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CONTENT HIGHLIGHTS:

Sergei I. Syrbu, Michael B. Cohen
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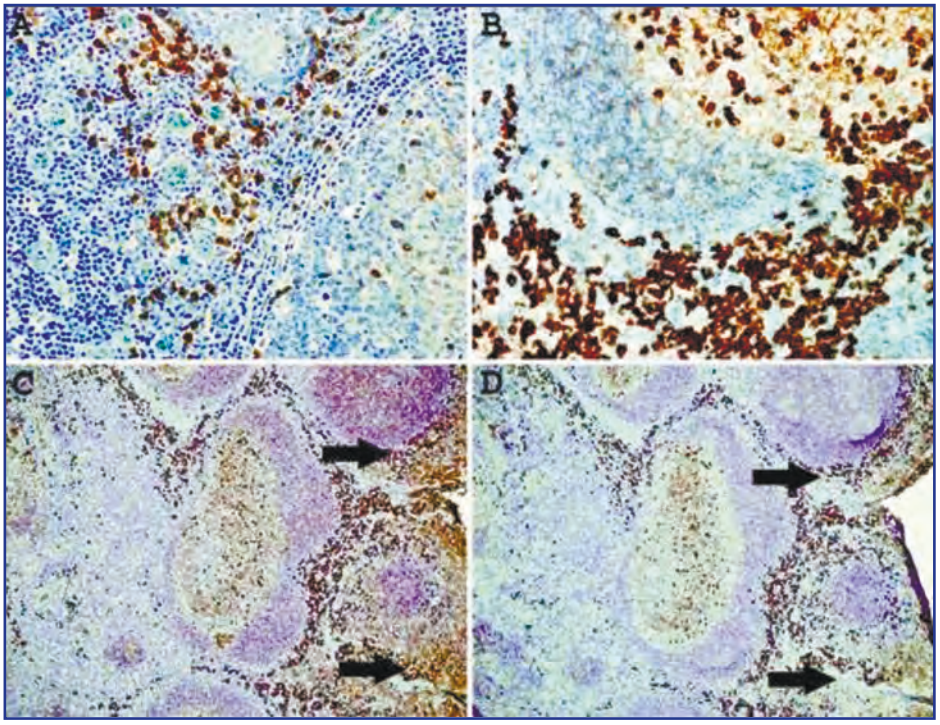

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Mod de administrare: Pentru uz oral, poate fi luat indiferent de mese. **Contraindicații:** Hipersensibilitate la lisinopril sau la orice alt inhibitor al enzimei de conversie a angiotensinei (IECA).Hipersensibilitate la indapamidă sau la orice alți derivați de sulfonamide.Hipersensibilitate la oricare dintre excipienții.Antecedente de angioedem, inclusiv antecedente de angioedem asociat cu terapia anterioară cu inhibitori ai ECA.Angioedem ereditar sau idiopatic.Insuficiență renală severă (clearance-ul creatininei < 30 ml/min).Encefalopatie hepatică sau tulburări severe ale funcției hepatice.Hipokaliemie. Utilizarea concomitentă a Diroton Plus cu produse care conțin aliskiren este contraindicată la pacienții cu diabet zaharat sau insuficiență renală ușoară sau severă (GFR<60 ml/min/1,73 m2).Utilizarea concomitentă cu antagoniști ai receptorilor de angiotensină II (ARAII) la pacienții cu nefropatie diabetică.Utilizare concomitentă cu inhibitori neutri de endopeptidază (de exemplu, produse care conțin sacubitril) din cauza riscului ridicat de angioedem.Sarcina si alaptarea.Copii și adolescenți cu vârsta sub 18 ani (eficacitatea și siguranța nu sunt stabilite).Intoleranță la lactoză, galactozemie, deficit de lactază, malabsorbție la glucoză-galactoză. **Atenționări și precauții speciale pentru utilizare:** Stenoză de aortă, cardiomiopatie obstructivă hipertrofică, boli cerebrovasculare (inclusiv insuficiență circulatorie cerebrală), boală cardiacă ischemică, insuficiență coronariană, boli sistemice autoimune severe ale țesutului conjunctiv (inclusiv lupus eritematos sistemic, sclerodermie), mielosupresie, diabet zaharat, hiperkalemie, stenoză bilaterală a arterelor renale, stenoză a unei singure artere renale, afecțiune după transplant renal, insuficiență renală, azotemie, aldosteronism primar, dietă cu limitare de sare, afecțiuni asociate cu scăderea volumului sanguin circulant (inclusiv vărsături și diaree), pacienți vârstnici, insuficiență hepatică. Pacienți slăbiți sau pacienți aflați în terapie combinată cu alte antiaritmice și medicamente cunoscute ce cauzează prelungirea intervalului QT, precum și aritmie torsada vârfurilor, utilizare concomitentă cu produse care conțin aliskiren sau antagoniști ai receptorilor de angiotensină II (ARA II) (risc crescut de hipotensiune arterială, hiperkaliemie și insuficiență renală în blocarea dual a RAAS)", dezechilibru hidric și electrolitic, pacienți cu interval QT prelungit pe ECG; hemodializa cu membrane cu permeabilitate mare sau desentizare, inainte de afereza LDL concentratie mare de acid uric, hiperparatiroidism, la pacienții negri, la sportivi (control doping pozitiv). **Reacții adverse:** Rezumatul profilului de siguranță: Frecvența reacțiilor adverse este dată separat pentru lisinopril și indapamidă.Următoarele reacții adverse la medicamente (RAM) au fost raportate în timpul tratamentului cu lisinopril și indapamidă independent: *Efectele secundare ale lisinoprilului:* Cele mai frecvente efecte secundare sunt amețeli, dureri de cap, oboseală, diaree, tuse uscată și greață.Frecvente (≥1/100 până la <1/10): amețeli, cefalee, scădere severă a TA, efecte ortostatice, tuse, diaree, vărsături, disfuncție renală. *Legate de indapamidă:* Cele mai frecvente reacții adverse au fost: reacții de hipersensibilitate, în principal reacții cutanate la pacienții cu predispoziție la reacții alergice și astmatice și erupții cutanate maculopapulare. Majoritatea reacțiilor adverse (descoperiri de laborator și clinice) sunt dependente de doză. Frecvente (≥1/100 până la <1/10): hipersensibilitate, erupții cutanate maculopapulare. **Data și numărul autorizației de punere pe piață:** Nr.29135; Nr. 29134; Nr.29133 din 24.06.2023. **Statutul legal:** cu prescripție medicală. **Data revizuirii textului:** Iunie 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/*

***Referințe:* 1.** *Gorokhovskaya G et al. Current perspectives on fixed-dose ACE inhibitor diuretic combination therapy in the management of arterial hypertension. Медицинский совет. 2019;(16):10–18.* **2.** *https://nomenclator.amdm.gov.md/.* **3.** *RCP Diroton Plus 24.06.2023.*

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RESEARCH ARTICLE

Monoclonal antibodies to human kappa and lambda light chains suitable for staining of formalin-fixed, paraffin-embedded tissue

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ABSTRACT

Introduction. The hallmark of most B-cell neoplasia is proliferation of B-cells and clonal rearrangement of the immunoglobulin gene, which is lambda or kappa light chain restricted. Since fresh tissue is not always available to determine clonality, immunohistochemical (IHC) staining for kappa and lambda light chains is often performed on formalin-fixed, paraffin-embedded (FFPE) tissue. Commercially available polyclonal antibodies typically produce high background staining and have a significant false negative rate, which makes it difficult to prove clonality. We developed a series of monoclonal antibodies to human kappa and lambda light chain constant regions by immunizing mice with purified kappa and lambda immunoglobulin light chains.

Materials and methods. Screening of the hybridoma populations and clone selection was performed in two steps. Primary screening was done by ELISA in plates coated with purified human kappa and lambda light chains fixed with 10% formalin. The positive populations were then selected by IHC staining of FFPE containing B-cell neoplasms with known immunophenotype.

Results. We selected eight hybridomas that produced highly specific monoclonal antibodies (mAbs) directed to the constant regions of kappa (four clones) and lambda light chains (four clones). The antibodies were used for IHC staining and direct immunofluorescence microscopy on FFPE tissues. Plasma cell neoplasms could be stained without antigen retrieval. For uniform and reproducible staining of diffuse large B-cell lymphomas and some marginal zone B-cell lymphomas with plasmacytoid differentiation, a heat-based antigen retrieval procedure was necessary. Compared with commercial polyclonal antibodies to kappa and lambda light chains these mAbs demonstrated lower background staining and produced fewer false negative results.

Keywords: monoclonal antibodies, kappa and lambda immunoglobulin light chains, immunohistochemical staining, formalin-fixed, paraffin-embedded tissue.

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Key messages

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

For the first time we used an innovative approach to produce and select novel monoclonal antibodies to human kappa and lambda light chains suitable for immunohistochemical staining and direct immunofluorescent microscopy on formalin-fixed, paraffin embedded tissues. Main principal - the hybridoma initial screening by ELISA was performed in wells coated with kappa or lambda light chains and fixed with 10% formalin. Positive clones were selected by IHC staining of tissue microarray from FFPE tissue blocks.

The research hypothesis

By fixing the antigen used for mice immunization in ELISA plates with 10% formalin can prove useful to select monoclonal antibod-

ies which recognize the antigenic epitopes either unaffected or minimally affected by cross-linkage of formaldehyde groups.

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

This novel approach may prove useful in production of monoclonal antibodies to a variety of hematopoietic and non-hematopoietic antigens for research or diagnostic/immunophenotyping purposes.

Introduction

The majority of plasma cell neoplasms and B-cell lymphoproliferative processes are characterized by clonal immunoglobulin gene rearrangement and expression of cytoplasmic or surface immunoglobulin with kappa or lambda light chain restriction.

Heavy immunoglobulin gene rearrangement can be demonstrated by polymerase chain reaction on formalin-fixed, paraffin-embedded (FFPE) tissues although the procedure is time-consuming, expensive, and has a variable (65-73%) sensitivity [1, 2]. An alternative way to prove clonality is immunological detection of light chain restriction, which is largely performed on cells in suspension by flow cytometric immunophenotyping or direct immunofluorescence microscopy (DIFM) on frozen tissues. Since fresh tissue is not always available, immunohistochemical (IHC) staining for kappa and lambda light chains is often performed on FFPE tissues [3-6]. Commercially available polyclonal antibodies to human kappa and lambda light chains have high background and false negative rates, which makes it difficult to prove clonality. In addition, polyclonal antibodies and the majority of monoclonal antibodies (mAbs) available on the market require digestion of tissue sections with proteolytic enzymes, which may alter tissue morphology and give false negative results due to protein over-digestion, particularly if specimens are poorly fixed. We report here a series of new mAbs to constant regions of human kappa and lambda light chains suitable for IHC staining and DIFM of FFPE tissues. Epitope enhancement was performed at a constant and uniform temperature in a water bath and does not require the use of proteolytic enzymes [7].

Materials and methods

Monoclonal antibody production

Monoclonal antibodies directed to the constant region of human immunoglobulin kappa and lambda light chains were produced by immunization of BALB/c mice with purified human immunoglobulin light chains (Bence Jones protein provided by Dr. A. Solomon from University of Tennessee, Knoxville, and from Bethyl Laboratories, Inc., Montgomery, TX) in the presence or absence of CpG oligodeoxynucleotide 1826 (InvivoGen, San Diego, CA). After final boost, the spleens were removed and splenocytes were fused with NS1 myeloma cells, as previously described [8]. Primary screening of the hybridoma cell lines was performed by a modified enzyme-linked immunosorbent assay (ELISA). In brief, the 96-well ELISA plates were coated with 0.2 mg/well purified human kappa or lambda light chains fixed with 10% formalin. Hybridoma cell lines secreting antibodies that would react only with free kappa and lambda light chains were then excluded by ELISA using plates coated with purified human IgG (Sigma, St. Louis, MO). Positive hybridoma populations were selected by immunohistochemical staining of FFPE tissue without antigen retrieval. For this purpose, we used tissue microarray constructs containing multiple 3 mm tissue cylinders retrieved from donor blocks of plasma cell neoplasms with known immunophenotype. Hybridoma cell lines staining plasma cell neoplasms on FFPE tissues without antigen retrieval were subcloned and used for production of ascitic fluids in mice.

Antibody purification and labeling

Monoclonal antibodies from ascites were purified by ion exchange chromatography using DEAE Sepharose (Sigma-Aldrich, St. Louis, MO). Purified antibodies were labeled with Alexa Fluor 488 or R-PE by using Alexa Fluor 488 Protein Labeling Kit (Molecular Probes, Eugene, OR) and AnaTag R-Phycoerythrin Protein Labeling Kit (AnaSpec, Inc., San Jose, CA), respectively.

Immunohistochemical staining and direct immunofluorescence microscopy

4-um thick sections of FFPE tissues mounted onto plus slides were deparaffinized in xylene, rehydrated in graded alcohols, and endogenous peroxidase activity was blocked with 1% hydrogen peroxide in distilled water. The water bath (Isotemp 205, Fisher Scientific) containing the staining dish with 250 ml of epitope enhancement buffer was pre-heated to 97°C. The slides were immersed in the staining dish and incubated for 45 minutes. The reduction of high background staining on certain types of tissues ("bloody specimens") was achieved by treating the tissue sections for 10 min with ReBlot Plus mild antibody striping solution (Chemicon, Temecula, CA) immediately after the epitope enhancement procedure. The sections were then washed in buffer solution (DakoCytomation, Carpinteria, CA) for three minutes and incubated with 30 ng/ml mAbs to kappa or lambda light chains for 30 minutes. After washing twice for 5 minutes the bound primary antibodies were detected with EnVision+ System-HRP from DakoCytomation (Carpinteria, CA) with diaminobenzidine chromogen substrate. For DIFM studies, tissue sections after epitope enhancement were incubated for 30 minutes with 70 ng/ml Alexa Fluor 488 and/or R-PE labeled mAbs to kappa and lambda light chains and examined under fluorescence microscope.

Results and discussion

Production and characterization of mAbs to human immunoglobulin constant- region light chain isotypes. Our first attempt to generate mAbs by immunizing mice with a mixture of kappa and lambda light chains in the pres-

ence of CpG oligodeoxynucleotide 1826 (CpG ODN) as an adjuvant [9] failed to detect hybridoma cell lines secreting antibodies to kappa protein. The great majority of hybridoma populations secreted antibodies to lambda protein. In contrast, when mice were immunized with purified kappa light chains without CpG ODN a large proportion of hybridoma cell lines secreted antibodies to kappa protein. Since the main goal of this study was to generate mAbs to antigenic epitopes of kappa and lambda light chains either unaffected or minimally affected by formalin fixation, we utilized a novel strategy for screening and selection of antibody-producing hybridoma populations.

Primary screening was performed by ELISA in plates coated with kappa and lambda light chains that were fixed with 10% formalin after coating the wells. The rationale for formalin fixation was to select hybridoma populations secreting antibodies to antigenic epitopes either unaffected or minimally affected by cross-linkage of formaldehyde groups. After the first screen, approximately 120 hybridomas were selected from each of the kappa or lambda fusions. The second screen was performed to exclude hybridoma populations that secreted antibodies that would react with variable-region subgroups, free light chains not associated with immunoglobulin molecules, and antibodies cross-reacting with both kappa and lambda proteins. This goal was achieved by testing antibody reactivity with kappa and lambda light chains from patients with different variable subgroups and purified human polyclonal IgG. As a result, 41 hybridoma populations secreting antibodies to kappa (21) and lambda (20) light chains were selected for immunohistochemical staining of FFPE tissues without antigen retrieval.

IHC was performed on sections of tissue microarray constructs containing nine 3 mm tissue cylinders retrieved from donor blocks of plasma cell neoplasms with known immunophenotype. By IHC we selected eight hybridoma populations that produced highly specific mAbs to kappa (4 populations) and lambda light chains (4 populations) for subcloning and ascites production in mice. All of the mouse mAbs were either of IgG1, 2a or 2b isotypes.

Immunohistochemical staining of FFPE tissues

For all IHC and DIFM studies the culture media, the mixture of purified and labeled antibodies to kappa (4 clones) or lambda (4 clones), were diluted to a final concentration of approximately 30 ng/ml (IHC) or 70 ng/ml (DIFM). Staining the sections of FFPE normal tonsil without epitope enhancement detects primarily plasma cells located in interfollicular areas and submucosa (Fig. 1A). When a heat-based epitope enhancement procedure is used, the detection of plasma cells and large B-cells (transformed cells) in interfollicular areas is markedly enhanced (Fig. 1B). Moreover, a subset of large centroblasts located in germinal centers is also detected. The staining of small mature lymphocytes in mantle zones or centrocytes in germinal centers is equivocal, probably due to a low density of B-cell receptors on these cells.

Immunoglobulin proteins are widely present in all tissues and interstitial fluid, which inevitably give high background staining and make the interpretation in some instances very difficult. In the tonsil, immunoglobulins are also present on epithelial cell surfaces and are readily revealed by IHC staining with anti-kappa or anti-lambda antibodies. Since the immunoglobulins present in the interstitial fluid and on epithelial surface are not an integral part of

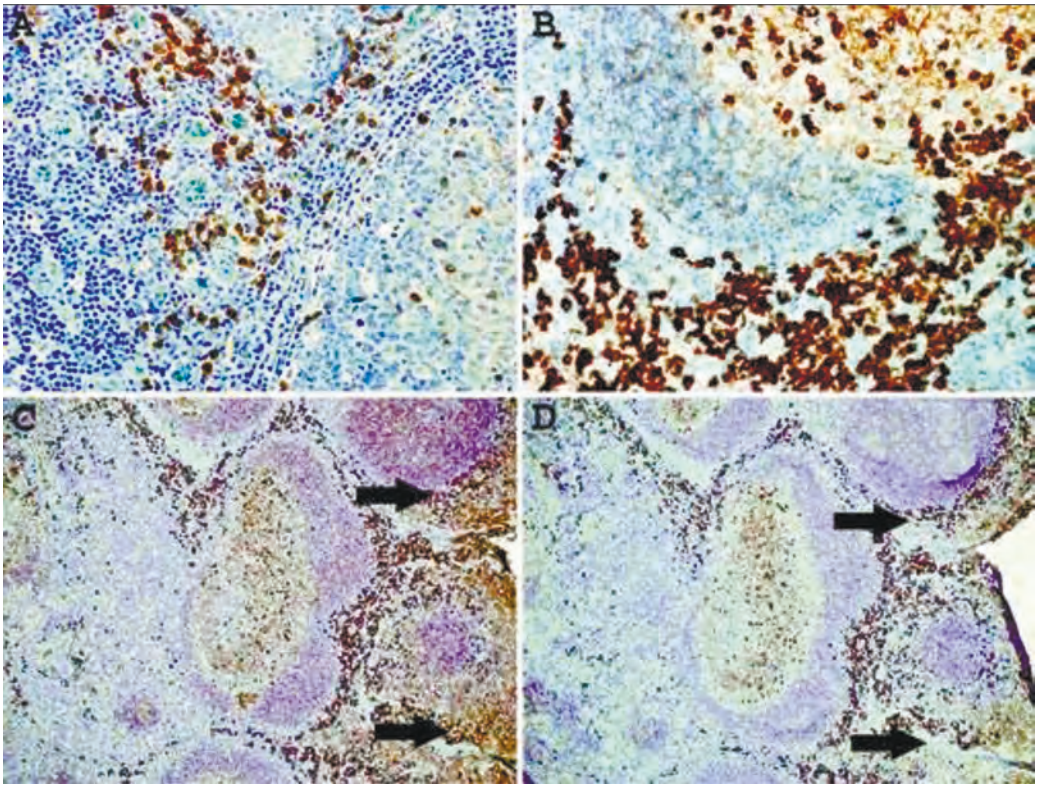

Fig. 1 Immunohistochemical staining of formalin-fixed, paraffin-embedded normal tonsil with a mixture of mAbs to kappa and lambda light chains.
A – no epitope enhancement; B – heat-based epitope enhancement. Original magnification 40X. C - untreated and treated (D) sections of the tonsil for 10 min with ReBlot Plus mild antibody striping solution after epitope enhancement. Arrows indicate high background staining. Note attenuation of the mantle zone staining after striping procedure. Original magnification 10X.

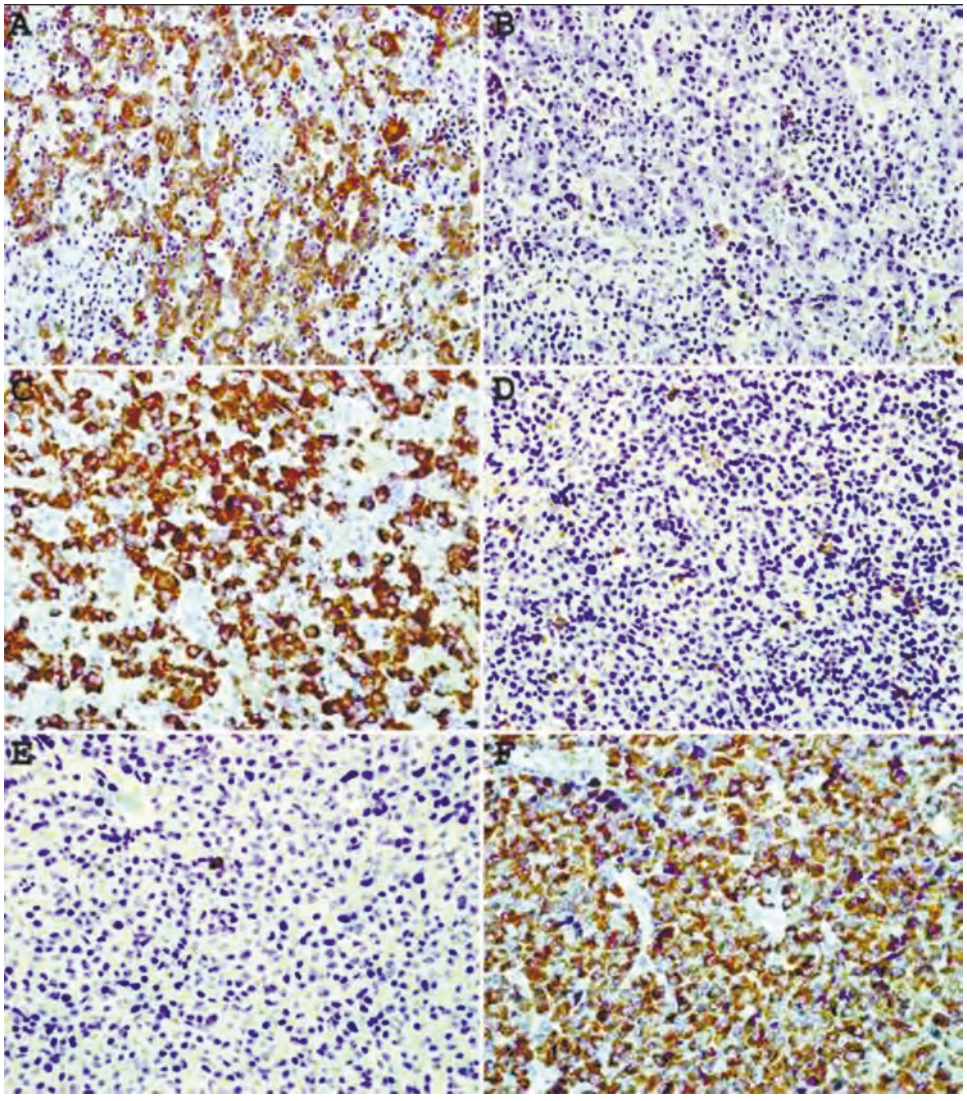

Fig. 2 Immunohistochemical staining of plasma cell neoplasm with mAbs to kappa (A, C, E) and lambda (B, D, F) light chains.
A, B – no epitope enhancement. C-F – heat- based epitope enhancement. Original magnification 40X.

cell membranes, we hypothesized that these proteins may be partially removed by pre-treating the tissue sections with stripping agents. Fig. 1C shows a normal tonsil stained with mAbs to kappa and lambda light chains, which demonstrate high background staining of the mucosal surface. Pretreatment of the section for 10 min with ReBlot Plus mild antibody striping solution significantly reduced the background staining of mucosal lining without affecting the staining of plasma cells and large B-cells (Fig. 1D). However, it appears that this pretreatment attenuates staining of B-cell in the mantle zones.

Staining of plasma cell neoplasms with our mAbs usually does not require epitope enhancement (Fig.2 A, B) although after epitope enhancement the staining is more uniform and intense with minimal increase in background staining (Fig. 2 C-F). Clonality of some B-cell neoplasms, in particular lymphomas composed of large cells with abundant cytoplasm or plasmacytic differentiation can also be readily demonstrated by IHC staining. As can be seen from Fig. 3 A, B, lambda light chain restriction is appreciated in a diffuse large B-cell lymphoma (DLBCL) with plasmablastic features. An example of large B-cell lymphoma with anaplastic features showing lambda light chain restriction is demonstrated in Fig. C, D. A case of angioimmunoblastic T-cell lymphoma associated with monoclonal expansion of large B-cells (immunoblasts) is shown in Fig. 3 E, F. Flow cytometry immunophenotyping on cell suspension from this lymph node stained with our mAbs conjugated with Alexa Fluor 488 and R-PE revealed a kappa light chain restricted lymphoid population with a kappa to lambda ratio of 8:1. Approximately the same kappa to lambda ratio is demonstrated on IHC staining with mAbs (Fig. 3E, F).

Direct immunofluorescence microscopy of FFPE tissues

In a large portion of diffuse large B-cell lymphomas and in a subset of plasma cell neoplasm no immunoglobulin expression can be demonstrated by IHC. This may be due to a high background or lack of surface immunoglobulin on a large subset of diffuse large B-cell lymphomas [10, 11]. We

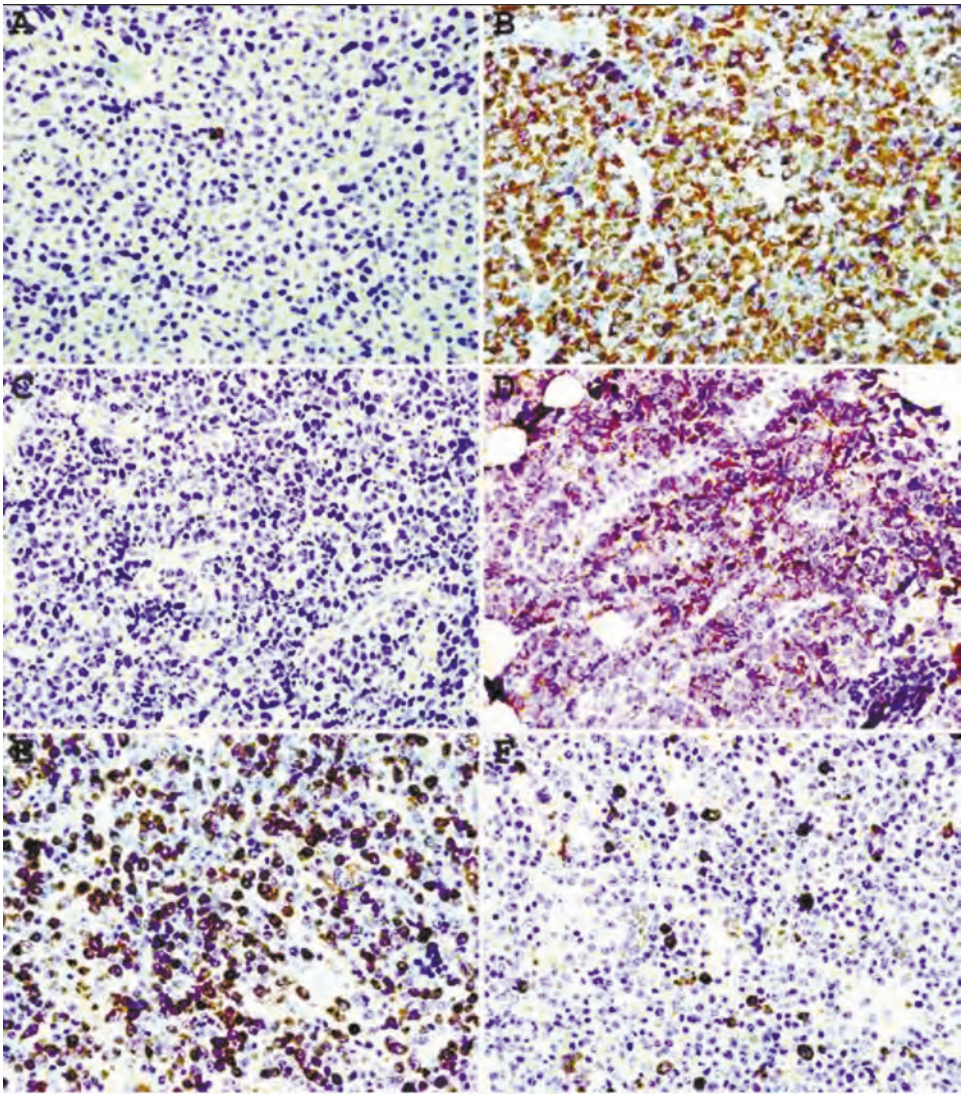

Fig. 3 Immunohistochemical staining with epitope enhancement of a diffuse large B-cell lymphoma with plasmablastic features

(A, B), highly pleomorphic plasma cell myeloma (C, D), and angioimmunoblastic T-cell lymphoma associated with clonal immunoblastic proliferation (E, F). A, C, E – mAbs to kappa light chain; B, D, F – mAbs to lambda light chain. Original magnification 40X.

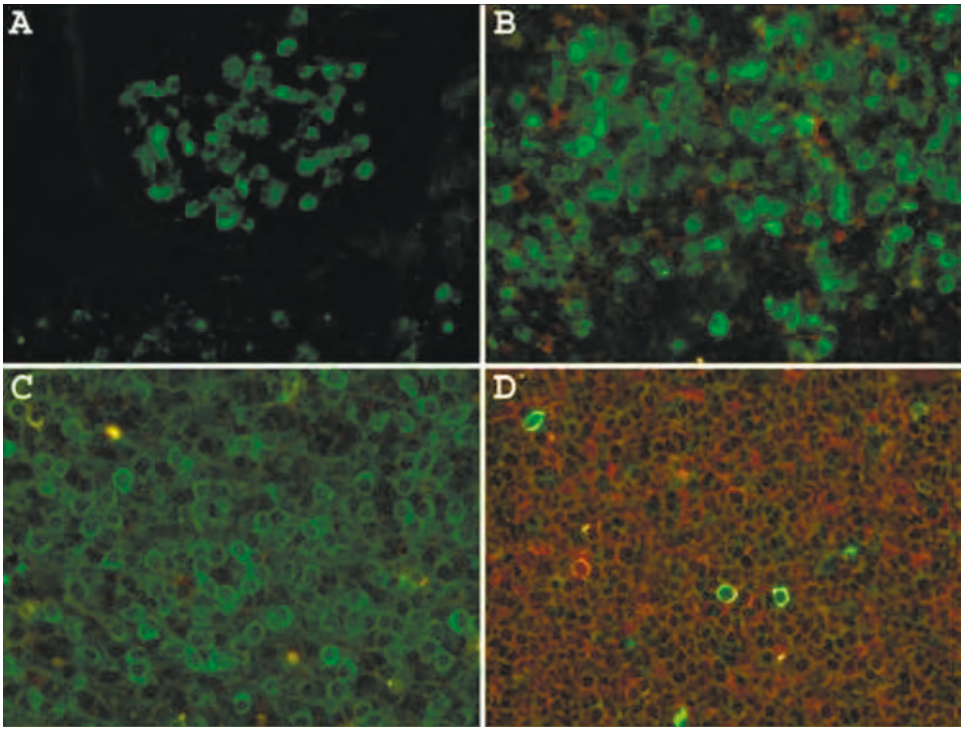

Fig. 4 Direct immunofluorescence microscopy on formalin-fixed, paraffin-embedded tissue using a heat-based epitope enhancement procedure.

Green fluorescence represents mAbs to kappa light chain labeled with Alexa Fluor 488 and in red fluorescence are mAbs to lambda light chain labeled with R-Phycoerythrin.

A. Normal tonsil stained with anti-kappa. B. Normal tonsil stained with anti-kappa and anti-lambda. C. Diffuse large B-cell lymphoma kappa light chain restricted; D. Diffuse large B-cell lymphoma, lambda light chain restricted. Original magnification 60X.

labeled antibodies to kappa and lambda light chains with Alexa Fluor 488 and R-PE, respectively, and used them for DIFM of FFPE tissues.

Staining a normal tonsil with anti-kappa antibodies labeled with Alexa Fluor 488 shows a lower background staining intensity in surface epithelium (Fig. 4A) compared to IHC staining (Fig. 2A). Combining anti-kappa and anti-lambda antibodies clearly illustrates the polyclonal nature of plasma cells and large B-cells in reactive lymphoid hyperplasia on the same section of the tonsil (Fig. 4B). This antibody cocktail was also used to demonstrate kappa (Fig. 4C) or lambda (Fig.4D) light chain restriction in two cases of DLBCL.

Comparison of monoclonal antibodies with commercially available polyclonal antibodies to kappa and lambda light chains

Twenty-two cases of known plasma cell neoplasms were selected from the files of the Department of Pathology, University of Iowa Hospitals and Clinics. All cases were stained at the time of diagnosis with commercial polyclonal antibodies after proteolytic digestion of deparaffinized sections with Protease K. Seven cases were kappa and 5 cases were lambda-restricted, respectively; 4 cases were "indeterminate" or "equivocal"; 6 cases were negative; and one case was very weakly positive for lambda light chain (Table 1). Four cases with equivocal staining had a high background when stained with polyclonal antibodies to kappa and lambda light chains, which made the interpretation very difficult. Interestingly, all equivocal and 5/6 negative cases showed lambda light chain restriction by IHC staining with or without antigen retrieval when our mAbs were utilized. Serum protein immunofixation electrophoresis (SIFE) on 8/9 of these equivocal and negative cases showed the presence of a monoclonal protein, either IgG- (5 cases) or IgA- (3 cases). For case 19 the SIFE results were unknown. Case 12 stained very weakly with both polyclonal and monoclonal antibodies. A case of CD30-positive plasmablastic lymphoma (case 23) was completely negative with polyclonal and monoclonal antibodies to both light chain isotypes. This data suggests that polyclonal antibodies to lambda light chains have a high false negative rate (5 out of 15 cases) compared to anti-kappa antibodies.

In conclusion, our new mAbs have lower false negative rates and lower background staining compared to commercial polyclonal antibodies. In some specimens ("bloody tissues") the high background staining may be significantly reduced by treating the sections with ReBlot Plus mild antibody striping solution. It also appears that DIFM is more sensitive and has a lower background staining compared to IHC and may be used for immunophenotyping of large B-cell neoplasms.

Table 1. Comparative immunohistochemical staining of FFPE tissues of plasma cell neoplasms with commercial polyclonal antibodies and monoclonal antibodies to kappa and lambda light chains

Patients	PA with EE		mAbs with EE		mAbs without EE	
	anti-κ	anti-λ	anti-κ	anti-λ	anti-κ	anti-λ
1	-	+	-	+	ND	ND
2	-	+	-	+	ND	ND
3	-	-	-	+	ND	ND
4	+	-	+	-	ND	ND
5	+	-	+	-	ND	ND
6	+	-	+	-	ND	ND
7	Ind	Ind	-	+	ND	ND
8	-	-	-	+	ND	ND
9	-	-	-	+	ND	ND
10	-	-	-	+	-	+
11	-	-	-	+	-	+
12	-	+/-	-	+/-	-	+/-
13	-	+	-	+	-	+
14	+	-	+	-	ND	ND
15	+	-	+	-	+	-
16	+	-	+	-	+	-
17	-	+	-	+	-	+
18	Ind	Ind	-	+	-	+
19	Ind	Ind	-	+	-	+
20	+	-	+	-	ND	ND
21	Ind	Ind	-	+	-	+
22	-	+	-	+	ND	ND
23	-	-	-	-	ND	ND

Note: FFPE – formalin-fixed, paraffin-embedded, PA – polyclonal antibodies; EE- epitope enhancement; Ind – indeterminate; ND- not done; mAbs – monoclonal antibodies

Competing interests

None declared.

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RESEARCH ARTICLE

Impact of umbilical cord pathology on perinatal outcomes: risk factors and clinical implications

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ABSTRACT

Introduction. Umbilical cord pathology can contribute to neonatal asphyxia, stillbirth, and postnatal death in numerous cases. However, the diagnosis of umbilical cord pathology remains imperfect, and its impact on pregnancy course and outcome is often underestimated. Therefore, prenatal diagnosis of umbilical cord pathology is becoming increasingly important in preventing intra- and postnatal morbidity and mortality. Early detection of these abnormalities enables the development of necessary strategies for optimal pregnancy and delivery management.

Material and methods. The study included 190 patients divided into 2 groups: L_1 – 95 patients with UC abnormalities, and L_0 – 95 with a normal UC. A p-value of less than 0.05 was regarded as statistically significant.

Results. The development of umbilical cord pathology was frequently observed in pregnant women exposed to harmful workplace factors (psychological and emotional stress, p=0.01), harmful habits (smoking, p=0.04), and primiparas (p=0.005) with complicated gynecological and somatic histories, as well as those with a history of UC pathology in previous pregnancies (p<0.0001). Pregnancy and labor progression in patients with cord pathology showed a high rate of complications compared to control group, including urinary tract disorders (p=0.02), preterm labor at 27-28 weeks (p=0.01), polyhydramnios, which was ten times more frequent (p=0.002), and fetal growth restriction (p=0.02). In the study group, a prolonged second stage of labor was observed (p=0.01), along with acute fetal hypoxia, which required urgent pregnancy termination (p=0.01) through vacuum extraction (p=0.0009) or C-section (p=0.04).

Conclusions. The analysis of the anamnestic and clinical peculiarities of the perinatal period in patients with UC pathology, compared to pregnant women without this pathology, confirmed that this commonly encountered obstetric condition represents a considerable risk factor for perinatal complications.

Keywords: umbilical cord, perinatal complications, risk factors, obstetric management, pregnancy.

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Key messages

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

The precise mechanisms by which various maternal factors contribute to the development of umbilical cord pathologies, as well as the exact relationship between these pathologies and specific adverse perinatal outcomes, such as preterm birth and fetal growth restriction, remain unclear. There is limited understanding of the long-term impacts of early detection and intervention in managing these pathologies and of how different diagnostic approaches compare in predicting outcomes.

The research hypothesis

The authors consider that umbilical cord pathologies to be significantly linked to adverse perinatal outcomes, such as preterm birth and intrauterine growth restriction. Specific maternal or fetal fac-

tors, such as smoking or a history of cord abnormalities, increase the risk of these pathologies.

The novelty added by manuscript to the already published scientific literature

The new insights into the relationship between umbilical cord pathologies and adverse perinatal outcomes focus on under-explored risk factors like maternal smoking and a prior history of cord abnormalities. The research explores how these pathologies might influence preterm birth and intrauterine growth restriction, contributing to a more comprehensive understanding of their clinical significance.

Introduction

The umbilical cord (UC) is essential for fetal survival, facilitating necessary exchanges between the mother and fetus. Umbilical cord pathologies, such as true knots, hypercoiling, velamentous insertion, cord entanglement, and cord prolapse, are often associated with fetal hypoxia and can significantly impact perinatal outcomes, leading to complications and even fetal death [1-3]. Recent studies show that umbilical cord abnormalities may be associated with an increased risk of preterm birth and the need for intensive neonatal care, highlighting the importance of identifying and monitoring these pathologies during pregnancy [4]. Placental and umbilical cord examinations in cases of fetal death indicate that a significant portion of fetal deaths can be attributed to cord pathologies [5]. In this context, understanding the underlying causes of umbilical cord pathologies can aid clinical management by identifying etiopathogenesis and improving perinatal outcomes [6, 7].

Nonetheless, existing research provides only limited insight into predictive factors and effective management strategies for the risks associated with umbilical cord pathology, as well as the complex relationships between umbilical cord structure, obstetric outcomes, and perinatal health. Despite ongoing research, there is still a lack of comprehensive understanding in the literature regarding these predictive factors and management strategies. Additionally, the complex interactions between the structure of the umbilical cord, obstetric outcomes, and perinatal health remain insufficiently explored. This research could enable a deeper understanding of pathological mechanisms and support the development of strategies that could improve prenatal care and reduce the incidence of complications associated with umbilical cord pathologies.

The aim of the study is to determine the clinical and evolutionary characteristics of pregnancies and deliveries in patients with umbilical cord pathology.

Material and methods

The study was conducted at the Department of Obstetrics and Gynecology, Nicolae Testemițanu State University of Medicine and Pharmacy, in the Republic of Moldova. A total of 190 patients participated, divided into two groups: L1 – 95 patients with umbilical cord (UC) abnormalities and L0 – 95 with normal UC. This prospective cohort study included data on demographics, medical history, pregnancy progression, and perinatal outcomes. The data were analyzed using statistical and mathematical methods with SPSS 23.0, SAS 9.4, and Microsoft Office Excel 2016. These tools enabled the calculation of rates, mean values, and proportion indicators, as well as the determination of statistical significance at a 95% confidence level. The Pearson chi-square test ($\chi 2$) was applied to discrete variables, and odds ratios (OR) with confidence intervals (CI) were calculated. The study aimed to investigate the relationship between umbilical cord pathology and pregnancy complications and delivery outcomes.

Results

Most women in both groups were between 20 and 34 years old (mean age 29.09±4.85 years in first group vs. 27.86±4.36 years in the second one, p>0.05), with a coefficient of variation of 15.66% for L0 and 16.66% for L1. The assessment of socioeconomic status between the groups did not show statistically significant differences (p>0.05).

In addition to age and socioeconomic factors, we analyzed the determinants of umbilical cord pathology that influence its development (Table 1). The evaluation of harmful workplace factors for patients in group L1 revealed that these factors were recorded more frequently. However, only psychological and emotional stress showed a correlation with umbilical cord pathology (CO), with $\chi^2 1df = 5.9047$, Cramer's V = 0.21, and p=0.01.

Table 1. Determinant factors for umbilical cord pathology

Factor	Study group (n=95)	Control group (n=95)	OR	95% CI	p
Psychological and Emotional Stress at Work	26	14	2,5536	1,1861-5,4976	0,01
Harmful Habit (Smoking)	10	3	1,0824	1,0012-1,1701	0,04
Primiparity	49	28			0,005
Umbilical Cord Pathology in Previous Pregnancies	34	20			<0,0001
Infertility	9	1			0,02
Urinary Tract Disorders	40	25	1,2727	1,0323-1,5692	0,02
Polyhydramnios	11	1	2,1190	2,0375-2,2070	0,002
Urgent Cesarean Section	11	2	5,0769	1,9287-27,7546	0,04

***Note**: OR – odds ratio; CI – confidence interval.*

The analysis of behavioral risk factors found a high incidence of harmful habits in patients with umbilical cord pathology, with smoking being a significant determinant ($\chi^2$1df=4.0461, Cramer's V=0.2; p=0.04). Regarding parity, the samples were heterogeneous, with primiparity emerging as a key factor that correlated with the risk of umbilical cord pathology ($\chi^2$2df=10.2928, Cramer's V=0.23; p=0.005). An indirect Pearson correlation (rxy=-0.33; p=0.001) was established between the number of spontaneous abortions in the patient's history and the number of umbilical vessels observed on prenatal ultrasound in the current pregnancy, suggesting a risk of vascular anomalies in the umbilical cord for women with a history of spontaneous abortions.

The study's data on patients' personal medical history revealed a relatively high percentage of urinary tract disorders (chronic pyelonephritis, bacteriuria, chronic cystitis, hydronephrosis, nephroptosis, renal colic, pyeloectasia, nephrolithiasis, double kidney), which were more prevalent in the L1 group, with 40 (42.11%) cases compared to 25 (26.32%) cases in the L0 group ($\chi^2$1df=5.2615, Cramer's V=0.2; p=0.02).

During the study, it was found that one in every three to four women in L1 had at least one episode of imminent pregnancy termination, with such incidents occurring more frequently around the 27-28-week gestation mark (p<0.05). Additionally, polyhydramnios was detected 10 times more frequently in L1 compared to the control group, confirming a significant link between polyhydramnios and umbilical cord pathology (p<0.05).

As for fetal growth restriction (FGR), it was observed only in the L1 group ($\chi^2$1df=5.1351, Cramer's V=0.2; p=0.02), with a predictive model showing AUC=0,8679 (Fig. 1). It is important to emphasize that the role of umbilical cord pathology should not be overlooked, as such conditions often lead to profound changes in the fetoplacental complex, resulting in fetal distress.

Following the evaluation of the duration of the anhydramnios period, it was found that in patients with umbilical cord pathology, this period was prolonged – up to 109 hours and 45 minutes, with a mean of 9.17±17.76 hours (95% CI 5.11-

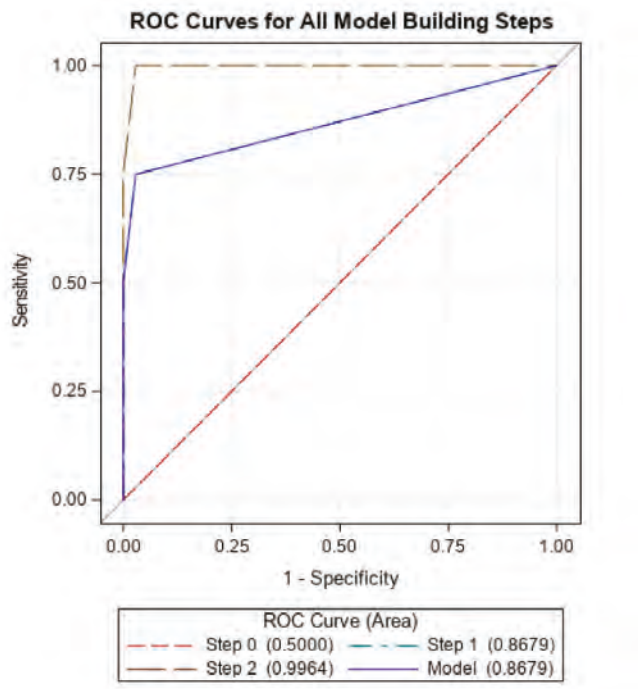

Fig. 1 The ROC curve of the predictive model for the probability of developing fetal growth restriction in children with umbilical cord pathology, based on perinatal morbidity

13.23). In the L0 group, the duration ranged from 5 minutes to 18 hours and 15 minutes, with a mean of 3.69±3.65 hours (95% CI 2.88-4.49), with p<0.05. The significant difference in the duration of the anhydramnios period between the compared groups is also clearly illustrated in Figure 2.

In evaluating the delivery outcomes for the women in the study groups, it was found that vaginal delivery predominated: 81 (85.26%) cases in L0 vs. 71 (74.73%) in L1. Cesarean sections were performed more often in the L1 group: 24 (25.26%) cases vs. 14 (14.74%) in L0 ($\chi^2$1df=2.1934, Cramer's V=0.1; p=0.01), with urgent cesarean sections being more common: 11 (11.58%) in L1 vs. 2 (2.11%) in

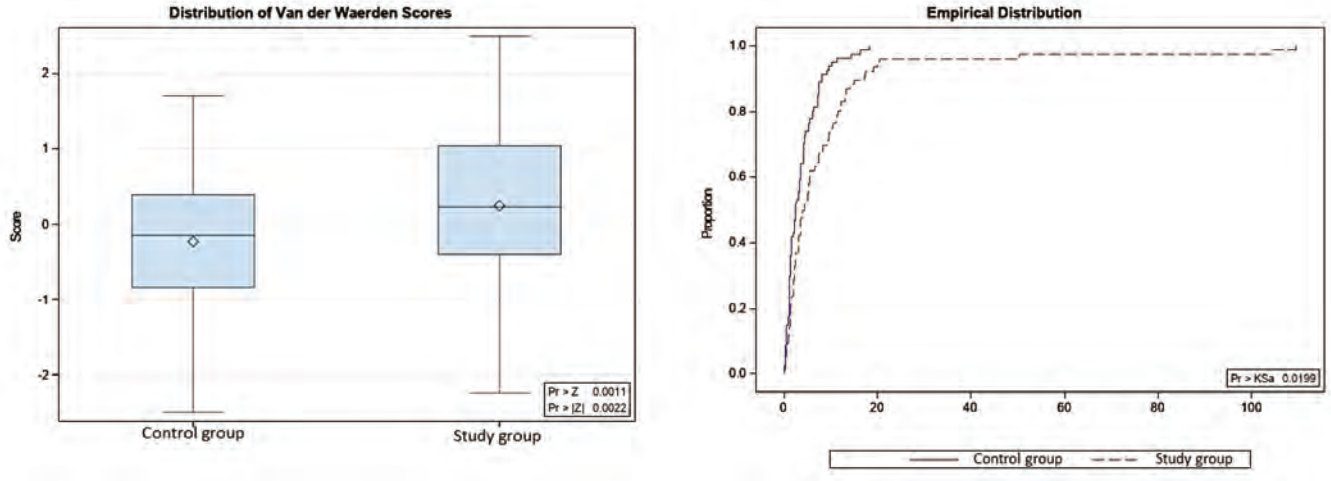

Fig. 2. Distribution of study groups by the duration of the anhydramnios period (hours/minutes).

L0 ($\chi^2$1df=3.9100, Cramer's V=0.32; p=0.04). Fetal distress during the second stage of labor, resolved via vacuum extraction, occurred in 12 (12.63%) patients in L1 compared to 1 case in L0 ($\chi^2$1df=11.1143, Cramer's V=0.26; p=0.0009).

Postpartum hemorrhage (>500 ml) was more prevalent in patients with umbilical cord pathology: 31 (32.55%) cases compared to 16 (16.80%) cases in those with a normal umbilical cord (p<0.05).

The analysis of anamnestic and clinical features of the perinatal period in patients with umbilical cord pathology, compared to those without this pathology, highlights that the presence of this condition is associated with psychological and emotional stress, a higher number of spontaneous abortions, a history of imminent pregnancy termination, complicated somatic history (nephro-urinary conditions), polyhydramnios, and intrauterine fetal growth restriction. These factors should be carefully considered in the antenatal care and the clinical management of delivery.

Discussion

Umbilical cord pathology constitutes a major issue in contemporary obstetrics. Examining its determining factors allows for a detailed understanding of the pathogenesis of various umbilical abnormalities that negatively impact perinatal outcomes [4].

Our study data show that the age of the pregnant women ranged between 20 and 34 years, with no statistical differences between the two groups. Similar findings have been published by other authors, who confirmed that the age of the participants varied between 20 and 35 years and did not constitute a risk factor for the development of umbilical cord pathology [8, 9].

The data from our study also demonstrate that pathological conditions of the umbilical cord are more frequently observed in primiparas, serving as a determinant factor ($\chi^2$2df =10.2928, Cramer's V=0.23; p=0.005). The extragenital medical history of the patients included in the study was compromised by the presence of 2-3 pathologies, with urinary tract conditions being the most prevalent [OR=1.2727; 95% CI 1.0323-1.5692; p=0.02]. Similar data were reported in a recent systematic review and meta-analysis, where these conditions were identified as risk factors for the development of umbilical abnormalities [10].

The evolution, clinical management, and complications during the current pregnancy were examined. It was found that one in three or four women in the study group had at least one episode of imminent pregnancy termination, most frequently occurring around 27-28 weeks of gestation (p<0.05). A relevant study by Meyer et al. (2020) confirms that women with umbilical cord pathology are at an increased risk of premature pregnancy termination. According to this study, there is a strong association between umbilical cord abnormalities and an increased risk of preterm contractions and premature rupture of membranes, particularly in the second half of pregnancy, between weeks 24 and 28 of gestation [11]. Similarly, Sanchez et al. (2021) observed in a cohort of pregnant women with umbilical cord pathology that the incidence of pregnancy loss and the risk of prematurity were significantly higher among those with structural abnormalities of the umbilical cord, such as cord knots or cord entanglements around the fetal neck. These pathologies can lead to impaired blood circulation through the umbilical vessels, thereby increasing the risk of placental insufficiency and, consequently, premature birth [12]. Furthermore, Zhang et al. (2023) reported that umbilical cord pathology is a significant predictor of prematurity, especially during the 27–28-week gestation period, when the fetus is particularly vulnerable to circulatory disturbances. This period represents a critical stage for fetal organ development, particularly for the respiratory system, and any complication affecting umbilical circulation can rapidly lead to severe perinatal risks, including preterm birth [13]. The study by Dubetskyi (2023) examined the structural and functional aspects of the umbilical cord in relation to pregnancy outcomes and placental characteristics. Four patient groups were analyzed postnatally: Wharton's jelly edema (10 samples), velamentous cord insertion (10 samples), single umbilical artery (10 samples), and a control group with physiological pregnancies (10 samples). A combined presentation of umbilical cord characteristics (51.1%) was common, such as the absence of Wharton's jelly alongside velamentous insertion or a "thin" cord with a single umbilical artery. Additional factors like maternal age, obesity, nicotine use, and history of IVF were associated with increased incidence of umbilical cord abnormalities, prematurity, preeclampsia, and placental dysfunction. Clinical complications included prematurity (24.4%), oligohydramnios (45.6%), preeclampsia (37.8%), placental dysfunction (44.4%), low birth weight (18.9%), neonatal pathologies (22.2%), and a higher rate of cesarean sections (32.2%) [14].

This finding underscores the importance of careful monitoring and evaluation of pregnant women, especially considering the perinatal risks more frequently encountered during the intermediate weeks of pregnancy.

Our study revealed a higher incidence of fetoplacental system pathologies in the group of patients with umbilical cord abnormalities, compared to those with a normal UC. These pathologies included polyhydramnios, oligohydramnios, fetal growth restriction, and intrauterine infection. Other studies have also highlighted that amniotic fluid acts as a protective factor, helping prevent compressions, as long as the liquid environment is sufficient and clear. Otherwise, it becomes a major risk factor for complications, leading to reduced blood flow in the UC [15].

FGR was diagnosed in cases of marginal and velamentous insertion, AOU, umbilical cord coiling, thin umbilical cord, hypocoiled, or torsioned cord. Notably, the research group recorded nearly twice as many cases of UC pathology in previous pregnancies (p<0.0001), placing these patients at higher risk for complications in their current pregnancies as well. Studies by Li et al. (2022) and Zhang et al. (2023) demonstrated that marginal and velamentous insertion of the umbilical cord, combined with twisting or hypocoiling, can impair placental function and negatively affect fetal development, leading to an increased risk of FGR and preterm birth.

Additionally, umbilical cord wrapping around the fetal neck and disrupted circulation are significant contributors to complications associated with FGR and higher miscarriage risk [16, 17]. Acute fetal distress due to umbilical abnormalities required urgent delivery using obstetric forceps (p=0.0009). Research by Lavric I. et al. (2021) also emphasized that indications for forceps extraction included umbilical cord coiling (66%) and short umbilical cord (16%) [18].

Conclusions

The results of the study suggest that psychoemotional stress, smoking, primiparity, adverse obstetric history with spontaneous abortions, the presence of polyhydramnios, and fetal growth restriction in the current pregnancy represent significant risks for the presence of umbilical cord pathology. Early identification of these and other risk factors, along with careful monitoring of pregnancies and early antenatal diagnosis of umbilical cord pathology, are crucial for minimizing perinatal complications that may arise from this condition.

Competing interests

None declared.

Authors' contributions

IuD proposed the study area, conceived the study design, and oversaw the main aspects of its implementation. AA participated in the study design, included subjects in the research, performed the statistical analysis of the collected data, and drafted the manuscript. HC critically reversed the manuscript. All authors critically reviewed the work and approved the final version of the manuscript.

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Patient consent

Obtained.

Ethics approval

The Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy approved the study **-** Minutes no. 95 from 21.06.2017.

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RESEARCH ARTICLE

Development and validation of a questionnaire for hygienic estimation of the impact of risk factors on morbidity due to osteoporosis

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ABSTRACT

Introduction. Osteoporosis remains a major public health issue, particularly affecting the elderly, and is characterized by decreased bone mass and deterioration of bone structure, increasing the risk of severe fractures. The development of specific and precise tools that allow for the identification and estimation of the influence of modifiable factors on the development of osteoporosis is an important step in early intervention for at-risk individuals.

Material and methods. The initial version of the questionnaire was created to identify modifiable factors contributing to the development of osteoporosis, including the type and frequency of drinking water consumption, water quality, knowledge about its mineral composition, dietary preferences, alcohol and tobacco use, physical activities, and stressful situations. The questionnaire initially contained 65 questions, organized into three sections. Five experts reviewed the content validity, evaluating the clarity, cultural and linguistic relevance, structure, and coherence of the questions. The feedback led to adjustments, reducing the questionnaire to 52 questions. A pre-test was conducted with 30 adults from the Republic of Moldova, confirming the validity and internal consistency of the questionnaire, with a Cronbach's Alpha coefficient of 0.768.

Results. To ensure content validity, experts evaluated the questionnaire, and based on their comments and suggestions, semantic and syntactic reformulations and modifications were made. As a result, 13 questions were eliminated, reducing the second version of the questionnaire to 52 questions. During the pre-testing stage, the order and content of the questions were deemed appropriate by the respondents. The average time required to complete the questionnaire was 14.19 minutes (SD ± 1.567), with a minimum of 11.90 minutes and a maximum of 17.33 minutes. The sample used in the pre-testing stage included 30 respondents, the majority of whom (93.3%) were women. The respondents' ages ranged from 25 to 72 years, with a mean age of 50.37 ± 2.6 years. The distribution of respondents was balanced between urban (53.3%) and rural (46.7%) areas. Regarding education level, 56.6% had higher education, 26.7% had secondary vocational education, and 16.7% had no higher education.

Conclusions. The study demonstrated that the new questionnaire is a valid and reliable instrument for assessing the impact of modifiable risk factors on morbidity due to osteoporosis. Ongoing research is necessary to refine and further validate the questionnaire within the broader population.

Keywords: osteoporosis, risk factors, modifiable factors, non-modifiable factors, prevention, validation, pre-testing.

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Key messages

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

The present study is innovative by developing a complex tool aimed at elucidating the causal relationship between osteoporosis

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morbidity and its determinants, with a focus on mineralization indices of drinking water.

The research hypothesis

The research hypothesis is that the mineral composition of drinking water has a significant impact on the development of osteoporosis among the population of the Republic of Moldova, compared to other modifiable factors.

The novelty added by manuscript to the already published scientific literature

The study makes an innovative contribution to the existing scientific literature by establishing a research concept on population health in relation to environmental factors, using the questionnaire method. The novelty of the study lies in the validation of an original questionnaire, designed to assess the quality and nature of drinking water consumption among individuals affected by osteoporosis. This research instrument, through its specific items, offers a new perspective on the interaction between environmental factors, particularly the composition of drinking water, and bone health, thus contributing to a better understanding of the determinants of osteoporosis.

Introduction

Education-based interventions should be evaluated with a focus on promoting behaviors related to the identification, prevention, and management of risk factors, leading to an effective reduction in the incidence of osteoporosis.

Osteoporosis represents a major public health issue globally, particularly affecting the elderly population. It is characterized by a reduction in bone mass and the deterioration of bone tissue structure, which increases the risk of fractures. According to the World Health Organization, osteoporosis affects millions of people worldwide, and its incidence is rising due to the aging population [1]. The risk factors for osteoporosis are numerous and include age, sex, genetic factors, lifestyle, diet, and exposure to certain toxic substances or environmental conditions [2, 3].

Preventing osteoporosis involves a comprehensive approach to managing risk factors. Identifying and managing these risk factors are essential for the prevention and control of the disease. They can include genetic elements as well as environmental and lifestyle factors. Adequate intake of calcium and vitamin D, a healthy diet, and consuming drinking water with an appropriate mineral salt composition are essential for maintaining bone health. Adopting a healthy lifestyle, avoiding smoking, and limiting alcohol consumption are important for preventing osteoporosis.

In the context of osteoporosis prevention and management, the hygienic evaluation of the impact of risk factors plays a crucial role. Developing specific tools that allow for the identification and estimation of the influence of modifiable factors on the development of osteoporosis is an important step in early intervention for at-risk individuals. The importance of such a tool is supported by the need for precise data to guide preventive interventions and to optimize public health strategies aimed at reducing the incidence of osteoporosis. Previous studies have demonstrated that using questionnaires to assess osteoporosis risk can significantly contribute to early risk identification, improve clinical outcomes, and reduce the disease burden [4-7].

The present study focuses on the development and validation of a questionnaire designed for the hygienic assessment of the impact of risk factors, especially modifiable ones, on morbidity due to osteoporosis. This research aims to provide a useful tool for public health professionals and researchers, thereby facilitating the more effective identification and management of risks associated with osteoporosis.

Material and methods

To achieve the proposed aim, both secondary and primary studies were conducted as part of this research.

Secondary research. The narrative synthesis involved collecting information from international open-access databases – PubMed and Google Scholar – using the following keywords: "osteoporosis", "drinking water", "mineral composition of drinking water", "risk factors", "modifiable factors", "non-modifiable factors", "prevention", and "calcium". Boolean operators – *AND* and *OR* – were used to refine the search domain. To enhance the efficiency of database querying, the metacharacters *.ti* and *.ab* were employed. The metacharacter *$* was used to search for plural forms of terms. A total of 67 bibliographic sources were analyzed, with 26 sources retained based on relevance and completeness of information. The languages of the studied bibliographic sources included English, French, Russian, and Romanian.

Primary research. A primary study was planned, which involved the development and validation of a questionnaire for the hygienic estimation of the impact of risk factors on morbidity due to osteoporosis. This study was conducted in five stages: (i) development of the questionnaire, (ii) con-

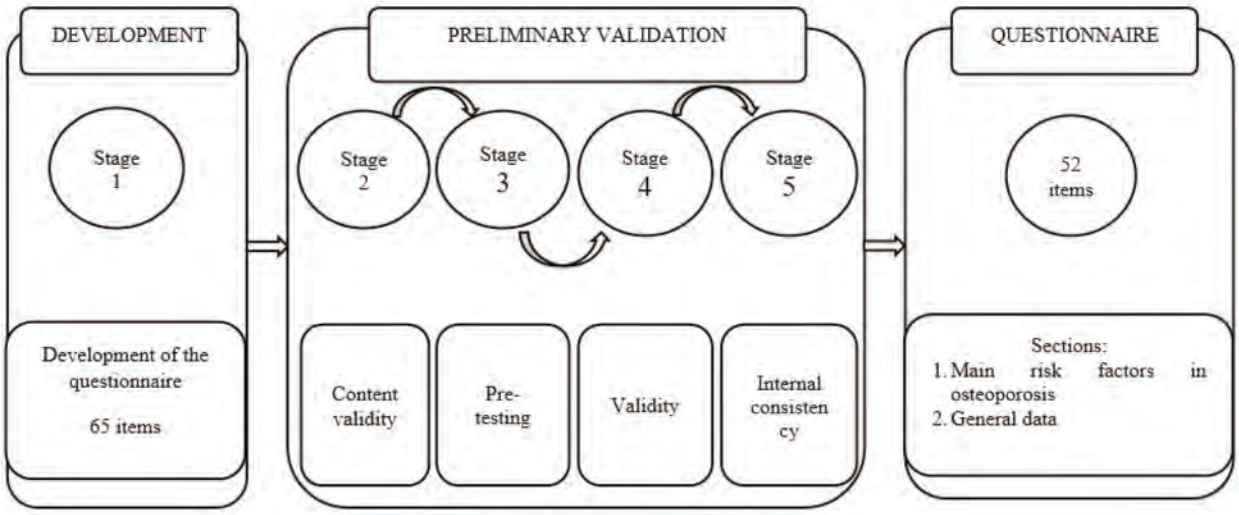

Fig. 1 Design of the pre-testing and validation study.

tent validity, (iii) pre-testing, (iv) validity, and (v) internal consistency (Fig. 1).

This study was conducted in accordance with the guidelines established in the Declaration of Helsinki, and all procedures involving participants in the research study were approved by the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy in the Republic of Moldova, minutes No.01, dated June 28, 2023. The questionnaire process was conducted face-to-face. Participants were informed about the purpose and outcomes of the study, as well as the potential benefits and risks. Written consent to participate in the study and agreement to answer questions were obtained from each participant.

Stage 1. Development of the questionnaire.

To achieve the research goal, a new working tool was developed titled "Hygienic Estimation of the Impact of Risk Factors on Morbidity Due to Osteoporosis." The criteria followed for developing the first version of the questionnaire were: (i) Identification of modifiable factors responsible for the development of osteoporosis, including the frequency of drinking water consumption and type of water, the volume and quality of consumed drinking water, knowledge about the mineral composition of drinking water, dietary preferences, alcohol consumption, intake of foods rich in mineral compounds and vitamins, smoking, frequency and intensity of physical activities, and presence of stressful situations; (ii) Identification of specific characteristics in individuals diagnosed with osteoporosis, including knowledge about osteoporosis, diagnosis established and confirmed by a doctor, history of fractures, fracture locations, hereditary history, adherence to prescribed treatment, and data on menopause; (iii) General data: age, biological sex, living environment, body weight, and height. The questionnaire consisted of 3 sections: (i) Modifiable Risk Factors – 44 questions; (ii) Specific Questions for Individuals with Osteoporosis – 14 questions; (iii) General Data – 7 questions. At this stage, the questionnaire included 65 questions.

To minimize measurement errors, the short closed-format questions were designed to be concise and clearly understandable. Additionally, definitions and examples were provided to better clarify the purpose of the questionnaire.

Stage 2. Content validity

This stage involved reviewing the item bank to ensure it measured exactly what was intended. The initial version of the questionnaire was based on the opinions of five medical experts (a rheumatologist, a laboratory physician, a hygiene specialist, a social medicine and management specialist, and a medical imaging specialist) and on a literature search focused on other behavior-based questionnaires and the relationships between risk factors and their impact on the development of osteoporosis. Each expert received a copy of the initial version of the questionnaire via email and was asked to evaluate the tool according to the following criteria: content validity, clarity and comprehensibility, cultural and linguistic relevance, structure and order of questions, and coherence and consistency of the questions. Experts also provided additional comments and suggestions for each item in the questionnaire. As a result, thirteen questions were removed, and eight questions were reformulated, but no new questions were added. At the end of the second stage, the second version of the questionnaire was produced, which included 52 questions, structured as follows: (i) Main Risk Factors in Osteoporosis – 44 questions; (ii) General Data – 6 questions.

Stage 3. Pre-testing

At this stage, both quantitative and qualitative analyses of the questions were conducted to assess their suitability for inclusion in the questionnaire, focusing on validity and internal consistency. A sample of 30 adults from the Republic of Moldova was recruited, who presented themselves at the medical center for the Dual Energy X-ray Absorptiometry (DEXA) investigation, at the doctor's recommendation. Of these participants, 93.3% were female and covered a wide age range. Notably, 21 individuals (70%) had a diagno-

sis of osteoporosis. The sample was significantly skewed by gender, with an overrepresentation of female participants. The number of subjects included for pre-testing was based on similar studies [8-11]. Data collection occurred from October 2023 to February 2024.

Stage 4. Validity

Participants answered questions from the second version of the questionnaire and a brief ad-hoc questionnaire with three open-ended questions to provide additional feedback on the new questionnaire regarding the ease of understanding and clarity of the questions on fundamental aspects. At this stage, all questionnaires were analyzed based on the degree of completion for each question. All questionnaires met the completion criteria, and none were excluded from the study. For validity, frequencies were calculated, and open-ended responses provided by participants were analyzed to improve the instrument.

Stage 5. Internal consistency

To determine the internal consistency and reliability of the questionnaire, the Cronbach's Alpha coefficient was calculated based on the linear correlation model. The value of Cronbach's Alpha ranges between 0 and 1. To be considered consistent, an instrument should achieve a value as close to 1 as possible, with 0.70 generally accepted as a threshold by most researchers. The interpretation of the Cronbach's Alpha coefficient is provided in Table 1 [12]:

Table 1. Interpretation of Cronbach's Alpha coefficient values (Lee J. Cronbach, 1951).

Values of the coefficient	Consistency
Greater than 0.9	Excellent
Between 0.7 and 0.9	Good
Between 0.6 and 0.7	Acceptable
Between 0.5 and 0.6	Poor
Less than 0.6	Unacceptable

Note: Cronbach's alpha (Cronbach'sα), also known as tau-equivalent reliability or coefficient alpha, is a reliability coefficient and a measure of the internal consistency of tests and measures. It assesses how closely related a group of items is as a whole; a higher value indicates greater reliability. Generally, a Cronbach's alpha of 0.70 or above is considered acceptable, while values above 0.90 may indicate excellent reliability. It was named after the American psychologist Lee Cronbach.

Since the questionnaire was designed to identify risk factors for osteoporosis and the questions were not segmented into distinct domains, a consistency analysis of the entire instrument was performed. The reliability test result was 0.768. As a result of this stage, the final version of the questionnaire did not undergo any changes, and no questions were excluded, as all questions were deemed relevant to the research. The Cronbach's Alpha value indicated an acceptable range of internal reliability for the instrument.

Statistical processing

Statistical processing of the data and determination of the Cronbach's Alpha coefficient were performed using the licensed IBM SPSS Statistics 27 software. In the statistical analysis, various methods were employed to describe and summarize the data. Descriptive measures such as the mean and standard deviation were calculated to assess central tendency and data dispersion. Additionally, minimum and maximum values, as well as percentages and percentiles, were determined to provide a detailed view of the data distribution. Absolute values were used to illustrate the frequencies or magnitudes of measurements within the analyzed sample.

Results

Development of the questionnaire

The first version of the questionnaire contained 65 questions organized into three sections: (i) Identification of Modifiable Factors Responsible for the Development of Osteoporosis; (ii) Identification of Specific Characteristics in Individuals Diagnosed with Osteoporosis; and (iii) General Data.

The elements of the first section, "Identification of Modifiable Factors Responsible for the Development of Osteoporosis", included 44 questions: open-ended questions, dichotomous questions, and closed-ended questions with predetermined answers, ranging from 2 to 11 response options. The section "Identification of Specific Characteristics in Individuals Diagnosed with Osteoporosis" included 14 questions: open-ended questions, dichotomous questions, and closed-ended questions with predetermined answers, ranging from 2 to 6 response options. The elements of the "General Data" section included 7 open-ended and closed-ended questions. Respondents had the option to choose multiple answers, but all questions were mandatory.

Content validity

Based on the evaluations and comments from experts, syntactic and semantic reformulations and modifications were made to the three sections of the questionnaire, including the removal of 13 irrelevant or repetitive questions. Specifically, 11 questions were removed from the section "Identification of Modifiable Factors Responsible for the Development of Osteoporosis," one question was removed from the section "Identification of Specific Characteristics in Individuals Diagnosed with Osteoporosis," and one question was removed from the section "General Data." As a result, the second version of the questionnaire had a total of 52 questions, restructured into two sections: (i) Major Risk Factors for Osteoporosis and (ii) General Data.

Pre-testing and validity

At the pre-testing stage, according to the respondents' opinions, the order and content of the questions were found to be good. In face-to-face questioning, all respondents (100%) stated that the questions were clear and easy to understand. However, in 4 (7.7%) questions, respondents suggested replacing some specialized terms with synonyms. As a result, minimal changes were made, specifically related to the terms used to denote certain symptoms. These terms were corrected before the final administration of the questionnaire. The average time for face-to-face questioning was 14.19 minutes. The time range, from a minimum of 11.90 minutes to a maximum of 17.33 minutes, suggests that there is relatively little variation in the duration of the interviews, all falling within a margin of approximately 5.43 ± 1.567 minutes (M ± SD), indicating that most of the interview durations are relatively close to the average of 14.19 minutes.

The questionnaire was revised several times regarding syntax and semantics to avoid ambiguity and errors, after considering the respondents' reviewed results, pre-testing comments, and experts' suggestions and opinions. These measures were taken to ensure that the questions in the final questionnaire could competently evaluate each subject and address the study's objectives.

Characteristics of the sample

The socio-demographic characteristics of the individuals who participated in the pre-testing phase of the questionnaire are presented in Table 2 and Table 3.

Table 2. The demographic and anthropometric characteristics of the respondents: pre-testing stage.

	Mean	± SD	Min	Max
Age, years	50.37	2.6	25	72
Body weight, kg	70.75	2.03	43.5	94.0
Height, cm	165.03	1.25	152.0	182.0

Note: SD - Standard Deviation; Min – minimum; Max – maximum; kg – kilograms; cm – centimeters.

The respondents' ages ranged from 25 to 72 years, with a mean age of 50.37 ± 2.6 years. The average body weight of the participants was 70.75 kg, with a standard deviation of ± 2.03 kg, indicating moderate variation in body weight among individuals. The lowest recorded weight was 43.5 kg, and the highest was 94.0 kg. This variability may reflect different levels of physical activity, types of body constitution, and possibly different dietary habits among participants. The average height of the participants was 165.03 cm, with a standard deviation of ± 1.25 cm. The minimum recorded height was 152.0 cm, and the maximum was 182.0 cm.

Table 3. Demographic and educational profile of respondents: pre-testing stage.

	No	**%**
Gender		
- Male	2	6.7
- Female	28	93.3
Total Gender	30	100
Living Environment		
- Urban	16	53.3
- Rural	14	46.7
Total Living Environment	30	100
Education		
- No education	5	16.7
- Secondary vocational education	8	26.7
- Higher education	17	56.6
Total Education	30	100

Note: No - the absolute number; % - percent

As shown in Table 3, the gender distribution of a sample of 30 respondents was analyzed. Of these, 28 (93.3%) were female and 2 (6.7%) were male. To better understand the data distribution, the median, 25th percentile (Pr25), and 75th percentile (Pr75) were calculated. The gender distribution analysis indicates a clear predominance of the female gender. Most central and dispersion values (median, Pr25, and Pr75) are "Female," reflecting the imbalanced gender distribution in this sample. It is noteworthy that respondents were similarly sampled from both urban (53.3%) and rural (46.7%) environments. Most respondents (56.6%) reported having higher education, followed by those with secondary vocational education (26.7%) and those with no education (16.7%).

Internal consistency

Out of the 52 questions subjected to the consistency analysis, 5 open-ended questions and 6 questions from the "General Data" section, which could not be coded, were excluded. The Cronbach's Alpha coefficient was 0.768, demonstrating good consistency.

Table 4. Statistical analysis of questionnaire items.

	Mean Scale if Item is Excluded	Scale Variance if Item is Excluded	Corrected Item-Total Correlation	Cronbach's Alpha if Item is Excluded	Decision
Q1	106.43	116.286	0.575	0.757	
Q2	106.57	121.619	0.097	0.767	
Q3	106.71	122.571	0.000	0.768	
Q4	106.71	122.571	0.000	0.768	
Q5	106.57	121.619	0.097	0.767	
Q6	106.57	121.619	0.097	0.767	
Q7	106.43	116.286	0.575	0.757	
Q8	106.57	123.619	-0.142	0.771	Item retained
Q9	106.71	122.571	0.000	0.768	
Q10	106.57	123.619	-0.142	0.771	Item retained
Q11	106.71	122.571	0.000	0.768	
Q12	106.71	122.571	0.000	0.768	
Q13	106.43	119.286	0.286	0.763	
Q14	106.43	121.619	0.066	0.768	
Q15	105.57	123.619	-0.092	0.780	Item retained
Q16	105.57	104.286	0.677	0.739	
Q17	105.71	116.571	0.111	0.776	Item retained
Q18	106.57	115.286	0.880	0.754	
Q19	103.43	115.619	0.393	0.758	
Q20	103.14	116.143	0.533	0.757	
Q21	103.57	114.619	0.506	0.755	
Q22	106.00	118.000	0.409	0.761	
Q23	106.14	115.143	0.623	0.755	
Q24	106.43	116.286	0.575	0.757	
Q25	106.43	121.619	0.066	0.768	
Q26	106.71	122.571	0.000	0.768	
Q27	106.00	118.000	0.409	0.761	
Q28	106.00	117.333	0.285	0.762	
Q29	106.29	108.238	0.838	0.741	
Q30	106.00	119.000	0.313	0.763	
Q31	105.57	106.619	0.577	0.745	
Q32	105.57	118.286	0.135	0.769	Item retained
Q33	106.71	122.571	0.000	0.768	
Q34	106.00	117.333	0.285	0.762	
Q35	106.57	115.286	0.880	0.754	
Q36	106.00	122.000	0.031	0.769	Item retained
Q37	106.71	122.571	0.000	0.768	
Q38	106.71	122.571	0.000	0.768	
Q39	105.57	121.619	0.097	0.767	
Q40	105.71	117.905	0.226	0.764	
Q41	106.43	115.286	0.673	0.755	

Note: Q - question

It should be noted that excluding certain questions may increase the Cronbach's Alpha coefficient (Table 4). If questions Q8 and Q10 are excluded, the coefficient could potentially rise to 0.771. For question Q15, it could increase to 0.780; if question Q17 is excluded, it could rise to 0.776; and if questions Q32 and Q36 are excluded, it could be 0.769. Given that these questions are relevant to the research and will provide unique and qualitative information, the decision was made to retain them in the current version of the questionnaire without any modifications.

Discussions

This study aimed to develop and test a new questionnaire designed for the hygienic assessment of the impact of risk factors, particularly modifiable ones, on morbidity due to osteoporosis. Our results indicate that the new instrument was considered by experts to have good clarity and relevance. Respondents found the questionnaire acceptable and easy to understand, demonstrating its validity. Additionally, the Cronbach's Alpha coefficient was 0.768, which further confirms that the responses across the questions are well correlated.

In the context of recent literature [13, 14], new questionnaires have been developed and validated to assess people's knowledge and perceptions about osteoporosis. Generally, these questionnaires have focused on studying knowledge, attitudes, and practices regarding osteoporosis from various perspectives [15-20]. However, few studies focus on developing questionnaires aimed at highlighting modifiable risk factors, such as the mineral composition of drinking water. Most frequently, studies provide information about both modifiable and non-modifiable risk factors for osteoporosis, contributing to its development. They also highlight common risk factors such as age, sex, family history, dietary habits, physical activity level, smoking, and alcohol consumption [20].

In the study that evaluated the knowledge, attitudes, and practices of adult patients at Bashair Hospital, Sudan, regarding osteoporosis [21], it was revealed that factors such as age, education level, and sex had a notable influence on the levels of knowledge and practices concerning some modifiable risk factors responsible for the development of osteoporosis. Our results are consistent with these findings, highlighting the acceptability and clarity of the questionnaire among respondents from various living environments and education levels.

Another study conducted by Barik S. *et al.* (2022) focused on the translation and adaptation of the OKAT (Osteoporosis Knowledge Assessment Tool), which consisted of 20 questions designed to assess knowledge about osteoporosis, risk factors, prevention, and treatment options. The Cronbach's Alpha coefficient was calculated to measure internal consistency, obtaining a value of 0.892. The results showed good readability and consistency of the OKAT instrument. The average score obtained by participants was 11.3 ± 2.1, indicating a low level of knowledge about osteoporosis. Significant differences in scores were observed based on education level and family history of osteoporosis or fractures [22]. In comparison, the Cronbach's Alpha coefficient of 0.768 indicates good internal consistency, suggesting that our questionnaire is a reliable tool for measuring modifiable risk factors.

These findings highlight the importance of developing reliable and valid assessment tools for measuring knowledge and practices related to osteoporosis, considering modifiable risk factors. The implementation of such questionnaires can help improve educational programs and preventive strategies, thereby contributing to the reduction of morbidity due to osteoporosis [23].

The development of the questionnaire included several stages, such as pre-testing and content validation, which led to the elimination and reformulation of some questions to improve their clarity and relevance. The socio-demographic characteristics of the individuals who participated in the pre-testing phase showed a diversity of age, gender, and living environment, contributing to the overall validity of the instrument.

These findings are consistent with those reported in specialized literature. Previous studies have emphasized the importance of developing reliable and valid assessment tools for measuring knowledge and practices related to osteoporosis. Moreover, research has shown that modifiable risk factors, such as diet and physical activity, play a significant role in the prevention of osteoporosis [24-26]. Our study results support these conclusions and suggest that the new questionnaire can be an effective tool for evaluating and improving osteoporosis prevention strategies.

Conclusions

The study demonstrated that the new questionnaire is a valid and reliable tool for assessing the impact of modifiable risk factors on morbidity due to osteoporosis. It can be used in various contexts to enhance preventive knowledge and practices, thereby contributing to a reduction in osteoporosis incidence. Further research is needed to refine and validate this questionnaire in broader populations to ensure its applicability and utility on a larger scale.

Competing interests

None declared.

Authors' contributions

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

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Patient consent

Obtained.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy in the Republic of Moldova, Minutes No.01, dated June 28, 2023.

Limitations

A significant limitation of this study is the inability to establish criterion validity, defined as the degree of agreement between the new questionnaire and another measure of the

construct that serves as a benchmark or gold standard. This limitation arises because no standardized analogous questionnaire was identified in the specialized literature. Most available questionnaires in the field are of the KAP (Knowledge, Attitudes, and Practices) type, which prevented the use of a recognized benchmark for criterion validation of the new questionnaire. In this research, the pilot testing phase of the questionnaire was not conducted because the overall sample size calculated for the main study was 296 individuals diagnosed with osteoporosis. Recruiting the required number of individuals with osteoporosis proved challenging due to time constraints and additional costs.

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RESEARCH ARTICLE

Disability-adjusted life years associated with liver-related complications among patients with Delta viral infection in Republic of Moldova

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ABSTRACT

Introduction. Disability-adjusted life years (DALY) is a multidimensional measure used to quantify specific tasks of the disease. Chronic liver disease attributed to hepatitis Delta virus (HDV) is one of the major causes that contribute to morbidity and mortality in our country. The DALY estimate in HDV-induced liver disease has the potential to highlight both fatal and nonfatal outcomes of the disease and thus, help in policy making and allocation of health resources.

Material and methods. This was an analytical prognostic study conducted in the 2021–2024 period, with the enrollment of 104 patients with HDV-induced liver disease. YLL was predictively rated using Child-Pugh's estimate of the remaining years of life. The chronic liver disease questionnaire was used to evaluate patients' disabilities along with the disability weights of the Global Burden of Disease study, calculating YLD. The impact of DALY was interpreted according to the patient's safety indicators.

Results. 104 patients with HDV, originating in different areas of the country, were evaluated, with an average age of 56 ±4.5 years. Substantial YLD losses caused by HDV were mainly reported in working class. The greatest losses of YLD and DALY were caused by cirrhosis of the liver, followed by hepatocellular carcinoma. A great rate of mortality attributed to HDV was seen in the 45-59 and 60-74 age group. A total of 739.1 YLL in male gender and 719.5 in females were recorded for cirrhosis. Overall, men attest a DALY value of 1358.44 total/29.53 per person, and women registered – 1477.3total /25.47 per person.

Conclusions. Chronic liver disease attributed to HDV is a medical challenge in Republic of Moldova. Loading the national medical system with serious and overwhelmed patients, make the DALY calculation approach a priority setting.

Keywords: Disability Adjusted Life Years, cirrhosis, Global Burden of Disease.

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Key messages

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

Although chronic liver disease attributed to hepatitis Delta virus is considered an orphan disease worldwide, Republic of Moldova represents an endemic region. The existing epidemiological tools are considered to be suboptimal in describing the general and social impact of the disease.

The research hypothesis

The research of DALY in chronic liver disease induced by HDV should lead to a quantitative understanding of the loss suffered by

patients in social and health resource allocation aspects and thus raising awareness among researchers and policymakers.

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

The DALY estimate in HDV-induced liver disease has the potential to highlight both fatal and nonfatal outcomes of the disease and can formulate global and national health policies by projecting and comparing various disease burdens.

Introduction

Chronic hepatitis Delta virus (HDV) -induced liver disease continues to be a major cause of premature mortality despite impressive advances in the prevention of chronic viral infections [1].

HDV prevalence is globally descending, a phenomenon explained by the introduction of primary prophylaxis with the approval of HBV vaccination programs. But despite this, endemic areas are still registered. Republic of Moldova is a region of „endemic pocket" for HDV, according to the latest international statistics [1-3]. The course of this infection is dependent on the condition of infection with HBV (coinfection or superinfection), as well as a multitude of viral and host factors such as age, gender, immune status, HBV genotype and/or HDV genotype, HBeAg status that will direct the disease to possible moderate or even fulminant progression or induce chronic liver process. The peculiarities of chronic liver disease attributed to HDV in the evolutionary-progressive framework, note multiple fatal complications in the younger age of the patient [4-6].

According to the National Liver Transplant Registry, about 70% of patients on the waiting list have terminal HDV-induced liver disease [7]. Although it is associated with high mortality, the decompensation of HDV-induced liver disease is one of the preventable causes of death worldwide [1, 5]. The main objective of this study is to estimate the DALY of HDV liver disease and make a comprehensive quantification of the burden of the disease in terms of years lived with disability. It can be used effectively to educate people about the year lost due to the premature death of a patient who develops HDV-induced cirrhosis and the time these patients lose from disability cause. This step can allow policy makers to understand the severity of the problem more explicitly and encourage more effective public spending on health and disease prevention [8].

We mention that the course of infection with the delta hepatitis virus also depends on the condition of infection, according to which we may have coinfection between HBV and HDV and superinfection between HDV and chronic HBV infection. Coinfection between these two viruses more commonly develops forms of severe or fulminant hepatitis than acute viral infection B. On the other hand, acute infection with HDV is imperceptible to an ordinary infection with HBV, from a clinical and histological point of view [2, 3, 5]. Thus, the clinical course of chronic HDV infection is still dependent on the course of liver disease induced by chronic B viral infection. Chronic viral hepatitis Delta is more severe and rapid-progressive versus other chronic viral hepatitis known. This leads to cirrhosis in about 70% of patients within 5 to 10 years, usually involving the young working class. The risk of developing cirrhosis is 3 times higher in case of a HDV infection than in case of a mono-HBV infection [5, 9-11].

Material and methods

For the social impact assessment based on the DALY indicator, 104 patients (46 men and 58 women) with HDV-induced liver disease, mean age of 56±4.5 years, were selected. Demographic distribution of patients Central area – 54%, South area – 36%, North area – 10%. There were also analyzed aspects of the natural evolution of the disease with the progression to cirrhosis of the liver, taking into account further and installed complications that can affect the quality of life, such as ascites, hepatic encephalopathy, variceal bleeding, development of hepatocellular carcinoma (HCC) or death. This is an analytical prognostic study. The representative research batch was calculated in the F tests Program - ANOVA: Fixed effects, omnibus, one-way Analysis: Calculations are based on the following parameters: the confidence interval for 95.0% of significance of the results, statistical power – of 80.0%. Result: noncentality parameter = 9.9375000, critical F = 3.0540042, numerator df = 2, denominator df = 175, total for research = 194. The given study is part of the research protocol that obtained a favorable opinion from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes No.5, dated 17.06.2022). The study is still continuing. The patients enrolled in the study were hospitalized in the Hepatology Department of a tertiary Centre and Therapy section of the Medical Service of Municipal Centre Chisinau for 2021-2024 years interval. Data on the onset of the disease, duration until liver cirrhosis, progression in months/years until the registration of complications or death were collected from the discussion with patients, by collecting anamnestic data, analysis of the medical archive, and the medical record of the patient from the stationary 003/e form.

Clinical informatics data were being analyzed in Microsoft Excell and SPSS statistical analysis software. Several data processing steps were undertaken.

Qualitative representation of the progression of the disease by ordering all relevant health results related to the disease over time, with reflection of the intense dynamic effect of the disease were taken into account. The DALY components, namely the years of life lost due to premature death (YLL) and the years lived with disability (YLD), were calculated separately for all health outcomes included in the final tree, initially stratifying the impact in patients with moderate liver disease then the impact on progression to

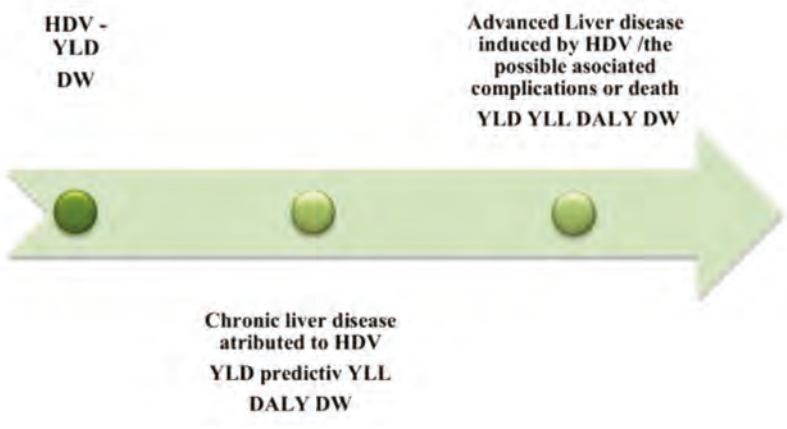

Fig. 1 HDV-induced liver disease and the determinants needed Note: DALY (Disability-adjusted life years) components, namely the years of life lost due to premature death (YLL), the years lived with disability (YLD), predictive YLL, DW-Dissability weight* according to GBD

advanced liver disease, then the association of complications or death. Thus, we aim to mark the scale of disease progression and its impact in years of life, at each stage of the disease (Fig.1).

Next, we are going to analyze the social impact produced by HDV-induced liver disease through the following methodology: initially, the WHO-proposed indicator for this purpose is YLD – years lived with disability or altered behavior and/or motor faculties. In order to estimate YLD for a particular disease over a certain period of time, the number of cases in this period is multiplied by the average duration of the disease and the impact coefficient of disability, and, reflecting the severity of the disease on a scale from 0 (perfect health) to 1 (death). The disability impact coefficient is found in the Global Burden of Disease (GBD) source [12-14]. The basic formula without applying the social aspects for calculating YLD includes the following

$$YLD = I \times L \times DW$$

In this formula I – number of incident cases in the population, DW – disability weight of specific condition, and L – average duration of the case until remission or death (years).

How much a medical condition affects a person is called the *disability weight* (DW). This is determined by disease or disability and does not vary with age. Tables have been created for thousands of diseases and disabilities. In the GBD terminology, the term disability is used broadly to refer to departures from optimal health in any of the important domains of health in GBD. According to research analysis of the Global Burden of Disease 2019 Study, leading causes of global DALYs, percentage of total DALYs, and percentage change in number of DALYs and age-standardized DALY rates from 1990 to 2019 for both sexes combined for all ages, cirrhosis of the liver ranks as 16th leading causes of disability-adjusted life years, in the < 49 age group being the 12th cause. Cirrhosis ranked seventh among those aged 50-74 years in 2019. With low-cost treatments available to low-income and middle-income countries, there is an opportunity to eradicate hepatitis C, but still remains a problem regarding HDV. Childhood vaccinations for hepatitis B will eventually also reduce cirrhosis (and HCC) outcomes, but the full effect will probably not be apparent for years. Its contribution to the global disease burden has increased from 24.3 million in 1990 to more than 31 million in 2019, which represents a 27% increase in DALYs. But while health has improved, after accounting for population growth and ageing, the absolute number of DALYs has remained stable [14].

Results

Referring to our study compared to the average duration of a case of disease in a patient with Delta viral hepatitis in males – 18.86, is recorded a total YLD of 127.86, and in the case of liver cirrhosis at an average duration of a disease case of only 6.20, there is a total YLD of 619.34 and a media of 14.90 (95% Cl: 3.95-33.77), this means that advanced liver disease shows a substantially impact on patients. Table 1 provides an average age of onset of advanced liver disease in men 45.35, and 45.87 women with an average duration of one case from its onset to enrolment in the study of 6.2 in the male gender and 6.56 in women. The highest value of YLD is recorded in the group of men within age category of 60-74 years being 23.43 YLD, while in women a higher YLD number remarks in 30-44 category with a value of 20.69 YLD

Table 1. Years lived with disability due to HDV-induced liver disease by gender-dependent age groups (in YLD)

No.	Age group	Patients gender	Nr. Of cases	Mean age of the onset of the disease	Average duration of the case	DW	Years lived with disability YLD
1	30-44 (16)	M	10	33.4	5.2	0.716	124.35/12.43
		F	6	34.67	5.0	0.716	124.11/20.69
2	44-59 (45)	M	24	45.17	6.58	0.716	212.80/8.86
		F	21	46.71	6.71	0.716	312.70/14.89
3	60-74 (43)	M	12	57.5	6.83	0.716	282.19/23.43
		F	31	56.25	7.97	0.716	320.99/10.35
Total/Average		M	46	45.35	6.20	-	619.34/14.90
	F		58	45.87	6.56	-	757.80/15.31

Note: YLD - Years lived with disability due to HDV-induced liver disease, DW - Disability weight according to GBD [2,14]

Compared to the average duration of a male patient's disease case at an average duration of a disease case of only 6.20, there is a YLD total of 619.34 and a per person media of 14.90 (95% Cl: 3.95-33.77), this explains the severity of the disease, with substantial social impact. Thus, among female patients at an average duration of a case of 6.56 with a total YLD of 757.80 and an individual media of (95% Cl : 2.43-28.18). When the disease progresses to mortality 17 patients had an YLD with a value of 1668.7 in men and 1102.67 in women.

As a component in math formula for DALY specifically important is YLL – the life years lost as a result of premature deaths practically correspond to the number of deaths caused by a disease multiplied by the indicator of life expectancy at the age at which death occurs. The basic formula for YLL (not including other social aspects) is the following for a particular disease, age and gender; includes the following: YLL = N x L where: N – number of deaths, L – life expectancy at the age of death in years [8].

Although the YLL measure was proposed in the 1940s, the creation of the GBD and its associated methodological developments have increased awareness and understanding of its application, although many issues are still being challenging. First, it is important to articulate that the 'true' YLL can never be observed and as such, a language indicating that YLL estimates have been under-estimated or over-estimated or maybe pseudo-interpreted. From the perspective of public health policy information, the element to be applied in the YLL estimate is that of an ideal, aspirational standard based on low mortality risks [15]. Premature mortality refers to the anticipated death for the age at which the person could have survived if they were part of a model-standardized population with a life expectancy equal to that of a maximum long-term population in the given region, information reflected individually by each country, specific data of the Republic of Moldova is generated by the National Bureau of Statistics last edition 2022 [13-15]. To estimate population health loss due to advanced liver disease is a largely contested fact. This has led to the application of different approaches for estimating lost life years due to premature mortality (YLL). The YLL was predicted by using the Child-Pugh estimate of the remaining years of life. Based on recent laboratory reports of bilirubin, albumin, prothrombin time (INR), and clinical assessment of ascites and encephalopathy, the Child-Pugh Score calculator identifies patients in one of three risk classes: class A (5 to 6 points): Life expectancy is 15-20 years, class B (7 to 9 points): Life expectancy is 4-14 years, class C (10 to 15 points): Life expectancy 1-3 years.

The Child-Pugh score was used to estimate the remaining life years and this number was subtracted from the ideal age-specific life expectancy to allow us to calculate YLL not just for the 16.34% of patients from the total group, this data reflecting the mortality rate.

The index of YLL due to early mortality first was calculated for the 17 deceased patients, the mean age of onset of liver cirrhosis was 45.84 years with mean disease duration of about 16.95 years. Preterm mortality associated with advanced liver disease induced by HDV with the highest incidence was attested in the 30-44-year age group (Table 2). Of the total age groups, 45–59-year-olds had the highest YLL index for both women and men with a total of 164 YLL. For women, the highest rate of major premature mortality associated with advanced HDV-induced liver disease is recorded in the 44-59-year group – 79.2 YLL. The data for 30-44-year group, men lost 94.2 YLL. The data presented reveal that premature mortality associated with advanced HDV-induced liver disease and the fact that the disease occurs more frequently in the 45–59-year age group, the lowest values of YLL were in the 60–74-year-old group where the YLL number was 46.0 for men and 56.4 for women. Overall, men lost 225.6 years as a result of premature mortality, and women – 135.6 years. By the age of about 59 the proportion of YLL increases, after which a slight decrease is subsequently perceived (Table 2). Moreover, we are seeing an increase in the burden of male mortality, which may indicate the fact of a later addressability. This situation underlines the need for effective prevention policies aimed at men's health.

Thus, with all the above mentioned, we set out to calculate the DALY index, by predictive YLL, in living patients according to the severity of the disease by means of Child Pugh classification, this phenomenon being also applied in other studies. So we were able to establish a predictive YLL in 46 males with a total of 246.36 YLL in a mean age of death 58.34 and in 58 women a value of 239.83 predictive YLL in a mean age of death 64.55 (Table 3).

Table 2. Life years lost as a result of premature mortality associated with advanced liver disease induced by HDV by gender-dependent age groups, for deceased patients (in YLL)

No.	Age group	Gender	N. of cases	Mean age of death	Life expectancy at the age of death in years*	YLL
1	30-44	M	3	38.66	31.6	94.8
		F	0	0	0	0
2	45-59	M	4	51.25	21.2	84.8
		F	3	52.57	26.4	79.2
3	60-74	M	4	66.5	11.5	46.0
		F	3	61.33	18.8	56.4
Total/Mean		M	11	52.11	20.4	225.6/20.5
F			6	56.95	23.0	135.6/22.6

Note: YLL - Life years lost as a result of premature mortality

**according to the data of the National Bureau of Statistics of the Republic of Moldova 2022 [16]*

Table 3. Life years lost as a result of premature mortality associated with advanced liver disease induced by HDV by gender-dependent age groups, (in predictive YLL)

No.	Age group	Gender	N. of cases	Mean age of death	Life expectancy at the age of death in years*	Predictive YLL
1	30-44 (16)	M	10	45.30	25.7	250.70
		F	6	48.50	30.0	180.00
2	45-59 (45)	M	24	59.41	15.6	374.40
		F	21	69.71	12.7	266.70
3	60-74 (43)	M	12	70.33	9.5	114.00
		F	31	75.45	8.8	272.80
Total/Mean		M	46	58.34	16.93	246.36/5.35
F		58	64.55	17.16	239.83/4.13	

Note: Predictive YLL-predictive life years lost as a result of premature mortality,
**according to the data of the National Bureau of Statistics of the Republic of Moldova 2022 [16]*

Here in the context of the disease state with evolution in death we allow ourselves to calculate DALY the indicator proposed by WHO for this purpose – (Disability Adjusted Life Years). DALYs are calculated by taking the sum of these two components: DALY = YLD + YLL.

The DALY relies on an acceptance that the most appropriate measure of the effects of chronic illness is time. One DALY, therefore, is equal to one year of healthy life lost [14].

Table 4. DALY associated with advanced liver disease induced by HVD by age groups dependent on person-gender

No.	Age group	Gender	N. of cases	YLD	Predictive YLL	DALY	
						Total	Per person
1	30-44	M	10	124.35	250.70	375.05	37.50
		F	6	124.11	180.00	304.11	50.68
2	45-59	M	24	212.80	374.40	587.20	24.47
		F	21	312.70	266.70	579.40	27.59
3	60-74	M	12	282.19	114.00	396.19	33.01
		F	31	320.99	272.80	593.79	19.15
Total by groups		M	46	619.34	739.10	1358.44	29.53
F		58	757.80	719.50	1477.30	25.47	
Media by groups		M		206.44	246.36	452.81 (95% Cl: 162.51-743.11)	
F			252.60	239.83	492.43 (95% Cl: 86.89-897.97)		

Note: *YLD - Years lived with disability, predictive YLL- Life years lost as a result of premature mortality predictive, DALY Disability Adjusted Life Years*

Based on the above data, the value of the DALY indicator lost by the three groups according to age can be determined (Table 4).

Thus, it was established that male patients aged 30-44 years lost 375.05 DALYs and per person 37.5 DALY. Men aged 45-59 years lost 587.2 DALYs per person – 24.47 DALY; in the age group of 45-59 years female gender losses were: 579.4 DALY. At the age group of 60-74 years it was determined that the men lost – 396.19 DALYs, per person – 33.01 DALY; and in women of this age – 593.79 DALYs and 19.15 per person. With a total of 1358.44 DALY men, that is, in the environment each of them losing about 29.53 DALY as a result of disability during the period they were sick and because of premature death caused by the disease. While the 58 women lost 1477.3 DALYs or an average of 25.47 per person. The media per groups reflected in table indicate 452.81 (95% Cl: 162.51-743.11) in males, and 492.43 (95% Cl: 86.89-897.97) in females. We attest a DALY value per person higher in the group of young people, people able to work.

17.64% of the total number of enrolled patients developed HCC, with installation at an average age of 61 years, of which 35.29% resulted in death within the first 3 months of diagnosis. In males a media value of 20.24 (95% Cl: 14.59-25.89) YLL, in females 18.28 (95% Cl: 14.13-22.44) YLL. While the value of YLD With the advancement of the hepatic process, with progression towards the onset of complications such as the presence of liver cancer, the study attests a DALY value per person increasing – DALY per person in males being 32.01 and females 39.69 (Table 5) compared to patients without any, DALY per person male 31.66 and female-32.47 (Table 4).

YLL contributes in over 51% of the total DALY, so as YLD 48%, meaning that we have impairment in quality of life determined by associated disabilities, and an early mortality (Table 6).

Composite indicators, such as DALY, is a multipurpose tool for health planning that allows more transparency in showing the impacts of mortality and morbidity of HDV induced liver disease. Thus, health outcomes are potentially highlighted with the aim of making better health-related decisions in the field of current national hepatology.

Table 5. DALY indicator in HCC on the background of HDV-induced liver cirrhosis by gender-dependent age group (in YLD)

No.	Age group	Gender	Onset medium age	Average duration of a case	DW**	YLD	YLL	DALY	DALY
								Total	Per pers
1	44-59	M (6)	28.50	19.66	0.857	101.09	144.8	245.89	40.98
		F (2)	46.00	7.00	0.857	11.99	51.20	63.19	31.59
2	60-74	M (3)	51.33	12.33	0.857	31.70	37.4	69.1	23.03
		F (6)	24.42	37.28	0.857	191.69	95.1	286.79	47.79
Total/Media F (8)		M (9)	39.91	28.47	-	132.79 14.75	182.2/l 20.24	314.99	32.01
			35.21	22.14	-	203.68 25.46	146.3/ 18.28	349.98	39.69

Note: *YLD - Years lived with disability, YLL- Life years lost as a result of premature mortality, DALY- Disability Adjusted Life Years*
***according to the data of Global Burden Disease 2019 [2, 14].*

Table 6. The relative share of YLD and YLL in total DALYs.

	Sum	% from DALY
YLD	459.04	48.57%
YLL	486.19	51.43%
DALY	945.24	100%

Note: *YLD - Years lived with disability, YLL - Life years lost as a result of premature mortality, DALY Disability Adjusted Life Years*

Discussions

The World Health Organization proposed the goal of eliminating viral hepatitis by 2030 as a target that's unfortunately still untouched [1]. There are several studies that reflect the actual worldwide epidemiological status of viral hepatopathy, being of particular interest different ways that hepatitis indifferent of etiology HBV (hepatitis B Virus), HCV, HDV or others impacts the patient and society. A prominent leader in the global hepatitis community is Su Wang, scientist reflects the impact of hepatitis and states that one of the most common form of hepatitis is being HBV. In one of the biggest studies Wang et al. mentioned that hepatitis B-related deaths are increasing significantly in countries with high socio-demographic index, such as the UK, the US and Canada, and the social impact of the disease is of substantial interest [5] that's why the Viral Hepatitis Programs are still considered methods to influence the problem from the root, but we should reconsider the individual, family and society impact of the disease.

The data from China, reflected in a study that aimed to reveal the three-decade dynamics of the natural history of HBV infection in the 1990-2019 period, age-standardized incident rate, age-standardized DALY rate, was used, and the age-standardized mortality rate to quantify the burden of HBV disease per 100,000 person-years. The incidence of cirrhosis and other chronic liver disease decreased significantly between 2010-2015 and remained stable from 2015-2019. The age-standardized incident rate and liver cancer age-standardized death rates remained stable over the period 2010-2019 expect the incidence of HBV-related liver cancer in the period 2019-2044 to decrease by 39.4% also demonstrates, a 36.5% increase in the burden of hepatocellular carcinoma from the total number of DALYs lost due to chronic hepatitis B infection and a 25.8% increase in the total burden of acute viral hepatitis, although there was a decrease of 46.1% in this country on the social impact/ hepatic cirrhosis burden [2].

In Brazil, medical scientists have also proposed to evaluate the social impact of liver pathology (referring to hepatitis C, hepatitis B and alcohol-related diseases). Carvalho together with the associations [12] concluded that chronic viral hepatitis and cirrhosis of the liver are responsible for a significant burden in Brazil, mainly affecting men and individuals in the producing age. 57,380 DALYs (30.3 per 100,000 inhabitants) are declared attributable to chronic hepatitis B and cirrhosis due to hepatitis B, with 41,262 DALYs in men. Most of the weight is caused by YLL (47.015 or 24.8/100.000), rather than by YLD (10.365 or 5.5/100.000) [12].

Due to the paucity of information related to HDV, the latter has not been analyzed.

Overall estimates of HBV infection recorded in 2022 report about 258 million chronically infected people [15]. The epidemiological condition in relation to HDV infection is quite robust. Registered in some global regions with orphan disease status, other regions are "enjoying" with intense popularity. The known situation is that HDV is a RNA satellite virus that requires HBV to assemble and introduce de novo viral particles. But as a continuation of the natural progression of the disease, infection with HDV dramatically accelerates the progression of liver disease to liver failure or HCC about 3 times higher than in patients with HBV monoinfection, with a manifest contribution to premature mortality, with a 2 times higher risk [9, 17, 18].

Several studies have used the DALY indicator to estimate the impact of chronic hepatitis B and C in different countries or regions [12-17]. According to the global study of the burden of diseases, a 25% increase in the level of viral hepatitis B burden worldwide to a thousand population, between 1990 and 2010. In the Wang et al. study, the authors proposed assessing the burden of the disease through hepatitis B attributable to globally and regionally modifiable risk factors in 204 countries and territories between 1990 and 2019 [5]. One third of the total burden of the disease is attributed to smoking status, alcohol consumption and a high body mass index (BMI), which is increasing during the considered period. Although the burden of hepatitis B attributed to tobacco and alcohol consumption decreased, regional disparities in disease burden and time trends differed. Statistically, the study reflects 33.73% of standardized hepatitis B deaths and 34.52% of DALY deaths attributable

to smoking status, alcohol consumption and a high BMI. A similar pattern was observed for HCC secondary to HBV, in which about one-third of deaths and DALY were attributed to modifiable lifestyle risk factors [5].

Another study reflecting the state of HBV in China mentions the incidence and declining viral hepatitis DALYs from 1990 to 2016. However, the prevalence still remains at a high level. In 2016, age-standardized DALY rates for hepatitis B were about 9.1/100,000 for mid-high-level regions according to the socio-demographic index 17.4/100,000 for mid-level regions and 56.4/100,000 for medium-low level. Although the age-standardized prevalence rate remains high, the DALY rate fell sharply by 85.5% from 1990 to 2016. This could be partly explained by widely used antiviral therapy. The highest absolute number of DALYs was observed in the 15-49-year-old group in 2016. The highest rate of DALY occurred in men aged 50-69 years and in women aged ≧70 years [13].

The continued increase in the global burden of liver cirrhosis and HCC requires collaborative prevention and control from government, prevention and control departments at multiple levels. Surveillance of patients affected by the HDV continues to be suboptimal, thus masking the real burden of this disease. In the context of poor clinical management, which continues to be a challenge, it remains only to opt for a balanced patient monitoring program, based on the maximum prophylaxis of the installation of complications and slowing down the progression of the disease. As a stage of prevention of HDV infection, we consider justification by promptly implementing measures designed to control HBV. Further efforts are needed, including supplier awareness, patient education and measures to improve the quality of the health system. Let us not forget about the peculiarity that distinguishes HDV, from the bitterness of the rate of evolution of the natural history of the intensely progressive infection. Thus, future medicine in the field of HDV a=induced liver disease relies on the development of surveillance strategies, a phenomenon really depending on strategies of political influencing factors such as Health Policy programs, so in order to direct such programs, we need to prove the existing social impact of the disease in math formulas and stable epidemiological instruments such as DALY, YLD, YLL, their implementation, with expectations of decreasing the social impact of the disease [13, 19-20]. As we mentioned earlier the HDV infection is associated with a higher risk of advanced liver disease in a short period of time, health management and early screening should be strongly applied to this patients [12]. Analyzing the burden of HCC in the enrolled patients, we attest an impairment in men at the productive age. We believe that our study will help to elucidate the characteristics of the national burden of HCC and influence more effective and target prevention strategies.

Health interventions to decrease and control viral hepatitis HDV would cut down the burden of advanced liver disease and reduce the total life years lost to this disease by the community.

Conclusions

1) Premature mortality associated with viral cirrhosis B and D in the study, with the highest incidence is attested in the age group 30-44 years. Patients aged 45-59 years had the highest YLL index for both women and men with a total YLL of 641.1. Overall, men lost more years of their lives compared to women.
2) Males exhibited a greater health burden (29.53 age-standardized DALYs per person) than females (25.47 DALYs per person). One DALY representing the loss of the equivalent of one year of full health.
3) The results obtained oblige us to adapt to the health system, with a strategic planning of medical services and the provision of timely intervention measures, especially for working age groups where the value of the YLL and DALY indicator is increased.

Competing interests

None declared.

Patient consent

Obtained.

Ethics approval

This study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes no.5 from 17.06.2022).

Authors' contribution

AT and OS conceived the study and participated in the study design. EC participated in the study design, recruited eligible patients, completed the study questionnaire, analyzed the data in Excel, performed the statistical analysis, and drafted the manuscript. All authors have read and approved the final version of the article.

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RESEARCH ARTICLE

The importance of mucogingival flaps in guided bone regeneration in the jaws

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ABSTRACT

Introduction. Guided bone regeneration (GBR) is a surgical method that allows the formation of new bone in areas of atrophy of the maxillary bone. The integration of the graft or the augmented bone under the mucosal flap and the primary healing of the soft tissues are essential conditions for preventing the exposure of the regeneration site and infectious complications that inevitably lead to the failure of the GBR procedure.

Materials and methods. This study presents the results of a cohort study that includes 70 patients who underwent GBR. The research involved techniques for forming muco-periosteal flaps: the Modified Periosteal Releasing Incision (MPRI) according to the principle of the double flap technique (DF) and the coronal advanced lingual flap (CALF). The study group included patients who underwent GBR using perforated titanium membrane, while the second group underwent GBR using bioresorbable poly-4 hydroxybutyrate (P4HB) synthetic mesh. The patients were evaluated periodically to monitor postoperative progress, the cases of dehiscence of the area related to augmentation site were recorded, measured and classified according to Fontana. Statistical results were generated and processed by the R Studio program.

Results. In the study groups, a total of 8 cases of gingival dehiscence were registered, 4 cases in each group. In two cases, partial removal of the titanium membrane was performed by milling it, and the remaining 6 cases of dehiscence were remedied with rinses with oral antiseptic solution and scheduled visits for local care until the appearance of granulation tissue and epithelization.

Conclusions. Protective membrane, flap formation, thread tension and suture relaxation all play crucial roles in guided bone augmentation without dehiscence. In our study, the small number of dehiscence cases recorded as complications did not provide significant statistical results, namely due to the technique of performing the flap according to contemporary methods, a fact also described by the specialized literature.

Keywords: guided bone regeneration (GBR), flap.

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Key messages

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

This study argues that the implementation of a new bone augmentation method guided by an absorbable barrier membrane is reliable and does not present more complications than the already existing method.

The research hypothesis

New techniques for forming mucogingival flaps are crucial in the prevention of dehiscence in guided bone regeneration operations regardless of the chosen technique.

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The novelty added by manuscript to the already published scientific literature

This article has demonstrated the importance of correct formation of mucogingival flaps, regardless of the type of membrane.

Introduction

Guided bone regeneration is a surgical method that allows obtaining a new bone in places of atrophy of the maxillary bone. The technique aims to restore sufficient bone volume to insert dental implants in an adequate way. The integration of the graft or the augmented bone under the mucosal flap and the primary healing of the soft tissues are essential conditions for preventing the exposure of the regeneration site and infectious complications that inevitably lead to the failure of the Guided Bone Regeneration (GBR) procedure. In the opinion of Thoma *et al.* the exposure of the membrane in the oral cavity has a significant impact on the overall result of the surgical procedure, leading to a reduction in the height of the augmentation between 40% and 60% [1, 2].

Wang and Boyapati introduced 4 minimal key principles that must be respected for GBR surgery to be successful: primary wound healing; angiogenesis; blood clot stability and maintaining augmentation space [3]. To ensure primary wound healing, flap shape and mobilization techniques play a crucial role in achieving complete closure of the augmented site. According to the authors Machtei *et al.* [4], these principles must be respected especially when using the GBR technique and performing vertical augmentations in the posterior area of the mandible, where the soft tissues are thin, and the edges of the flaps must be stretched. In these areas, the exposure of the membrane occurs most frequently [5, 6]. Therefore, optimal soft tissue management is required to achieve tension-free primary flap closure and to prevent infection of the membrane and augmentation material [7].

Burkhardt *et al.* reported a risk of dehiscence of 10% if the flap tension before suturing is less than 10 g and increases substantially to 40-100% if the flap tension exceeds 10 g [8]. All flaps sutured at more than 25.5 g show dehiscence, a low-tension flap suture can be achieved by combining an external-internal suspended suture, which greatly reduces tension at the flap edge, sutured with a second tension-free suture closer to the edge wounds [8]. Suture material and proper wound management with release incisions are also of considerable importance.

The shape of the flap is key to reducing post-bone augmentation complications. Zazou *et al.* recommended that for the coronal advance, it is necessary to perform a deperiosteal incision at the junction of the fixed and mobile mucosa in the case of the vestibular flap and to carry out tight muscle debridement in the case of the lingual flap [9]. The author argues this modification of the incision provides a larger keratinized gingival sleeve to the lingual flap. Another important factor highlighted by Kim *et al.* proposes to make the flap without discharge incisions, they explain this particular approach preserves vascularization and venous return supply of the flap [2].

Dehiscence is known to be a major problem in bone regeneration surgery, according to authors such as Canullo *et al.* the important factor is not the technique of performing bone regeneration, but the shape and type of mucogingival flap prepared and advanced coronally, which will cover the regeneration [10]. Other authors such as Gallo *et al.* in a study conducted in 2019 on 80 cases obtained about 68% complications with exposure; they believe that the separation device is responsible for the occurrence of dehiscence [11]. By simply using collagen membranes over the titan devices, Urban *et al.* significantly decreased the rate of dehiscence to 3% of 65 sites undergoing regeneration [12]. Lately there has been increased emphasis on research that includes new techniques for the preparation of mucogingival flaps [13, 14].

Materials and methods

This study presents the results of a cohort study, that includes 70 patients who underwent guided bone regeneration operations. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki, approved by the Research Ethics Committee of *Nicolae Testemițanu* USMF, minutes No. 43 of 13.02.2020. The research is a multicenter one, conducted during the period 2019-2024, enrolled patients who applied for surgical treatment of guided bone augmentation within the *Arsenie Guțan* Department of Oro-Maxillo-Facial Surgery and Oral Implantology at the clinical base in Chisinau Municipal Dental Center and at the "MASTERDENT" SRL university clinical base.

The research included the use of the techniques for forming muco-periosteal flaps: the Modified Periosteal Releasing Incision (MPRI) according to the principle of the double flap technique (DF) and the coronal advanced lingual flap (CALF).

Patients were divided into two groups of 35 subjects each, as calculated using EpiInfo 7.2.2.6 program, "StatCalc – Sample Size and Power" compartment for analytical study based on the following parameters: 95.0% confidence interval a statistical power of 80.0%.

Inclusion criteria:

70 patients, aged between 20 and 60, both female and male, were included in the study. The subjects included in the research were patients who presented atrophy of the alveolar process, diagnosed clinically and paraclinically, making it impossible to insert a dental implant.

Exclusion criteria:

- Patients with coagulation disorders
- Pregnant and breastfeeding women

- Smokers more than five cigarettes a day
- Patients with unsatisfactory hygiene
- Patients with chronic otorhinolaryngological pathologies
- General pathologies such as: diabetes, glycemic values >7-8 mmol/l, oncological pathologies, diseases of the hematopoietic system, autoimmune diseases, liver cirrhosis, cardiac and respiratory pathologies.

I group – included patients to whom it was performed GBR using perforated titanium membrane;

II group – GBR was performed by using bioresorbable poly-4 hydroxybutyrate (P4HB) synthetic mesh.

The patients were evaluated periodically to monitor postoperative progress. Cases of dehiscence of the area related to augmentation site were recorded, measured and classified according to Fontana *et al.* [14]. Statistical results were generated and processed by the R Studio program. The following descriptive statistics were estimated for the numerical variables: minimum value, maximum value, mean value with standard deviation, median value with interquartile deviation (AI). For all the statistical tests applied in the study, the threshold value (p) was considered to be 0.05, completed with 95% confidence intervals for the relative frequencies.

Results

In our research, 70 patients underwent guided bone regeneration using resorbable polymer meshes and titanium membranes. All patients were operated with the creation of the mucogingival flap according to modern methods, using the "MPRI" technique to create vestibular flaps and the "CALF" technique for lingual flaps. Of these, 25 patients were operated on the lower jaw- mandible using the "MPRI" vestibular flap method and the lingual "CALF" method, another 45 patients were operated on the upper jaw using the "MPRI" vestibular flap method (see table 1).

Table 1. Demographic and clinical data

Number	70
Gender: Male Female	 24 (34.3%) 46 (65.7%)
Age	53.1 (range, 27 – 60)
Lower jaw operated patients "MPRI" vestibular flap combined with "CALF" technique for lingual flaps	25
Upper jaw operated patients "MPRI" vestibular flap	45

When analyzing the data, a total of 8 cases of complications with gingival dehiscence were registered, of which 4 cases were grade I dehiscence and 4 cases were grade II dehiscence according to Fontana.

In the control group where the titanium membrane was used, three cases of dehiscence were recorded, accounting for 5.7% (95% CI 1.21). Of these, grade I dehiscence – one case, located in the upper jaw where it was performed the „MPRI" flap and constituting 2.9% (95% CI 0.15,17). Grade II dehiscence occurred in two other cases, constituting 5.7% (95% CI 1.21), located on the mandible where the „MPRI" vestibular flap and „CLAF" lingual flap were performed. The remaining 32 cases in this group did not show any dehiscence, the ratio being 91.4% (95% CI 76 .98).

In the study group – five cases of dehiscence were observed as follows: grade I dehiscence were recorded in three clinical cases, constituting 8.6% (95% CI 2.2, 24), among which two cases were located in the mandible where was performed „MPRI" vestibular flap combine with „CLAF" lingual flap and one case in the upper jaw where it was performed the „MPRI" flap. Grade II dehiscence were in two cases – 5.7% (95% CI 1.21), both located in the mandible where it was performed the „MPRI" vestibular flap and „CLAF" lingual. The remaining 30 cases did not show any form of dehiscence, the proportion being 85.7% (95%CI 69, 95).

The management of dehiscence were grouped according to the degree of complications, as follows:

- I group (control group) – dehiscence grade II were treated by partial removal of the membrane; the titanium membrane was performed by milling it, as recommended by Al-Ardah *et al.* [15] figure 1 (A).
- II group (resorbable mesh group) – dehiscence grade I (4 cases, 5.7% (95% 1.8,15)) of gingival dehiscence were remedied with rinses with „Loroben" oral antiseptic solution and scheduled visits for local processing with 10% Polyvidon Iodine solution and 0.05% Chlorhexidine solution until the appearance of granulations and epithelization, the grade II dehiscence was treated by partial removal of the mesh by surgical scissors, figure 1(B).

It should be specified that all cases of dehiscence were treated early.

The remaining 62 respondents did not develop dehiscence complications and constituted 88.6% (95% CI 78, 95).

In table 2 we can see the general characteristics of the entire research group of dehiscence complications, and we can state that a total of 8 cases of dehiscence were recorded in the research.

Table 2. Representation of "Gingival dehiscence" values

Variable	N = 70	95% CI
Dehiscence grade		
Grade 0	62 (88.6%)	78%, 95%
Grade I	4 (5.7%)	1.8%, 15%
Grade II	4 (5.7%)	1.8%, 15%

Note: N- number of patients, CI -confidence interval

For categorical variables, absolute frequencies and relative frequencies were estimated, supplemented with 95% confidence intervals for relative frequencies. Visualization was performed using barplot graphics (bar diagram). Hypotheses testing was conducted using Pearson's Chi-square test (Monte Carlo variant with 10,000 repetitions). In the study, the dehiscence association between

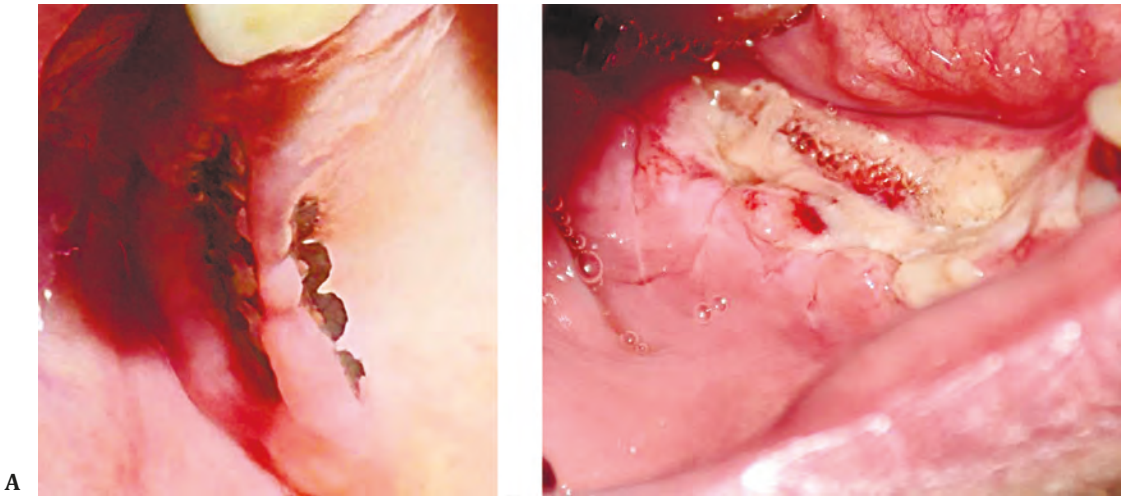

Fig. 1 Types of dehiscence, by following techniques:
A. Dehiscence occurring after GBR treatment with titanium membrane in the upper right jaw.
B. Dehiscence after GBR treatment with P4HB in the lower jaw on the right.

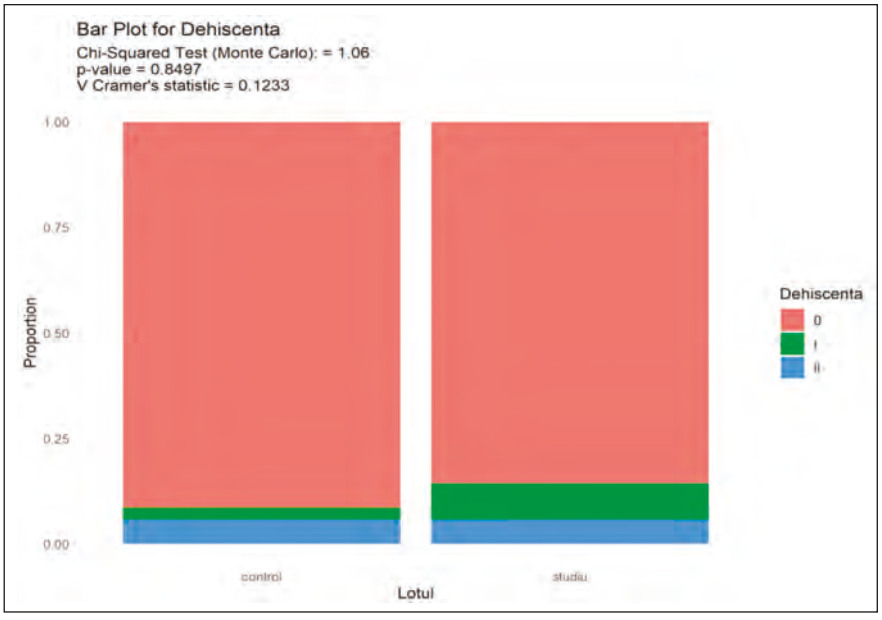

Fig. 2 Bar plot for the association values of flap formation technique and occurrence of dehiscence.

Note: *Applying the χ2 test (Monte Carlo variant) = 1.06, p=0.84, which means that there are no significant statistical differences, the result being unchanged even after applying the correction for multiple comparisons using the Hochberg method where p>0.9. Cramer statistical test - 0.12, which shows a value with no practical importance.*

the performed bone regeneration techniques and the appearance of dehiscence was evaluated (see figure 2). The null hypothesis formulated was that there is no association between the applied technique and the appearance of gingival dehiscence, the alternative hypothesis being the presence of the association.

Applying the χ^2 test (Monte Carlo variant) = 1.06, p = 0.84, which means that there are no significant statistical differences, the result being unchanged even after applying the correction for multiple comparisons using the Hochberg method where p > 0.9 (see figure 2).

The Cramer statistical test applied to estimate the effect size showed a value of 0.12, which shows a value with practical importance and presents a reduced phenomenon in our research.

Discussion

Recently, several techniques of flap formation have been proposed to achieve proper wound closure. Ronda *et al.* proposed a flap technique to achieve coronal displacement of the lingual flap in augmentation of the mandibular bone ridge in the posterior areas – a full-thickness lingual flap must be raised and partially detached from the mylohyoid muscle [16, 17].

Urban *et al.* describes this technique, dividing the surgical approach of the lingual flap into 3 different areas, as illustrated in figure 3 [18, 19]:

- zone I: tunneling and elevation of the retromolar lampon.
- zone II: separation of the flap with preservation of the mylohyoid muscle.
- zone III: anterior periosteal release, semibont.

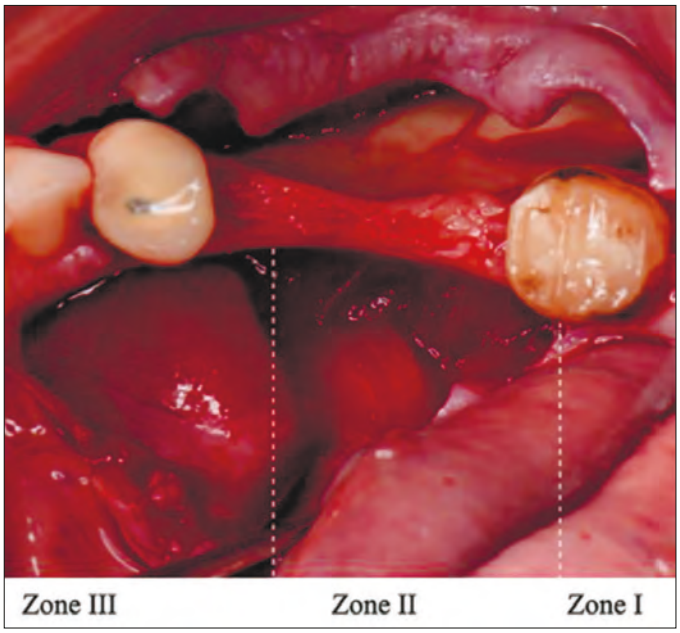

Fig. 3 The shape of the flap and the location of the surgical access areas.[20]

This approach ensures adequate advancement of the lingual flap, which facilitates tension-free wound closure. Lingual flap mobilization is currently an imperial requirement in vertical bone augmentation of the mandible [19]. The lingual region of the mandible is considered a danger zone due to the anatomical elements located in the mandibulo-lingual groove, severe complications such as hemorrhages, hematomas, swellings have been reported in GBR implantation operations at the level of the mandible [20, 21]. The main cause is the specific anatomical area of the aforementioned groove, especially the sublingual artery which is a branch of the lingual artery, and which passes between the mylohyoid muscle and the genioglossus, it can give some small branches that supply the gum and can be easily damaged during flap preparation intervention [22]. Although the terminal branches of the lingual artery can be found in the lingual flap, the lingual artery is not at risk, provided that blunt dissection of the flap is performed. According to Urban *et al.* the most common complication is hemorrhage and is the result of trauma to the superficial periosteal terminal branches described above [19].

To achieve optimal wound adjustment and flap mobility, several flap processing techniques are described. Recently introduced flap advancement techniques may be superior to the classic periosteal release incision technique in large augmentation procedures. Periosteal release incision is reported to lead to several postoperative complications and may affect the blood supply to the flap. Buser *et al.* proposed the lateral flap incision technique in staged bone augmentation [23].

In the opinion of Porcaro *et al.,* damage or excessive stress of the flap during surgery, a thin tissue biotype, and surgeon inexperience are factors that increase the risk of complications [21]. Maridati *et al.* specify that the flap must present a sufficiently large surgical field and allow complete coverage of the membrane with soft tissues and adequate vascularization. Flap repositioning should be tension free and not damaged during periosteal incisions to prevent soft tissue necrosis [24]. Fontana *et al.* mention that the most frequently used technique to allow the preservation of the blood supply to the flap is the incision of the flap through its entire thickness and with access to the upper part of the alveolar ridge [25].

Some surgeons like Kim *et al.* suggest making vertical release incisions to improve tissue mobility, while others report that this procedure reduces blood supply [2]. In many cases, the availability of soft tissues limits the potential for bone regeneration, and surgical augmentation of the soft tissue volume becomes necessary before the GBR procedure [26, 27]. The main disadvantages associated with the coronal advancement of the flap are the reduction of the depth of the vestibular fornix, the dislocation of the keratinized mucosal band and the mucogingival junction, and the generation of tissue tension. Reduction of the keratinized mucosal strip can compromise the aesthetic outcome of the future prosthetic construction and make oral hygiene procedures more difficult [28]. To reduce these risks, other flap formation techniques have been introduced by avoiding excessive sectioning of the periosteum, namely the periosteal release incision. The authors propose the following techniques for forming mucogingival flaps.

1. The double flap (DF) incision involves dividing the flap into two layers: an inner periosteal layer and an outer mucosal layer. The mucosal layer extends coronally and exceeds the height of the neighboring teeth to achieve primary closure, without traumatizing the periosteum, it is detached from the submucosa and the muscular insertion. It must be specified that the release of the periosteum starts from the level of the incision of the flap (the middle of the alveolar ridge) and is advanced towards the apex until the necessary release of the vestibular flap is achieved, technique illustrated in figure 4 [1, 9].
2. The coronal advanced lingual flap (CALF) involves the detachment of the insertion of the mylohyoid muscle from the periosteum of the lingual flap, making it free to move coronally. It should be noted that the authors specify that the detachment of the muscle have to be done with a blunt instrument, atraumatic and without damaging the periosteum. Coronal advancement should be carried out at a height twice the height of the crowns of the neighboring teeth, technique illustrated in figure 5 [10, 16].
3. Modified Periosteal Releasing Incision (MPRI) introduced by Hur *et al.* in 2015 presents vestibular flap formation, and involves a superficial periosteal release incision similar to DF technique, but differs in that the level of incision and release of the periosteum begins at the level of the junction of the fixed and mobile mucosa and thus creates two segments: a

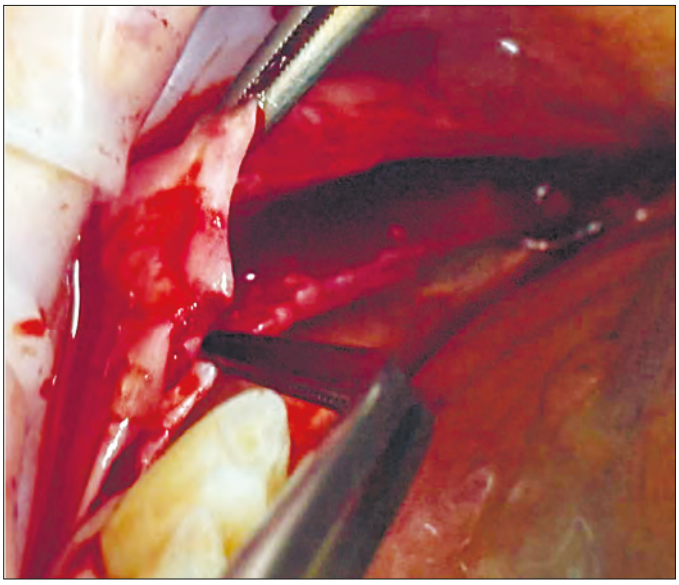

Fig. 4 Double flap (DF) technique.
The buccal flap clearly reflects two separate layers, the periosteum separated bond and vestibular mucosa.

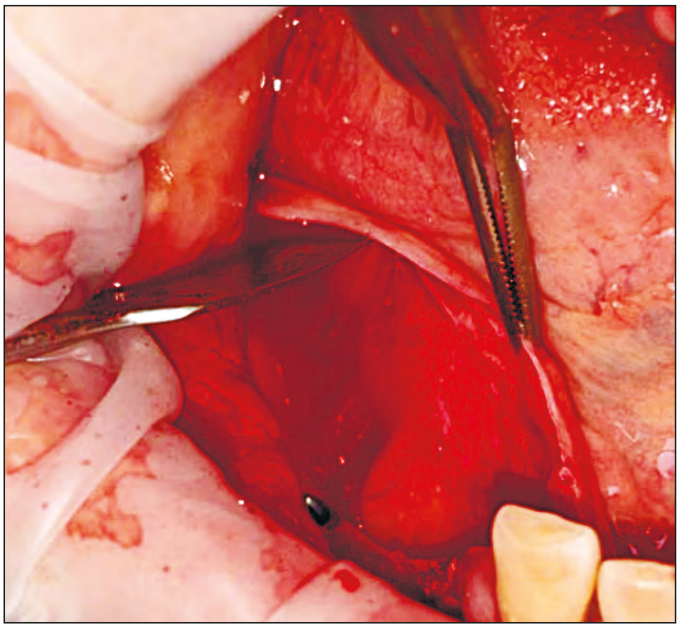

Fig. 5 Coronal advanced lingual flap (CALF), with its blunt release from the mylohyoid muscle.

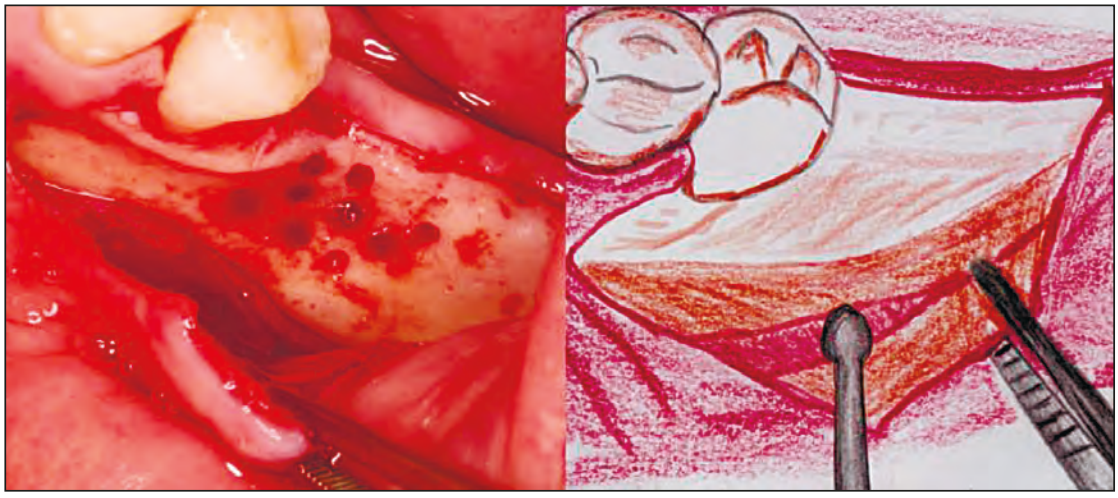

Fig. 6 Modified Periosteal Releasing Incision (MPRI) [9]

segment of mucosa with coronal periosteum and the second, the apical periosteum which separated by abutment dissection with lateral extension according to the technique described in "DF" [28, 29]. The modified technique for performing the periosteal release flap is illustrated in figure 6 [9].

According to the authors, in these sectional techniques the periosteum was involved less. They reported fewer complications compared to the classic periosteum release incision. A study comparing the types of flaps in GBR shows the following: according to Zazou *et al.* the coronal advanced lingual flap reported the highest advancement, the lowest post-intervention edema score and the lowest subsequent exposure [9]. Therefore, it is recommended that in large augmentation procedures in the posterior areas of the mandible, experienced manipulation of the lingual flap and knowledge of the anatomy are crucial to avoid injury to any vital structures in the given area. Noguera-Mutlló *et al.* in his work obtained that the detachment of the mylohyoid muscle from the lingual flap allows a significant increase in its extension, 2.5 times. Thus, this maneuver could be indicated in posterior mandibular bone augmentation procedures that require tension-free closure [20]. The results reported by Fugazzotto *et al.* show that with modified periosteal release technique and double vestibular flap formation achieved approximately 14.5 mm of its advancement, reporting the lowest mean pain score. This technique can be used in large bone augmentation operations with predictable results when used in patients with thick gingival tissue phenotype [30, 31].

Conclusion

The GBR technique is a complex method of bone augmentation, the success of which depends on several factors such as: protective membrane, flap formation, thread tension, as well as suture relaxation, which play key roles in guided bone growth. Strict adherence to the steps GBR minimizes the rate of complications, which are inevitable. In our research we demonstrated through statistical data that the occurrence of dehiscence has no correlation with the GBR technique used in our case, as the results were without statistical significance. Therefore, we can say with certainty that the small amount of dehiscence is mainly due to the shape and the formation disorder of the relaxed mucogingival flap, a fact also described in the specialized literature [30, 32]. Regardless of the chosen technique, it is important to consider the type and viability of the tissues used to cover these GBR elements.

Competing interests

None declared.

Authors' contributions

VZ **-** conceptualization, investigation, methodology, writing - original draft, writing - review & editing, visualization, project administration, data curation, resources, DC **-** investigation; visualization, formal analysis. NC **-** investigation, project administration, validation, supervision, data curation. GC **-** supervision, data curation. All authors critically reviewed the work and approved the final version of the manuscript.

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Patient consent

Obtained.

Ethics approval

The Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy approved the study **-** Minutes no. 43 from 13.02.2020.

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RESEARCH ARTICLE

Analysis of spironolactone in compound powder by ultraviolet-visible spectrophotometry

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ABSTRACT

Introduction. Spironolactone (Spir) is a selective and competitive antagonist of aldosterone that increases the excretion of water and sodium while decreasing the excretion of potassium (K+ sparing diuretic). The substance was studied to develop qualitative and quantitative methods of analysis and to validate them according to documents regulating the quality of active pharmaceutical ingredients in the development of pharmaceutical forms.

Material and methods. A new dosage form (powder) with Spir was developed and analyzed by a spectrophotometry method using a UV-Vis spectrophotometer (Agilent 8453, USA) with 10.0 mm matched quartz cells at 238±2nm, with methanol as the blank. The method was validated for specificity, linearity, precision, accuracy, robustness, LOD and LOQ.

Results. The method was found to be linear in the drug concentration range of 5.0 to 30.0 µg/ml, with a correlation coefficient (R^2) of 0.9994 for Spir. The LOD of Spir was 0.5 µg/ml and the LOQ was 1.4 µg/ml, indicating the method's sensitivity. The method was established as accurate (mean recovery values of concentration at 80%, 100%, 120% ranging between 99.9 and 101.7%). Repeatability precision and intermediate precision %RSD values amongst six sample solutions were from 0.13% to 0.25% for Spir (less than 2%). The accuracy (recovery) ranged between 99.9% and 101.7%, with standard deviations ranging from 0.08% to 0.17%.

Conclusions. In the presence of common excipients, such as microcrystalline cellulose, lactose monohydrate, and stearic acid, no interferences were observed. This method was found to be suitable for the routine analysis of Spir from the newly developed pharmaceutical form.

Keywords: spironolactone, UV-Vis spectrophotometry, validation.

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Key messages

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

A new pharmaceutical dosage form was developed with spironolactone and other active ingredients (potassium orotate, potassium and magnesium aspartate). Until now, no method for dosing spironolactone in such a combination has existed. An easy, accurate, and efficient assay method for the simultaneous determination of spironolactone in this pharmaceutical dosage form was elaborated, which can be applied for routine analysis.

The research hypothesis

If the results obtained by this developed method comply with validation requirements (specificity, linearity, precision, accuracy, robustness), then it can be applied to routine quality control, quantitative analysis, and stress stability testing.

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

Spironolactone is combined for the first time in the same pharmaceutical form with potassium aspartate, magnesium aspartate, and potassium orotate. Thus, the spectrophotometric dosing technique for spironolactone in this combination is developed for the first time. The results obtained indicate that the proposed method is suitable for the analysis of spironolactone in the newly developed pharmaceutical form in the presence of other active ingredients, with excellent recovery, precision, and linearity.

Introduction

Spironolactone is a selective and competitive antagonist of aldosterone, due to its structural similarity to aldosterone. Chemically, spironolactone is 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone (Figure 1). Spironolactone works by competing with aldosterone for interactions with aldosterone receptors in the collecting duct. This antagonistic effect increases the excretion of water and sodium while decreasing the excretion of potassium (K^+ sparing diuretic) [1]. Unfortunately, spironolactone works slowly, requiring several days to develop its pharmacologic action, and similarly, its effect diminishes slowly [1].

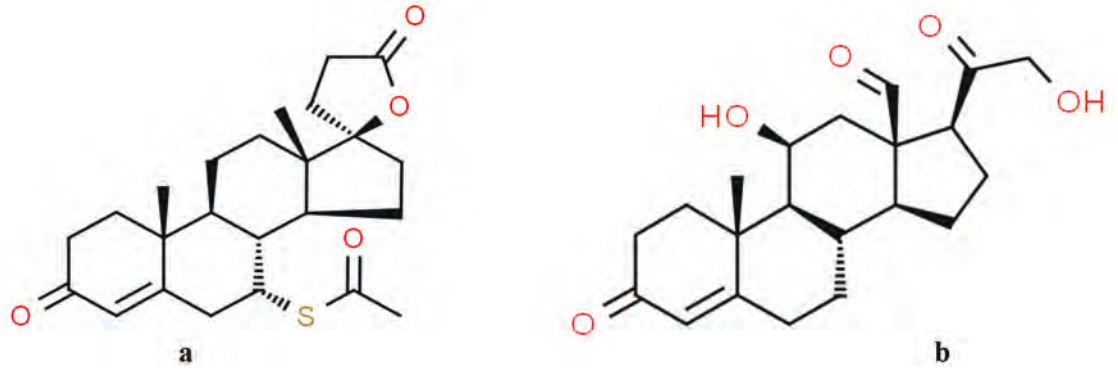

Fig. 1 Chemical structure of spironolactone (a) and aldosterone (b) [2, 3].
Data from http://www.chemspider.com/Chemical-Structure.5628.html
Data from http://www.chemspider.com/Chemical-Structure.5633.html

To expand the portfolio of effective and harmless potassium-containing products with mechanisms of action at the molecular level, a new composition was developed, consisting of potassium orotate (OK), potassium and magnesium aspartate (AsMg, AsK). Excipients were included in the pharmaceutical form to improve technological parameters for facilitating the manufacturing process, as well as to enhance stability, bioavailability, and efficacy of the final medicine. Moreover, a validated method is necessary to analyze spironolactone in various dosage forms, such as suspension (5mg/ml), tablets (25mg), capsules (50mg), and the newly developed powder. The developed assay method was validated according to International Conference on Harmonization (ICH) Guidelines and was successfully employed for the analysis of spironolactone in pharmaceutical dosage forms, based on analytical performance characteristics: Linearity, Precision, Accuracy, Ruggedness, Low Limit of Detection (LOD), and Low Limit of Quantitation (LOQ) [4-8]. The validated assay methods allow for the testing of spironolactone in stability studies, using stress-factors such as acid, base, temperature, light, oxidizing agents, and susceptibility across a range of pH values, as well as dissolution testing.

The aim of this study was to develop a simple, sensitive, accurate, precise, and low-cost UV-spectrophotometric assay method for estimating spironolactone (S) in a combined powder that also contains potassium orotate (OK), potassium and magnesium aspartate (AsMg, AsK), and auxiliary substances.

Materials and methods

The method was validated according to International Conference on Harmonization (ICH) guidelines.

A single beam Ultraviolet-Visible spectrophotometer (Agilent 8453, USA) with 10.0 mm matched quartz cells was used. All absorbance measurements were carried out at 25±1ºC and at 238±2nm. All weights were taken on an electronic balance (Model Radwag), and the samples were sonicated using an ultrasonic bath (Sapfir).

Potassium aspartate, magnesium aspartate, and potassium orotate (Sigma-Aldrich), spironolactone (Acros Organic and European Pharmacopoeia Reference Standard), and auxiliary substances such as microcrystalline cellulose, lactose monohydrate (Himedia), and stearic acid (Chem-Lab), as well as analytical grade reagents and solvents (Chem-Lab), were used to carry out the study.

Preparation of the test sample

Twenty powders were mixed, weighed, and the average weight of each powder was determined. The mass of one

powder (approximately 1.991 mg) was weighed and placed into a 25-ml volumetric flask. Approximately 10 ml of methanol was added, and the mixture was ultrasonicated for a minimum of 15 seconds before being made up to the mark with methanol. The sample was filtered through filter paper; 1 ml of this solution was transferred to a 10-ml volumetric flask and made up to the mark with methanol, resulting in a concentration of 10 µg/ml. Three series of final sample solutions were analyzed by UV spectrophotometry at 238 nm and calculated according to the Formula 1:

(1)

$$C, \% = \frac{A_{pr} * m_{st} * V_{pr} * P * (100 - U)}{A_{st} * m_{pr} * V_{st} * 100} * 100\% \quad (1)$$

A_{pr} – the mean value of 3 series of absorbance of sample solutions;
A_{st} – the mean value of 3 series of absorbance of standard solutions;
m_{pr} – mass of sample substance, g;
m_{st} – mass of standard substance, g;
P – standard substance content, %;
V_{pr} and V_{st} – volumes of sample and standard solutions, respectively.

Preparation of standard stock solution

A spironolactone standard stock solution containing 100 µg/ml was prepared in a 25-ml volumetric flask by dissolving 2.5 mg of spironolactone reference standard in methanol. The solution was sonicated for a minimum of 15 seconds and then made up to the mark with methanol. Three series of standard solutions were analyzed by UV spectrophotometry at 238 nm and calculated using Formula 1.

Preparation of placebo solution

The auxiliary ingredients were weighed in quantities necessary to prepare 20 powders, mixed, and homogenized in a mortar. About 1.528 mg was weighed and placed into a 25-ml volumetric flask. About 10 ml of methanol was added, and the mixture was ultrasonicated for a minimum of 15 seconds before being made up to the mark with methanol. The sample was filtered through filter paper; 1 ml of this obtained placebo solution was transferred to a 10-ml volumetric flask and made up to the mark with methanol. It was then analyzed by UV spectrophotometry.

Validation method

Linearity

Standard solutions at five different concentrations were prepared and analyzed for linearity studies to determine the linearity within the concentration range by calculating linear regression equations and regression coefficient values (Pearson, R^2). Linearity test solutions were prepared at levels from 50 to 300% of assay analyte concentration (5, 10, 15, 20, and 30 µg/ml). Each solution was prepared in triplicate.

Preparation of standard calibration curves: Aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 3 ml from the spironolactone standard stock solution were placed into 10-ml volumetric flasks and diluted to 10ml with methanol to obtain final concentrations of spironolactone at 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 30 µg/ml, respectively. The obtained standard solutions were analyzed at 238 nm using a spectrophotometer, with methanol as a blank. The graphs were plotted with the concentration of standard spironolactone against the response (absorbance).

Limits of detection and limits of quantification

The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected, while the limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantitated. Both LOD and LOQ were experimentally verified and calculated using the following equation: LOD = 3.3 (SD/Slope) and LOQ = 10 (SD/Slope).

Precision

The precision of the method was evaluated through repeatability and intermediate precision, according to intra-day and inter-day precision, and reported as percent relative standard deviation (%RSD ≤ 2). The repeatability precision was analyzed by performing six spironolactone test sample preparations made on the same day (intra-day). Intermediate precision was evaluated by 2 analysts performing the same procedure on a different day (inter-day) under the same experimental conditions.

Accuracy

The accuracy of the assay method was evaluated through a recovery study by adding a known amount of spironolactone standard to a pre-analyzed test sample solution at 3 different concentrations: 80%, 100%, and 120%. Various concentrations of standard spironolactone solutions of 6, 10, and 14 µg/ml were added to a fixed concentration of test sample solution (10 µg/ml) in a 1:1 ratio.

Robustness

The robustness of the method was determined by analyzing the sample solution (10 µg/ml) at two different wavelengths (± 4 nm) and by a single analyst performing the analysis on two different instruments, while maintaining other spectrophotometric conditions constant. The effect of these changes was studied based on the percent recovery and standard deviation of spironolactone.

The ***specificity*** of the method was assessed by evaluating the spectra of the placebo solution (containing all the

excipients of the powder except the active ingredients) to confirm the lack of interference or overlap with Spir at the analytical wavelength.

Results

The assay method for spironolactone in pharmaceutical forms was developed and validated according to ICH Guidelines in terms of linearity, accuracy, precision, LOD, LOQ, and robustness. Analyses were conducted using a UV-visible single-beam spectrophotometer (Model: Agilent 8453) at a wavelength of 238±2 nm, with methanol as the blank. The results are shown in Figure 2.

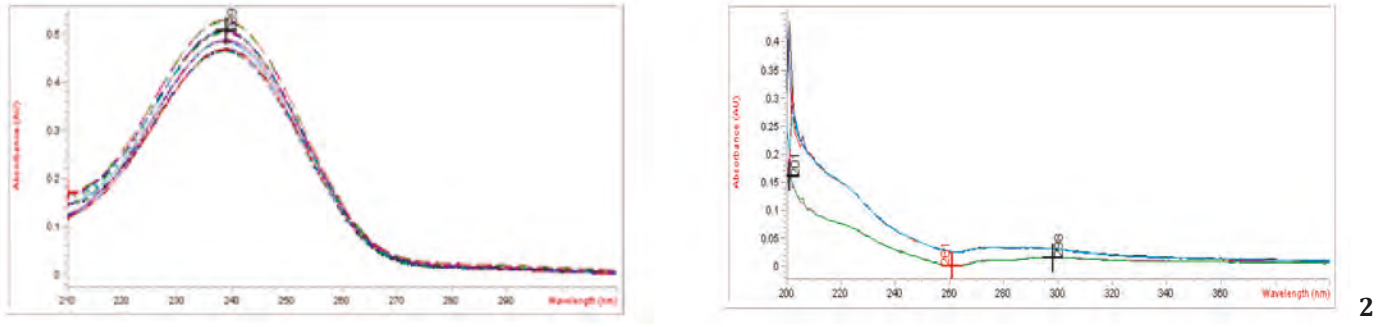

Fig. 2 Ultraviolet absorbtion spectra of spironolactone sample (1) and placebo (2)

Linearity test solutions were prepared at 5 concentration levels, ranging from 50 to 300% of the assay analyte concentration (5, 10, 15, 20, and 30 µg/ml), and repeated 3 times (Tab.1). Linearity was established over the concentration range of 5-30 µg/ml for spironolactone. The linear regression equation was found to be: y = 0.0558x + 0.022, with an R^2 of 0.9994. The y-intercept (constant of regression) was 0.022, and the slope (coefficient of regression) was 0.0558. The results are represented in Figure 3.

Table 1. Calibration data for spironolactone spectrophotometric determination

	Ast_1	Ast_2	Ast_3	Ast_4	Ast_5	Ax_{med}
5 µg/ml	0.279	0.280	0.279	0.283	0.279	0.280
10 µg/ml	0.596	0.595	0.595	0.596	0.595	0.595
15 µg/ml	0.844	0.844	0.845	0.845	0.844	0.845
20 µg/ml	1.145	1.146	1.144	1.146	1.145	1.145
30 µg/ml	1.673	1.673	1.688	1.698	1.698	1.686

Note: Ast-- Absorbance of the spironolactone standard solution at different concentrations

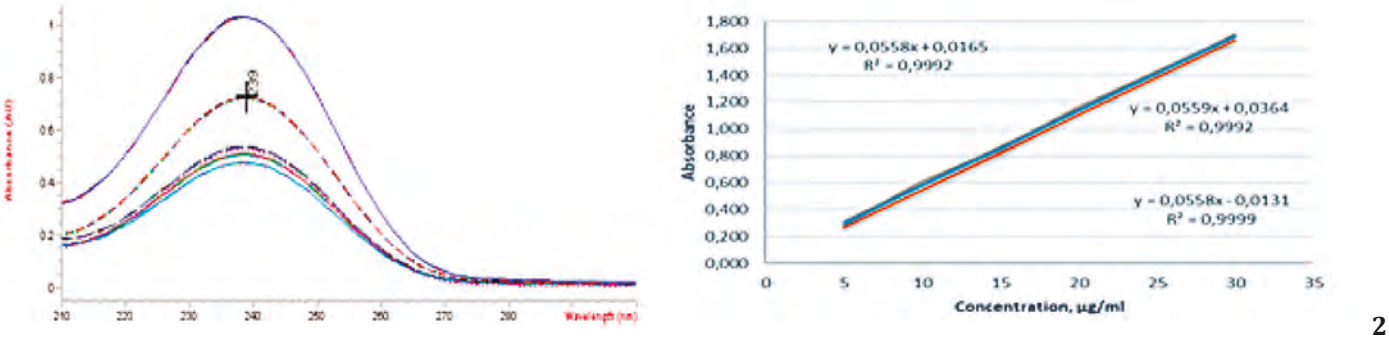

Fig. 3 Ultraviolet spectra of standard solutions of spironolactone at different concentrations (1) and calibration curves for 3 series of determinations (2)

The determined LOD of spironolactone (0.5 µg/ml) and LOQ (1.4 µg/ml) showed the sensitivity of the method.

Precision was assessed by integrating the absorbance of a 10µg/ml test solution in six replicates on the same day and over multiple days, repeated 3 times. The precision studies showed that the % relative standard deviation was within acceptable limits (RSD < 2). The results are shown in Tables 2 and 3.

Table 2. Results of the repeatability precision for the assay of spironolactone

Run	Absorbance	Amount found, %
1	0.5261	100.4
2	0.5259	100.4
3	0.5275	100.7
4	0.5275	100.7
5	0.5273	100.6
6	0.52686	100.6
Mean	0.527	100.6
RSD, %	0.13	0.13

Note: *RSD - Relative standard deviation*

Table 3. Results of the intermediate precision for the assay of spironolactone

	Intra-day				Inter-day	
Run	Absorbance of the 1st analyst	Amount found, %	Absorbance of the 2nd analyst	Amount found, %	Absorbance of the 1st analyst	Amount found, %
1	0.5261	100.4	0.5273	100.6455	0.5259	100.4
2	0.5259	100.4	0.5269	100.5691	0.5287	100.8
3	0.5275	100.7	0.5267	100.531	0.5274	100.7
4	0.5275	100.7	0.5274	100.6646	0.5295	101.1
5	0.5273	100.6	0.527	100.5882	0.5279	100.8
Mean	0.527	100.6	0.5	100.6	0.5	100.7
RSD, %	0.15	0.15	0.05	0.05	0.25	0.25

***Note*:** *RSD - Relative standard deviation*

The accuracy of the developed method was determined by adding standard solutions (6, 10, and 14 µg/ml) to a fixed concentration of test sample solution (10 µg/ml). Accuracy was evaluated at 80%, 100%, and 120% levels of the standard solution (SD). The mean percentage recovery was calculated, and the results are shown in Table 4.

Table 4. Results of the accuracy for the assay of spironolactone

Spiked Level (%)	Amount of Spir from taken sample (µg/ml)	Amount of Spir added standard (µg/ml)	Total amount of Spir (µg/ml)	Amount found of Spir from taken sample (µg/ml) ±SD	Recovery, %	RSD, %
80	10	6	8	*9.99*±0.08	99.9	0.82
100	10	10	10	*10.07*±0.12	100.7	1.23
120	10	14	12	*10.17*±0.17	101.7	1.67

Note: RSD - Relative standard deviation, Spir - spironolactone

The robustness of the method and the influence of deliberate variations in the analytical parameters on the absorbance of Spir were examined. Parameters such as the analytical wavelength (238 ± 4 nm) and the type of instrument were modified. The test solution of 10 µg/ml Spir was applied to this parameter It was determined that these changes in the spectrophotometric determination conditions did not result in changes in the quantitative content of Spir, confirming that the developed method is robust. The results are reported in Table 5.

Table 5. Results of the robustness for the assay of spironolactone

	Parameter	RSD, %	Assay, mg	Assay, %
Wavelength	234 nm	0.17	23.68	100.8
	238 nm	0.15	24.14	100.6
	242 nm	0.07	23.69	99.1
Instrument	Agilent 8453	0.15	24.14	100.6
	Shimadzu 1800 UV	0.13	24.64	102.68

Note: RSD - Relative standard deviation, Spir - spironolactone

In the presence of common excipients such as microcrystalline cellulose, lactose monohydrate, and stearic acid, no interferences were observed (Figure 2). This confirms the specificity of the developed method.

Discussions

In this study, a validated method was developed for the analysis of spironolactone from a new compound powder containing spironolactone, potassium orotate, potassium and magnesium aspartate, and excipients. This combination of active pharmaceutical substances is specific and can be successfully applied for the treatment of hypopotassemia. According to other reported studies, different solvents (such as ethanol, methanol, acetonitrile) and wavelengths have been used to analyze spironolactone from other medicines by UV-Vis spectrophotometry. Based on the performed analyses, we selected the most satisfactory conditions to validate the method in compliance with ICH guidelines for specificity, linearity, precision, accuracy, and robustness. This method can be applied for routine quality control, quantitative analysis, and stress stability testing. The UV-Vis spectrophotometric method for the analysis of spironolactone from the compound powder was developed for the first time and demonstrated good accuracy, precision, LOD, LOQ, robustness, and specificity. The results of the analysis were statistically validated using SPPS, and the RSD (%) values for all measurements were less than 2.

Conclusion

The proposed UV-Vis spectrophotometric method was found to be simple, rapid, precise, and low-cost. Validated according to ICH guidelines, the method demonstrated ex-

cellent linearity, accuracy, precision, LOD, LOQ, robustness, and specificity. This new analytical method was developed for the routine simultaneous determination of spironolactone in the presence of OK, AsK, and AsMg in the newly developed pharmaceutical form – combined powder.

Competing interests

The author declares that there is no conflict of interest in the manuscript.

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The research was conducted at the Scientific Center for Drug Research within *Nicolae Testemiţanu* State University of Medicine and Pharmacy. The study was initiated by the author.

Authors' contributions

EM conducted the literature review, wrote the manuscript, revised the final text, and approved the final version of the manuscript.

Ethics approval

Not needed for this study

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REVIEW ARTICLE

The use of artificial intelligence in coordinating COVID-19 prevention measures at the territorial level

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ABSTRACT

Introduction. The Coronavirus Disease 2019 (COVID-19) pandemic presented a significant challenge for global society, leaving a profound impact across the board. Although COVID-19 cases are still reported, they are no longer at previously high levels. One of the key tools in combating the pandemic was Artificial Intelligence (AI), which played a vital and advancing role throughout the pandemic. AI contributed significantly to the gradual reduction in COVID-19 cases. Effective coordination of the pandemic response, timely management, and the integration of AI into the medical system were crucial factors in achieving success.

Materials and methods. A comprehensive literature review focusing on publications from 2019 to 2024 was conducted using Google Scholar, PubMed, and Science Direct. Twenty publications were selected for their relevance to AI in the COVID-19 response, based on criteria such as accessibility, language, and publication type.

Result. The review focused on the significant role of AI during the COVID-19 pandemic, highlighting its impact on public health and medical systems. In countries like the USA, China, and South Korea, AI was crucial in tracking the virus, predicting infection trends, and optimizing resource allocation. AI models helped identify outbreak hotspots and enabled targeted interventions, while natural language processing efficiently managed extensive data. Conversely, in countries such as Brazil, Mexico, India, and many African nations, AI was used less extensively due to limitations in technological infrastructure and data availability. The pandemic drove a closer integration of AI with medical services, streamlining processes and saving time. AI also enhanced laboratory efficiency and supported the development of new medications and vaccines. Despite its potential, the uneven adoption highlighted disparities in technological readiness and resource allocation during the crisis.

Conclusions. The COVID-19 pandemic has once again highlighted that we live in an era of advanced technology and underscores the need for closer integration between healthcare systems and artificial intelligence. This integration allows for more effective and timely management of current and future health challenges. AI contributes to a more rapid and high-quality response to emergencies, providing innovative solutions for both existing and upcoming challenges.

Keywords. Artificial Intelligence, COVID-19 response, pandemic management, machine learning.

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Key messages

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

AI's role in pandemic response is acknowledged; however, there is limited data on its effectiveness across various countries and its capacity to address technological disparities. This lack of data makes it unclear how AI's benefits can be applied consistently in regions with different levels of technological infrastructure.

The research hypothesis

AI significantly improves pandemic management by optimizing resource allocation, predicting infection trends, and enhancing diag-

nostics, though its effectiveness is influenced by regional technological capabilities and data availability.

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

Research results underscore the advancement of AI technologies during the pandemic and their potential for future public health crises, addressing gaps in understanding AI's role in pandemic preparedness and response.

Introduction

Throughout the 21st century, numerous breakthroughs in the field of Artificial Intelligence (AI) have significantly improved our lives. With the onset of Coronavirus Disease 2019 (COVID-19), which has caused approximately 6.9 million deaths globally since its emergence in December 2019, according to the World Health Organization (WHO) [1], these technological advancements have become indispensable tools for managing and coordinating the pandemic response. COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had profound impacts on public health, economies, and daily life worldwide [2].

The rapid spread of COVID-19 necessitated innovative solutions for monitoring, predicting, and controlling the outbreak. AI technologies have been pivotal in predicting infection trends, optimizing resource allocation, facilitating drug discovery, and enhancing diagnostic capabilities. For instance, machine learning algorithms have been used to forecast outbreak hotspots, while natural language processing tools have managed the overwhelming amount of data generated during the pandemic [3].

The integration of AI into the COVID-19 response has provided critical support across many fields, highlighting its potential to enhance pandemic management and prepare for future public health emergencies. Despite various challenges and controversies surrounding AI use, recent literature demonstrates the effectiveness of different AI systems in managing COVID-19. For example, these algorithms can assist doctors in designing personalized treatment plans, leading to faster patient recovery. Furthermore, AI's applications extend beyond COVID-19 management, offering improvements in handling other diseases, including non-communicable ones, and enhancing the accuracy, efficiency, and speed of medical systems [4].

This study explores the effective integration of AI into the coordination and leadership of medical systems, highlighting the advancements achieved during the COVID-19 pandemic. It illustrates how AI contributes to a more efficient medical system, not only in the context of COVID-19 but also in preparing for future medical crises.

Materials and methods

This study conducted a thorough review of existing literature using a range of key resources. Initially, Google Scholar was utilized, followed by academic databases such as PubMed and ScienceDirect (Elsevier), which were pivotal for the search.

The search strategy employed keywords including "Artificial Intelligence," "COVID-19 response," "Pandemic management," and "Machine learning". Only publications from the years 2019 to 2024 were considered, resulting in approximately 297 articles.

Articles that were not freely accessible, unavailable through the Scientific Medical Library of the *Nicolae Testemițanu* State University of Medicine and Pharmacy (SUMPh), duplicates, or inaccessible in Moldova were excluded. The remaining articles were then refined using advanced search filters to focus exclusively on English-language publications with full text, systematic reviews, and meta-analyses. This process ensured the inclusion of highly relevant and high-quality sources.

From the filtered articles, 20 studies were selected based on their relevance to the application of artificial intelligence in coordinating the COVID-19 response. These studies were meticulously analyzed to evaluate their contributions and insights into how AI technologies were employed during the pandemic.

Result

AI use in COVID-19 management. During the COVID-19 pandemic, countries like the United States of America (USA), China, and South Korea extensively used AI for tracking the virus, predicting infection trends, optimizing resources, and enhancing diagnostics. AI models forecasted outbreak hotspots, enabling targeted interventions, while natural language processing managed vast amounts of data, improving information extraction. Conversely, countries such as Brazil, Mexico, India, and many African nations used AI to a lesser extent or not at all. This disparity is due to differences in technological infrastructure, data availability, and governmental support [5, 6].

Initial limitations of AI. While AI provided significant support, it is important to note that it was not fully prepared for such a global health crisis initially. Until the end of 2020, AI was not fully utilized for tracking and predicting COVID-19 cases due to the lack of large amounts of historical data necessary for training AI models. Consequently, early studies published shortly after the global COVID-19 outbreak reported results of limited relevance, primarily due to insufficient data for adequately training AI techniques and the poor quality of the available data [7].

Preparation for future pandemics. It is surprising that the world had already received a stark warning about the need for better pandemic preparedness. In 2015, in the wake of the Ebola epidemic, Bill Gates, through the Gates Foundation, emphasized the critical need for global warning and response mechanisms to more effectively prepare for future pandemics. However, this call to action went

largely unheeded. It seems that the Ebola crisis, despite its severity, was not enough to galvanize global action. The world required an even more catastrophic event–the COVID-19 pandemic–to fully grasp the importance of AI and advanced technological preparedness in managing global health crises [8].

AI evolution and impact. The rapid spread of COVID-19 meant that sufficient data and extensive labeled datasets were initially unavailable. Training models on unrepresentative datasets led to poor and misleading results, as the fast-evolving nature of the problem made it difficult to make informed model and parameter selections. This significantly affected the performance and accuracy of prediction models. However, today, the availability of COVID-19 surveillance data, such as daily and cumulative numbers of cases, deaths, and recoveries, is no longer an issue. In fact, years after the COVID-19 outbreak, multiple collections of detailed data are available from different sources, such as those gathered by the Johns Hopkins University Coronavirus Resource Center. These data are essential for improving the accuracy and performance of AI models, highlighting the crucial importance of AI in pandemic management [7].

Machine Learning (ML), a subfield of AI, was extensively used during the COVID-19 pandemic. ML involves developing algorithms that allow computers to learn and make predictions or decisions based on data. Initially, existing AI-based facial recognition software and cameras were used to identify individuals who were not adhering to self-isolation or quarantine guidelines. Over time, these technologies evolved, leading to the creation of new systems capable of independently identifying individuals wearing masks versus those who were not and determining whether social distancing guidelines of one meter were being followed. Similar computer vision systems were developed for hospitals to monitor interactions with bedridden COVID-19 patients, document the healthcare workers who entered the rooms, and track the duration and proximity of these interactions [9].

AI was utilized on a broad scale at various levels and proved valuable for decision makers. ML was employed to help decision-makers understand adherence to non-pharmaceutical interventions in near real-time. While AI usage was prevalent in developed and some developing countries, the high costs associated with these systems were not necessarily a barrier. Some system creators allowed the release of the complete code stack and system design, enabling others to quickly replicate and improve the systems. This approach was particularly beneficial in resource-limited settings and low- to middle-income countries. These opportunities were available from the early stages of the pandemic, indicating that the primary reasons for not using AI were not necessarily due to prohibitive costs but rather other regional or territorial factors [9, 10].

Challenges and solutions. Despite AI's potential to protect vulnerable communities in low-income countries by providing timely access to care, addressing structural and systemic barriers to AI implementation is a necessary step in these settings. This is especially critical as global efforts are made to prevent the reemergence of COVID-19 and its variants [11, 12].

As mentioned, AI played a leading role in coordinating COVID-19 responses; however, the previously discussed systems alone are insufficient for successful pandemic management. While AI automated tasks that would have been impossible to manage manually in a short time, it is important to recognize that AI systems in healthcare were implemented to support decision-making processes. There remains a gap in developing data analysis and AI methods for better healthcare supply chain management. These supply chains were disrupted at an incredible speed during the pandemic, creating numerous bottlenecks. It is essential to address these issues when operating in rapidly changing environments. This is particularly true for inventory planning in healthcare supply chains, which significantly impacted healthcare services during the pandemic [13, 14].

An exemplary case of effective management is represented by Swedish Health Services, a healthcare organization in the USA, which developed a platform for healthcare workers to report real-time data on COVID-19 patient volumes, personal protective equipment, staffing, ventilator usage, and other resource information. This data was shared among its hospitals to monitor unit status, allocate healthcare resources, and increase hospital bed capacity [15, 16].

In the fight against the COVID-19 pandemic, the rapid development of vaccines has been astonishing, due in part to AI. Numerous research laboratories continuously worked to create vaccines and medications against COVID-19 during the pandemic. AI was employed to evaluate existing drug compounds and determine their efficacy in combating the coronavirus. Countries such as South Korea and the United States utilized AI-based systems to discover repurposed drugs with potential for treating COVID-19 [17, 18].

Although the pandemic has caused global stagnation, it has paradoxically accelerated modernization and advancements in healthcare. Future plans include the enhanced automation of medical systems, primarily aimed at protecting and facilitating human work. For example, several types of robots have been developed to meet specific needs. Cylindrical robots are designed to move through hospital corridors, assisting healthcare workers by checking the temperature, blood pressure, and oxygen saturation of COVID-19 patients on ventilators. Another type of robot, designed to move vertically, disinfects hospital interiors using UV rays. Some remote-controlled drones transport infectious samples to external laboratories for testing. Currently, research laboratories and medical companies are developing remote-controlled robots capable of collecting blood samples and oral swabs for COVID-19 testing without human contact. Additionally, in various hospitals and nursing homes, robots are used to interact with patients,

maintaining a safe distance. Although a range of robots is already in use, future plans include expanding automation in the medical system, which is expected to play a crucial role in pandemic coordination [19, 20].

Discussion

The COVID-19 pandemic showcased the transformative potential of AI in enhancing public health management. This study highlights AI's crucial role in predicting infection trends, optimizing healthcare resource allocation, and supporting real-time decision-making. By integrating AI technologies, public health systems were able to respond more swiftly, improve patient outcomes, and reduce strain on healthcare resources.

An additional example of AI's application is the UK Government's Bluetooth-based app, which alerted the non-infected population about potential exposure to high-risk areas or COVID-positive individuals. This tool demonstrated AI's value in infection prevention, but it also revealed challenges, such as data privacy concerns and the need for improved technological infrastructure in certain regions [17].

Ultimately, the study underscores the importance of expanding AI's use in future health crises, addressing gaps in technology access, and ensuring ethical governance for broader and more equitable implementation.

Conclusions

The extensive use of AI during the COVID-19 pandemic significantly facilitated more efficient and rapid coordination of the response. AI technology helped avoid mass errors in database management and prevented additional cases of illness among healthcare workers. The implementation of AI proved to be a valuable asset, particularly by enabling robust management of the pandemic and preventing larger-scale crises.

Competing interests

None declared.

Authors' contributions

DD contributed significantly to the conception and design of the review, the analysis and interpretation of the literature, as well as drafting and revising the manuscript. OL actively participated in the literature search, critically reviewed the scientific content, and provided essential contributions to the final conclusions.

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REVIEW ARTICLE

Parasitic infestations and their influence on joint inflammation

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ABSTRACT

Objective. The objective of this study was to conduct a bibliographic analysis of current data regarding the impact of parasitic infestations on immune status and the progression of osteoarticular diseases within the context of parasitic infections.

Material and methods. This was a qualitative analytical study presented as a narrative literature review. Relevant primary sources published between 2016 and 2022 were identified and selected using data extraction and analysis methods.

Results and discussion. The concept of "parasitic therapy" has generated considerable interest among researchers, the public, and patients for whom standard treatments have been ineffective or offered limited results. Although studies exploring the role of parasitic infections in arthritis are less common than in other fields, animal models suggest that parasitic infections may alleviate joint inflammation. However, further research is needed across different forms of arthritis, including clinical data collection and double-blind, controlled clinical trials.

Conclusions. While only a few studies have demonstrated that parasitic infections may worsen preexisting diseases, the scientific consensus is that parasitic infections can create an immunoregulatory environment, reducing the severity of coexisting conditions. Finally, more rigorous animal studies are required to thoroughly investigate immunomodulatory mechanisms and potential side effects of parasitic infections in the presence of other diseases.

Keywords: parasitosis, parasitic arthritis, parasitic immune status.

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Key messages

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

The mechanisms and systemic effects of parasitic therapy on joint inflammation are still unclear. More research is needed to assess its safety, long-term impact, and therapeutic potential in autoimmune diseases.

The research hypothesis

Parasitic infections can modulate immune responses and reduce joint inflammation, offering a potential therapeutic approach for autoimmune diseases, including rheumatoid arthritis.

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

The manuscript provides novel insights by exploring the immunomodulatory effects of parasitic infections on joint inflammation, a relatively under-researched area. It suggests new perspectives on using parasitic therapy as a potential treatment for autoimmune diseases, particularly rheumatoid arthritis, while emphasizing the need for personalized approaches and identifying molecular pathways involved in immune regulation.

Introduction

Parasitosis represents a persistent global health challenge, with its prevalence being particularly high in developing countries. According to a World Health Organization report from 2010, there were an estimated 48.4 million cases of parasitic infections, with 59,724 deaths recorded [1, 2].

In the Republic of Moldova, parasitic infections hold a significant share among infectious diseases, following only acute respiratory infections and acute diarrheal illnesses in prevalence. In the first six months of 2015, 7,645 individuals were diagnosed with various helminthic infections, marking a 9.5% decrease compared to the same period in the previous year [3, 4].

While gastrointestinal, visceral, encephalic, and cutaneous manifestations are commonly associated with parasitic diseases, the musculoskeletal system can also be affected [5, 6].

The main types of parasites responsible for such conditions include:

- Cestodes (e.g., *Taenia spp.*, *Echinococcus spp.*);
- Trematodes (e.g., *Schistosoma spp.*, *Opisthorchis spp.*);
- Nematodes (e.g., *Toxocara spp.*, hookworms, *Strongyloides spp.*, filarial worms);
- Protozoa (e.g., *Giardia lamblia*, *Toxoplasma gondii*).

Involvement of the musculoskeletal system may present with various clinical features, such as:

- Arthritis, myositis, enthesitis, and tendinitis;
- Soft tissue swelling, trophic ulcers, and muscle necrosis;
- Elephantiasis, bone or muscle cysts, pathological fractures;
- Calcification of soft tissues;
- Migration of larvae through subcutaneous tissues;

Joint damage caused by parasitic infections occurs through three key mechanisms:

1. Direct invasion of the parasite into the joint, triggering an inflammatory response (arthritis). This inflammation may be further complicated by a secondary bacterial infection introduced either by microbial agents attaching to the parasite or entering the joint through pathways created by the parasite [2].
2. Periarticular deposition of parasites, leading to secondary joint inflammation [5].
3. Immune-mediated reactions resulting from the body's response to the presence of the parasitic agent [2, 6].

Over the past decade, significant progress has been made in parasitology research. New discoveries related to interleukins and immune cell networks have reshaped our understanding of how parasites interact with the human immune system. These insights demonstrate how parasites may either evade immune responses to persist in the host or modulate immune reactions to prevent reinfection. However, despite these advances, many challenges remain. Weaknesses in infection control systems continue to impact public health and diminish the quality of life, especially in underprivileged populations.

Many parasitic worms thrive at the expense of children's development, particularly in areas with poor living conditions and limited healthcare access. Additionally, diarrheal diseases caused by parasites such as *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Cyclospora cayetanensis* remain a persistent burden, particularly in developing countries, where even basic sanitary measures are insufficient [5].

Though musculoskeletal complications from parasitic infections are more commonly associated with tropical regions, they are increasingly encountered in non-endemic areas, such as the Republic of Moldova, due to migration and seasonal travel. Although Moldova is not classified as an endemic zone for severe parasitic diseases affecting the musculoskeletal system, exotic infections have become more frequent.

The most reported parasitic infections in Moldova that have shown musculoskeletal involvement are:

- *Echinococcus granulosus*: The national morbidity rate for echinococcosis over the past decade averaged 4.3% [3, 7].
- *Giardia lamblia*: This infection exhibited an average prevalence of 4.86% in the same period [3, 4].

Despite numerous reports documenting musculoskeletal involvement in parasitic infections, there is still a lack of systematic research that can provide comprehensive insights. Such research is essential not only for national and international recognition but also for developing early intervention strategies in the diagnosis and treatment of musculoskeletal disorders related to parasitosis.

The aim of this study was to conduct a bibliographic analysis of the most recent data concerning the impact of helminthic infestations on immune function and the progression of osteo-articular diseases in the context of parasitic infections.

Material and methods

This qualitative and analytical study focused on primary research published between 2016 and 2022. The objective was to identify musculoskeletal biomarkers relevant to the diagnosis, disease progression, and complications associated with parasitic infestations.

To achieve the research goals, scientific databases such as PubMed, NCIB, Google Search, and Medscape were explored using the following key terms:

- Parasitic arthritis;
- Parasitic biomarkers;
- Diagnosis of parasitic infestation;
- Prediction of disease progression in parasitic infections.

A total of 74 reference sources were identified, out of which 19 studies were deemed most relevant and selected for detailed analysis.

Results and discussion

Modulating joint inflammation through parasitic infections

Chronic autoimmune conditions like rheumatoid ar-

thritis (RA) pose significant challenges to musculoskeletal health, requiring innovative treatments beyond conventional therapies. Parasitic infections, known to influence immune function in their hosts, have drawn considerable interest in parasite-mammal model studies. These models aim to uncover pathways and molecules that could inspire novel therapies for autoimmune and idiopathic diseases. Although much research has focused on mucosal inflammation in parasitic infections and gastrointestinal diseases such as inflammatory bowel disease (IBD), the impact of these infections on joint inflammation remains underexplored. This paper highlights the potential for parasitic infections to alleviate joint inflammation and reviews relevant literature supporting this hypothesis.

Immune modulation by parasitic infections

Parasitic infections trigger an immune response characterized by type 2 helper T cell (Th2) activation [8-11]. This response plays a regulatory role, often suppressing the more aggressive Th1-driven immune responses. As a result, individuals with parasitic infections may experience milder symptoms of Th1-mediated inflammatory diseases. Numerous studies have shown that parasites residing in mucosal tissues, such as trematodes, cestodes, and nematodes, can reduce inflammation in experimental models of colitis and airway hyperreactivity [5, 12-14]. However, limited research has investigated the systemic effects of parasitic infections on organs beyond the primary site of infection, including joints. This article discusses the potential for parasitic infections to regulate immune responses and alleviate joint inflammation.

The growing burden of autoimmune diseases

Over the last few decades, the prevalence of autoimmune diseases, including inflammatory bowel disease (IBD), multiple sclerosis, diabetes, and rheumatoid arthritis (RA), has increased substantially, especially in developed nations. RA, a chronic and progressive autoimmune disorder, affects approximately 1% of the North American population [15, 16]. The socio-economic impact of RA is significant; in 2003, arthritis and other related conditions cost the United States around $127.8 billion, equating to 1.2% of the national GDP [15, 16].

Limitations of current treatments for RA

Current treatments for RA rely on nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), and disease-modifying antirheumatic drugs (DMARDs). While NSAIDs reduce inflammation, they do not stop disease progression and often result in gastrointestinal, renal, and neurological side effects [17].

GCs are potent anti-inflammatory agents but carry significant risks with long-term use, including reduced bone mineral density, obesity, diabetes, and cataracts [17, 18]. DMARDs, such as methotrexate (MTX), are effective in slowing joint destruction but can cause severe toxic effects in the liver, respiratory system, gastrointestinal tract, and other organs [19].

Given the limitations and side effects of current treatments, there is an urgent need for alternative therapies. Advancing these therapies requires a thorough understanding of the immune mechanisms driving joint inflammation. Although animal models cannot perfectly replicate human RA, they provide valuable insights into the disease's immune processes. Notably, research on mice paved the way for the development of anti-TNFα therapies, now a cornerstone of RA treatment [18].

Parasitic therapy and immune regulation

Infections with parasites induce immune responses dominated by IL-4, IL-5, IL-9, IL-10, and IL-13 [6, 12, 20]. These responses are associated with mast cell hyperplasia, eosinophilia, and increased IgE production [21, 22]. Parasites also secrete molecules that can modulate the host's immune system by reducing inflammation or redirecting immune responses. Parasitic infections are believed to shift immune responses from a Th1-dominated state to Th2 pathways, potentially mitigating autoimmune inflammation.

For example, the ES-62 glycoprotein, derived from *Acanthocheilonema vitea*, has demonstrated anti-inflammatory properties. It inhibits the activation of pro-inflammatory cytokines and suppresses mast cell degranulation [23]. In murine models, ES-62 reduced the severity of collagen-induced arthritis by lowering levels of TNFα, IL-6, and IFNγ, while increasing IL-10 production [23, 24].

Research on parasitic infections and joint inflammation

Several studies have shown that parasitic infections can reduce joint inflammation. One early observation involved rats infected with *Syphacia obvelata*, which developed a milder form of arthritis after receiving CFA injections [23, 24]. In another study, mice infected with *Heligmosomoides polygyrus* and *Nippostrongylus* exhibited a reduced incidence of arthritis, although some joint damage persisted [25].

Recent research found that infection with *Schistosoma mansoni* reduced collagen-induced arthritis in mice by decreasing joint swelling and synovial hyperplasia [26]. Similarly, infection with the tapeworm *Hymenolepis diminuta* alleviated arthritis symptoms, with effects comparable to those of steroids and NSAIDs [5, 25].

Despite promising results, parasitic therapy faces significant challenges. Not all parasites are suitable for therapeutic use; for example, *Schistosoma mansoni* causes severe disease and is unsuitable for clinical application. The risk of iatrogenic infections and the potential for comorbid conditions also need to be carefully considered. Some studies have raised concerns that parasitic therapy might not be appropriate for patients with diseases characterized by eosinophilia [6, 26, 27].

Another challenge is the difficulty in isolating specific immunomodulatory molecules from parasites. However, advances in mass spectrometry and other technologies have improved our ability to extract and analyze these molecules. This progress offers hope for the development of new anti-inflammatory drugs based on parasitic molecules.

While research on parasitic therapy remains in its early stages, evidence suggests that parasitic infections can mod-

ulate immune responses and reduce joint inflammation. However, further studies, including clinical trials, are needed to confirm these findings and explore their potential for human treatment. The ultimate goal is to identify specific parasitic molecules that can serve as templates for developing novel therapies for autoimmune diseases.

Conclusions

Although a limited number of studies have indicated that parasitic infections may sometimes aggravate pre-existing conditions, the prevailing consensus is that parasitic infections can create an immunoregulatory environment that helps reduce the severity of concurrent diseases. While research on the impact of parasitic infections on arthritis remains limited, findings from animal models and concurrent parasitic infections suggest that "parasitic therapy" holds promise as a potential strategy for managing inflammatory joint diseases.

This field represents a fascinating area of translational research, with the potential to yield important discoveries about immunomodulatory mediators, anti-inflammatory cell functions, and pathological mechanisms. These insights could pave the way for the development of novel treatment strategies targeting autoimmune and idiopathic diseases in humans. The idea of using specific parasitic organisms as therapeutic agents, especially for individuals whose immune profiles have been carefully studied, aligns with the concept of personalized medicine. However, at present, this remains a theoretical possibility rather than a clinical reality.

It is important to highlight that individuals should not attempt to self-infect with parasitic organisms in pursuit of potential health benefits. Lastly, animal studies must be conducted with greater rigor to better understand all aspects of immunomodulation induced by parasitic infections and to assess potential adverse effects when such infections occur in conjunction with other diseases.

Competing interests

None declared.

Authors' contributions

Study conception and design: MG, LG, GP. Data acquisition: MG, GP. Analysis and interpretation of data: MG, ER. Drafting of the manuscript: MG, LG. Significant manuscript review with significant intellectual involvement: LG. Approval of the „ready for print" version of the manuscript: LG, MG, GP, ER**.**

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REVIEW ARTICLE

Mechanisms of niacin skin test pathogenesis in patients at clinical high risk for psychosis and schizophrenia

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ABSTRACT

Introduction. Elevated or imbalanced levels of markers of oxidative stress and inflammation are often observed in various somatic pathologies and mental disorders, including schizophrenia.

Purpose of the study. This study aims to investigate the mechanisms of pathogenesis and the evidence supporting the use of niacin skin and oral tests in patients with schizophrenia.

Materials and methods. A literature review was conducted on the specific reactions to the niacin skin or oral test in patients with schizophrenia, first-episode psychosis, and those at clinical high risk for psychosis (CHR-P). Evidence-based data up to and including 2024 were reviewed, with 48 literary sources selected.

Results. An attenuated niacin-induced flush, coupled with low vitamin B3 levels, an imbalance in the Redox-Ratio and omega-3/omega-6 fatty acids, and elevated phospholipase A2 levels, are the main evidence-based findings associated with schizophrenia.

Conclusions. The niacin skin and oral tests in patients with schizophrenia and those at high risk for psychosis are characterized by an abnormal response to niacin. Additional markers may further validate positive test results for niacin.

Keywords: niacin, skin test, pathogenesis, psychotic disorders, high-risk populations, schizophrenia.

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Key messages

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

There are ongoing challenges in the timely diagnosis of schizophrenia, particularly during prodromal states. Current diagnostic criteria are primarily based on assessment tools that are applicable only when
symptoms of schizophrenia are well manifested, leaving a gap in early detection.

The research hypothesis

The use of the niacin test as a diagnostic method may provide additional insights, especially in the pre-psychotic stage, potentially aiding in earlier diagnosis.

The novelty added by manuscript to the already published scientific literature

This manuscript introduces the novelty of exploring inflammatory imbalance, phospholipid dysregulation, and NAD+ deficiency in schizophrenia. These factors may contribute to enhanced diagnostic strategies and offer possibilities for adjuvant treatment options for schizophrenia.

Introduction

Many mechanisms of the pathogenesis of mental and somatic disorders share common points of contact, related to the increase in markers of oxidative stress and inflammation. Oxidative stress and decreased antioxidant activity play important roles in the development of these disorders. Violations of mitochondrial functions, depletion of NAD+ (Nicotinamide adenine dinucleotide oxidized form), NAD+ -induced signaling cascades, and increases in levels of reactive oxygen and nitrogen species are the basis of this process [1]. Zapata-Pérez R. et al. (2021) describe the relationship between the oxidized (NAD+) and reduced (NADH) forms of nicotinamide adenine dinucleotide, the redox balance, and the biosynthesis of fatty acids and nucleic acid [2]. The balance between the synthesis and breakdown of NAD+ is required to maintain cellular homeostasis and physiological functioning. It is known that NAD+ deficiency, which can be genetically determined and also due to low levels of its precursors as a result of dietary deficiencies and synthesis issues, leads to serious somatic and mental disorders. For example, niacin deficiency is associated with pellagra symptoms such as diarrhea, dermatitis, and dementia [3]. Sitarz R. et al. (2023), describe the mechanisms underlying metabolic imbalances due to fatty acid and vitamin B3 deficiency. The authors reported the effectiveness of omega-3 fatty acids in the onset of mental disorders, even when there are no clear indications for the initiation of psychotropic therapy [4]. The nicotinate phosphoribosyl transferase gene (NAPRT1) encodes a key enzyme for niacin metabolism. According to Periyasami S. et al. (2019), niacin deficiency is associated with schizophrenia-like symptoms [5]. Therefore, the antioxidant and anti-inflammatory properties of some food additives, such as omega-3 fatty acids, vitamin B3, and NAD (Nicotinamide adenine dinucleotide), can be used as alternative or adjuvant therapies for mental diseases and comorbid somatic pathology caused by inflammatory reactions and oxidative stress. Such therapies may include the use of NAD+ precursors like nicotinamide mononucleotide and nicotinamide ribose, which quickly increase NAD+ levels in the brain and periphery [1, 6]. The disturbances of neurotransmission constitute one of the pathogenetic elements of the membrane phospholipid hypothesis in schizophrenia [7]. Impaired prostaglandin signaling in schizophrenia is established, with several pathways explaining this mechanism: reduced levels of arachidonic acid, a precursor of prostaglandins; increased activity of the enzyme phospholipase A2, and abnormal expression of niacin receptors in cutaneous capillary walls. Skin flushing in response to niacin administration is also considered to be influenced by prostaglandins [8]. David F. Horrobin, the proponent of the prostaglandin signaling imbalance hypothesis in schizophrenia, stated in 1977 that it "does not necessarily contradict modern theories of the transmitters of schizophrenia, since prostaglandins alter the secretion and action of mediators." He supported his hypothesis with clinical observations, noting that the increased pain threshold and decreased inflammation in patients with schizophrenia could be attributed to prostaglandins. Additionally, he noted that the increase in prolactin due to antipsychotic treatment might be associated with prostaglandin synthesis. However, it remains unclear why "all effective antischizophrenic drugs stimulate prolactin secretion" but "high doses of drugs recently shown to be prostaglandin antagonists cause schizophrenia-like syndromes [9]. Clinical practice shows that high doses of antipsychotics are less associated with symptom exacerbation. Another important metabolic criterion and risk factor is the omega-3 index, which represents the percentage of red blood cells with omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in erythrocytes [10, 11]. According to David F. Horrobin et al. (1994), "in schizophrenia, the metabolism of phospholipids may be impaired both in the brain and in red blood cells" [12]. The authors note that the patients with negative symptoms have had "the concentrations of arachidonic acid (AA) and DHA in erythrocyte membrane phospholipids deviated sharply from the norm"[12]. Ya-Hui Yu et al. (2022) also report the membrane hypothesis of schizophrenia, according to which low levels of polyunsaturated fatty acids, including omega-3 arachidonic acid (AA), lead to dysfunctional signaling, changes in the structure, rigidity, and conformation of cell membranes, and pathology of receptor activity, proteins, prostaglandins, cyclooxygenases, and ion channels in these patients [13]. Reporting an abnormal response to niacin in patients, Ranpiao Gan et al. (2022) conclude that this response occurs in the "early stage of psychosis" and during "disease progression," linking it to PUFAs (polyunsaturated fatty acids) [14]. Zhang T. et al. (2023) stated a significant correlation between attenuated niacin response and core negative symptoms in patients with first-episode psychosis. According to the authors, this indicates that an attenuated niacin response (ANR) may be a potential biomarker for certain subtypes characterized by negative symptoms and poor symptomatic remission [15].

The aim of the research

The aim of this paper was to study the mechanisms of pathogenesis and the evidence supporting the use of niacin skin and oral tests in patients with schizophrenia, first-episode psychosis, and those at clinical high risk for psychosis (CHR-P). Additionally, the research aimed to examine the rationale for the use of vitamin B3 as an additional or preventive therapy in these groups of patients.

Materials and methods

A literature review was conducted on the occurrence of specific reactions to the niacin skin or oral test in patients with schizophrenia, first-episode psychosis, and those at clinical high risk for psychosis (CHR-P). Information was sourced from the PubMed, MEDLINE, Medscape, Scopus, Cochrane Library, and research4life.org databases up to and including 2024, from which 48 sources were selected. The inclusion criteria were studies published in English and human studies only. The literature was analyzed concerning mechanisms of action, risk factors, and evidence supporting the utility of the niacin skin or oral test in high-risk psychosis states and schizophrenia.

Table 1. Niacin or methylnicotinate tests in patients with schizophrenia-control group studies

Author, year	Number of the patients/control	Diagnose	Dose /route of administration	Result
Basant K Puri et al., 2002 [22]	27/26	Schizophrenia	Niacin	Flushing response is reduced in schizophrenia, with a sensitivity of 75% and a specificity of 65%
Nilson B.M.,2006 [23]	30/17	Schizophrenia	200 mg niacin /orally	The patients showed a delayed temperature increase after niacin ingestion (P=0.002) and a higher frequency of electrodermal non-responding (P<0.05).
Karakula-Juchnowicz H. et al., 2020 [24]	56/45	Schizophrenia	Methylnicotinate 0.1M; 0,001M; 0.0001M	The absent response was detected in 60.7% of individuals suffering from schizophrenia, and the proposed method could predict schizophrenia with 71% sensitivity and 66% of specificity.
Arzanlou M., 2021 [25]	36/33	Schizophrenia	Niacin patches 0.1M; 0,001M; 0.0001M	Flush responses to niacin are more impaired in patients with schizophrenia. At 10 min, the highest test accuracy was reported when a 0.001 M niacin solution was used (Sensitivity=94%, specificity=50%, PPV= 51%, and NPV= 94%). At 15 min, the highest test accuracy was observed with a 0.01 concentration (Sensitivity=52%, specificity=92%, PPV=79%, and NPV=77%).
Dan-Dan Wang et al., 2021 [26]	307 schizophrenia /148	Schizophrenia, bipolar disorder	Niacin	Attenuation and a delay of the niacin-induced skin flushing were characterized in the largest cohort of patients with schizophrenia. Patients with either schizophrenia or affective disorders were identified from the healthy control group with a sensitivity of 55.28%, a specificity of 83.56%, and a positive predictive value of 93.66%.
Ya-Hui Yu et al., 2022 [13]	46/37	Schizophrenia	Methyl nicotinate 0.1M; 0,001M; 0.0001M before and after 2 months follow-up period	Healthy controls exhibited enhanced niacin-induced flush at the 2-month follow-up, whereas patients with schizophrenia did not show significant changes in their flushing response.
Gan Ranpiao, 2022 [14]	105CHR/57FES/ 52 HCs	CHR FES	Methylnicotinate 0.1M; 0,001M; 0.0001M	CHR individuals showed attenuated niacin-induced flushing responses characterized by a modest level of severity that was intermediate between those of HCs and patients with FES. The niacin flush response is more blunted in CHR converters to full psychosis at 2 years of follow-up compared to non-converters.
Carena F. et al., 2023 [27]	21/20	Schizophrenia	375 mg crystalline niacin orally	Abnormal niacin response was observed in 90,5% of the patient group and 0% in the control group (non-randomized clinical trial).
TianHong Zhang et al., 2023 [28]	60/60	Clinically high-risk psychosis (CHR)	Methylnicotinate	The CHR group exhibited significantly higher half-maximal blood flow response - $LogEC_{50}$ (t = 3.650, P < .001) and minimal blood flow response - Span (t = 2.657, P = .009) values than the HC group. These findings indicate a significant association between niacin response and psychosis conversion outcomes in individuals with CHR.

Note: *CHR*- Clinically high-risk psychosis, M- Methylnicotinate, PPV-positive predictive value, NPV-negative predictive value, FES-first episode of schizophrenia, HC-healthy control group.

Results and discussions

„Niacin is a general term used to define vitamin B3 and derivatives, including nicotinic acid, nicotinamide, and related compounds, such as nicotinamide riboside" [2]. Pathological processes at the level of membrane phospholipids are considered an essential factor in the pathophysiology of schizophrenia. These processes are believed to have a direct influence on the imbalance of neurotransmitters within the central nervous system [16]. It is known that niacin and its amide, nicotinamide, are considered precursors of vital coenzymes that participate in metabolic reactions. They play a key role in the Krebs cycle and the recovery of nicotinamide adenine dinucleotides (NAD, NADH, NAD+, and NADP+). These coenzymes are involved in DNA repair, detoxification, and the synthesis of steroid hormones [16]. Abram Hoffer (1998) recommends the use of vitamin B3, specifically niacin, in doses ranging from 1 to 12 grams per day for treating psychotic states, including schizophrenia. If adverse reactions occur with niacin, niacinamide can be administered as an alternative, as it generally produces a more moderate effect [17]. The recommended dose for niacin is 2 to 4 mg for infants, 6 to 8 mg for children, 12 mg for teenagers, 16 mg for men, 14 mg for women, and 17 and 18 mg for lactating and pregnant women [18, 19]. In a randomized clinical trial involving patients with schizophrenia who had negative symptoms, the patients failed to flush and had significantly reduced levels of arachidonic and docosahexaenoic acids. Over a period of six months, supplementation led to a conversion from non-flushing to flushing. This conversion was predicted by an increase in arachidonic acid levels in red blood cell membranes, regardless of the nature of the supplementation [20]. Niacin enters the body through food and can also be synthesized from the amino acid tryptophan with the help of vitamin B6. Studies have shown that 1 mg of nicotinamide is produced from 67 mg of tryptophan [21]

In Table 1, the results of several niacin or methylnicoti-

nate skin flush test studies in patients with schizophrenia can be seen.

Hoffer A. and Osmond H. (1966) proposed the NAD-deficiency hypothesis of schizophrenia, concluding that „schizophrenia is an NAD-deficiency disease". They explained this position by stating that „large doses of nicotinic acid and nicotinamide are effective in schizophrenia", quickly improving the clinical condition of patients. The authors drew a parallel between schizophrenia and pellagra, suggesting that patients with schizophrenia „are not able to synthesize NAD as effectively" as the control group [29]. Sang-Young Kim et al. (2017) provide evidence of "redox disturbances" in schizophrenia and mood disorders. The authors identify elements of pathogenesis in schizophrenia, which, in their opinion, include the "immune-oxidative" pathway, oxidative stress, mitochondrial dysfunction, neuroinflammation, and cell-mediated immune response [30]. Ramachandran P. et al. (2012) propose a hypothesis for the therapeutic treatment of schizophrenia using vitamin B3 (niacin), based on the adrenochrome theory of schizophrenia by Hoffer (1981). According to this theory, a pathological product of oxidative metabolism, an adrenaline derivative called adrenochrome, is involved in the productive symptoms of schizophrenia, which have hallucinogenic properties. Vitamin B3, by limiting the production of adrenaline, reduces the synthesis of adrenochrome [31, 32]. In schizophrenia, a high level of adrenochrome accumulates in the central nervous system. Vitamin B3, as a precursor of nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH), participates in these transformations under normal conditions and in schizophrenia. With sufficient levels of NAD and NADH, coenzyme B3 enables adrenochrome to convert back into adrenaline. Hoffer (1981) discussed the influence of stress on changes in the neurotransmitter system: „Stress is harmful for two reasons. The increase in the production of noradrenaline and adrenaline will lead to an increase in adrenochrome," and genetically predisposed individuals might experience an„increase in adrenolutin", a toxic metabolite of adrenochrome [32]. Many authors have studied the hypothesis that early life stress is associated with the development of schizophrenia later in life. This is linked to changes in the hippocampus during stressful situations, leading to neuroinflammation, microglial activation, the expression of proinflammatory mediators (IL-1β, TNF-α, IL-6), the loss of hippocampal neurons, and schizophrenia-like behavior. NAD therapy reverses these changes. It is believed that NAD has neuroprotective activity and could be used during puberty to prevent neuronal loss and improve hippocampal function in people exposed to early life stress [30-33]. The biological importance of NAD metabolites extends beyond participation in carbohydrate metabolism. They also play a role in oxidative stress processes and are involved in many metabolic pathways. NAD+ supports various processes, including the conversion of glucose and other nutrients into energy, energy metabolism and modulation, repair of damaged DNA and gene expression, support of cell defense systems and immunological functions, calcium homeostasis, regulation of the sleep-wake cycle, and antioxidant activity. Additionally, it participates in apoptosis, the aging process, and carcinogenesis [30]. Taking the tablet form of NADH helps increase wakefulness, attention, daily performance, and vitality [31]. Indications for prescribing NADH include the prevention of fatigue and maladjustment when changing time zones, as well as improving memory, attention, and other aspects of cognitive functioning of various causes. These causes include stress-related maladjustment, aging, and metabolic syndrome. Improvement is observed in cases of asthenia of any origin, chronic fatigue syndrome, Parkinson's and Alzheimer's diseases, depression, and the normalization of mood swings [31].

The Redox Ratio as an index for oxidative stress

According to Ryszard Sitarz et al. (2023), the niacin skin test is a simple and inexpensive method "used to assess the fatty acid content of cell membranes" and is "a possible indicator in the diagnosis of mental disorders" [4]. The niacin skin patch test is characterized by a decrease in skin hyperemia in patients with schizophrenia, manifesting as an abnormal response to niacin (niacin response abnormality - NRA). This serves as an endophenotype for schizophrenia, compared with the control group, where redness is pronounced [7, 34, 35]. The practical significance of this test involves the rapid screening of patients at risk for schizophrenia at the onset of the disease, even before clinical symptoms appear, allowing for timely intervention [7]. The NAD+/NADH ratio plays a crucial role in maintaining redox homeostasis. Sang-Young Kim et al. (2017) describe redox balance as the ratio of NAD+ to NADH concentrations, expressed as the Redox Ratio (RR) = [NAD+]/[NADH]. According to authors, the Redox Ratio serves as an index for determining oxidative stress levels in the brains of individuals with schizophrenia, as measured by magnetic resonance spectroscopy [30].

Interaction of the GPR109A receptor and phospholipase A2 in individuals with schizophrenia

Oxenkrug G. and Forester B. (2024) report the interaction between the GPR109A receptor and phospholipase A2 in individuals with schizophrenia. The GPR109A receptor exhibits anti-inflammatory and neuroprotective activities. In the brains of individuals with schizophrenia and those at high risk of developing psychosis, phospholipase A2 levels are elevated. Phospholipase A2 regulates the conversion of arachidonic acid to prostaglandins, contributing to pathological activity in schizophrenia by producing excess prostaglandins and depleting arachidonic acid levels. This enzyme can destroy the myelin layer of axons, thereby reducing neuroprotective functions and disrupting nerve conduction. The authors found correlations between the upregulation of the GPR109A receptor and the inhibition of phospholipase A2. One of the mechanisms influencing the niacin test result, as noted by the authors, is attributed to a genetic mutation in GPR109A. This mutation leads to a decreased blood flow response to niacin exposure, which is a characteristic of individuals with schizophrenia [34]. Tavares et al. (2003) conducted a study involving 38 individuals

with schizophrenia and 28 individuals in a control group. They found that phospholipase A2 levels were significantly increased in the schizophrenia group compared to the control group (344±115 pmol/ml/min vs. 290±71 pmol/ml/min; p=0.03). Over 8 weeks of antipsychotic treatment, phospholipase A2 levels were reduced to approximately 267±39 pmol/ml/min (p=0.001). Additionally, 4 out of 13 individuals who previously showed no response to the niacin test exhibited a positive response after treatment [36].

Inflammatory imbalance, phospholipid dysregulation, and the „Two hit hypothesis of schizophrenia"

In schizophrenia, there is an imbalance between pro-inflammatory and anti-inflammatory metabolites. Levels of polyunsaturated fatty acids, anti-inflammatory prostaglandins PGE1 and 15d-PGJ2 are reduced, while the level of the pro-inflammatory prostaglandin PGE2 is increased. According to the "two-hit hypothesis," changes in membrane phospholipids can contribute to the pro-inflammatory pathogenesis of schizophrenia and act as a vulnerability factor [37]. Jeffrey K. Yao (2016) discussed mechanisms underlying the abnormal niacin response in individuals with schizophrenia, focusing on signaling pathways involving cell membrane phospholipids, arachidonic acid, and eicosanoids. G protein-coupled receptors, such as GPR109A or HM74A, located on dermal macrophages and adipocytes, activate phospholipase A2, releasing arachidonic acid from cell membranes. This arachidonic acid is then converted into prostaglandins PGD2 and PGE2, which have vasodilatory effects [35]. Proinflammatory responses in patients with schizophrenia activate microglia, leading to neuronal apoptosis. According to Fillman, S.G., et al. (2014), "excessive activation leads to increased synthesis of cyclooxygenases, causing inflammatory reactions". Microglia synthesize various cytokines and respond to stimuli, which also occurs in patients with schizophrenia, but with a shift towards pro-inflammatory activity [38]. Keith A. Feigenson also reports the "Two-Hit Hypothesis of Schizophrenia," which posits that "early immune activation of microglia may sensitize them to later activation." This hypothesis suggests that prenatal risk factors, stress, and early infections lead to the formation of pathological microglia that are easily "activated" in the long term, for example, at puberty in response to a new trigger [37].

The Niacin Test and High Risk for Psychosis. Correlation between PUFA and Phospholipase A2

Oxenkrug G. and Forester B. (2024) also highlight the antipsychotic effect of nicotinic acid in clinical studies [34]. Similar conclusions are drawn by Nadalin S. et al. (2010), who establish the niacin test as a potential marker in schizophrenia. They assert that it "may be useful for further research into the genetics and pathophysiology of schizophrenia," considering it "an indicator of vulnerability to the development of psychosis" and a "simple, non-invasive, and easily reproducible method for the study of schizophrenia" [7]. The practical relevance of the niacin skin test is emphasized by Sabrina H. Ansarey (2021), who states that "the niacin skin flush test is useful in identifying patients with ultra-high-risk schizophrenia," and "may offer effective treatment". The author also notes that reducing the skin redness reaction depends on the interaction of neurons and microglia [39]. As mentioned previously, the niacin test has been proposed as a biomarker for schizophrenia or as a "potential factor" for screening for schizophrenia [7, 24, 40]. However, according to Berger, G. E., et al. (2016), an atypical "significantly increased response in patients" with ultra-high risk for schizophrenia is also possible [40]. They found that "sensitivity was inversely correlated with the levels of omega-3 and -6 fatty acids, but positively correlated with phospholipase A2 (PLA2)," leading the authors to associate the cause of psychosis with a "pro-inflammatory state" [40]. Ryszard Sitarz et al. (2023) propose the hypothesis that "the reduction of skin reaction in patients with schizophrenia can be caused by the deficiency of arachidonic acid in the cell membrane" [4]. It has been established that the most important omega-3 polyunsaturated fatty acids are alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid [41]. Arachidonic acid refers to omega-6 polyunsaturated fatty acids. Its adequate level in the body is very important, as the omega-3/omega-6 ratio should ideally be 1:1. However, in reality, omega-6 usually prevails in these ratios, and a lower omega-3 ratio increases the risks of somatic and mental diseases. Both omega-3 and omega-6 play an important role, as they are part of the phospholipids of cell membranes. They can change the fluidity of membranes and the activity of ion channels, membrane receptors, and neurohormones [7]. Deficiency of both polyunsaturated fatty acids can lead to phospholipase A2 activation, excessive release of these polyunsaturated fatty acids from cell membranes, cyclooxygenase-2 activity and prostaglandin synthesis, accumulation of oxidative stress products, free radicals, lipid peroxidation, synthesis of pro-inflammatory mediators of arachidonic acid, and pathological activity of desaturases. These processes have been related in other publications concerning patients with schizophrenia [7, 13, 35]. One meta-analysis found that levels of the omega-3 fatty acids docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), and the omega-6 fatty acid arachidonic acid (AA) were reduced in the red blood cells of patients with schizophrenia, "especially DHA and AA" [42]. Yu Y-H et al. (2022) related that the abnormal response to niacin in patients with schizophrenia may "reflect dysfunctional signaling" due to low AA levels [13]. Additionally, they concluded that, with a probability of $p < 0.05$ compared with the control group "adjusted for age, sex, and smoking," patients with schizophrenia had higher delta-5 desaturase (D5D) and delta-6 desaturase (D6D) activity [13]. Furthermore, D5D and D6D, encoded by the FADS1 and FADS2 genes, are rate-limiting enzymes for the conversion of polyunsaturated fatty acids [43]. This indicates a connection between the niacin skin test and omega fatty acids.

Kynurenine pathway in schizophrenia

An important component in the pathophysiology of schizophrenia is the kynurenine pathway. Tryptophan, an essential amino acid, is vital for the development of the serotonin and kynurenine pathways. The World Health Organization recommends a daily intake of tryptophan of

about 3.5–6.0 mg/kg of body weight [44]. In the serotonin pathway, metabolism ends with the production of serotonin and melatonin. In the kynurenine pathway, the production of nicotinic acid occurs, followed by nicotinamide, and finally NAD+[45]. Plitman et al. (2017) attribute about 90% of tryptophan metabolism to the kynurenine pathway [46]. Increased levels of kynurenic acid and cytokines in the brain have been reported post-mortem in patients with schizophrenia [47]. The kynurenine pathway influences behavior, the immune system, energy metabolism, and serotonin balance. Dysregulation of this pathway is also associated with gut and brain inflammation [45].

In Table 2, the most common mechanisms underlying the abnormal response to the niacin test in schizophrenia are highlighted:

Table 2. Mechanisms of abnormal niacin response in patients with schizophrenia

Mechanism	Description	References
Endophenotype of skin reactions	Reduction of skin hyperemia and decreased vasodilation response in patients at risk for schizophrenia	[4, 5, 7, 8, 12, 14, 15, 32, 33]
Prostaglandin levels	Reduced levels of anti-inflammatory prostaglandins (PGE1 and 15d-PGJ2) and increased level of pro-inflammatory PGE2	[33, 35]
Immune activation and inflammation	Excessive immune activation of microglia, expression of pro-inflammatory mediators (IL-1β, TNF-α, IL-6), loss of hippocampal neurons, and schizophrenia-like behavior	[28. 30, 31]
Fatty scid levels	Reduced levels of docosahexaenoic acid (DHA) and the omega-6 fatty acid arachidonic acid (AA) in red blood cells	[4, 12, 13]
Kynurenine pathway dysregulation	Dysregulation of the kynurenine pathway, affecting serotonin balance and immune response	[43-45]
Omega-3/omega-6 imbalance	Imbalance in the omega-3/omega-6 ratio, affecting cell membrane fluidity and signaling pathways	[11, 14]
Phospholipase A2 (PLA2) activity	Increased activity of the enzyme phospholipase A2 (PLA2), leading to excessive release of polyunsaturated fatty acids from cell membranes	[34]
GPR109A receptor and phospholipase A2 correlation	Correlations between the upregulation of the GPR109A receptor and the inhibition of phospholipase A2 (PLA2)	[34]
NAD+/NADH Ratio Reduction	Significant reduction in the NAD+/NADH ratio in chronically ill schizophrenia patients compared to healthy controls; Redox Ratio (RR) = [NAD+]/[NADH] serves as an index for determining oxidative stress levels	[2, 27, 28]
Delta-5 Desaturase (D5D) and Delta-6 Desaturase (D6D) activity	Higher activity of delta-5 desaturase (D5D) and delta-6 desaturase (D6D), enzymes involved in polyunsaturated fatty acid conversion	[41]

Note: *IL-interleukin, NAD+ - Nicotinamide adenine dinucleotide oxidized form, NADH - Nicotinamide adenine dinucleotide reduced form*

There are publications describing oral niacin reactions in schizophrenia. Francisco Carena et al. (2023) state that even with this route of niacin administration, patients with schizophrenia have an abnormal response to niacin compared to controls and individuals with other mental illnesses. The prevalence of abnormal niacin response is higher in patients with schizophrenia. According to the authors' studies, the prevalence of this abnormal response in patients with schizophrenia is 90.5%, while in the control group it is 0%. Additionally, the abnormal response to oral niacin in these patients is dose-dependent [27].

A genome linkage scan of niacin skin flush response

A study conducted by Yin-Ju Lien et al. (2011) on 115 families, including 226 affected individuals, 137 unaffected individuals, and 94 healthy controls (HCs), established that both affected and unaffected individuals had lower niacin flush scores compared to HCs for moderate (0.01 M) and high (0.1 M) concentrations of niacin in skin patches. The authors revealed a linkage region with a significant signal (3.39 at 14q32.12). They identified two genes located in the 14q32.12 region that could be related to schizophrenia: ataxin 3 (AT3) and chromogranin A (CGA). Both genes are not related to phospholipase A2, which is encoded by the PLA2G6 gene located in the 22q13.1 region. The authors concluded that the 14q32.12 region could be responsible for the response to niacin in schizophrenia [48].

Conclusions

1. The abnormal niacin response in schizophrenia is linked to metabolic disturbances such as imbalances in the omega-3/omega-6 and NAD+/NADH ratios, along with low levels of vitamin B3. These imbalances contribute to a pro-inflammatory state, oxidative stress, and phospholipid dysregulation.
2. Vitamin B3 (niacin) and its derivatives, along with omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), show potential as adjunctive therapies for schizophrenia by reducing oxidative stress, inflammatory responses, and improving membrane fluidity.
3. Genetic factors, including mutations in the GPR109A receptor and dysregulation of the kynurenine pathway, play a significant role in the pathophysiology of schizophrenia. These genetic influences contribute to the observed metabolic imbalances and inflammatory responses.
4. The niacin test has a high degree of sensitivity and specificity, making it an easy and cost-effective screening tool for identifying clinical high-risk (CHR) individuals, patients with schizophrenia, or those at risk for developing schizophrenia. Assessing additional parameters could enhance preventive or adjunctive interventions.

Competing interests

None declared.

Authors' contribution

LB conceived the original draft preparation and was responsible for the conception and design of the review. LB and IN were responsible for the data acquisition, collection and assembly of the articles/published data, as well as their inclusion and interpretation in this review. Both authors reviewed the manuscript and approved the final version.

Informed consent for publication

Not needed for this article.

Ethics approval

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CASE STUDY

A case report of primary hepatic amyloidosis manifesting as severe cholestasis and acute liver failure

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ABSTRACT

Introduction. Hepatic amyloidosis is characterized by the deposition of fibrillar amyloid proteins, which result from light chain amyloidosis (AL) immunoglobulin fragments, in the extracellular space and the vessel walls of the liver. A case of primary hepatic amyloidosis without evidence of a primary or secondary cause of amyloid deposition is rare. This case was unique to the Republic of Moldova, presenting a diagnostic and therapeutic challenge for clinicians. Because the liver is rarely affected, this pathology remains underdiagnosed and is associated with a reserved prognosis.

Clinical case presentation. An unusual case of primary hepatic amyloidosis is reported in a previously asymptomatic 59-year-old woman who presented at admission with peripheral edema, ascites, and hepatomegaly. Biochemical tests revealed severe cholestasis with normal bilirubin levels and acute liver failure. Liver damage caused by viral hepatitis or autoimmune diseases was excluded. A percutaneous bone marrow biopsy was normal, and Bence Jones protein was negative, indicating no evidence of primary amyloidosis. The definitive diagnosis was based on liver biopsy, which revealed apple green birefringence on polarizing microscopy after positive Congo red staining.

Conclusions. The reported case highlights the need to differentiate between infiltrative diseases, such as amyloidosis, when a patient presents with rapidly progressive severe cholestasis and acute liver failure. Future studies should focus on the availability of specific therapies for primary amyloidosis to improve the survival rate of these patients.

Keywords: amyloidosis, hepatic insufficiency, cholestasis

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Key messages

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

Hepatic amyloidosis often goes undetected due to its rarity and heterogeneous manifestations, leading to delayed diagnosis, a reserved prognosis, and a lack of effective treatment options.

The research hypothesis

Clinicians must recognize the significance of this rare yet potentially deadly disease when a patient presents with severe cholestasis and acute liver failure.

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

A case of primary hepatic amyloidosis with no identifiable primary or secondary cause of amyloid deposition is uncommon. Progressive cholestatic syndrome and liver failure presented uniquely in a previously asymptomatic patient, posing a particularly challenging situation for the diagnosis of primary liver amyloidosis.

Introduction

Amyloidosis consists of a heterogeneous group of diseases in which proteins resulting from light chain amyloidosis (AL) immunoglobulin fragments are deposited in the extracellular space of organs and tissues, with the heart and kidneys being the most frequently involved. These amyloid deposits lead to the disorganization of the normal architecture of the affected organ or tissue, resulting in its progressive dysfunction [1, 2].

Amyloidosis is classified according to the biochemical nature of the fibrillar material derived from the precursor protein. Several heterogeneous types of amyloidosis are recognized, including primary amyloidosis, in which the protein precursor consists of immunoglobulin light chains, more commonly lambda (AL); secondary amyloidosis, where the precursor is serum protein A (AA); familial amyloidosis, with transthyretin (prealbumin) as the precursor protein; and the senile form (ATTR). Primary amyloidosis is part of the group of plasma cell dyscrasias characterized by clonal expansion, while the secondary form (AA) is a complication of chronic diseases such as rheumatic or inflammatory bowel diseases, tumors, and tuberculosis [3, 4].

From a clinical perspective, primary amyloidosis is differentiated into two subgroups: a) AL amyloidosis associated with multiple myeloma (about 20% of cases), which contains the Kappa light chain; and b) primary AL amyloidosis not associated with multiple myeloma (approximately 80% of cases), characterized by the lambda monoclonal chain [3].

Next, a unique clinical case for the Republic of Moldova is described involving a patient with primary hepatic amyloidosis not associated with multiple myeloma, presenting with acute liver failure and severe cholestasis that rapidly evolved into a fulminant state. Isolated liver damage in amyloidosis is a rare phenomenon, and this clinical case serves as an example of an atypical evolution of the disease.

Clinical case presentation

A 59-year-old woman, previously asymptomatic, was hospitalized in the Timofei Moşneaga Clinical Republican Hospital, Hepatology service, due to the onset of ascites, leg oedema, cholestatic syndrome, and severe liver insufficiency syndrome, which were detected incidentally in the biochemical profile conducted on an outpatient basis.

She had been considered ill since the beginning of December 2023, complaining of postprandial epigastric pain. An ambulatory upper digestive endoscopy demonstrated a healed duodenal ulcer, which was subsequently treated with proton pump inhibitors, leading to further remission of the symptoms. However, within a short period, her condition worsened due to the appearance of leg oedema and increased abdominal size. The patient had an uncomplicated medical history, with no history of alcohol consumption, smoking, or use of substances or medication.

Physical examination revealed a pale-brown complexion, clear sclera, and an enlarged abdomen due to ascitic fluid, along with massive leg oedema. Abdominal palpation demonstrated hepatomegaly, with the liver having an elastic consistency, and splenomegaly. The patient was hemodynamically stable.

Paraclinical investigations on admission revealed a minimal cytolytic syndrome and severe cholestatic syndrome, with elevated alkaline phosphatase (ALP) at 1245.90 U/L (norm: 30 - 120 U/L), gamma-glutamyl transpeptidase (GGT) at 599.40 U/L (norm: 9 - 39 U/L), and total cholesterol at 12.30 mmol/l (normal: 0 - 5.2mmol/l), while bilirubin levels remained normal at onset. Severe liver insufficiency with coagulation disorders was also observed: albumin at 20.80 g/L (norm: 35 - 53 g/L), total protein at 44.20 g/L (norm: 66 – 83 g/l), prothrombin index at 39.10%, and International Normalized Ratio (INR) at 1.86.

Liver damage from viral and autoimmune causes was ruled out by testing for viral hepatitis markers, dsDNA antibodies, antimitochondrial antibodies (AMA-M2), antinuclear antibodies, and anti-PR3 antibodies (ANCA), all of which were within reference values. The cytology of the ascitic fluid was unremarkable, with no atypical cells identified. Urinary protein electrophoresis with immunofixation for Bence Jones proteins was negative.

Abdominal ultrasound confirmed the presence of ascites and hepatosplenomegaly. The mechanical etiology of cholestasis was assessed via magnetic resonance cholangiopancreatography (MRCP), which revealed only significant biliary sludge. Contrast-enhanced computed tomography confirmed these findings. An outpatient abdominal MRI with contrast demonstrated significant diffuse ascites and hepatosplenomegaly, with no evidence of portal hypertension. However, a dysplastic Li-RADS III nodule was identified in segment 4 (S4) without signs of malignancy.

Given the patient's deteriorating condition and inconclusive clinical and paraclinical findings, a diagnostic laparoscopy with liver biopsy was performed. Macroscopic examination revealed an enlarged liver with sharp edges, a brownish-violet color, and a firm texture. In S4b, a tumor mass approximately 2 cm in diameter was observed.

Histological examination of the liver biopsy sample revealed altered trabecular architecture, with hepatocellular oedema and swelling accompanied by protein degeneration. The hepatocytes exhibited hyperchromatic nuclei of uneven size, a slight increase in the nucleocytoplasmic ratio, occasional intranuclear inclusions, and mildly prominent nucleoli. Diffuse capillarization of the sinusoids was identified using Masson's trichrome stain, while the reticular architecture remained preserved as shown by Reticulin staining (Fig. 1a, b.).

The positive Congo Red staining test was validated by a control test under polarizing microscopy, which confirmed amyloid deposits in the vascular wall, capsule, and perisinusoidal areas. Under polarizing microscopy, these deposits exhibited characteristic apple-green birefringence (Fig. 2 a, b), creating a microscopic picture indicative of hepatic amyloidosis.

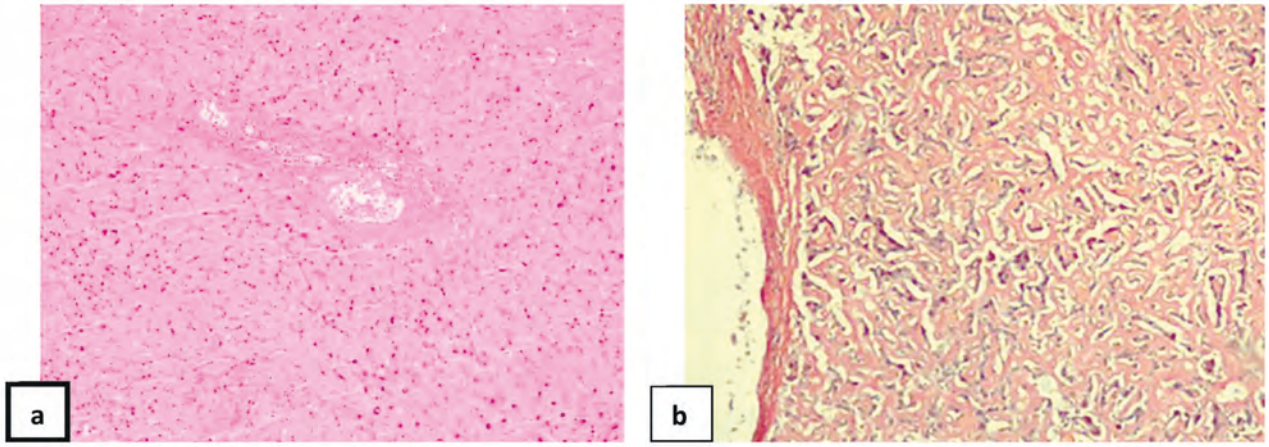

Fig. 1 AL hepatic amyloidosis.
a. Perisinusoidal pattern of amyloid deposit (staining: hematoxylin-eosin).
b. Disruption of liver architecture marked by diffuse extracellular and vascular wall accumulations of amorphous, acellular eosinophilic material, causing noticeable atrophy of focal hepatocytes (staining: Congo red).

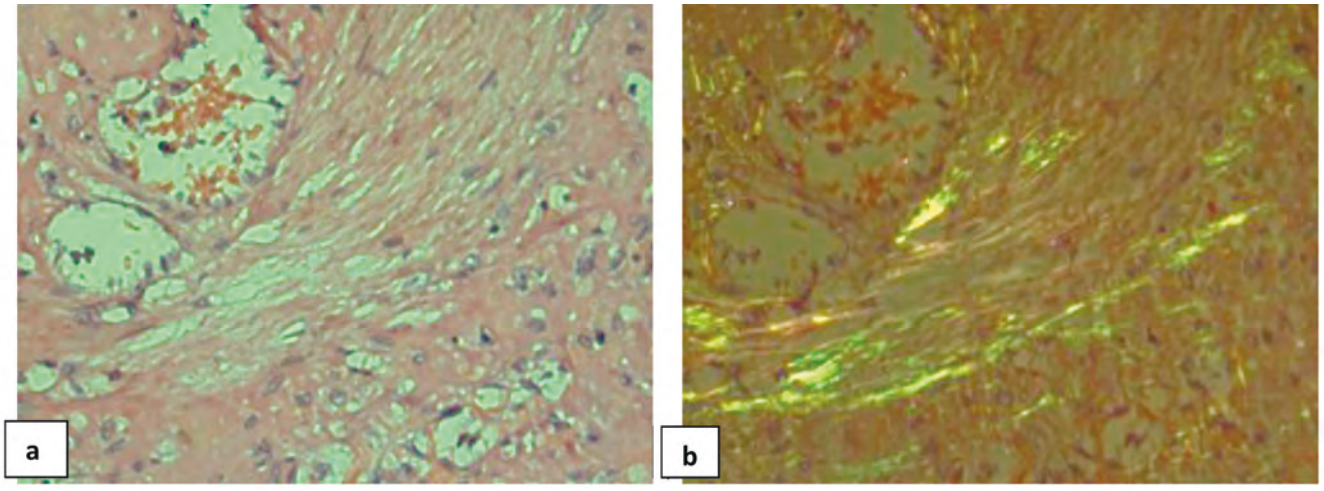

Fig. 2 AL hepatic amyloidosis.
a. Vessel with amyloid deposits observed under optical microscopy (staining: Congo red);
b. Amyloid deposits in the vessel under polarizing microscopy.

Given the frequent association of primary amyloidosis with multiple myeloma and Waldenstrom's macroglobulinemia, the patient underwent a percutaneous bone marrow biopsy. The myelogram revealed a hypocellular bone marrow, with the granulocytic series constituting 69.0%, segmented neutrophils accounting for 57%, and multiple macrophages observed. Megakaryocytes were within normal limits, and platelets showed a slight increase.

In the 3-7 weeks following hospitalization, the patient's condition deteriorated, leading to the development of hepatorenal syndrome, with creatinine levels reaching 446 µmol/L (norm: 44 – 115 µmol/L), and urea at 36.2 mmol/L (norm: 2.8 – 7.2 mmol/L), accompanied by oliguria. Liver failure and cholestasis continued to progress. The patient subsequently developed septicemia, with blood cultures positive for Escherichia coli, and was diagnosed with mycotic esophagitis during upper digestive endoscopy. Additionally, bilateral basal pneumonia and left-sided pleurisy emerged as complications over time.

Treatment included corticosteroids, substitution therapy, antibiotic therapy, antifungal treatment, and supportive care. Despite the efforts of a multidisciplinary team consisting of a gastroenterologist, hematologist, oncologist, and radiologist, the patient passed away two months after admission. Liver transplantation and chemotherapy were not recommended due to concurrent hospital-acquired infections and multi-organ failure.

Discussion

A case of a patient with acute liver failure and severe cholestasis as the primary manifestations of AL amyloidosis is reported. The patient presented with jaundice, hepatomegaly, leg oedema, ascites, preserved renal function, hepatic failure, and severe cholestatic syndrome, character-

ized by alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) levels exceeding 20 times the upper limit of normal.

Involvement of the liver in primary amyloidosis is reported in only 3.9% of cases, frequently being asymptomatic at onset [5]. The clinical picture varies, with pronounced physical asthenia, peripheral oedema, and headache being the most common manifestations. Jaundice is present in about 5% of cases, typically associated with moderate to severe cholestatic syndrome [2].

In a retrospective clinical study evaluating 98 patients with hepatic amyloidosis, 88% of them presented with proteinuria, 86% with increased ALP, 81% with hepatomegaly, and 62% with hyposplenism on peripheral blood smear. Similarly, the patient had hepatomegaly and elevated ALP but no other associated features [6]. In another study, Nakano Y. et al. reported that 80% of patients with hepatic amyloidosis exhibited hypercholesterolemia. As demonstrated in this clinical case, the patient had a total cholesterol level of 12.30 mmol/L. Therefore, physicians should consider amyloidosis as a potential cause of secondary hypercholesterolemia, especially after ruling out nephrotic syndrome [7].

Imaging findings are nonspecific. On contrast-enhanced computed tomography, liver areas infiltrated by amyloid typically appear as hypoattenuating focal lesions. In this clinical case, however, the liver parenchyma presented multiple lesions with increased contrast up to +80 HU, more suggestive of dysplastic regenerative nodules, well-known indicators of liver cirrhosis [8].

A positive bone marrow biopsy for amyloid is reported in 95% of cases in patients with primary hepatic involvement. However, in this case, the bone marrow biopsy was negative for amyloid [9].

Primary hepatic amyloidosis presents with a heterogenous clinical profile that can mimic other hematological diseases, such as lymphoma, or various liver diseases, including drug-induced liver injury and Budd-Chiari syndrome. Diagnosis is confirmed by histopathological examination where the liver parenchyma will test positive for Congo red and show apple-green birefringence under polarized light microscopy. In this case, spectroscopic analysis could not be performed due to technical issues in identifying the type of amyloid protein [9, 10]. Early diagnosis of amyloidosis is crucial for effective therapy, as it allows for better chemotherapy tolerability [11].

Primary amyloidosis has a poor prognosis, with an average survival rate of up to 9 months despite chemotherapy treatment [12]. When patients develop jaundice with bilirubin levels exceeding 34 umol/L, the prognosis worsens considerably, with median survival ranging from one to 3.3 months [13, 14]. In this case, despite high-dose corticosteroid treatment, replacement therapy, and palliative care, the patient survived for 1.5 months following the progression of cholestatic syndrome.

Data on liver transplantation in patients with primary amyloidosis is limited. A retrospective study reported that 9 patients who underwent transplantation had 1- and 5-year survival rates of 33% and 22%, respectively [15].

Conclusions

Due to nonspecific clinical and imaging findings, early diagnosis of primary hepatic amyloidosis is challenging and requires a multidisciplinary approach. Severe cholestasis and liver failure are indicators of poor prognosis and limited treatment response. A meticulous differential diagnosis, including infiltrative diseases such as amyloidosis, is essential when a patient presents with cholestasis and deteriorating liver function. Future studies should focus on developing specific therapies for primary amyloidosis to improve survival rates in these patients.

Competing interests

None declared.

Authors' contribution

ET conceived the study and contributed to the study design. AMB also conceived the study, participated in the study design, and helped draft the manuscript. COS assisted in drafting the manuscript and made a substantial contribution to data acquisition. RP performed immunofluorescence tests and participated in staining. EB critically reviewed the article for significant intellectual content. KB made a substantial contribution to data acquisition. All authors critically reviewed the work and approved the final version of the manuscript.

Informed consent for publication

Obtained.

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CASE STUDY

Unusual presentation of MALT lymphoma. A case report

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ABSTRACT

Introduction. MALT lymphoma is the most common type of extranodal non-Hodgkin lymphoma. In two-thirds of cases, it originates in the stomach and is strongly associated with *Helicobacter pylori* infection. It presents a complex clinical picture, that can resemble multiple conditions, and typically follows a benign course.

Clinical case presentation. This report discusses an uncommon case of a 52-year-old female who presented to the oncologist with insignificant weight loss, episodes of melena and fatigue. The disease started in 2018 with upper gastrointestinal bleeding from a gastric ulcer caused by *H. pylori*, which was later eradicated. The patient underwent multiple upper endoscopies over the years, with no evidence of malignancy. During the most recent investigation, an ulcerated mass of about 20 mm with infiltration of the adjacent mucosa was found. The biochemical and serological examination was within normal values. Abdominal computed tomography revealed unexplained perigastric and intestinal lymphadenopathy. The patient underwent total gastrectomy and lymph node dissection due to suspected gastric cancer. Histological and immunohistochemical examinations confirmed advanced MALT lymphoma with tumour cells positive for cluster of differentiation 45, 20 (CD45, CD20) and B-cell lymphoma 2 (BCL2). Given the advanced stage of the disease, adjuvant polychemotherapy was also administered, and the patient's condition improved significantly. **Conclusions.** MALT lymphoma is a rare condition that requires close attention and a high index of suspicion, even in unusual cases like the one presented. Endoscopic examination performed by an experienced specialist, accompanied by proper biopsy and a multidisciplinary team approach increases the survival rate of patients.

Keywords: MALT lymphoma, *Helicobacter pylori*.

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Key messages

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

MALT lymphoma is classified as an indolent type of non-Hodgkin lymphoma that typically progresses in a benign fashion. In routine medical practice, clinical suspicion for gastric MALT lymphoma is often low, as patients typically present with a variety of vague, nonspecific symptoms.

The research hypothesis

Early recognition is essential for achieving high survival rates and ensuring appropriate patient counselling.

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

The following article presents a case of severe, infiltrative gastric MALT lymphoma initially complicated by upper gastrointestinal bleeding, resistant to *Helicobacter Pylori* eradication. The diagnostic workup took over 5 years and required a multidisciplinary approach, ultimately managed by total gastric resection and followed by adjuvant chemotherapy.

Introduction

Marginal zone lymphomas (MZLs) are a group of slow-growing B-cell tumours, the second most common non-Hodgkin lymphomas (NHL lymphomas). There are three types of marginal zone lymphomas: the extranodal MZL (EMZL) of mucosa-associated lymphoid tissue (MALT or gastric GALT), the splenic MZL, and the nodal MZL [1]. The gastrointestinal tract is the most affected site, with the involvement of the stomach in 2/3 of cases [2].

MALT lymphoma, or extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, accounts for 50% of gastric lymphomas and represents the most common type of extranodal non-Hodgkin lymphoma. It can develop anywhere in the gastrointestinal tract, but most cases start in the stomach. Frequent non-gastric sites include orbit, lungs, and skin [3].

Tumour development is strongly associated with *Helicobacter pylori* (*H. pylori*) Cytotoxin-associated gene A strain-positive infection, according to a specific strain-host-organ process, with generally benign evolution. Eradication of *H. pylori* infection ensures complete remission of the gastric tumour in 75% of cases. Other recognised risk factors are hepatitis B virus (HVB), human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV) infections [3-5].

In daily medical practice, clinical suspicion of gastric MALT lymphoma is practically absent, with patients presenting a wide range of vague, unspecific symptoms from dyspepsia to the presence of alarm signs. The appearance on white light endoscopy picture is not specific, with mucosal changes imitating different clinical entities, such as gastritis, peptic ulcer, polyps, early or advanced gastric cancer and subepithelial lesions, so this diagnosis is often overlooked [6].

In the following report, a unique case is presented of an advanced MALT lymphoma patient, unresponsive to *H. pylori* eradication treatment, with HBV hepatitis and an atypical onset of upper digestive bleeding.

Clinical case presentation

A 52-year-old woman presented in March 2023 to Institute of Oncology in Chisinau with repeated episodes of melena over the last month, progressive asthenic syndrome, and insignificant weight loss over the last half a year.

She has suffered from gastrointestinal tract disease since 2018, being admitted that year to the emergency room with syncope and severe anaemia. At that time, the patient was diagnosed with gastric ulcer Forrest IIb. of *H. pylori* origin. The biopsy of the lesion did not reveal the presence of tumour cells. For differential diagnosis and to exclude gastric neoplasia with submucosal extension, an abdominal computed tomography (CT) scan was performed, revealing an unexplained gastric and retro-pancreatic lymphadenopathy. The complicated peptic ulcer was treated conservatively, and the *H. pylori* infection was subsequently eradicated with bismuth subsalicylate quadruple therapy. From 2019 to 2022, due to persistent epigastric discomfort and lymphadenopathy, the patient underwent multiple upper endoscopies with histological examination of gastric biopsies and abdominal CT, but without the identifying a specific pathology.

The anamnesis revealed a history of untreated hypertension and HBeAg-negative chronic HBV infection. There was no history of smoking or alcohol consumption, and the patient denied a family history of upper gastrointestinal bleeding, oesophageal, or gastric cancer.

On physical examination, the patient was hemodynamically stable with blood pressure of 120/70 mmHg, heart rate of 70 bpm, and an oxygen saturation of 96% on room air. No stigmata of chronic liver disease were observed. The abdomen was soft, tender to palpation in the epigastrium, and supple, with normal bowel sounds. Rectal examination showed no evidence of melena on examination. Laboratory investigations revealed no significant changes; general blood analysis, biochemical examination, and tumour markers were within reference values.

Upper gastrointestinal endoscopy revealed an area of hard submucous infiltration with deep ulcerative destruction, about 20 mm in size, covered with yellowish fibrin, adjacent foveolar hyperplasia with a relatively regular pattern, erythema and petechiae, localized in the proximal area of the gastric body, on the lesser curvature and anterior wall. On palpation, the area of infiltration was excessively hard. Multiple biopsy specimens were taken from the lesion and the stomach. Histologic examination revealed a severely expressed lymphocytic inflammatory infiltrate. The Rapid Urease Test was negative. Endoscopic images of the gastric mucosa can be visualized in Figure 1.

Non-Hodgkin's lymphoma or proximal gastric cancer was suspected. An abdominal computed tomography with contrast, performed 1 month previously, was used to stage the tumor, which showed perigastric, peripancreatic, and intestinal lymphadenopathy up to 1.9 cm in size. For confirmation of the diagnosis, the patient underwent median laparotomy with total gastrectomy and Roux-en-Y loop reconstruction following the double-tract method and lymph node dissection. During surgery, a firm, infiltrative tumor of about 8x10 cm was determined. Along the common hepatic artery - lymphadenopathy up to 3.5 cm. Histopathological examination of the resection specimen revealed an extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue with primary involvement of the stomach characterized by proliferation of small lymphoid cells of B-cell morphology. Further immunophenotyping tests were performed with the following results: tumor cells diffusely positive for CD45(+), CD20(+), BCL2(+) and negative for BLC6(-), CD5(-), CD23(-), Cyclin D1(-), with transmural involvement of the gastric wall. Ten lymph nodes along the lesser curvature were investigated. In only one nodule tumor infiltration of lymphoid tissue was found. A diagnosis of MALT lymphoma, Ann-Arbor stage IVA, with involvement of the stomach, perigastric, peripancreatic, and intestinal lymph nodes was established.

The postoperative course was uncomplicated, the patient was discharged 11 days after gastrectomy. Because

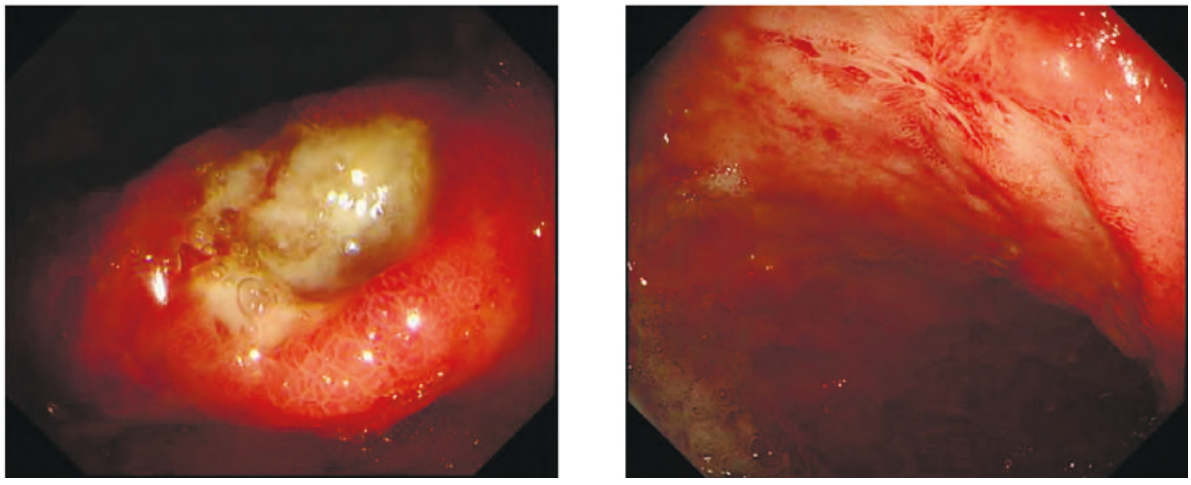


Fig. 1 Endoscopic examination of the gastric mucosa
a. Ulcerative lesion; adjacent mucosal infiltrate; foveolar hyperplasia.
b. Erythema and petechiae adjacent to the ulcerative lesion.

of the risk of lymphomatous lesions at other mucosal sites and the advanced stage of the disease, adjuvant polychemotherapy (PChT) was administered, consisting of 8 courses of PChT: one COP session (cyclophosphamide, vincristine, prednisone) visit, two R-COP sessions (rituximab, cyclophosphamide, vincristine, prednisone) and five R-CHOP sessions (rituximab, doxorubicin hydrochloride, cyclophosphamide, vincristine, prednisone).

The patient continues to be monitored clinically and by imaging. 18F-Fluorodeoxyglucose Positron Emission Tomography-computed Tomography (FDG PET-CT) was performed 2 months (January 2024) after completion of induction chemotherapy. According to the classification of the glucose metabolism, it showed a reduced tracer uptake at the right axillary, lateral aortic and left mesenteric lymph nodes, corresponding to a Deauville score 2, considered a complete metabolic response (Figure 2).

Discussion

A clinical case was presented of advanced-stage MALT lymphoma with an unusual onset of upper gastrointestinal bleeding. The comprehensive diagnostic workup spanned over 5 years, entailing the collaborative efforts of gastroenterologists, oncologists, radiologists, and hematologists.

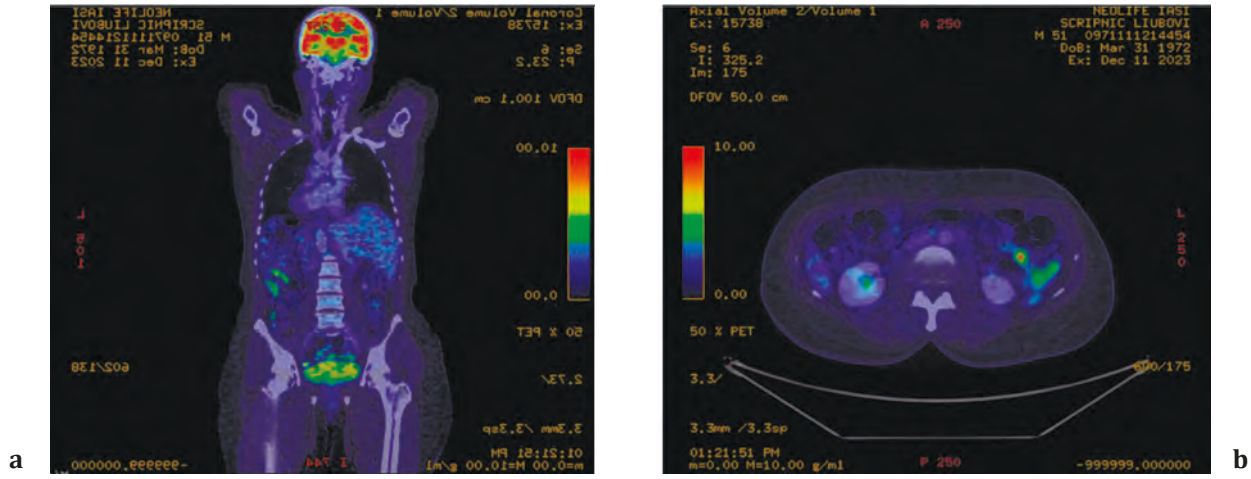
The median age of onset of MALT lymphoma is 57 years, similar to the patient presented, with a male/female ratio of 1.27:1. The clinical presentation is diverse and often asymptomatic, with the presence of warning signs, such as weight loss, hemorrhage, fever in only 42.1% of cases. Only a few cases of MALT lymphoma are characterized by the presence of peptic ulcer, with dyspepsia and pain syndrome. Disease onset with HDS as in this patient is not a usual manifestation and has been reported in only 15.6% of cases in some retrospective studies [7, 8]. It is the clinician's responsibility to consider gastric lymphoma as the potential cause of such symptoms, particularly in patients with persistent chronic ulcers and recurrent bleeding despite targeted treatment. The patient's case serves as an instructive example in this regard.

Endoscopic features in patients with gastric MALT lymphoma are varied, categorized as ulcerative (34- 69%), polypoid (26- 35%), diffuse infiltrative (15- 40%) and other types. The use of narrow-band imaging (NBI) during endoscopy may be helpful. Another endoscopic characteristic of MALT lymphoma is the presence of abnormally large capillaries "tree trunk-like, with long empty branches". With the advent of endoscopic microscopy, the presence of intraglandular aggregation of cellular components and small-sized nuclei in gastric lymphoma and, the presence of lymphoepithelial lesions were detected, but additional studies are needed [9, 10]. On upper gastrointestinal endoscopy performed on this patient, foveolar hyperplasia with a regular vascular pattern was determined.

However, the primary method for diagnosing the disease is histologic examination. It is important to mention the need to perform multiple biopsies, at least 10 biopsies in and outside the lesion to increase the chances of detection and to exclude the association of other precancerous conditions [11].

Given the association of MALT lymphoma with *H. pylori* infection, the ideal treatment option in patients with gastric lymphoma of low malignancy is its eradication, which results in complete remission in about 78% of cases. MALT lymphoma is currently the only cancer that can be treated with antibiotics. Resistance to treatment is associated with advanced stage III/IV disease, absence of infection, proximal localization of the tumour in the stomach, and the presence of infiltrative endoscopic type [3, 9, 12].

In this patient, treatment for *H. pylori* infection was performed incidentally in the context of managing the first episode of upper gastrointestinal bleeding, which was considered to be due to a complicated gastric ulcer. Eradication of the infection was confirmed with a negative *H. pylori* antigen test in the stool sample. However antibacterial treatment did not demonstrate efficacy in tumor regression, reflecting the uniqueness of the case. It can only be speculated that the lack



a b

Fig. 2 FDG PET-CT reduced accumulation of 18-fluorodeoxyglucose, complete metabolic response.
a. sagittal section, b. cross-section

of results was motivated by the advanced stage of the disease as early as 2018, tumour localization in the proximal 1/3 of the gastric body and infiltrative endoscopic form.

The second-line treatment for gastric MALT lymphoma, following the failure of eradication treatment and in cases of complications such as upper gastrointestinal bleeding, is total resection of the stomach. In some limited cases, radiotherapy may be recommended, while chemotherapy may be used for patients with extensive and aggressive forms of the condition. The chemotherapy protocol used in this patient, namely R-CHOP and R-COP, is considered effective and well-tolerated in clinical trials as well [12, 13].

FDG PET-CT is recommended for monitoring treatment efficacy in aggressive MALT lymphoma. The Deauville scale is a simple scoring system based on visual interpretation of FDG uptake with 2 reference points: mediastinum and liver. A Deauville score of 2, as in the patient in question, is considered to be a mild uptake associated with a complete metabolic response. This denotes the absence of a malignant process in the lymph nodes analysed and a low risk of recurrence. However, the prognostic value of FDG PET-CT is uncertain and requires further research [14].

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue are considered exceptionally indolent tumours. Most patients can expect their longevity to be unaffected by disease-associated complications. However, MALT lymphoma may have a heterogeneous course of progression. In a retrospective study with analysis of 593 patients with MALT lymphoma, the 5- and 10-year mortality rates were 2.8% and 4.3%, the identified independent risk factors were age over 70 years and advanced stage of disease [15].

The prognosis of this patient is positive, given the PET-CT findings and lack of other risk factors, but careful monitoring remains necessary.

Conclusions

Gastric MALT lymphoma is a rare condition that, in the early stages of the disease, can be managed solely by antibiotic therapy. Defined by a multitude of nonspecific symptoms andoften asymptomatic, this pathology can also be termed a “big masquerader” that requires maximum vigilance in making the diagnosis. A thorough endoscopic examination, with an adequate number of biopsies, along with the use of NBI technology, carried out by a skilled specialist is essential for both detection and subsequent monitoring. Although it generally follows a favourable clinical course, it requires a multidisciplinary approach and chemotherapeutic treatment in advanced stages.

Competing interests

None declared.

Authors’ contribution

ET conceived the study, participated in the study design and reviewed the article. COS participated in the study design and drafted the manuscript. MS made a substantial contribution to the data acquisition. AT revised critically the article, ensuring accuracy and validation. LA, MC conducted the investigation process and evidence collection. All the authors reviewed the work critically and approved the final version of the manuscript.

Informed consent for publication

Obtained.

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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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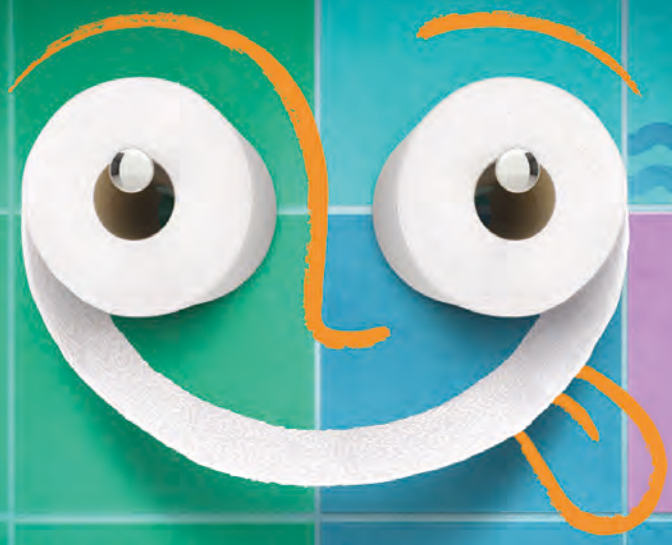

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Nu este recomandat să luați o doză dublă pentru a compensa doza uitată.*Vârstnici (peste 65 de ani)*:Pacienții vârstnici trebuie tratați cu prudență.Nivelul creatininei plasmatice trebuie monitorizat la pacienții geriatrici, în funcție de vârstă, greutate 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 (indusiv 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 m^2 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 serice > 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 hymenoptera:* 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.

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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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