiseptic solutions (Fig. 2a).

Immediate post-extraction implants (diameter: 4 mm, lengths: 12 mm and 14 mm) were placed. The insertion axis was palatalized to ensure optimal engagement with cortical bone (Fig. 2b).

Under-drilling protocols were employed to enhance primary stability. The integrity of alveolar walls was carefully evaluated, with particular attention to the vestibular plate.

Measurements

Implant stability was assessed using a torque wrench, with insertion forces measured at 50 Ncm.

Periotestometry was performed, yielding values of -5 (mesial implant) and -6 (distal implant), indicative of excellent primary stability.

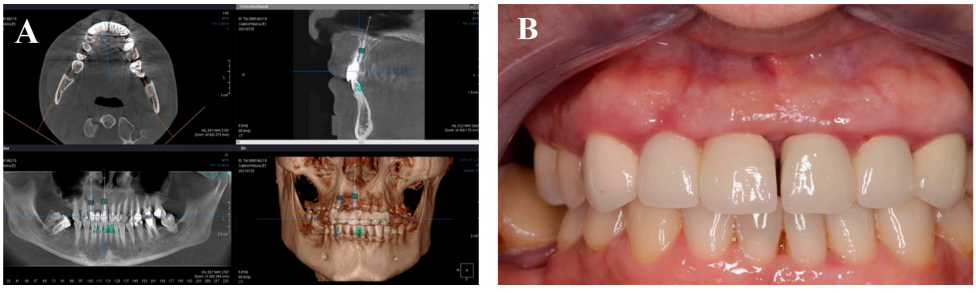

Fig. 1 Preoperative appearance.
A. Tomography analysis, and digital surgery planning.
B. Intraoral aspect of the prosthetic work.

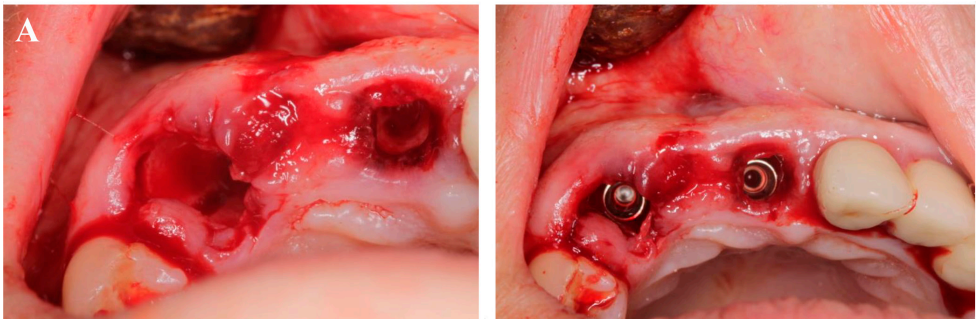

Fig. 2 Post-extraction socket.
A. Evaluation of post-extraction socket, and bone wall integrity
B. Implant placement palatal, for better soft tissue result

Restoration

Provisional acrylic crowns were fabricated and loaded within 48 hours. These restorations were designed to avoid occlusal contact during mastication, preventing undue stress on the implants (Fig. 3b).

Soft tissue modeling was facilitated by the provisional crowns, ensuring optimal gingival contour preservation (Fig. 4a).

Data collection included quantitative measurements (torque, Periotest values) and qualitative assessments (patient-reported outcome measures focusing on comfort, aesthetics, and functionality).

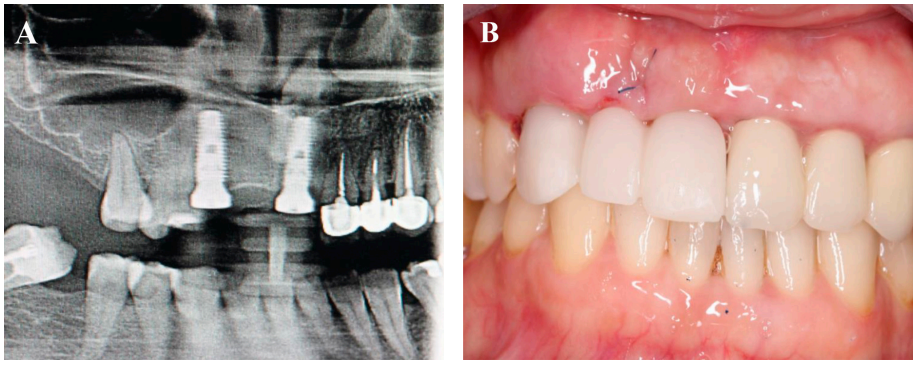

Fig. 3 After surgery photo.
A. Radiological evaluation after implant placement.
B. Fixation of immediate provisional crowns.

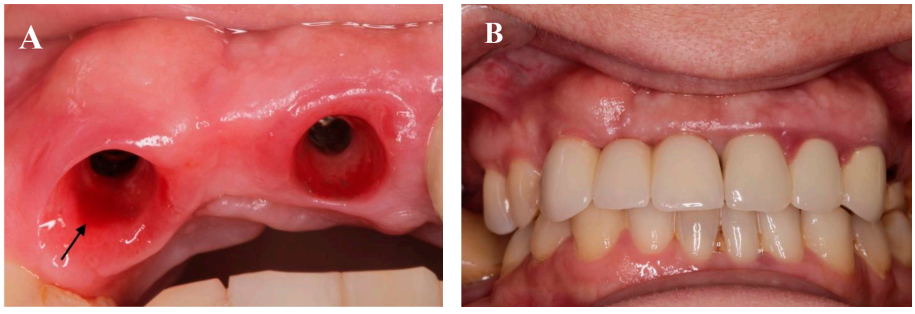

Fig. 4 Implant evaluation after osseointegration period.
A. Soft tissue appearance and bleeding evaluation by Mombelli after osseointegration.
B. Fixation of final work, and emergency profile evaluation.

Results

-Primary Stability:

Insertion torque exceeded the recommended threshold of 35 Ncm, ensuring immediate loading feasibility.

Periotest values of -5 and -6 corroborated excellent primary stability and osseointegration potential.

- Aesthetic and Functional Outcomes:

Gingival contours were preserved throughout the treatment period, with no signs of soft tissue recession or inflammation.

Provisional restorations provided satisfactory aesthetics, meeting patient expectations for anterior zone rehabilitation.

-Patient Satisfaction:

Post-treatment surveys indicated 85% satisfaction with comfort and appearance. The remaining 15% expressed mild concerns regarding initial adaptation to the provisional restorations, which were resolved within two weeks.

Literature reviews supported these findings, with success rates for immediate loading reported at 94-98%. Biomechanical studies emphasized maintaining occlusal forces below 150 Newtons to prevent micromovements during healing. Follow-up evaluations at 12 months confirmed implant stability, with repeated Periotest measurements remaining consistent.

According to postextraction implantation requirements, the implants were applied subcortically, to have enough space for the soft tissues. After the healing period, according to the measurements on the radiological image, we observed an insignificant bone remodeling with bone loss of 0.8 mm from the mesial and 0.7 mm from the distal. The platform of the implants being below the crestal level anyway, which demonstrates the effectiveness of the treatment method.

Discussion

Immediate loading minimizes treatment time while maintaining patient comfort and aesthetics. Atraumatic extraction techniques are pivotal in preserving alveolar bone integrity, particularly the vestibular plate, which is essential for achieving optimal aesthetic results. Temporary crowns play a crucial role in maintaining gingival contours and facilitating soft tissue adaptation [9].

The case study emphasizes the importance of achieving primary stability and avoiding lateral occlusal forces during the healing period. Statistical data support the protocol's predictability, aligning with broader findings in contemporary literature [3, 6]. Challenges, such as patient-specific anatomical variability and bone density, underscore the need for tailored approaches.

Future research should explore advanced implant materials, surface modifications, and digital planning techniques to enhance outcomes further. The integration of these advancements could address current limitations and improve the predictability of immediate loading protocols.

Conclusions

Immediate loading of dental implants is a reliable and effective treatment method that reduces treatment time, ensures psychological comfort, and delivers stable, long-term results. Adherence to atraumatic techniques, precise implant placement, and meticulous soft tissue management is essential for success. This case study validates the protocol's predictability and highlights its role in advancing patient care.

Competing interests

None declared.

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Ethics approval

Not needed for this study.

Informed consent for publication

Obtained.

Provenance and peer review

Not commissioned, externally peer review.

References

1. Albrektsson T, Branemark PI, Hansson HA, Lindström J. Osseointegrated titanium implants. Requirements for ensuring long-lasting direct bone-to-implant anchorage in humans. Acta Orthop Scand. 1981;52(2):155-170. doi: 10.3109/17453678108991776.
2. Barzilay I. Immediate implants: their current status. Int J Prosthodont. 1993;6(2):169-175.
3. Chele N. Implantarea imediată: riscuri şi beneficii. Studiu preliminar [Immediate implantation: risks and benefits. Preliminary study]. Medicina Stomatologică. 2014;(3):56-64. Romanian.
4. Chele N. Factorii determinanţi ai osteointegrării în implantologie [Osseointegation deteminant factors in implantology]. Medicina Stomatologică. 2015;(3):29-32. Romanian.
5. Chele N, Motelica G, Zănoagă O, Slabari E. Extracţia dentară – tehnici, accidente şi complicaţii [Tooth extraction – techniques, accidents, and complications]. Chişinău: [s. n.]; 2022. 144 p. Romanian.
6. Chele N, Topalo V, Sîrbu D. Instalarea imediată ghidată a implantelor dentare de stadiul doi [Guided immediate installation for stage two dental implants]. Medicina Stomatologică. 2015;(2):33-38. Romanian.
7. Mostovei A. [Evaluation of the integration of endosseous dental implants installed in one session through flapless surgery] [dissertation]. Chişinău: Nicolae Testemitanu State University of Medicine and Pharmacy; 2014. 141 p. Romanian.
8. Mostovei A. Formarea spaţiului biologic periimplantar în tehnica *flapless* în dependenţă de tipul mucoasei [The biological width formation related to flapless technique and gingival biotype]. Bul Acad Sci Mold. 2013;(3):180-184. Romanian.
9. Mostovei A, Topalo V, Chele N, et al. One-step vs. two-step e*arly flap*less placement of two-stage dental implants. Clin Oral Implant Res. 2013;24(Suppl 9):139-140.

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ANNIVERSARY

Professor Nicolae Fruntaşu at 90: A Life in Service of Alma Mater

Medicine is a fascinating field that demands sacrifices. Those who are passionate about this profession devote their entire lives to studying for the benefit of human health and conducting profound research, so that later their scientific contributions may be reflected in innovation and medical practice. One such individual is Professor Nicolae Fruntaşu, who turns 90 and who, alongside his teaching career, has dedicated himself to morphological research, an area in which he has distinguished himself as a renowned specialist.

Nicolae Fruntaşu was born on January 13, 1935, in Cuhureştii de Sus, Soroca County, Romania, into a family of farmers for whom hard work was paramount.

In 1942, he began his primary education, and in 1949, he graduated from the local General Education School. That same year, he was admitted to the Medical College in Soroca. In 1952, Nicolae Fruntaşu enrolled at the State Medical Institute in Chişinău.

After graduating in 1958, Nicolae Fruntaşu was assigned to Ciutuleşti, Floreşti district, where he began working as chief physician. Medical services were provided to six villages within a radius of 15–16 km, serving a population of approximately 10,000 residents. He then worked for three years as a district physician, a period that became a true "university of life" for him.

Gaining clinical experience, Nicolae Fruntaşu felt ready to embrace teaching. Thus, in 1961, he was hired as an assistant professor in the Department of Human Anatomy. His first steps in morphology were guided by Professor Boris Perlin, an experienced specialist, erudite scholar, and dedicated mentor, from whom he learned the techniques and methods of teaching the discipline.

A year later, he was offered the opportunity to research "The Innervation of the Human Knee Joint". He pursued the topic with passion and diligence, and within a year and a few months, he presented his research project to his supervisor. In 1964, he successfully defended his doctoral dissertation in medical sciences. That same year, he was transferred to the Department of Topographic Anatomy and Operative Surgery as an assistant professor, and three years later, he earned the title of Associate Professor. In 1967, he was appointed head of the Topographic Anatomy and Operative Surgery Course at the Faculty of Continuous Medical Education. Starting in the 1970s, Professor Fruntaşu shared his knowledge of human anatomy with colleagues from the department, as well as with those from clinical departments, dedicating himself to the continuous education of medical professionals for the next 41 years.

Professor Nicolae Fruntaşu's methodological and teaching activities included developing curricula and thematic plans for continuing medical education courses. Introduced in the 1990s based on the Russian school model, these courses covered various medical fields: surgery, traumatology, otorhinolaryngology, neurosurgery, urology, anesthesiology, dentistry, endoscopy, and ultrasonography.

Nicolae Fruntaşu continuously improved his knowledge and professional competencies. For this purpose, collaborations were established with the Department of Clinical Anatomy at the Central Institute for Medical Advancement in Moscow, allowing faculty and scientific staff to attend continuous medical education development courses, including international ones, every two years.

Upon being transferred to another faculty and at the suggestion of Academician Vasile Anestiadi, Nicolae Fruntaşu decided to change the focus of his research for his habilitation thesis to "The Biomorphosis of the Human Aorta". He employed complex study methods to investigate histochemical and morphometric manifestations in the aortic wall components under the influence of aging factors. In 1994, he was awarded the title of Doctor Habilitated in Medical Sciences, and in June of that year, he received the academic rank of Professor. From 2008 to 2020, he served as a consulting professor.

Professor Nicolae Fruntaşu's scientific activity has resulted in 230 publications, including three monographs, numerous methodological works, five inventions, and 76 innovations. He has supervised three doctoral students and guided the development of several master's theses. He actively participated in numerous congresses, conferences, and symposia, presenting scientific papers and theses.

Beyond his professional responsibilities, Nicolae Fruntașu played an active role in the social life of his institution and country. He led a team of students in establishing a recreational camp in Sergheevka, Ukraine. For seven consecutive years, he was a member of the admissions committee of the State Medical Institute in Chișinău; for five years, he served as the president of the Union Office of the Faculty of General Medicine; for ten years, he was vice-president of the Specialized Scientific Council for Doctor Habilitated Thesis Defense (DH 14.92.06 – theoretical profile); and he participated in organizing central and local government elections 11 times, including nine times as chairman of the electoral commission.

For several decades, Nicolae Fruntașu actively participated in intra- and inter-university sports competitions as a member of faculty teams in basketball, volleyball, and checkers.

Professionally, he stood out for his responsibility, integrity, passion, and dedication, devoting 59 years of his life to Alma Mater and the education of medical specialists. Reflecting on his multifaceted career, he considers his dreams fulfilled, as he accomplished everything he set out to do in teaching, research, and social engagement.

For his outstanding contributions to the development of higher medical education, his substantial role in training highly qualified specialists, and his remarkable methodological, teaching, and scientific activities, in 2020, Professor Nicolae Fruntașu was awarded the honorary title of "Emeritus Person".

On this momentous occasion, on behalf of the entire university community, we express our profound respect and appreciation for Professor Nicolae Fruntașu. We wish him good health, peace, and tranquility, prosperity, and many more joyful moments with his loved ones.

Happy birthday!

Emil Ceban, rector of the *Nicolae Testemițanu* SUMPh
Dr. hab. med. sciences, university professor,
corresponding member of the ASM

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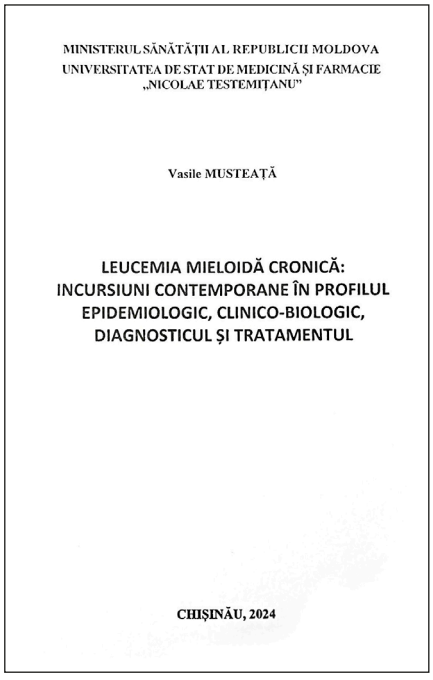
MONOGRAPH REVIEW

„Chronic myeloid leukemia: contemporary insights into the epidemiological, clinical-biological profile, diagnosis and treatment”

Author: Vasile Musteaţă, PhD, associate professor
Discipline of Hematology, *Nicolae Testemiţanu* State University of Medicine and Pharmacy

Monograph details: Musteaţă V. Leucemia mieloidă cronică: incursiuni contemporane în profilul epidemiologic, clinico-biologic, diagnosticul şi tratamentul [Chronic myeloid leukemia: contemporary insights into the epidemiological, clinical-biological profile, diagnosis and treatment]. Chişinău: Medicina, 2024. 167 p. ISBN 978-9975-82-395-1. Romanian.

Chronic myeloid leukemia (CML) is a chronic myeloproliferative neoplasm, which derives from clonal myeloid proliferation because of malignant transformation of hematopoietic stem cells. The median age of the patients is lower than the respective indicator in other chronic malignant myeloproliferations and amounts to 52-53 years, which denotes the predominant involvement of socially active and workable population. Approximately 2.5% of CML cases are attributed to the age group below 20 years and 7.4% to the age group between 20-34 years. That disease represents a complex and actual problem of clinical hematology and public health. The clinical-hematological picture of CML is characterized by splenomegaly or spleno- and hepatomegaly, progressive leukocytosis, constitutional, hypercatabolic symptoms, commonly relapsing evolution, which can also be found in other leukemic processes, raising questions of differential diagnosis. Despite major advances in induction and maintenance therapy, which have led to the increased hematologic and molecular response rates, as well as overall survival, a significant proportion of CML patients relapse and die from the disease progression in the form of acute phase – blast crisis. Regardless of the geographical area, the insidious and latent onset of the disease, the late addressing of the patient, the reduced vigilance and lack of specific competency of physicians of the general network can cause delay in diagnosis and treatment, when the evolution of the tumor process progressively decreases the chances of recovery. Patients diagnosed in the chronic phase, from the low-risk group, have a high potential for recovery. Therefore, detailed training of students, residents, postdoctoral fellows and trainees in the field of this hematologic malignancy is welcome both from a clinical and scientific point of view.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UNIVERSITATEA DE STAT DE MEDICINĂ ȘI FARMACIE
„NICOLAE TESTEMIȚANU”

Vasile MUSTEAȚĂ

LEUCEMIA MIELOIDĂ CRONICĂ:
INCURSIUNI CONTEMPORANE ÎN PROFILUL
EPIDEMIOLOGIC, CLINICO-BIOLOGIC,
DIAGNOSTICUL ȘI TRATAMENTUL

CHIȘINĂU, 2024

That monograph covers the basic topics necessary for students, the medical and academic community for detailed knowledge of epidemiology, etiopathogenesis, establishing the diagnosis of CML, processing the differential diagnosis and selecting personalized treatment tactics.

The monograph is properly structured, presented in a standard manner and composed in literary medical language. The respective manuscript consists of 5 chapters, the synthesis of clinical cases, includes 20 tables and 19 figures. Each chapter comprizes the actuality of the studied theme compartment, the purpose, the research materials and methodology, the author’s research results, the international achievements in the field in the form of discussion – narrative synthesis. In the first chapter, the world literature data on epidemiology, etiopathogenesis, clinical picture, positive diagnosis, differential diagnosis and treatment of CML are presented. Chapter II is dedicated to the contemporary epidemiological and management aspects of CML patients. The monograph describes contemporary diagnostic, management and treatment algorithms and options used

worldwide in clinical hematology. The importance of molecular-cytogenetic investigations in the diagnosis and monitoring of treatment results, as well as the advantages of personalized treatment with tyrosine kinase inhibitors (TKI), are demonstrated. Low- and middle-income countries have been found to bear a considerable burden of CML, which is mainly driven by their limited access to targeted TKI therapy. Globally the burden of CML remains relatively stable due to population growth in developing countries and population aging in developed countries. Chapter III reflects clinical-biological patterns and therapeutic-management approaches in relapses and refractory forms of CML. The results of the author's postdoctoral research and those at the regional, international level in the field of relapses and resistant forms of CML are presented. The author found that elevated LDH values can suggest CML activity both during diagnosis and relapse. In the present study, T315I and K222R/665A mutations of the BCR-ABL1 gene and elevated LDH values were associated with a higher rate of relapses and resistance to imatinib. TKI medication has been found to significantly improve overall survival rates and ECOG-WHO score of CML patients, regardless of treatment line, as well as in relapsed, refractory forms. Chapter IV highlights the particularities and achievements of the management of patients with CML and deep molecular response. Interruption of TKI treatment may be considered as a promising option in patients with CML in chronic phase (especially in early chronic phase), with long-lasting complete molecular response, with low baseline BCR-ABL1 p210 transcript expression and in cases of absence of the BCR-ABL1 p190 transcript. According to the reviewed literature, once patients have discontinued treatment, they should be asked about withdrawal symptoms or the need for support to manage these symptoms. CML patients with minor molecular relapses can achieve a 2nd complete molecular response after restarting treatment with increased doses or newer generation TKI. The prospective practical importance of the study is to highlight the priority options for CML management by assessing the risk and patterns of molecular relapses in patients with complete molecular response after discontinuation of TKI therapy. Chapter V describes infection of COVID-19 in patients with CML – impediments and managerial options, the results of their treatment. The studies reflected in the monograph indicated the predominance of COVID-19 in CML patients of working age, caused by immunocompromised status and comorbidities. COVID-19 frequently affects unvaccinated CML patients who are male and older than the average age of CML patients. SARS-CoV-2 infection is recorded more frequently in CML patients than in the general population, but less frequently than in other malignant hemopathies, not being influenced by the Sokal score. The last compartment includes clinical cases, which will allow students, residents, PhD students and trainee doctors to evaluate their acquired knowledge. All the goals outlined in the monograph, thus, have been achieved.

I would like to conclude that the monograph **Chronic myeloid leukemia: contemporary insights into the epidemiological, clinical-biological profile, diagnosis and treatment**, represents a mature, actual and finalized scientific-methodical work, with potentially beneficial impact on teaching, curative and research activities within the medical and academic community. It reflects the author experience with long-term activity and special professional training. His own experience and careful narrative study of the monograph allowed him to propose both the scientific opinions and a synthesis of contemporary views on the researched problem.

Valentin Țurea, MD, PhD
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[Revised May, 2023]

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	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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- **nivel scăzut al riscului de sângerare**
- **previne eficient accidentul vascular cerebral, embolia sistemică, tromboza venoasă profundă și trombembolismul pulmonar**

Denumirea comercială a medicamentului: KARDATUXAN 2,5 mg comprimate filmate. KARDATUXAN 10 mg comprimate filmate. KARDATUXAN 15 mg comprimate filmate. KARDATUXAN 20 mg comprimate filmate. **Compoziția calitativă și cantitativă:** KARDATUXAN 2,5 mg: fiecare comprimat filmat conține: rivaroxaban 2,5 mg. KARDATUXAN 10 mg: fiecare comprimat filmat conține: rivaroxaban 10 mg. KARDATUXAN 15 mg: fiecare comprimat filmat conține: rivaroxaban 15 mg. KARDATUXAN 20 mg: fiecare comprimat filmat conține: rivaroxaban 20 mg. **Indicații terapeutice:** KARDATUXAN 2,5 mg: Kardatuxan, administrat concomitent doar cu acid acetilsalicilic (AAS) sau cu AAS plus clopidogrel sau ticlopidină, este indicat pentru prevenirea evenimentelor aterotrombotice la pacienții adulți după un sindrom coronarian acut (SCA) cu valori crescute ale biomarkerilor cardiaci.Kardatuxan, administrat concomitent cu acid acetilsalicilic (AAS), este indicat pentru prevenirea evenimentelor aterotrombotice la pacienții adulți care prezintă boală arterială coronariană (BAC) sau boală arterială periferică simptomatică (BAP) cu risc crescut de evenimente ischemice. KARDATUXAN 10 mg: Prevenirea tromboemboliei venoase (TEV) la pacienții adulți care sunt supuși unei intervenții chirurgicale elective pentru substituția șoldului sau a genunchiului.Tratamentul trombozei venoase profunde (TVP) și al emboliei pulmonare (EP) și prevenirea recurenței TVP și a EP la adulți. KARDATUXAN 15 mg: *Adulți:* Prevenirea accidentului vascular cerebral și a emboliei sistemice la pacienții adulți cu fibrilație atrială non-valvulară cu unul sau mai mulți factori de risc, cum sunt insuficiența cardiacă congestivă, hipertensiunea arterială, vârsta ≥75 ani, diabetul zaharat, accidentul vascular cerebral sau atacul ischemic tranzitoriu în antecedente. Tratamentul trombozei venoase profunde (TVP) și al emboliei pulmonare (EP) și prevenirea recurenței TVP și a EP la adulți. *Copii și adolescenți:*Tratamentul tromboemboliei venoase (TEV) și prevenirea recurenței TEV la copii și adolescenți cu vârsta mai mică de 18 ani și cu greutatea cuprinsă între 30 kg și 50 kg după cel puțin 5 zile de la tratamentul anticoagulant parenteral inițial. KARDATUXAN 20 mg: *Adulți:* Prevenirea accidentului vascular cerebral și a emboliei sistemice la pacienții adulți cu fibrilație atrială non-valvulară cu unul sau mai mulți factori de risc, cum sunt insuficiența cardiacă congestivă, hipertensiunea arterială, vârsta ≥75 ani, diabetul zaharat, accidentul vascular cerebral sau atacul ischemic tranzitoriu în antecedente. Tratamentul trombozei venoase profunde (TVP) și al emboliei pulmonare (EP) și prevenirea recurenței TVP și a EP la adulți. *Copii și adolescenți:* Tratamentul tromboemboliei venoase (TEV) și prevenirea recurenței TEV la copii și adolescenți cu vârsta mai mică de 18 ani și cu greutatea peste 50 kg după cel puțin 5 zile de la tratamentul anticoagulant parenteral inițial. **Contraindicații:** KARDATUXAN 2,5 mg, 10 mg, 15 mg, 20 mg: Hipersensibilitate la substanța activă sau la oricare dintre excipienți; Hemoragie activă, semnificativă din punct de vedere clinic; Leziune sau afecțiune considerată a prezenta un risc semnificativ de sângerare majoră. Aceasta poate include ulcerația gastrointestinală curentă sau recentă, prezența neoplasmelor maligne cu risc crescut de sângerare, leziune recentă la nivelul creierului sau măduvei spinării, intervenție chirurgicală recentă cerebrală, spinală sau oftalmică, hemoragie intracraniană recentă, varice esofagiene cunoscute sau suspectate, malformații arterio-venoase, anevrism vascular sau anormalități vasculare intraspinale sau intracerebrale majore;Tratament concomitent cu orice alte anticoagulante, de exemplu, heparină nefracționată, heparină cu greutate moleculară mică (enoxaparina, dalteparina, etc.), derivate de heparină (fondaparina, etc.), anticoagulante orale (warfarina, dabigatran etexilat, apixaban etc.) exceptând situațiile specifice de schimbare a tratamentului anticoagulant sau când heparina nefracționată este administrată la dozele necesare pentru a menține deschis un cateter venos central sau arterial; Tratament concomitent al SCA cu terapie antiplachetară la pacienții cu accident vascular cerebral anterior sau accident ischemic tranzitoriu (AIT); Tratament concomitent al BAC/BAP cu AAS la pacienții cu accident vascular hemoragic sau lacunar în antecedente sau cu orice accident vascular cerebral în interval de o lună; Afecțiune hepatică asociată cu coagulopatie și risc hemoragic relevant din punct de vedere clinic, incluzând pacienții cu ciroză clasele B și C ale sistemului de clasificare Child Pugh; Sarcina și alăptarea.**Atenționări și precauții speciale pentru utilizare:** KARDATUXAN 2,5 mg: La pacienții cu SCA, eficacitatea și siguranța rivaroxaban 2,5 mg de două ori pe zi au fost investigate în administrare concomitentă cu medicamentele antiplachetare AAS în monoterapie sau AAS plus clopidogrel/ticlopidină.La pacienții cu BAC/BAP cu risc crescut de evenimente ischemice, eficacitatea și siguranța rivaroxaban 2,5 mg de două ori pe zi au fost investigate în asociere cu AAS. La pacienți după procedura recentă de revascularizare a membrului inferior din cauza BAP simptomatice, eficacitatea și siguranța rivaroxaban 2,5 mg de două ori pe zi au fost investigate în asociere cu medicamentul antiplachetar AAS în monoterapie sau AAS plus clopidogrel pe termen scurt. Dacă este necesar, tratamentul combinat antiplachetar cu clopidogrel trebuie să fie de scurtă durată; tratamentul combinat antiplachetar pe termen lung trebuie evitat. KARDATUXAN 10 mg, 15 mg, 20 mg: Se recomandă supravegherea din punct de vedere clinic pe toată durata perioadei de tratament. Risc hemoragic: pacienții trebuie monitorizați cu atenție pentru semnele de sângerare. Se recomandă să fie utilizat cu precauție în condiții cu risc crescut de hemoragie. Administrarea Kardatuxan trebuie întreruptă dacă apare hemoragie severă. Alți factori de risc hemoragic: rivaroxaban nu este recomandat la pacienți cu risc crescut de sângerare. Pacienți cu proteze valvulare:Rivaroxaban nu trebuie utilizat pentru tromboprofilaxie la pacienții care au trecut recent printr-o înlocuire percutanată de valvă aortică (TAVI).Pacienți cu sindrom antifosfolipidic: Rivaroxaban nu sunt recomandate la pacienții cu antecedente de tromboză, care sunt diagnosticați cu sindrom antifosfolipidic.Pacienți cu EP instabili hemodinamic sau pacienți care necesită tromboliză sau embolectomie pulmonară: Kardatuxan nu este recomandat ca alternativă la heparina nefracționată la pacienții cu embolie pulmonară care sunt instabili hemodinamic sau care pot beneficia de tromboliză sau embolectomie pulmonară, deoarece siguranța și eficacitatea rivaroxaban nu au fost stabilite în aceste situații clinice. KARDATUXAN 10 mg: Recomandări privind dozele înainte și după proceduri invazive și intervenții chirurgicale, altele decât intervențiile chirurgicale elective pentru substituția șoldului sau a genunchiului: Dacă este necesară o procedură invazivă sau o intervenție chirurgicală, trebuie oprită administrarea Kardatuxan 10 mg cu cel puțin 24 ore înainte de intervenție, dacă este posibil, precum și în funcție de opinia clinică a medicului. Dacă procedura nu poate fi amânată, trebuie evaluat riscul de sângerare comparativ cu gradul de urgență al intervenției. KARDATUXAN 15 mg, 20 mg: Recomandări privind dozele înainte și după proceduri invazive și intervenții chirurgicale: Dacă este necesară o procedură invazivă sau o intervenție chirurgicală, trebuie oprită administrarea Kardatuxan 15 mg sau Kardatuxan 20 mg cu cel puțin 24 ore înainte de intervenție, dacă este posibil, precum și în funcție de opinia clinică a medicului. Dacă procedura nu poate fi amânată, trebuie evaluat riscul de sângerare comparativ cu gradul de urgență al intervenției. **Reacții adverse:** KARDATUXAN 2,5 mg, 10 mg, 15 mg, 20 mg: Reacțiile adverse raportate cel mai frecvent la pacienții la care se administrează rivaroxaban au fost hemoragiile. Hemoragiile raportate cel mai frecvent au fost epistaxisul (4,5%) și hemoragia de tract gastrointestinal (3,8%). Frecvente (≥1/100 și <1/10): Anemie (inclusiv parametri de laborator corespunzători); Amețeli, cefalee; Hemoragie oculară (inclusiv hemoragie conjunctivală); Hipotensiune arterială, hematom; Epistaxis, hemoptizie; Sângerare gingivală, hemoragie la nivelul tractului gastrointestinal (inclusiv hemoragie rectală), dureri gastrointestinale și abdominale, dispepsie, greață, constipație, diaree, vărsături; Creștere a valorilor serice ale transaminazelor; Prurit (inclusiv cazuri mai puțin frecvente de prurit generalizat), erupție cutanată tranzitorie, echimoză, hemoragie cutanată și subcutanată; Dureri la nivelul extremităților; Hemoragie la nivelul tractului urogenital (inclusiv hematurie și menoragie), insuficiență renală (inclusiv creșterea creatininei serice, creșterea ureei serice); Febră, edem periferic, scădere a tonusului general și a energiei (inclusiv fatigabilitate și astenie); Hemoragie după o procedură (inclusiv anemie postoperatorie și hemoragie la nivelul plăgii), contuzie, secreții la nivelul plăgii. **Data și numărul autorizației de punere pe piață:** 2,5 mg - Nr.29430; 10 mg -Nr. 29431; 15 mg - Nr.29432; 20 mg - Nr.29433 din 30.11.2023. **Statutul legal:** cu prescripție medicală. **Data revizuirii textului:** Aprilie 2024. *Acest material publicitar este destinat persoanelor calificate să prescrie, să distribuie și/sau să elibereze medicamente. Pentru informații complete vă rugăm să consultați rezumatul caracteristicilor produsului. Informații detaliate privind acest medicament sunt disponibile pe site-ul Agenției Medicamentului și Dispozitivelor Medicale (AMDM) http://nomenclator.amdm.gov.md/*

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