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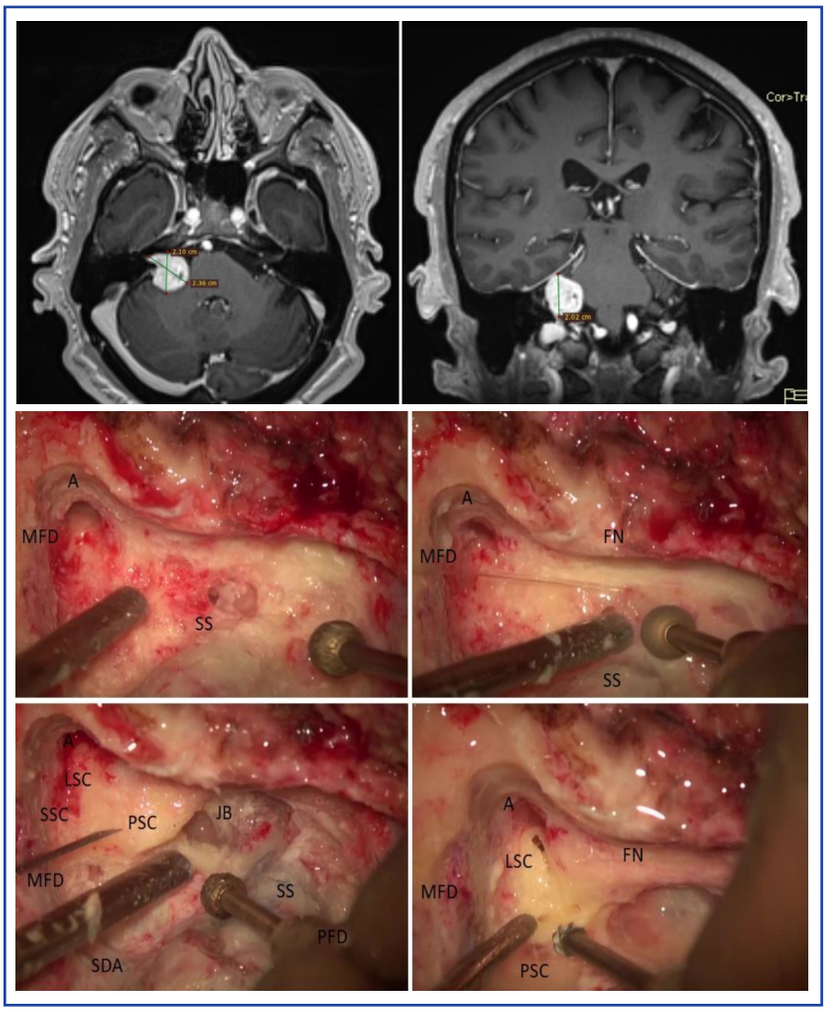

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RESEARCH ARTICLE

Clinical and paraclinical approach to community-acquired pneumonia in obese individuals

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ABSTRACT

Introduction. Obesity is a metabolic disease that presents a real challenge for the medical system due to the significant increase in the number of obese people in recent decades. Currently, 38% of the global population is overweight or obese. Obesity is an important risk factor for multiple chronic pathologies and lung infections, especially pneumonia. For obese subjects, chronic proinflammatory status due to an excess of fat cells is characteristic.

Material and methods. This prospective cohort study is based on clinical and laboratory examinations of patients hospitalized with community-acquired pneumonia in the Department of Internal Medicine at *Holy Trinity* Municipal Hospital, Chisinau, Republic of Moldova. The study included 210 patients with community-acquired pneumonia, divided into two groups: the base group (group 1) consisted of 105 patients with varying degrees of obesity, and the control group (group 2) consisted of 105 normal-weight patients. The research was conducted according to the principles of the Helsinki Declaration - WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The study was approved by the Research Ethics Committee of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, with the issuance of favorable opinion no. 46 from March 27, 2018. All patients were examined clinically and paraclinically (radiological examination, pulse oximetry screening, complete blood count, erythrocyte sedimentation rate, fibrinogen, LDH, C-reactive protein, oxidative stress markers). The obtained data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) version 20.

Results. According to the obtained data, the most common comorbidities associated with obesity were cardiovascular and metabolic diseases. The main symptom that prevailed in the obese was dyspnea (97%). Obese subjects showed more frequent signs of acute respiratory failure (86.7%), required oxygen therapy with an average duration of 7.62±6.23 days, showed increased serum levels of LDH (286.31±94.66 U/L) and C-reactive protein (66.08±71.44 mg/l), data that influenced the clinical course of pneumonia.

Conclusions. Patients with obesity and community-acquired pneumonia presented with infectious symptoms and acute respiratory failure, increased values of inflammatory markers, and required oxygen therapy more frequently compared to those of normal weight.

Keywords: pneumonia, obesity, clinical course, comorbidities.

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Key messages

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

The clinical evolution, pro-inflammatory status, and oxidative stress in obese subjects with community-acquired pneumonia are not sufficiently known. Some studies reported more severe evolution of pneumonia in obese patients, while others mentioned contradictory data.

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The research hypothesis

Community-acquired pneumonia in obese individuals is manifested by a more severe clinical course, complications, and marked inflammatory syndrome in comparison with normal-weight subjects.

The novelty added by manuscript to the already published scientific literature

Our research results report the clinical course of community-acquired pneumonia, commonly associated comorbidities, and possible complications in obese patients. The research data may contribute to the clinical management of patients with different degrees of obesity and pneumonia as well as predicting prognosis.

Introduction

The global increase in the number of obesity cases represents one of the most serious challenges for the public health system, the medical system, but also for the entire society. In 1997, due to the increase in worldwide prevalence, obesity was declared an epidemic by the World Health Organization. According to the 2023 *World Obesity Atlas* report, currently, 38% of the global population is overweight or obese, with a body mass index (BMI) greater than 25 kg/m^2 [1]. By 2035, it is estimated that the global prevalence of overweight and obese people will reach up to 51% [2].

Obesity is a metabolic disease, the consequence of a long-term imbalance between food intake and energy consumption. This clinical nosology arises as a result of the complex interaction between genetic, environmental and psychosocial factors [3]. Moreover, obesity is an important risk factor for cardiovascular diseases, diabetes mellitus, cancer, and sleep apnea syndrome. Also, the accumulation of excessive adipose tissue can attenuate the host's local pulmonary defenses. This contributes to the predisposition of obese subjects to lung infections, especially community-acquired pneumonia (CAP) [4]. This is explained by the fact that adipose tissue is a dynamic structure directly involved in various metabolic processes like lipid storage, production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-18), and hormone synthesis (leptin, adiponectin). White adipose tissue is mainly composed of preadipocytes, adipocytes, macrophages, dendritic cells, T cells, and B cells. Immune cells in adipose tissue maintain adipocyte integrity, metabolic function, and hormone sensitivity. Macrophages constitute up to 40–50% of all adipose tissue cells. In the obese, adipose tissue macrophages are transformed into proinflammatory (M1) macrophages that secrete proinflammatory cytokines. Due to adipocyte hypertrophy, adipose tissue hypoxia develops, which subsequently presents the source of activation of inflammatory processes. Consequently, systemic inflammation develops that compromises immune function, triggers changes in pro-inflammatory cells and oxidative stress, as well as increased susceptibility to infections, including pulmonary ones [5].

During the last years, due to the high prevalence among the general population, these two clinical entities (pneumonia and obesity), have garnered research interest in many clinical studies. Research data show that community-acquired pneumonia in obese subjects has a more severe clinical course, longer hospital stay, more expressed pro-inflammatory markers, more frequent complications, but also a higher mortality rate compared to normal-weight subjects. On the other hand, a series of meta-analysis studies present controversial data, such as obese patients hospitalized with pneumonia having better clinical results compared to normal weight or underweight individuals [6, 7].

Based on the literature data, but also considering that obesity has reached pandemic proportions and the evident role of associated comorbidities in the course of community-acquired pneumonia, we aimed to study clinical course, laboratory features, and comorbidities in community-acquired pneumonia in obese individuals compared to those with normal weight.

Material and methods

A prospective cohort study was conducted within the Department of Internal Medicine, Clinical Syntheses Discipline, *Nicolae Testemițanu* State University of Medicine and Pharmacy, with the clinical base at *Holy Trinity* Municipal Clinical Hospital, Chisinau, Republic of Moldova. The study included 210 patients with community-acquired pneumonia, divided into two groups: the base group (group number 1) constituted 105 patients with varying degrees of obesity, and the control group (group number 2) constituted 105 normal-weight patients. The research inclusion criteria were: age >18 years; the presence of clinical and radiological pulmonary consolidation syndrome; BMI for obese patients ≥ 30 kg/m^2 and BMI for normal-weight patients 18.5-24.9 kg/m^2.

The aim of the study was to assess the clinical course, laboratory findings, and comorbidities in community-acquired pneumonia in obese compared to normal weight patients.

The research was conducted according to the Principles of the Helsinki Declaration - WMA Declaration of Helsinki

- Ethical Principles for Medical Research Involving Human Subjects. The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, with the issuance of favorable opinion no. 46 from March 27, 2018.

All patients signed the agreement to participate in the study and were questioned about the history of the disease (the onset, presence of infectious signs, presence of respiratory symptoms). They were examined clinically and paraclinically (radiological examination, pulse oximetry screening, complete blood count, erythrocyte sedimentation rate, fibrinogen, LDH, C-reactive protein, oxidative stress markers). The clinical course of pneumonia was monitored during hospitalization.

The obtained data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) version 20. Results were expressed as percentages for categorical variables and mean ± SD for continuous variables. Variable analysis was performed using descriptive statistics. The correlation analysis of the variables was performed using the non-parametric test Spearman's Rho. A p-value < 0.05 was considered statistically significant.

Results

The mean age of patients participating in the study was 64.4±12.5 years for group 1 and 65.2±13.04 years for group 2, with a p-value of 0.906. Males constituted 39 (37%) in group 1 and 65 (61.9%) in group 2, with a p-value of <0.001. Among the associated comorbidities, cardiovascular and metabolic diseases showed statistical significance: chronic heart failure - 97 (92%) vs 83 (79%); p=0.005, grade III arterial hypertension - 63 (60%) vs 14 (13.3%); p<0.0001, coronary heart disease - 89 (84%) vs 73 (69.5%); p=0.009; dyslipidemia - 66 (62.9%) vs 35 (33.3%); p<0.0001, chronic kidney disease - 20 (19.9%) vs 7 (6.7%), p=0.006; diabetes mellitus - 42 (40%) vs 17 (16.2%), p<0.0001: and metabolic syndrome - 102 (97.1%) vs 27 (25.7%), p<0.0001, comparing group 1 to group 2.

Classical onset of community-acquired pneumonia was characteristic for 26 (24%) obese cases vs 41 (39%) normal weight cases, p=0.191. Presenting complaints at admission were: infectious symptoms in 67 (63%) vs 55 (52.4%), p=0.198; chills in 26 (24%) vs 31 (29.5%), p=0.268; dry cough in 40 (38%) vs 51 (48.6%), p=0.082; cough with mucous sputum in 22 (21%) vs 13 (12.4%); and cough with mucopurulent sputum in 26 (24%) vs 20 (19%), p=0.107; dyspnea in 102 (97%) vs 85 (81%), p<0.001, pleural pain in 34 (32%) vs 30 (28.6%), p=0.327, in groups 1 and 2, respectively.

The clinical data assessment revealed accentuated vocal fremitus in 19 (18%) cases vs 67 (63.8%) cases, p<0.0001; dullness on chest percussion in 24 (22.9%) vs 73 (69.5%), p<0.0001 diminished vesicular murmur on lung auscultation in 86 (81.9%) vs 89 (84.4%), p=0.356, comparing group 1 compared to group 2. Respiratory rate > 30 breaths/minute was observed in 24 (22.9%) vs 9 (8.6%), p=0.004, and peripheral O_2 saturation <90% in 46 (43%) vs 29 (27.6%), p=0.010, in obese compared to normal weight patients. Typical pulmonary consolidation syndrome was observed in 20 (19%) cases in group 1 vs 69 (65.7%) cases in group 2, p<0.0001. A severe general condition was more common in group 1 patients compared to group 2 - 21 (20%) vs 15 (14.3%), p=0.302, as was the severe form of pneumonia - 34 (32.4%) vs 29 (27.6%), p=0.274.

On radiological examination, bilateral extension of the inflammatory infiltrate was observed in 78 (74.3%) cases in group 1 compared to 60 (57.1%) cases in group 2, p=0.062. Control radiological examination revealed infiltrate resolution in 61 (58.1%) cases in group 1 vs 67 (63.8%) cases in group 2, p=0.504.

The mean hospital stay was 12.35±5.76 days in group 1 vs 11.69±4.58 days in group 2, p=0.355, and the mean duration of antibacterial therapy was 14.1±5.56 days vs 12.8±4.75 days, p=0.087, group 1 versus group 2. Transfer to ICU was required in 15 (14.3%) cases vs 10 (9.5%) cases, p=0.287, and the length of stay in ICU was 8.1±3.2 days vs 5.0±0.5 days, p=0.483, group 1 versus group 2.

The need for non-invasive ventilation was observed in 22 (21%) cases in group 1 vs 9 (8.6%) cases in group 2, p=0.009. Invasive mechanical ventilation was applied in 4 (3.8%) patients in group 1 vs 2 (1.9%) patients in group 2, p=0.341. Oxygen therapy via nasal cannula or face mask was required in 97 (92.4%) cases in group 1 vs 75 (71.4%) cases in group 2, p<0.0001, and the mean duration of oxygen therapy was 7.62±6.23 days in group 1 vs 4.66±5.77 days in group 2, p=0.001.

Examination of pro-inflammatory markers revealed: leukocytes (10^9/L) 10.79±5.09 vs 10.49±4.47, p=0.654; neutrophils (%) 72.65±13.36 vs 74.31±12.74, p=0.361; LDH (Units/L) 286.31±94.66 vs 215.89±110.16, p=0.001; fibrinogen (g/L) 5.9±8.21 vs 6.08±8.34, p=0.890; erythrocyte sedimentation rate (mm/h) 31.75±18.19 vs 27.89±18.23, p=0.127; and C-reactive protein (mg/l) 66.08±71.44 vs 40.85±58.82, p=0.006, in groups 1 and 2, respectively.

Pro-oxidative stress markers were also assessed: advanced oxidation products (μM/l) 97.51±6.33 vs 80.39±49.20, p=0.033; malondialdehyde (μM/l) 19.40±0.87 vs 20.68±1.03, p=0.346; AGE-verperlisin-like (μM/l) 737.98±34.56 vs 725.51±58.56, p=0.855; AGE-pentosidine-like (μM/l) 577.92±31.48 vs 485.82±24.50, p=0.022; nitric oxide (μM/l) 45.24±0.81 vs 44.65±0.69, p=0.585, in group 1 versus group 2.

Anti-oxidative stress markers did not show statistical significance between the groups: total antioxidant capacity (CUPRAC method) (μM/L) 29.14±3.97 vs 26.33±3.70, p=0.606; total antioxidant capacity (ABTS method) (μM/L) 128.04±1.90 vs 124.52±1.96, p=0.201; catalase (μM/L) 37.07±1.78 vs 37.34±2.69, p=0.925; thiolic compounds (μM/L) 7.21±0.35 vs 6.84±0.33, p=0.448, in group 1 compared to group 2.

Table 1. Clinical findings of community-acquired pneumonia in obese patients compared with normal weight patients

Clinical manifestations of community-acquired pneumonia in obese	Group 1 (Patients with obesity and CAP), n, %	Group 2 (Normal weight patients with CAP), n, %	P
Dyslipidemia	66 (62.69%)	35 (33.3%)	p<0.0001
Metabolic syndrome	102 (97.1%)	27 (25.7%)	p <0.0001
Classic onset of CAP	26 (24%)	41 (39%)	p=0.019
Infectious symptoms	67 (63%)	55 (52.4%)	p=0.198
Dyspnea	102 (97%)	85 (81%)	p <0.0001
Dry cough	40 (38%)	51 (48.6%)	p=0.082
Cough with mucous sputum	22 (21%)	13 (12.4%)	p=0.107
Severity of pneumonia	34 (32.4%)	29 (27.6%)	p=0.274
Typical pulmonary consolidation syndrome	20 (19%)	69 (65.7%)	p <0.0001
Respiratory rate >30/min	24 (22.9%)	9 (8.6%)	p=0.004
Bilateral/multilobar extension of inflammatory infiltrate on chest X-ray examination	78 (74.3%)	60 (57.1%)	p=0.062
O2 therapy	97 (92.4%)	75 (71.4%)	p <0.0001
Non-invasive ventilation	22 (21%)	9 (8.7%)	p=0.009
Invasive ventilation	4 (3.8%)	2 (1.9%)	0.341
Length of hospitalization	12.3±5.7	11.6±4.5	0.355
Length of ICU stay	8.1±3.2	5.0±0.5	0.483

Note: *CAP- community-acquired pneumonia, ICU – intensive care unit*

Community-acquired pneumonia complications were observed in both groups: acute respiratory failure - 91 (86.7%) vs 65 (61.9%), p<0.0001; acute respiratory distress syndrome - 17 (16.2%) vs 6 (5.7%), p=0.013; pleural effusion - 22 (21%) vs 28 (26.7%), p=0.209; disseminated intravascular coagulation syndrome and sepsis - 3 (2.9%) vs 1 (1%), p=0.311; hypercatabolic state - 41 (39%) vs 51 (48.6%), p=0.105; pulmonary thromboembolism - 6 (5.7%) vs 2 (1.9%), p=0.140; and death - 4 (3.8%) vs 2 (1.9%), p=0.249; group 1 compared to group 2.

Correlational analysis showed a positive correlation of obesity with dyspnea (rs=0.25), the need for oxygen therapy and the duration of oxygen therapy (rs=0.27, rs=0.24), the association of mixed dyslipidemia (rs=0.29) and diabetes mellitus (rs=0.26), increased serum LDH (rs=0.32), and C-reactive protein (rs=0.20).

Discussions

According to the literature data, obesity is an important risk factor for multiple chronic diseases. For example, the Framingham Offspring study presents data showing that 78% of new cases of essential hypertension in men and 65% in women were attributed to excess body fat [8]. Another study, which included 82,882 women followed prospectively for 14 years, showed that BMI was the strongest risk factor for the development of hypertension, with obese women having an incidence nearly five times higher than those with normal BMI [9]. Up to 70% of patients with obesity are concomitantly diagnosed with dyslipidemia, frequently manifested paraclinically by elevated serum low-density lipoprotein (LDL) and reduced high-density lipoprotein (HDL) levels [10]. Obesity is directly associated with the presence of metabolic syndrome, with the prevalence of metabolic syndrome among the obese being 62.4% [11]. Moreover, obesity is a significant factor in the association with diabetes, such that 85.2% of patients with diabetes are obese [12]. We obtained similar data in our research, showing that metabolic and cardiac diseases positively correlate with obesity.

Although literature sources report controversial data regarding the course of community-acquired pneumonia severity, length of hospitalization, and need for ventilation, there are studies demonstrating that obese patients who develop community-acquired pneumonia have a more severe clinical course, a longer length of hospitalization, and require ventilation compared to normal-weight subjects [13, 14]. The presented research shows that, although without statistical difference, obese patients had a longer period of hospitalization, a longer period of antibiotic therapy, and a need for mechanical ventilation.

Recent pandemic studies reveal multiple data indicating that respiratory failure was more pronounced in obese subjects with community-acquired pneumonia [15, 16]. Our data demonstrate that obese patients presented with acute respiratory failure more frequently compared to those with normal body weight.

Obesity is an underlying condition for the development of lung infections, including pneumonia, by impairing lung function and contributing to a reduced degree of chronic inflammation. At the same time, obesity as an associated comorbidity contributes to increased mortality from pneumonia [17, 18]. Multiple studies examining patients with different BMI have concluded that obese people have a marked pro-inflammatory status compared to those who are normal weight or underweight. This is explained by significantly elevated serum values of C-reactive protein and other inflammatory markers in the obese group patients [19].

Data from another study show that obese subjects have elevated serum lactate dehydrogenase values, which correlate with metabolic syndrome and increase the risk of cardiovascular complications [20]. According to the results of our study, obesity cases had a positive correlation with elevated C-reactive protein (CRP) values (rs=0.20) and LDH values (rs=0.32), supporting the relationship between inflammatory status and a high body mass index.

The results of our study revealed no significant differences in pro-oxidative and anti-oxidative stress markers, except for advanced oxidation products and AGE-verperlisin like markers. Similar data were also observed in other research, which demonstrate a more pronounced pro-oxidant status in obese individuals compared to normal weight [21].

Pulmonary thromboembolism is a complication observed more frequently in obese subjects with community-acquired pneumonia. The main cause of pulmonary thromboembolism in obese individuals is endothelial injury and hypercoagulable states. Some studies report an increased risk of thromboembolism of up to 2.4 times in obese patients compared to those with a normal BMI. Another common complication among obese individuals with pneumonia is acute respiratory distress syndrome, due to a more pronounced pro-inflammatory status, a condition that was also observed in our study [22].

Conclusions

Patients with obesity and community-acquired pneumonia had complaints of dyspnea, tachypnea, infectious signs, elevated inflammatory marker values, and required oxygen therapy more frequently compared to normal weight patients.

The most common comorbidities associated with obese patients were chronic heart failure, coronary artery disease, hypertension, diabetes mellitus, and metabolic syndrome, all of which influenced the clinical course of community-acquired pneumonia.

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 Research Ethics Committee of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy approved the study (Minutes no. 46 from March 27, 2018).

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RESEARCH ARTICLE

Outcome analysis of the traditional therapeutic approach in patients with bronchopulmonary cancer and advanced anesthetic-surgical risk

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ABSTRACT

Background. Lung cancer exhibits high global incidence and mortality rates. Surgical resection remains crucial to diagnosis, staging, and treatment of non-small cell lung cancer (NSCLC). However, advanced stages often require multimodal approaches. Prehabilitation, innovative perioperative techniques, and the exploration of immunotherapy hold promise for improving outcomes.

Materials and methods. This two-phase observational cohort study included a retrospective arm (100 patients, traditional treatment) and a prospective arm (100 patients, innovative perioperative management, and risk stratification). Descriptive and inferential statistics were used to evaluate data, identify risk factors, and compare the 2-year survival between treatment groups.

Results. Despite advances, lung cancer prognosis remains poor, with limited cure rates. Mean survival in the traditional approach was 1.3 years with a 5-year survival of only 1%. While prehabilitation and innovative techniques showed potential, no statistically significant differences in survival times were observed between the treatment groups. Patients with stage III (Charlson score 0-4) and stage I-II (Charlson score 5-12) demonstrated comparable outcomes, highlighting the importance of comorbidity burden.

Conclusions. Targeted screening protocols are of paramount importance for early detection and intervention. Population-wide smoking cessation programs, environmental protection measures, and the promotion of healthy lifestyles are vital for prevention and reducing incidence. Standard chemotherapy and radiotherapy offer limited therapeutic benefits in advanced lung cancer. This highlights a pressing need for breakthroughs in basic research to develop novel treatment paradigms that significantly improve outcomes. While this study did not reveal statistically significant survival differences between traditional and innovative perioperative approaches, prehabilitation and optimization techniques hold promise and deserve further research. The development of anti-tumor immunotherapy offers a significant potential in non-surgical lung cancer treatment, especially given the limitations in advanced-stage disease management. Harnessing the immune system to fight cancer represents a promising new frontier.

Keywords: lung cancer, surgery, perioperative management, prehabilitation, survival, immunotherapy.

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Key messages

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

The development of a multimodal perioperative approach for patients with bronchopulmonary cancer and advanced anesthetic-surgical risk aims to expand operability criteria, potentially increasing life expectancy and reducing postoperative complications.

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The research hypothesis

Multimodal prehabilitation for bronchopulmonary cancer with high anesthetic risk aims to expand surgical options and improve survival.

The novelty added by manuscript to the already published scientific literature

Innovative eligibility criteria and perioperative management, including risk modeling and prehabilitation, expand surgical options for high-risk lung cancer patients, significantly improving survival outcomes.

Introduction

Lung cancer exhibits alarmingly high incidence and mortality rates both globally and in the Republic of Moldova. In 2021, an estimated 2 million cases occurred worldwide, with 1.8 million deaths. The American Cancer Society projects 236.740 new cases and 130.180 deaths in the US for 2022 alone. Lung cancer tragically accounts for almost 20% of all cancer-related deaths [1-3].

Surgical intervention remains pivotal in diagnosing, staging, and treating non-small cell lung cancer (NSCLC). Lung resection is the gold standard approach for stage I-II NSCLC and a vital part of multimodal treatment in stage IIIA [4]. Standard resections (lobar, bilobar, pulmonary) with ipsilateral hilar and mediastinal lymph node revision are common practices. For early-stage cancers, reducing morbidity and mortality is the primary surgical objective [5]. In advanced stages or patients facing heightened surgical risks, careful selection is necessary to ensure that those most likely to benefit from surgery, potentially combined with radiotherapy or chemotherapy, are prioritized [6, 7].

A burgeoning trend in perioperative management is prehabilitation (respiratory, cognitive, motor, etc.). This multifaceted approach aims to enhance a patient's functional reserve, better equipping them to withstand surgical stress and optimize postoperative recovery. Additionally, the impact of techniques like fascial plane blocks and antifibrinolytics on thoracic cancer surgery outcomes remains understudied [8, 9].

Material and methods

This investigation employed a two-phased, observational cohort design: retrospective and prospective. The study protocol received approval from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy, Republic of Moldova (Protocol No. 04 of 12.11.2020). Written informed consent was obtained from all prospectively enrolled patients.

To achieve study objectives, two patient cohorts were established. The retrospective cohort (100 patients) was derived from the Oncology Institute database. Patients with lung cancer and documented high anesthetic risk were included if they met pre-defined enrollment criteria and were not offered surgery due to traditional contraindications. The prospective cohort (100 patients) included patients meeting the same enrollment criteria who underwent surgery based on the newly proposed approach. This approach incorporated prehabilitation, accelerated postoperative rehabilitation, anterior serratus fascial plane block, intraoperative antifibrinolytic therapy, and risk stratification using integrated scores (ASA, Th-RCRI, MET, Charlson).

Within the operated group, a sub-analysis was conducted to identify differences, risk factors, and predictors of postoperative mortality. Survivors were compared to early postoperative death. Finally, the long-term outcome (2-year survival rate) of surgically treated patients (under extended criteria) was compared to the historical cohort receiving only chemotherapy and/or radiotherapy (traditional approach). Descriptive and inferential statistical analyses were performed to evaluate the collected data. The results were subsequently utilized to develop a mathematical model for risk stratification. This model, based on identified and quantified risk factors (parameterized), aimed to refine surgical indications and contraindications. Additionally, it informed the formulation of general practice recommendations.

Two patient subcategories within the high anesthetic-surgical risk group emerged during analysis. These subgroups demonstrated comparable outcomes: (1) Stage III lung cancer patients with a Charlson score of 0-4 points; (2) Stage I-II lung cancer patients with a Charlson score of 5-12 points.

G*Power v. 3.1.9.6 (Franz Faul, University of Kiel) was used to determine the minimum sample size needed to detect a clinically meaningful difference of at least 15% in the two-year survival rate between groups. This calculation yielded a target enrollment of at least 184 patients. Data analysis was performed using GraphPad Prism software (version 9 trial). Statistical tests were chosen based on the specific research hypothesis, data distribution characteristics, and the number of data series involved. These included: (1) Parametric: t-Student test; (2) Non-parametric: Fisher exact test, Spearman/Pearson correlations, Mann-Whitney U test; (3) Survival Analysis: Kaplan-Meier curves with Mantel-Cox test; (4) Modeling: Logarithmic regression, probability calculations, multicollinearity testing.

Results are presented as mean ± standard deviation (with 95% confidence intervals where applicable) or relative frequencies. A p-value of <0.05 was considered statisti-

cally significant, with a study power of 80%, alpha error of 5%, and beta error of 20%.

Results

Given the limited cure rates associated with lung cancer, regardless of stage, therapeutic strategies primarily focus on extending life expectancy. Therefore, two critical outcome measures were used: (1) Life expectancy (in months): Measured from the date of diagnosis; (2) Survival rates at specific intervals: Commonly assessed at the 5-year mark. Results from patients receiving traditional therapy (the reference group) were analyzed using various covariates: (1) Stage of disease at diagnosis: A critical determinant of prognosis; (2) Charlson comorbidity index: Quantifies the burden of comorbid conditions; (3) Concurrent lung pathologies: Examines their impact on outcomes; (4) Treatment modalities utilized: Evaluates the effectiveness of existing therapies. Survival rates derived from this analysis will be compared to established literature data to assess the performance of the traditional management approach.

The frequency distribution highlights a stark reality: 40% survival at 1 year, 32.8% at 1.5 years, and only 8.2% at 2 years. Survival rates beyond 2 years remained at 8.0% (16 / 200 patients), with a mere 1% (2 / 200) reaching the 5-year mark. The mean survival time was 1.30±0.82 years. These findings underscore the urgent need for treatment innovations; even a 0.5-year increase in mean survival would be considered significant progress. Analyzing potentially modifiable factors (covariates) that influence survival may hold the key to such improvements. Key areas of exploration include the impact of comorbidities, concurrent lung pathologies, and the potential for optimizing existing treatment strategies (Table 1).

Table 1. Survival time, by disease stage and burden of comorbidities, expressed by the Charlson Comorbidity Index (CCI).

Parameters	**Duration between 'year of registration' and 'year of death', years**			
	Stage I n=2 / 200 (1%)	**Stage II** n=10 / 200 (5%)	**Stage III** n=51 / 200 (25.5%)	**Stage IV** n=137 / 200 (68.5%)
Charlson 0 points	-	-	0.8 (0.4 – 1.2)	0.9 (0.7 – 1.1)
Charlson 1-2 points	-	1.3 (0.6 – 2.1)	1.2 (0.9 – 1.5)	1.4 (1.1 – 1.6)
Charlson 3-4 points	1.7 (-7.7 – 11.2)	2.5 (-0.2 – 5.2)	1.2 (0.8 – 1.5)	1.4 (1.2 – 1.6)
Charlson 5≤ points	-	1.5*	1.6 (0.8 – 2.3)	1.2 (0.8 – 1.5)

***Note*:** *Data expressed in years, mean, and 95% confidence interval (95% CI). Defaults indicate the lack of patients with the given characteristics in the study group (certain stage versus CCI score). *- only one patient in the group with the given characteristics. "Stage IV" column - patients ineligible for surgical treatment. Grey box - patients with lung cancer and increased anesthetic/surgical risk.*

Given the disproportionate number of patients diagnosed with stage III and IV disease (94%), survival analysis primarily reflects outcomes for advanced stages. While stage I and II data represent individual cases, a focus on stages III and IV offers greater statistical relevance. Despite analyzing advanced stages, no significant differences in survival emerge, with mean survival ranging from 0.8 to 1.6 years. This emphasizes the poor prognosis associated with late-stage lung cancer and the urgent need for therapeutic advances across all stages.

Due to the small number of stage I and II cases, survival analysis from diagnosis to treatment initiation (radiotherapy or chemotherapy) is primarily descriptive for these early stages. Regarding advanced stages, neither chemotherapy nor radiotherapy alone appears to significantly impact survival. However, combining these modalities may yield an average survival gain of 6-8 months as presented (Table 2). These findings suggest a potential benefit of combination therapy and highlight the need for further investigation with larger sample sizes across all disease stages.

Table 2. Survival time, by stage of disease and by treatment (radiotherapy, chemotherapy, alone or in combination).

Treatment	Duration between 'year of registration' and 'year of death', years			
	Stage I n=2 / 200 (1%)	Stage II n=10 / 200 (5%)	Stage III n=51 / 200 (25.5%)	Stage IV n=137 / 200 (68.5%)
Isolated radiation therapy	2.5*	2.0*	-	2.0*
Isolated chemotherapy	-	-	0.9 (0.6 – 1.2)	1.4 (1.1 – 1.6)
Radio + chemotherapy	-	-	1.6 (0.9 – 2.3)	2.2 (1.1 – 3.3)

***Note*:** *Data expressed in years, mean, and 95% confidence interval (95% CI). Defaults indicate lack of patients with the given characteristics in the study group (certain stage vs. treatment). *- only one patient in the group with the given characteristics. Column "stage IV" - patients not eligible for surgical treatment.*

Certain lung cancer symptoms, while nonspecific, reflect the severity of underlying disease processes. These symptoms may indicate inflammation, metabolic disruptions, or consequences of tumor growth (invasion, compression). Advanced disease often necessitates complex compensatory mechanisms, potentially reducing patient resilience. We hypothesized that specific symptom profiles could be associated with shorter survival times. To investigate this, we analyzed data from the 200 lung cancer patients in our reference group (Table 3).

Table 3. Survival time, by stage of disease and by treatment (radiotherapy, chemotherapy, alone or in combination).

Symptom	Duration between 'year of registration' and 'year of death', years			
	Stage I n=2 / 200 (1%)	Stage II n=10 / 200 (5%)	Stage III n=51 / 200 (25.5%)	Stage IV n=137 / 200 (68.5%)
Fatigue	1.7 (-7.7 – 11.2)	1.8 (0.7 – 2.9)	1.2 (0.9 – 1.4)	1.2 (1.1 – 1.3)
Hemoptysis	1.0*	1.1 (0.4 – 1.8)	1.2 (0.9 – 1.4)	1.2 (1.0 – 1.4)
Chest pain	-	1.2 (-14.6 – 17.1)	1.1 (0.8 – 1.3)	1.3 (1.1 – 1.5)
Dry cough	-	2.0 (0.9 – 3.0)	1.3 (1.0 – 1.5)	1.3 (1.1 – 1.4)
Weight loss	1.7 (-7.7 – 11.2)	1.8 (0.7 – 2.9)	1.2 (1.0 – 1.4)	1.2 (1.1 – 1.3)
Dyspnea	1.7 (-7.7 – 11.2)	1.9 (0.8 – 3.0)	1.2 (0.9 – 1.4)	1.3 (1.1 – 1.4)

***Note*:** *Data expressed in years, mean, and 95% confidence interval (95% CI). Defaults indicate lack of patients with the given characteristics in the study group (certain stage vs. treatment). *- only one patient in the group with the given characteristics. Column "stage IV" - patients not eligible for surgical treatment.*

Our analysis of symptom presentation and survival (Table 3) revealed no statistically significant differences in survival time across various symptoms. Mean survival ranged from 1.1 to 2.0 years, with the majority of patients surviving 1.2-1.3 years on average. These findings suggest that symptom-based survival prediction in this context may be limited. This could be due to several factors: (1) Symptom Concurrence: Patients often experience multiple concurrent symptoms at different disease stages; (2) Tumor Heterogeneity: Tumor location and morphology can vary considerably. Therefore, further development of symptom-based prediction models in this specific patient population may be less promising. However, these results do not preclude the potential utility of symptom analysis in other contexts or alongside other prognostic factors.

We also investigated the relationship between various lung pathologies concurrent with lung cancer diagnosis (pleural effusion, pneumonia, pneumofibrosis, emphysema, spontaneous pneumothorax, hydrothorax, endobronchitis, and atelectasis) and survival time (Table 4). Our focus was to determine if specific associated pathologies could influence patient outcomes and potentially guide treatment decisions.

Table 4. Survival time, by stage of disease and lung pathological conditions, associated with lung cancer.

Parameters	Duration between 'year of registration' and 'year of death', years			
	Stage I n=2 / 200 (1%)	Stage II n=10 / 200 (5%)	Stage III n=51 / 200 (25.5%)	Stage IV n=137 / 200 (68.5%)
Pleural effusion	-	-	1.1 (0.07 – 1.2)	1.3 (1.0 – 1.5)
Pneumonia	-	1.2 (-1.9 – 4.4)	1.3 (0.7 – 1.9)	1.3 (0.7 – 1.9)
Pneumofibrosis	2.5*	2.3 (-0.4 – 5.2)	1.5 (0.2 – 2.7)	1.3 (0.9 – 1.7)
Emphizema	-	-	3.0*	0.8 (0.2 – 1.3)
Pneumothorax	-	-	1.0*	1.5 (-4.8 – 7.8)
Hidrothorax	-	-	-	0.7 (-2.4 – 3.9)
Endobronchitis	-	2.0*	1.4 (0.8 – 1.9)	1.5 (1.2 – 1.8)
Lung colaps	-	1.0*	1.1 (0.5 – 1.6)	1.3 (0.9 – 1.8)

***Note*:** *Data expressed in years, mean, and 95% confidence interval (95% CI). Defaults indicate lack of patients with the given characteristics in the study group (certain stage vs. lung pathological conditions). *- only one patient in the group with the given characteristics. Column "stage IV" - patients ineligible for surgical treatment.*

Analysis revealed that most associated lung pathologies manifest primarily in stage III lung cancer, becoming increasingly prevalent in terminal stages. While stages I-II exhibit occasional or limited occurrences of these conditions, survival times within this group show greater variability. Despite this, statistical analysis found no significant survival differences linked to specific pathologies. Median survival times ranged from 0.8 to 1.5 years, with a mode of 1.3 years. Our reference group comprised 200 lung cancer patients representing diverse disease stages at diagnosis. This group exhibited a range of comorbidities and associated lung pathologies. We conducted a multi-faceted analysis of these factors to establish comprehensive baseline group characteristics. The prospective study arm enrolled 86 patients who underwent lung cancer surgery following our innovative perioperative management protocol. We focused on two primary endpoints: survival time after diagnosis and the two-year survival rate. These illustrate potential outcomes for patients who would have been theoretically eligible for surgery under our protocol. For comparison, Figure 1 also includes outcomes from a subset of our reference group meeting specific criteria: age 38-75, stage I-III disease, and a Charlson Comorbidity Index (CCI) below 12.

Mantel-Cox analysis of Kaplan-Meier curves revealed no statistically significant differences in survival times for patients theoretically eligible for surgery under our innovative approach. Within this subgroup (combining stages I-III), median survival was 20 months, and the two-year survival rate was 38.2% (13/34). One patient achieved an exceptional survival time of 41 months (~3.4 years). Stratifying by disease stage, we observed a median survival of 12 months and a two-year survival rate of 35.0% (7/20) for

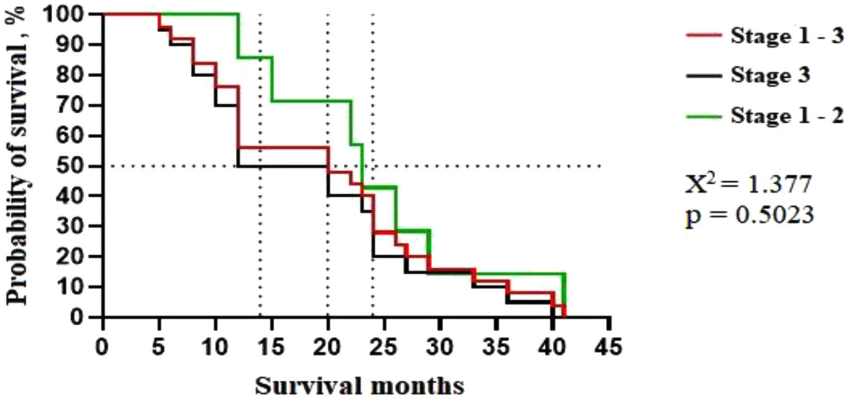

Fig. 1 Kaplan-Mayer survival duration curve of patients with lung cancer (stage 1-3), excluded SCLC, without severe comorbidities (ICC over 12 points), with an age limit of 38-75 years, from the reference group (patients treated without surgery).

Note: *X^2 - for linear trends (extended Hantel-Haenszel); p - survival analysis by Kaplan-Meier curves and log-rank (Mantel-Cox) test*

stage III patients. The mean survival was 18.3 months, with a range of 5 to 40 months (~0.4 - 3.3 years). For patients with stage I-II disease, median survival was 14 months, and the two-year survival rate was 42.9% (3/7), with a mean survival of 17.6 months and a range of 12 to 41 months (~1.0 - 3.4 years).

Discussions

Rjabov A *et al*. demonstrated the safety and efficacy of surgical treatment in selected lung cancer patients over 75 years of age. Key risk factors for postoperative complications included stage IIIb disease, lymph node involvement, and central cancers [10]. In this study of 73 patients, lobectomy was the most common procedure, with lymph node metastases noted in 32.9% of cases. Multivariate analysis identified the following significant risk factors: stage IIIb (OR=9.3, 95%CI=1.365-63.816; p=0.023), pN1 (OR=3.889, 95%CI=1.008-14.999; p=0.049), pN2 (OR=5.9, 95% CI=1.170-23.999; p=0.030), and central cancer (OR=7.572, 95%CI=1.742-32.884; p=0.007). Advances in diagnosis and treatment have significantly improved survival rates in NSCLC, which represents 80% of lung cancer cases [11].

While immunotherapy and targeted pharmacotherapy have significantly improved patient prognosis, the optimal treatment for advanced NSCLC remains an active area of research. Although surgery is generally not recommended for advanced NSCLC, particularly in cases of distant metastasis, some studies suggest potential benefits in selected patients with stage IV disease, especially those with oligometastatic tumors [12-15]. However, the overall benefit of surgery in this context continues to be debated, and the best surgical approach (local destruction, ablation therapy, sublobectomy, or lobectomy) requires further clarification [16].

Due to the rapid progression of lung cancer, standardized mortality rates closely mirror incidence rates for both sexes [17]. Unfortunately, over 75% of cases are diagnosed at advanced stages (IIIA-IV) [18]. While radiotherapy and chemotherapy, alone or in combination, offer significant improvements in long-term survival and symptom control for patients with locally advanced or metastatic disease [19, 20], surgery continues to play a crucial role in the management of advanced NSCLC.

Mounting evidence suggests potential survival benefits for carefully selected patients with advanced-stage lung cancer who undergo surgical resection. Studies have demonstrated improved survival outcomes in specific cases of stage IV NSCLC following surgery [21-23]. Notably, a large-scale analysis by Yang et al. in the United States revealed a significant 25% improvement in 5-year overall survival for patients with cT1-2, N0-1, M1 or cT3, N0, M1 disease who underwent surgery compared to those receiving non-surgical treatments (chemoradiation: 5.8%, chemotherapy: 5.9%, radiotherapy: 3.2%) [24].

Bauman JE *et al*. retrospectively investigated salvage lung resection in 24 stage IIIB patients. Procedures demonstrated technical feasibility with acceptable toxicity, even when performed after a delay following definitive radiotherapy. Key findings included a mean surgical duration of 5.5 hours, average blood loss of 250 ml, and a hospital stay of 8 days. While in-hospital mortality was 4% (morbidity 58%), the median overall survival of 30 months, and an estimated 3-year survival rate of 47% highlight the potential of this approach. Though encouraging, Bauman et al. emphasize the need for prospective validation in a well-defined patient population to determine the true efficacy of this strategy [25].

Sonnet J *et al*. prospectively evaluated the feasibility and safety of lung resection following induction therapy with concurrent chemoradiation at 45 Gy. The study included 40 patients with diverse preoperative stages (IIb-IV) and a significant proportion (13) with Pancoast tumors. Notably, the procedure was associated with no postoperative deaths. Importantly, pathological analysis revealed a high rate of complete response (45%) and significant reductions in residual disease burden (82.5% with no lymph node involvement). While overall and disease-free survival rates were promising (1 and 5-year rates exceeding 46%), the authors acknowledge the need for confirmation through larger, multi-institutional trials [26].

While surgical intervention offers clear benefits in early-stage NSCLC, its feasibility in stage III-IV disease remains

a subject of debate [27]. Prognosis in resectable stage III NSCLC patients undergoing surgery following neoadjuvant therapy is strongly linked to lymph node invasion [28]. Furthermore, stage IV NSCLC typically carries a limited life expectancy, leading to general discouragement for surgical intervention [29, 30].

In the United States, despite a significant improvement in the 5-year survival rate of NSCLC patients from 16.4% to 25.1% (1975-2015), nearly 55% eventually progress to advanced stages [11, 30]. While surgery offers proven benefits in early-stage NSCLC (stages I-II), its feasibility, and effectiveness in stages III-IV remain controversial [27]. For resectable stage III NSCLC, post-neoadjuvant therapy prognosis heavily depends on lymph node invasion [28]. Moreover, surgical intervention is often considered unsuitable in stage IV NSCLC due to limited life expectancy [29].

Ren J *et al*. demonstrated potential palliative benefits of surgery in stage IV NSCLC, revealing a doubling of average survival compared to the non-surgical group. Cox regression analysis identified surgery as an independent predictor of improved overall survival (OS) (HR=0.441; 95%CI: 0.426-0.456; p<0.001) and cancer-specific survival (CSS) (HR=0.397; 95%CI: 0.380-0.414; p<0.001). Importantly, the study suggests that lobectomy may offer survival advantages over local destruction or sub-lobectomy in this patient population (p< 0.001) [31].

Liang *et al*. observed a survival benefit in metastatic NSCLC patients who underwent prior primary tumor resection. Their model explored the hypothesis that surgical benefits in stage IV disease may depend on specific patient and tumor characteristics. Analysis of 30.342 stage IV patients revealed that 8.03% underwent primary resection, which was independently associated with a longer cancer-specific median survival (CSS) compared to non-surgical management (19 vs. 9 months, p<0.001). Importantly, 56.40% of the surgical cohort survived beyond 9 months [13].

Prehabilitation, a recently established practice, aims to optimize a patient's functional capacity before surgery and improve post-operative outcomes. This multifactorial approach encompasses medical optimization, exercise training, nutritional counseling, and psychological support to address perioperative stressors [32]. Surgical trauma, anesthesia, and perioperative therapies (neoadjuvant treatment, perfusion, ventilation) all contribute to stress alongside factors like malnutrition and anxiety. Patient resilience to these stressors depends on modifiable factors (comorbidities, smoking, physical fitness, psychological state) and non-modifiable factors (age, gender, cancer biology). Modifiable factors further interact with those related to the underlying malignancy, such as cachexia, malabsorption, and muscle wasting [32].

The efficacy of prehabilitation programs and their optimal duration remain areas of ongoing investigation. However, prehabilitation has the potential to expedite postoperative recovery, enhance quality of life, and improve tolerance to neoadjuvant therapies like chemotherapy [33]. Notably, prehabilitation is not a novel concept in thoracic surgery, where pulmonary prehabilitation has been implemented to improve functional capacity and reduce complications in high-risk patients undergoing lung transplantation or lung volume reduction surgery [34, 35].

In a pioneering study, Sekine *et al*. (2005) prospectively investigated the impact of a pulmonary prehabilitation program on 22 Chronic Obstructive Pulmonary Disease (COPD) patients undergoing lobectomy (FEV1/FVC ≤70%, >50%) [36]. When compared to a historical control group (n=60) matched for selection criteria, the prehabilitation group demonstrated a significantly lower incidence of postoperative pulmonary complications and a shorter hospital stay.

Jones *et al*. investigated 25 lung cancer patients, demonstrating significant improvements in VO2 max (3.3 ml/kg/min, p=0.006) and 6-minute walk distance (49 m, p=0.013) after training up to the day of resection [37]. These findings are mirrored in Bobbio et al.'s prospective study of 12 stage I/II NSCLC patients with COPD and compromised VO2 max (≤15 ml/kg/min). A 4-week pulmonary prehabilitation program yielded an average VO2 max increase of 2.8 ml/kg/min [38].

Tarumi *et al*. demonstrated the potential benefits of pulmonary prehabilitation initiated during induction chemoradiotherapy for lung cancer. Their study of 82 patients revealed significant improvements in both FVC (+6.4%, p=0.0096) and FEV1 (+10.4%, p<0.001) following participation in the 10-week program. Importantly, the most pronounced gains were observed in patients with initial respiratory compromise (FVC <80% or FEV1/FVC <70%), who showed a substantial increase in FVC (+13.9%, p=0.0025) and FEV1 (+22.5%, p<0.0001) [39].

Benzo *et al*. investigated the feasibility and impact of pulmonary prehabilitation on postoperative morbidity in patients with moderate-severe COPD undergoing curative lung cancer surgery [40]. Two randomized controlled trials were conducted, comparing prehabilitation programs to usual care. The initial 4-week program proved difficult to implement due to low recruitment. A revised 1-week, twice-daily prehabilitation program enrolled 19 patients. While statistically significant reductions in chest drain duration (mean 4.7 vs. 9.0 days, p=0.03) and prolonged drainage (>7 days) were observed in the prehabilitation group (11% vs. 63%, p=0.03), the study was ultimately limited by a small sample size and short program duration, preventing definitive conclusions about the impact of prehabilitation on postoperative morbidity [40].

Gao *et al*. (2015) investigated the effects of a preoperative pulmonary prehabilitation program on high-risk patients with resectable lung cancer. In their non-randomized study, 71 patients participated in the program (which included abdominal breathing exercises, respiratory device training, and lower limb resistance training) followed by lobectomy. These patients were compared to a control group of 71 patients who underwent lobectomy with conventional management alone [41].

Boujibar *et al*. investigated the potential of prehabilitation to improve surgical outcomes and reduce morbidity (as

measured by the Clavien-Dindo classification) in patients with resectable lung cancer and VO2 max ≤20 ml/min/kg. Their study compared 19 patients who underwent prehabilitation (exercise, muscle strengthening, education, and smoking cessation) to 19 patients who received standard care. Prehabilitation was associated with a significantly lower postoperative complication rate (42% vs. 80%, p=0.0382), particularly in terms of less severe complications (Clavien-Dindo score ≤2, p=0.0252). However, no difference in hospital stay was observed between groups [42].

Licker *et al*. conducted a randomized controlled trial investigating the impact of preoperative prehabilitation on patients with operable lung cancer. A total of 151 patients were randomized to either prehabilitation (high-intensity interval training, 2-3 sessions/week for an average of 25 days) or usual care. Primary outcomes were post-operative morbidity and mortality, while secondary outcomes focused on changes in cardiopulmonary exercise and 6-minute walk test performance. Despite significant prehabilitation-driven improvements in VO2 max and 6-minute walk test (6MWT) distance (+15%, p=0.003 and +15%, p<0.001 respectively), no significant difference in overall postoperative complication rates was detected (prehabilitation 35.5% vs. usual care 50.6%, p=0.080). However, a sub-analysis revealed a lower incidence of pulmonary complications in the prehabilitation group (23% vs. 44%, p=0.018) [43].

Licker *et al*. demonstrated the safety and feasibility of a short-term preoperative training program to improve aerobic performance. However, these improvements did not translate to a significant reduction in overall morbidity-mortality compared to usual care. This finding may be partially attributed to the study's inclusion of all resectable lung cancer patients without stratification by risk, potentially obscuring differences in postoperative complications. Additionally, the high proportion of open thoracotomies (>80%), despite many patients having early-stage disease, is a notable deviation from current practices where Video-Assisted Thoracic Surgery (VATS) is preferred. This factor may complicate the interpretation and generalizability of results [43].

Multimodal prehabilitation aims to optimize patients' resilience against surgical, anesthetic, and perioperative stressors, potentially improving long-term outcomes. While studies have demonstrated feasibility, safety, and improved muscle function, evidence of definitive clinical efficacy remains limited. These initial encouraging results justify the need for large-scale clinical effectiveness studies to fully establish the role and benefits of prehabilitation.

Conclusions

Our findings underscore several urgent priorities in healthcare policy and organization. Early detection through targeted lung cancer screening is crucial, given the late-stage presentation of most cases (94%). Alongside this, population-wide smoking cessation programs, environmental protection measures, and promotion of healthy lifestyles are vital for prevention. Standard chemotherapy and radiotherapy offer limited benefits in advanced lung cancer (stages III and IV), highlighting a pressing need for basic research breakthroughs and novel treatment paradigms.

The short average survival times from diagnosis (1.3-1.4 years) put pressure on basic research in particular. In addition to radio- and chemotherapy, the development of anti-tumour immunotherapy is seen as a new line in the non-surgical treatment of lung cancer.

Competing interests

None declared.

Authors' contributions:

Concept and design of study – IM; acquisition of data – IM, SG, IB; analysis and/or interpretation of data – IM, SG, IB; drafting the manuscript – IM, SG; revising the manuscript critically for important intellectual content – IM, SG. All authors have read and approved the final version of the manuscript.

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Patient consent

Obtained.

Ethics approval

The study protocol was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Protocol No. 04 of 12.11.2020).

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RESEARCH ARTICLE

Deep endometriosis – diagnosis and impact on quality of life

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ABSTRACT

Introduction. Deep infiltrating endometriosis (DIE) is considered the most painful form of endometriosis, responsible for reducing the women's quality of life (QoL). Its management presents difficulties in medicine. The #Enzian classification reflects locations of DIE and simplifies its medical management. International guidelines recommend studies of QoL in women with endometriosis.

Objective. To investigate the symptoms of DIE and determine its impact on QoL to optimize its diagnostics.

Materials and methods. A cohort study was conducted over 2 years at the *Gheorghe Paladi* Municipal Clinical Hospital, including 190 patients with endometriosis, who were divided into groups: main group - 85 patients with DIE, control group - 105 other endometriosis forms. To objectify the pain, Visual Analog Scale and Biberoglu and Behrman (B&B) were used. Endometriosis was staged with the #Enzian classification. For the analysis of QoL, three standardized questionnaires were used. Data were recorded in Excel and statistically calculated with the SPSS program.

Results. Pelvic pain syndrome according to the Visual Analog Scale and B&B scales in the main group was 3 times more pronounced than in the control group ($p < 0.01$). Lesions of DIE according to the #Enzian statistically correlated with chronic pelvic pain, dysmenorrhea, dyspareunia, dysuria, dyschezia >7 points (VAS), catamenial rectal tenesmus, defecation disorders, menometrorrhagia, hematuria, bladder tenesmus, hydronephrosis with ureteral stenting during pregnancy, catamenial cough and hemoptysis, chest pain and spontaneous pneumothorax, hemorrhagic scar, hiccups, and the frenicus symptom ($p < 0.05$). According to the questionnaires of QoL, DIE significantly influences life determinants by 44.27%, compared to the control group at 3.64% ($p < 0.01$), allowing realization of life determinants in a maximum of 58.54% vs. the control group's 92.18% ($p < 0.01$). Additionally, psychological well-being in patients with DIE is lower than that in the control group (44.29% vs. 81.38%, $p < 0.01$).

Conclusions. High-intensity pain syndrome and extragenital symptoms correlated with compartments of #Enzian will assist in the preoperative multidisciplinary diagnosis of DIE. The high influence on life determinants, the low realization of life potential, and the low psychological well-being confirm the significant impact of DIE on QoL, classifying it as a disability.

Keywords: endometriosis, deep endometriosis, diagnosis, pelvic pain, quality of life.

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Key messages

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

Association between clinical manifestations and the location of deep endometriosis according to the #Enzian classification with preoperative multidisciplinary diagnosis, the impact of deep endometriosis on pelvic pain syndrome, and the impairment of women's quality of life – these significant questions remain debatable.

The research hypothesis

Deep endometriosis is responsible for pronounced pelvic pain syndrome, a variety of extragenital symptoms, and a significant dete-

rioration in the quality of women's lives.

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

The study allowed for specifying the features of the clinical course of deep endometriosis depending on the localization of its foci, comparing the intensity and spectrum of the pain syndrome, as well as the impact of this pathology on the quality of life of women compared to patients with other forms of endometriosis.

Introduction

While the incidence of deep infiltrating endometriosis (DIE) continues to rise, the pathogenesis, clinical manifestations, diagnosis, and treatment of this pathology remain subjects of intensive research worldwide.

DIE is considered the most aggressive and painful form of pelvic endometriosis, constituting one-fourth of its three phenotypes [1]. Affects 2% of women of reproductive age, DIE is responsible for pelvic pain syndrome, complicated somatic and surgical anamnesis, and a reduced quality of life for these women [2, 3]. Its diagnosis and treatment pose an extremely challenging task for both the global medical community and healthcare professionals in the Republic of Moldova.

Although DIE was first described by C. Rokitansky in 1860, only in 2012 Koninckx proposed the first definition of this pathology, stating that DIE is the invasion of endometrial tissue to a depth of more than 5 mm beneath the peritoneum [4]. In 2015, the DIE lesions were discovered in the intestines, bladder, ureters, and diaphragm. In 2017, Balle and Dara proposed the modern definition of the DIE.

Thus, DIE is the pathology that involves fibromuscular infiltration of the organs and anatomical structures with the subperitoneal invasion of the endometrial tissues, regardless of the depth of infiltration [4, 5].

For clinical use in 2021, the following classifications were recommended: the revised American Society for Reproductive Medicine (rASRM), the Endometriosis Fertility Index (EFI), and the #Enzian classification. These classifications allow staging the pathological process and assessing the reproductive prognosis for each patient [6, 7].

Of particular interest for our study is the #Enzian classification, reflecting various locations of DIE [8]. Some studies suggest that this classification correlates with the clinical manifestations of DIE; however, randomized studies are needed for definitive conclusions. From a clinical standpoint, this unified reporting system simplifies the medical management of patients, avoiding multiple repeat surgeries and improving the quality of treatment and quality of life for DIE patients [8].

DIE most commonly affects the patients under 17 years old, with 20% of girls manifest the pathology simultaneously with menarche. According to the literature, the clinical manifestations of DIE include the 4 "D" symptoms: dysmenorrhea, dyspareunia, dysuria, and dyschezia, often in combination with infertility [9]. DIE is associated with infertility, primarily due to the distortion of normal pelvic organ anatomy. The intensity of pelvic pain syndrome in DIE often exceeds 6 points on the VAS (visual analog scale), and extragenital foci provoke the development of symptoms with a catamenial course, such as pain during defecation, intestinal obstruction syndrome, pain during urination, hematochezia and hematuria, recurrent cystitis, chest pain, pain in the operation scar with cyclic bleeding and gradual formation increase in this area, and others [10-15].

Based on the above information, it can be argued that DIE is the most aggressive and clinically vivid phenotype of endometriosis, significantly deteriorating the quality of life for the patients [16, 17].

Infiltration of DIE foci into adjacent organs increases the frequency of interventions with surgical and anesthetic risks, further diminishing the quality of women's lives [17]. Currently, evaluating the quality of life for patients is an essential element of the medical care, and studying the determinants of quality of life helps better understand the specific impact of a particular disease on the patients' well-being [18]. Research reports indicate that the work productivity of women with DIE is reduced by 38%, 50% of patients suffer from infertility, and 88% of patients experience anxiety disorders or depression [19]. International guidelines recommend initiating studies dedicated to examining the quality of life of women with endometriosis using specialized questionnaires (SF36, EIQ, etc.) that reflect all levels of patients' health [4].

Having reviewed the above information, it was decided to conduct a comparative clinical study aimed at investigating the diagnostic features of DIE and determining its impact on the quality of life of patients to optimize its preoperative diagnostics.

Material and methods

A single-center cohort clinical study was conducted over 2 years at the *Gheorghe Paladi* Municipal Clinical Hospital. The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes nr. 38, dated 21.05.2021). The study included reproductive-aged patients diagnosed with "Endometriosis," confirmed based on intraoperative findings or ultrasound/MRI indications, who consented to participate in this study. The exclusion criteria for this study were as follows: patients under the age of majority, virgin patients, retired patients, patients with endometriosis malignancy, patients with severe extragenital pathologies (hypertension, cardiovascular pathology, liver pathology, and others), patients with precancerous or cancerous conditions (cervical, endometrial, ovarian), patients who refused to partici-

pate in the clinical study. Each patient signed an informed consent form to participate in this study.

Thus, the 190 women enrolled in the study were divided into two groups: the study group comprised 85 patients with deep endometriosis, while the control group consisted of 105 women with other forms of endometriosis (the ovarian endometriomas and superficial endometriosis).

To objectify the pain syndrome, the Visual Analog Scale (VAS) and Biberoglu and Behrman (B&B) pain scales were used, categorizing pain as "mild," "moderate," "severe," and "very severe." Intraoperative data and protocol data from paraclinical studies were analyzed with the staging of endometrioid processes according to the #Enzian classification. To assess the impact of endometriosis on the quality of life, three questionnaires were utilized: the Endometriosis Impact Questionnaire (EIQ), the 36-Item Short Form Health Survey (SF-36), which identifies the impact of endometriosis on 8 determinants of quality of life, and the World Health Organization-Five Well-Being Index (WHO-5), which evaluates the psychological well-being of patients. Analyzed data were recorded in an Excel spreadsheet, and statistical calculations were performed using the SPSS program. For comparing quantitative variables in groups, the Mann-Whitney U test was utilized. For comparing qualitative variables in groups, the Pearson's Chi-square test (χ^2) was applied.

Results

Gynecological anamnesis data. Statistical comparison did not reveal any age difference between the patient groups (U = 4520.00, p = 0.879). The average age of women in the main study group was 32.39±0.81 years (95% CI [30.76 – 34.02 years]), while in the control group, it was 30.26±0.66 years (95% CI [28.91 – 31.60 years]). However, a significant difference in the age of onset of menarche was found between the study groups (U = 5697.00, p = 0.001), indicating an earlier manifestation of menstrual function among patients with deep infiltrating endometriosis (12.27±0.19 years; 95% CI [11.89 – 12.65 years]) compared to women with superficial and ovarian forms (13.18±0.20 years; 95% CI [12.77 – 13.59 years]). Statistical comparison of menstrual cycle regularity in the research groups revealed a significant difference ($\chi^2 = 11.206^a$, df = 1, p = 0.001), indicating a higher frequency of irregularities among women with deep endometriosis (40.0±5.4%; 95% CI [29.3 – 51.1%]) than those with other phenotypes of this pathology (18.1±5.4%; 95% CI [11.5 – 26.2%]). No statistically significant difference in menstruation duration was found between the research groups (U = 3840.50, p = 0.086), with a median of 5 days in both groups.

Medical history of patients. An analysis of the collected data revealed that in the main study group, patients' medical history was uncomplicated for only 16.5±4.1% (95% CI [8.6 – 25.3%]), whereas in the control group, this figure was 71.4±4.3% (95% CI [62.4 – 79.4%]), indicating a statistically significant differences ($\chi^2 = 81.844^a$, df = 6, p < 0.01) and indirectly suggesting reduced quality of life in women with deep endometriosis.

The time to diagnosis for patients with deep endometriosis exceeded 10 years in 67.1±5.3% (95% CI [56.5 – 76.7%]), while in the control group, the diagnosis was correctly made within 1 year of the disease in 56.2±4.9% (95% CI [46.3 – 65.6%]) of patients, indicating a statistically significant difference ($\chi^2 = 112.487^a$, df = 3, p < 0.01). This fact indirectly confirms the reduced quality of life in women with deep endometriosis.

Endometriosis symptoms. The results of assessing complaints from patients in this study showed the following frequency of symptoms: chronic pelvic pain – 67.89%, dysmenorrhea – 89.47%, dyspareunia – 54.74%, dysuria – 10.53%, dyschezia – 25.79%, menometrorrhagia – 28.42%, catamenial hematuria – 4.74%, catamenial tenesmus of the bladder – 4.21%, hydronephrosis, ureteral stenting during pregnancy – 2.11%, catamenial rectal tenesmus – 22.11%, catamenial defecation disorders – 20.00%, catamenial intestinal subocclusion – 2.63%, catamenial meteorism – 4.21%, catamenial cough and hemoptysis – 1.05%, catamenial breast pain and spontaneous pneumothorax – 1.05%, Benjamin's symptom – 50.00%, weakness – 40.00%, catamenial bleeding from the scar – 2.63%, hiccups – 1.58%, frenicus symptom – 1.58% (Fig. 1).

Pain syndrome. Pain levels on the VAS among women with DIE relative to the control group of patients were distributed as follows: the chronic pelvic pain – 7.90±0.21 points (95% CI; 7.47 – 8.34 points) vs the 2.41±0.45 points (95% CI; 1.49 – 3.33 points), the dysmenorrhea (Dm) – 9.02±0.17 points (95% CI; 8.67 – 9.38 points) vs the 5.31±0.52 points (95% CI; 4.24 – 6.37 points) (Figure 2a), the dyspareunia (Dp) – 7.85±0.33 points (95% CI; 7.18 – 8.53 points) vs the 2.18±0.47 points (95% CI; 1.23 – 3.13 points) (Figure 2b), the dysuria – 1.46±0.47 points (95% CI; 0.51 – 2.42 points) vs the 0 points, the dyschezia – 3.83±0.56 points (95% CI; 2.70 – 4.96 points) vs the 0 points. Statistical comparison showed significant differences between the study groups for chronic pelvic pain (U = 706.00, p < 0.01), dysmenorrhea (U = 1254.00, p < 0.01), and dyspareunia (U = 699.50, p < 0.01), while dysuria and dyschezia were identified as pathognomonic symptoms of deep endometriosis.

Objective pain assessment on the B&B score yielded the following results among DIE patients compared to the control group: the total symptom and sign severity score was 11.39±0.364 points (95% CI; 10.65 – 12.13 points) vs the 4.9±0.57 points (95% CI; 3.74 – 6.05 points) (Figure 3), the total pelvic pain score – 7.61±0.21 points (95% CI; 7.19 – 8.03 points) vs the 2.92±0.37 points (95% CI; 2.16 – 3.68 points), the total physical sign score – 3.78±0.22 points (95% CI; 3.34 – 4.22 points) vs the 1.98±0.26 points (95% CI; 1.46 – 2.49 points). Statistical comparison of pain intensity on the B&B scale between the study groups also revealed significant differences - U = 735.00, p < 0.01, confirming a more pronounced intensity of pain syndrome among women with deep infiltrating endometriosis.

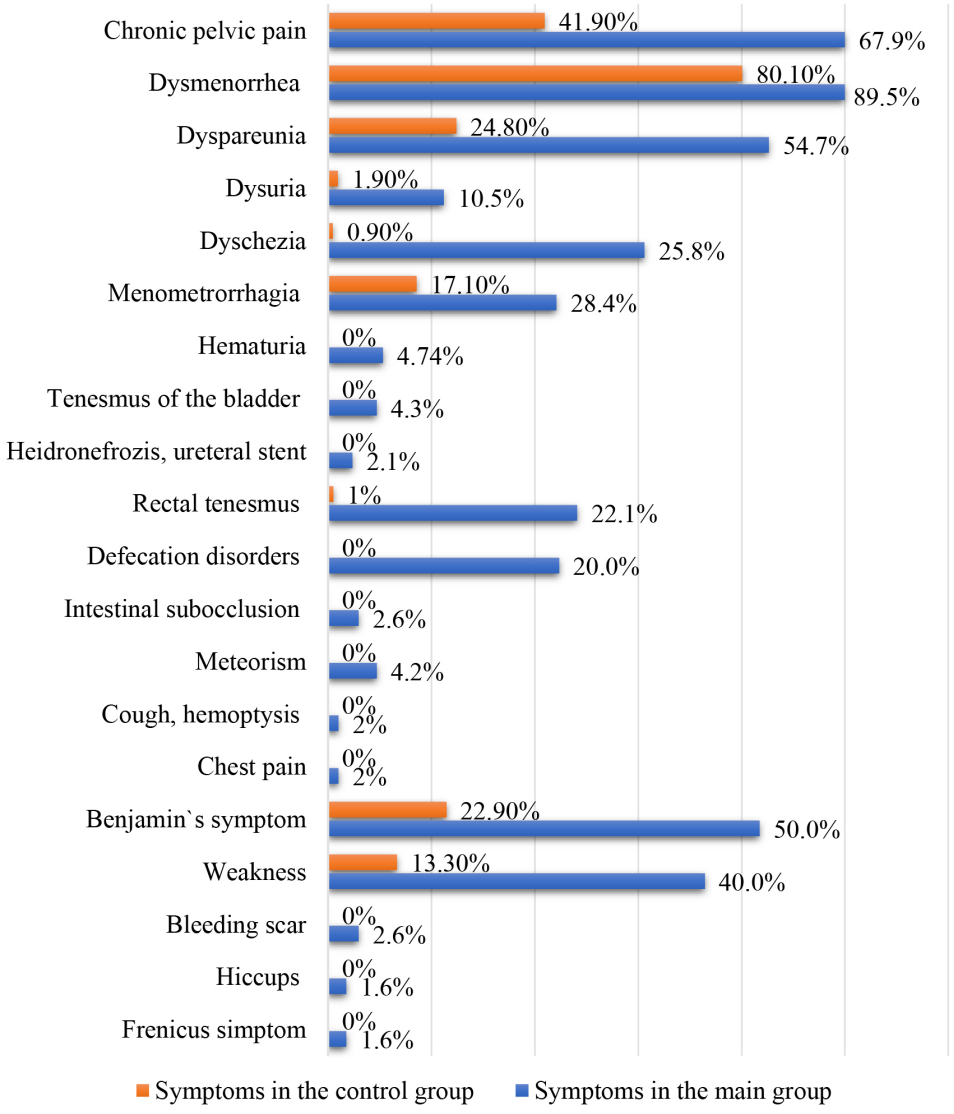

Fig. 1. Frequency of symptoms among patients in the cohort study

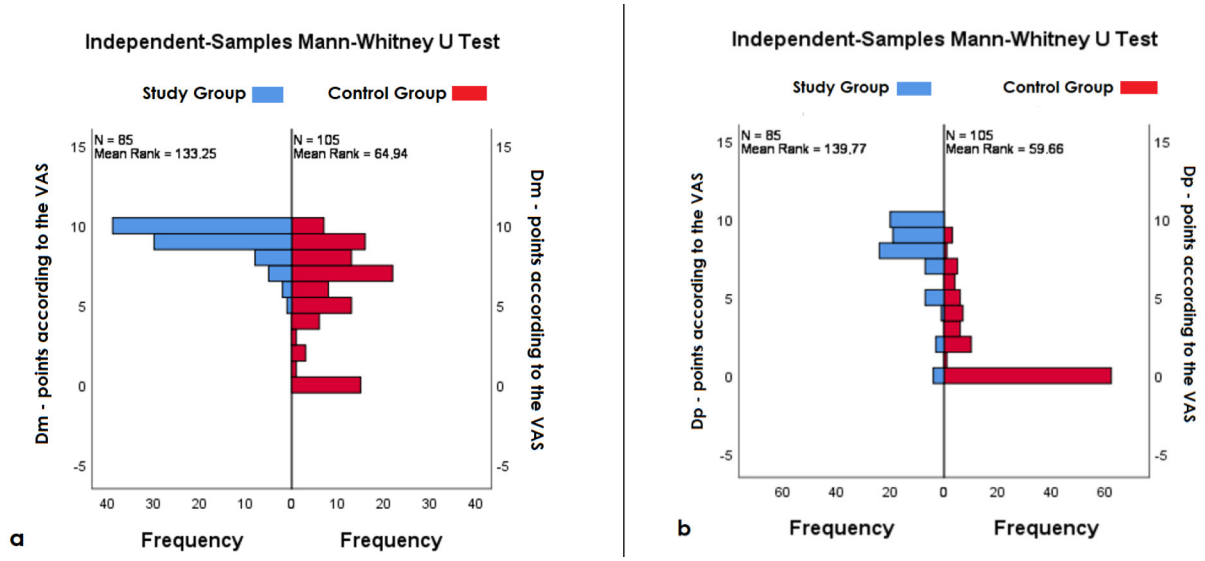

Fig. 2. The comparison of pain levels according to VAS dysmenorrhea Dm (a), dispareunia Dp (b) – in the research groups

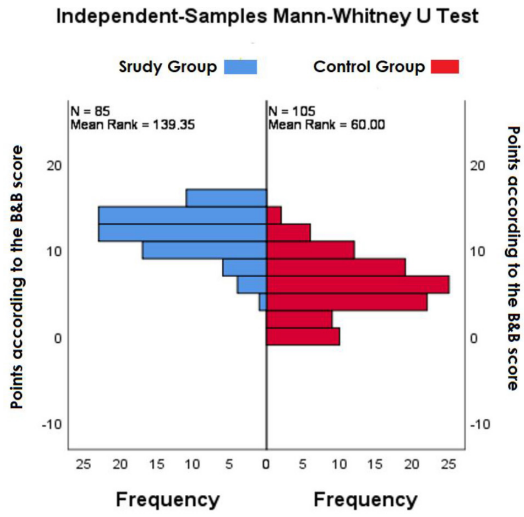

Fig. 3. Comparison of the pain level according to B&B scale in the research groups.

Localization of deep endometriosis according to the #Enzian classification. Analysis of the localization of deep endometriosis foci in the main research group showed the following frequency distribution of this pathology by compartments of the #Enzian classification: localization in compartment A (the rectovaginal septum and vagina) – 55.3±5.3% (95% CI [45.2 – 65.5%]); in compartment B (the uterosacral ligament and pelvic walls) – 8.2±2.9% (95% CI [3.4 – 14.4%]); in compartment C (the sigmoid colon and rectum) – 1.2±1.2% (95% CI [0.0 – 3.8%]); in compartment FA (adenomyosis) – 17.6±4.2% (95% CI [10.0 – 26.3%]); in compartment FB (the bladder) – 17.6±3.9% (95% CI [10.0 – 25.3%]); in compartment FI (the intestine) – 9.4±3.2% (95% CI [3.7 – 16.2%]); in compartment FO (other locations) – 12.9±3.7% (95% CI [6.0 – 20.5%]).

Correlation between #Enzian compartments and symptoms. Lesions of DIE in compartments A, B, FA, FI according to #Enzian classification statistically correlated with chronic pelvic pain, dysmenorrhea, dyspareunia and dyschezia > 7 points after VAS ($p < 0.05$). Additionally, lesions of DIE in compartment A according to #Enzian classification statistically correlated with catamenial rectal tenesmus, catamenial defecation disorders, Benjamin's symptom, and weakness ($p < 0.05$). At the same time, lesions of deep endometriosis in compartment B according to the #Enzian classification are statistically correlated with catamenial rectal tenesmus. The patient with a focus of deep endometriosis in compartment C according to the #Enzian classification presented with the following symptoms: chronic pelvic pain, dysmenorrhea, dyspareunia, and dyschezia > 7 points after VAS, catamenial rectal tenesmus, catamenial defecation disorders, Benjamin's symptom, and weakness. However, due to only one case, it would be inappropriate to discuss the statistical significance of these results. According to the #Enzian classification, lesions of endometriosis in compartment FA also show a statistically significant correlation with menometrorrhagia, catamenial rectal tenesmus, catamenial defecation disorders, Benjamin's symptom, and weakness ($p < 0.05$). Lesions of endometriosis in compartment FB, as per the #Enzian classification, demonstrate a statistically significant association with chronic pelvic pain, dysmenorrhea and dysuria > 7 points after VAS, catamenial hematuria, catamenial bladder tenesmus, hydronephrosis with ureteral stenting during pregnancy, Benjamin's symptom, and weakness ($p < 0.05$). Statistically significant correlations exist between lesions of endometriosis in compartment FI, categorized by the #Enzian classification, and catamenial defecation disorders, catamenial intestinal subocclusion, catamenial meteorism, Benjamin's symptom, weakness ($p < 0.05$). As per the #Enzian classification, lesions of endometriosis in compartment FO exhibit statistically significant correlations with chronic pelvic pain, dysmenorrhea, dyspareunia > 7 points after VAS, catamenial cough, hemoptysis, catamenial chest pain and spontaneous pneumothorax, catamenial hemorrhagic scar, hiccups, phrenic sign ($p < 0.05$).

Table 1 displays the frequency distribution of statistically significant symptoms depending on the localization of deep endometriosis, corresponding to the compartments of the #Enzian classification.

Quality of life in endometriosis. According to the results of the EIQ questionnaire calculation, deep endometriosis significantly affected the majority of life determinants in patients compared to the control group of women, negatively influencing the quality of life. The physical health of the main research group's patients was reduced by 64.49±3.39% (95% CI; 57.63 – 71.34%), whereas in the control group it was reduced by 14.13±2.40% (95% CI; 9.26 – 18.99%). The main research group's patients experienced a 70.27±3.06% (95% CI; 64.08 – 76.46%) reduction in mental health compared to a 14.10±2.71% (95% CI; 8.61 – 19.60%) reduction in the control group. The social function of the main research group's patients was reduced by 52.68±4.38% (95% CI; 43.81 – 61.55%), whereas in the control group it was reduced by 4.72±1.82% (95% CI; 1.03 – 8.40%). In comparison to the control group, the patients in the main research group showed a 64.9±4.53% (95% CI; 55.74 – 74.07%) decrease in sexual function, while the control group experienced a 12.74±2.97% (95% CI; 6.76 – 18.73%) decrease. The fertility of patients in the main research group deteriorated by 90.93±3.54% (95% CI; 83.77 – 98.09%), in contrast to a 79.90±5.45% (95% CI; 68.86 – 90.93%) deterioration observed in the control group. The main research group's patients experienced a 58.76±4.58% (95% CI; 49.50 – 68.02%) reduction in work capacity compared to a 4.77±1.52% (95% CI; 1.68 – 7.86%) reduction in the control group. Relative to the control group, patients from the main research group exhibited a 44.27±4.12% (95% CI; 35.95 – 52.59%) decrease in educational attendance, whereas the control group demonstrated a reduction of only 3.64±1.33% (95% CI; 0.95 – 6.33%). The life-

style of patients in the main research group deteriorated by 53.02±5.54% (95% CI; 41.83 – 64.22%) in contrast to a 2.28±1.24% (95% CI; -0.22 – 4.79%) deterioration observed in the control group. Comparison of EIQ questionnaire results revealed a statistically significant difference in the impact of pathologies in the research groups on physical health (U = 479.00, p < 0.01) and mental health of patients (U = 311.00, p < 0.01), on social function (U = 701.50, p < 0.01), sexual function (U = 690.50, p < 0.01), fertility (U = 1107.50, p = 0.001), work capacity (U = 841.00, p < 0.01), attendance of education (U = 567.50, p < 0.01), and lifestyle (U = 850.00, p < 0.01).

Table 1. Statistically verified correlation between #Enzian compartments and symptoms (p < 0.05).

	A	B	FA	FB	FI	FO
Chronic pelvic pain > 7 VAS	85.1%	100%	100%	86.7%	87.5%	90.9%
Dysmenorrhea > 7 VAS	97.8%	100%	100%	93.3%	100%	90.9%
Dyspareunia > 7 VAS	93.6%	100%	86.7%	-	75.0%	45.4%
Dysuria > 7 VAS	-	-	-	86.7%	-	-
Dyschesia > 7 VAS	53.2%	42.8%	46.7%	-	87.5%	-
Menometrorrhagia	-	-	100%	-	-	-
Hematuria	-	-	-	60.0%	-	-
Tenesmus vesical	-	-	-	100%	-	-
Hydronephrosis, ureteral stenting	-	-	-	26.7%	-	-
Tenesmus rectal	57.4%	71.4%	73.3%	-	-	-
Defecation disorders	57.5%	-	60.0%	-	100%	-
Intestinal subocclusion	-	-	-	-	62.5%	-
Meteorism	-	-	-	-	87.5%	-
Cough, hemoptysis	-	-	-	-	-	18.2%
Chest pain	-	-	-	-	-	18.2%
Symptom Benjamin	85.1%	-	80.0%	93.3%	100%	-
Weakness	74.5%	-	80.0%	73.3%	87.5%	-
Hemorrhagic scar	-	-	-	-	-	45.5%
Hiccups	-	-	-	-	-	27.3%
Phrenic sign	-	-	-	-	-	27.3%

Note: A – DIE in the rectovaginal septum and vagina; B – DIE in the uterosacral ligament and pelvic walls; FA - adenomyosis; FB – DIE in the bladder; FI – DIE in the intestine; FO – DIE in other locations

According to the SF-36 questionnaire calculation results, deep endometriosis significantly affected the quality of life of patients, as evidenced by the reduction in the realization of life determinants in women with deep endometriosis compared to similar data in the control group.

Thus, the realization of the general health potential (GH) among women in the main research group amounted to 42.15±3.42% (95% CI; 35.23 – 49.06%), compared to 76.23±3.02% (95% CI; 70.12 – 82.34%) in the research control group. The realization of the ability to perform

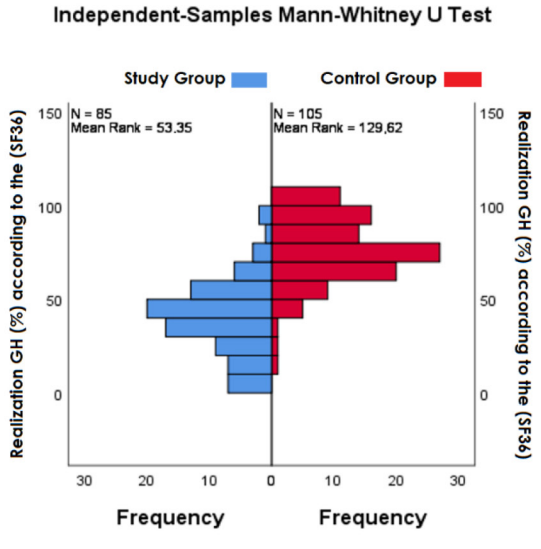

Fig. 4 Comparison of the general health (GH) by SF-36

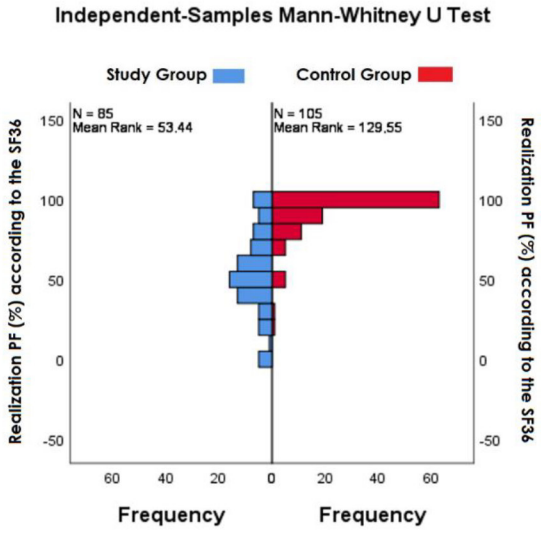

Fig. 5 Comparison of the physical functioning (PF) by SF-36

usual physical activities (PF) among women in the main research group amounted to 58.54±4.11% (95% CI; 50.22 – 66.85%), compared to 92.18±1.95% (95% CI; 88.23 – 96.13%) in the research control group. The realization of performance of work or other duties (RP) among women in the main research group was 39.63±5.16% (95% CI; 29.20 – 50.07%), as opposed to 89.10±3.41% (95% CI; 82.20 – 96.01%) in the research control group. The performance of work or other duties in the context of emotional limitations, denoted as RE, among women in the main research group was measured at 37.78±4.54% (95% CI; 28.60 – 46.96%), contrasting with 85.59±3.99% (95% CI; 77.50 – 93.68%) in the research control group. The level of social activity and impact of physical or emotional health on social life (SF), among women in the main research group was measured at 47.12±1.02% (95% CI; 45.05 – 49.20%), contrasting with 51.41±1.01% (95% CI; 49.35 – 53.47%) in the research control group. Among women in the main research group, the degree of physical activity influenced by pain, referred to as BP, averaged 28.32±3.35% (95% CI; 21.53 – 35.11%), in contrast to 77.46±4.34% (95% CI; 68.67 – 86.25%) in the research control group. The energy and vitality, designated as VT, among women in the primary research cohort, averaged 42.44±2.80% (95% CI; 36.78 – 48.10%), compared to 76.26±3.30% (95% CI; 69.56 – 82.95%) in the control cohort. Women in the main research group demonstrated mental well-being and mental health, termed as MH, of 47.32±2.37% (95% CI; 42.51 – 52.12%), while those in the control group exhibited a level of 77.23±2.96% (95% CI; 71.23 – 83.23%) for mental well-being. The statistically significant difference in the research groups was confirmed in GH (U = 8045.50, p <

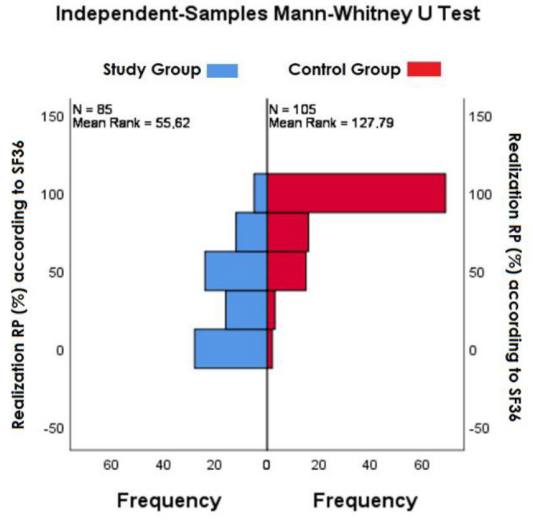

Fig. 6 Comparison of the role-physical health (RP) by SF-36

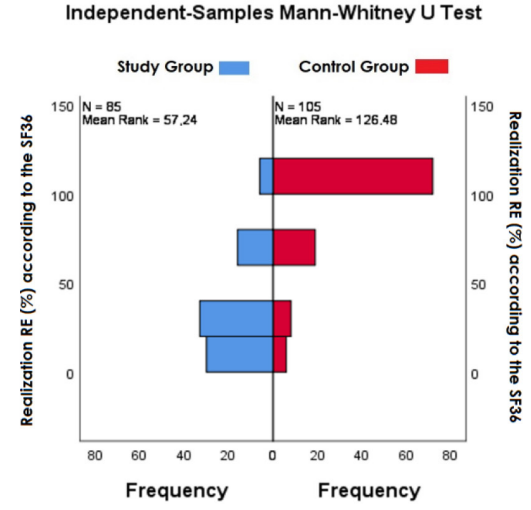

Fig. 7 Comparison of the role-emotional (RE) by SF-36

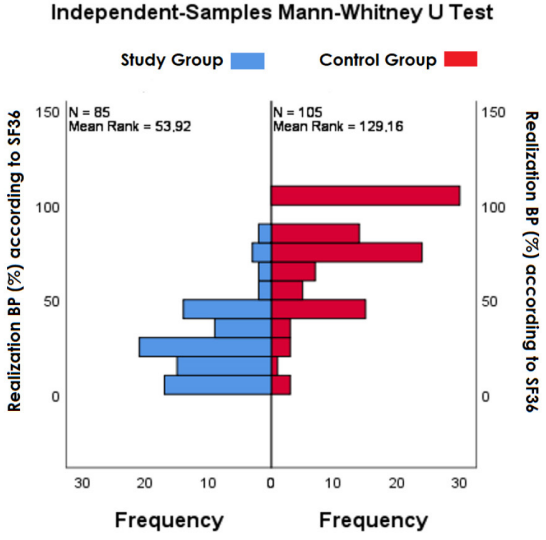

Fig. 8 Comparison of the bodily pain (BP) by SF-36

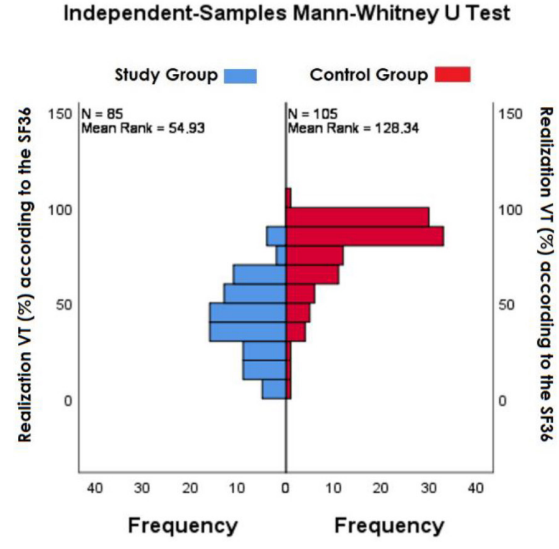

Fig. 9 Comparison of the vitality (VT) by SF-36

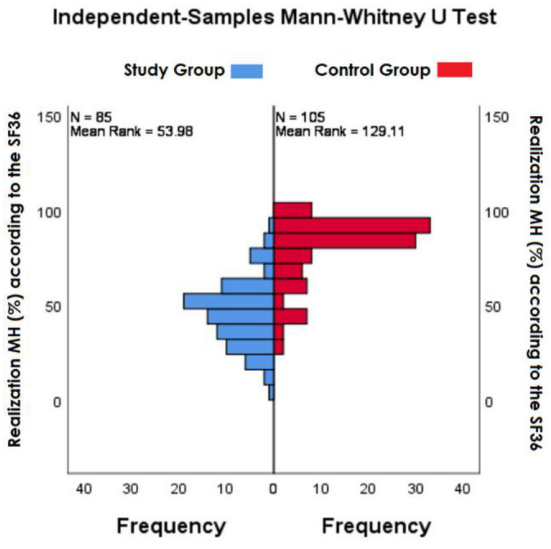

Fig. 10 Comparison of the mental health (MH) by SF-36

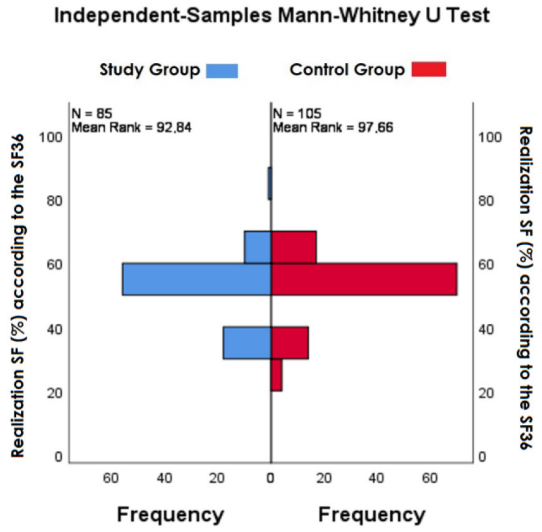

Fig. 11 Comparison of the social functioning (SF) by SF-36

0.01, Figure 4), PF (U = 8037.50, p < 0.01, Figure 5), RP (U = 7852.50, p < 0.01, Figure 6), RE (U = 7715.00, p < 0.01, Figure 7), BP (U = 7997.000, p < 0.01, Figure 8), VT (U = 7911.00, p < 0.01, Figure 9), MH (U = 7991.50, p < 0.01, Figure 10). And only for the level of SF, a statistically significant difference was not determined (U = 4689.00, p = 0.473, Figure 11).

According to the WHO-5 Well-Being Index questionnaire, the level of psychological well-being in patients with deep endometriosis compared to the control group of women was 44.29±2.05% (95% CI; 40.15 – 48.44%) vs. 81.38±2.72% (95% CI; 75.88 – 86.89%). Despite the fact that the average level of psychological well-being did not reach 100% for all women included in this study, in the group of patients with deep infiltrating endometriosis, this parameter was significantly lower, as confirmed by a statistically significant difference in psychological well-being in the research groups - U = 8277.00, p < 0.01 (Figure 12).

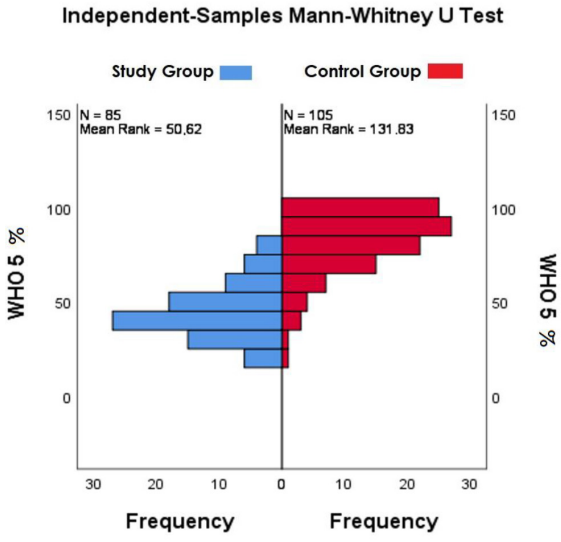

Fig. 12 Comparison of psychological well-being in research groups according to the WHO-5 Index.

Discussion

The results of our study offer valuable insights into the clinical and psychological characteristics of women with different phenotypes of endometriosis. Based on the data obtained, several key aspects can be highlighted.

Firstly, women suffering from deep infiltrating endometriosis (DIE) not only experience more intense pain, but also a wider spectrum of symptoms compared to patients with other phenotypes of this condition. This finding underscores the importance of early detection and accurate diagnosis of DIE to ensure timely treatment and improve the quality of life of patients. Secondly, our data indicate a high risk of recurrent surgical intervention in women with DIE, as this condition often may masquerade as extragenital pathologies. This highlights the importance of conducting more comprehensive clinical and paraclinical examinations when suspecting DIE to distinguish it from other nosological entities. Thirdly, our results confirm that severe pelvic pain syndrome is a characteristic feature of DIE and can be considered an important diagnostic indicator of this pathology. This emphasizes the need for an integrated approach to assessing pelvic pain syndrome in women suspected of having DIE. Fourthly, the results of our study, consistent with data from some international colleagues, have identified an association between the localization of DIE according to the #Enzian classification and clinical symptoms, which is an important aspect in the preoperative diagnostic process. These findings, together with existing literature, support recommending the #En-

zian classification as the primary method for staging and mapping foci of DIE for surgical treatment by a multidisciplinary team. Finally, our results allow us to better understand the impact of DIE on the quality of life of patients. Patients with this type of endometriosis have a lower level of quality of life and psychological well-being compared to other phenotypes of endometriosis. This underscores the need for developing individualized treatment and support approaches for patients with DIE.

Overall, our results have important clinical and practical implications and can serve as a basis for further research and improvement of approaches to the diagnosis, treatment, and support of patients with DIE.

However, it is important to acknowledge the limitations of our study. Firstly, despite being prospective, our sample size was relatively small, which may limit the generalizability of our findings. Secondly, the prospective nature of the study does not preclude the possibility of selection bias and reliance on medical records for data collection. Future studies with larger sample sizes and more comprehensive data collection methods are needed to further validate our results and address these limitations.

Conclusions

High-intensity pain syndrome and extragenital symptoms correlated with compartments of #Enzian will assist in the preoperative multidisciplinary diagnosis of DIE. The high influence on life determinants, the low realization of life potential, and the low psychological well-being confirm the significant impact of DIE on QoL, suggesting its classification as a disability.

Competing interests

None declared.

Authors' contributions

EI conceived the study and participated in the study design, contacted and included subjects in research, analyzed and calculated information from questionnaires, performed the statistical analysis of collected data, drafted the manuscript, reviewed the work critically, and approved the final version of the manuscript. NC proposed the study's area, conceived the study design, controlled main points of its realization, and reviewed the work critically. Both authors have read and approved the final version of the manuscript.

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Patient consent

Obtained.

Ethics approval

The study protocol was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes No. 38, from 21.05.2021).

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RESEARCH STUDY

Scrutinizing prognostic scores' effectiveness in non-Hodgkin's lymphomas with primary lymph node involvement

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ABSTRACT

Introduction. Non-Hodgkin lymphoma (NHL) encompasses a diverse group of malignancies arising from malignant proliferation of lymphocytes, each subtype characterized by unique epidemiological, etiological, and clinical features. Prognostication is essential for guiding treatment decisions and improving patient outcomes. Prognostic scores, including traditional and molecular systems, offer insights into survival prediction. The aim of the present study was to evaluate the applicability of traditional prognostic scores based on clinical markers and laboratory biomarkers in predicting the outcomes of patients with primary nodal NHL.

Materials and methods. This study included 78 NHL patients treated at the Chisinau Oncological Institute from 2017 to 2021. Clinical and biological data were collected, and the following prognostic scores were calculated: International Prognostic Index (IPI), The combined index of hemoglobin, albumin, lymphocyte, and platelet (HALP score), Platelet to Lymphocyte Ratio (PLR), Neutrophil to Lymphocyte Ratio (NLR), Albumin/Globulin ratio (AG), and Charlson comorbidity index (CCI). Statistical analyses, including descriptive statistics, ROC curve analysis, and Kaplan-Meier survival curves, were conducted.

Results. Of the patients, 40 (51.2%) were female, with a mean age of 57.1 ± 10.2 years. Peripheral lymph nodes were predominantly affected (84.6%), with diffuse large B-cell lymphoma being the most prevalent subtype (59.0%). Prognostic scores, including the International Prognostic Index (IPI), Hemoglobin, Albumin, Lymphocyte, and Platelet (HALP) score, and Charlson Comorbidity Index (CCI), demonstrated varying levels of discriminatory ability in predicting overall survival (OS). Notably, the HALP score (AUC = 0.650; p = 0.026), IPI (AUC = 0.745; p = 0.0002), and CCI (AUC = 0.636; p = 0.043) were statistically significant predictors of OS.

Conclusions. Traditional prognostic scores (IPI. HALP score, CCI) offer valuable prognostic information for NHL patients. Further research is needed to validate these findings and explore cost-effective prognostic strategies.

Keywords: non-Hodgkin lymphoma, prognostic scores, International Prognostic Index, HALP score, Charlson Comorbidity Index, overall survival.

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Key messages

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

Non-Hodgkin lymphomas represent a heterogeneous group of diverse lymphoproliferative tumors that vary clinically, immunophenotypically, and molecularly, with primary nodal involvement being the most common site (52-55%). Predicting response to treatment and survival is essential for tailoring treatment strategies effectively.

The research hypothesis

Classic prognostic scores, based on immunoinflammatory and nu-

tritional indicators such as IPI, HALP score, PLR, NLR, A/G ratio, and CCI, may be utilized as viable prognostic scores for predicting overall survival of nodal NHL.

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

Highlighting the possibility of using three of these scores (IPI, HALP score, and CCI) as highly sensitive scores in predicting overall survival of nodal NHLs.

Introduction

Non-Hodgkin lymphoma (NHL) is a lymphoid tissue tumor that develops from B cell progenitors, mature B cells, T cell precursors, and mature T cells. Non-Hodgkin lymphoma is divided into subgroups, each with its own epidemiology, etiology, immunophenotypic, genetic, clinical characteristics, and response to therapy [1, 2]. With an expected 544,000 new cancer cases in 2020, NHL was classified as the fifth to ninth most common malignancy in most countries worldwide [3]. NHL incidence in the Republic of Moldova is estimated to be 4.1 cases per 100,000 inhabitants [4, 5]. Based on data from the GLOBOCAN 2020 database provided by the World Health Organization's Global Cancer Observatory (GCO), 313 new cases of Non-Hodgkin's Lymphoma (NHL) were diagnosed in the Republic of Moldova in 2020. This ranked NHL as the 15th most common type of cancer in the country. These cases accounted for 2.2% of the total cancer diagnoses in the Republic of Moldova in 2020. The 5-year prevalence rate of NHL in the Republic of Moldova was 21.07 cases per 100,000 population [6].

Accurate prediction of the outcome of patients dealing with Non-Hodgkin's Lymphoma (NHL) is of paramount importance, as it plays a pivotal role in guiding the decisions surrounding their treatment approaches. This not only contributes to the potential improvement of patient outcomes but also ensures a more informed and effective management of their overall well-being and health. Arbitrarily, we can categorize prognostic scores into three main groups:

(a) *Classic Prognostic Scores (IPI* [7]*; R-IPI* [8]*; MIPI* [9]*; FLIPI* [10]). These encompass a combination of clinical and laboratory parameters that are accessible and commonly utilized in clinical practice.

(b) *Integrated Prognostic Scores (R/R IPI* [11]*; m7-FLIPI* [12, 13]*)*. These involve the fusion of classic prognostic scores with molecular biology data, thereby adding a new layer of insight.

(c) *Molecular-based Prognostic Scores (LymForest-25 Model* [14]*; IAC-FL* [15]). This category involves the incorporation of molecular data and the utilization of machine learning technologies to enhance the precision of prognostication.

Certainly, the latter prognostication scores undeniably showcase an elevated refinement and heightened sensitivity when juxtaposed with the traditional prognostic scores [16]. Nevertheless, a notable downside associated with these advanced systems is their tendency to impose substantial financial strain on healthcare systems, consequently restricting their widespread utilization.

The aim of this study was to evaluate the utility of conventional scores based on biological and nutritional data in predicting overall survival (OS) in patients with primary nodal NHL.

Materials and methods

This study included 78 adult patients with NHL diagnosed and treated in the Chisinau Oncological Institute, during the period 2017-2021.

Prior to data collection, written informed consent was obtained from each study participant. Ethical approval was obtained from the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes №1 from 03.07.2020).

Clinical-biological characteristics of NHL were collected from medical records. Additionally for each patient, prognostic scores (PS) were calculated in accordance with the recommendations of the original references. PS that were calculated are: International Prognostic Index (IPI) [7]; The combined index of hemoglobin, albumin, lymphocyte, and platelet (HALP score) [17]; Platelet to Lymphocyte Ratio (PLR) [18]; Neutrophil to Lymphocyte Ratio (NLR) [19]; Albumin/Globulin ratio (AG) [20] and Charlson comorbidity index (CCI) [21].

Statistical analysis of the results was carried out by using the standard packages of statistical programs SPSS for Windows (version 26). To create the database, the Microsoft Excel 7 version 2312 spreadsheet editor was used. To describe the nature of the distribution of quantitative features, standard methods of variational statistics were used with the determination of the arithmetic mean value of the variable (M) and the mean quadratic standard deviation (SD).

The average values in the study were presented in the form M ± SD. ROC curve analysis was used to determine the cutoff values of the evaluated PS in predicting mortality. To assess patient survival, Kaplan-Meier's life-table method of forming survival curves, was used. Differences were considered significant if $p < 0.05$.

Results

Out of the 78 patients participating in the study, 40 (51.2%) were women and 38 (48.8%) males. The mean age of the patients was 57.1 ± 10.2 years. The age categories most often affected by NHL in the study group were: age group 51-60 years – 22 (28.2%) patients, age group 61-70 years – 19 (24.4%) patients, followed by age group 41-50 years – 15 (19.2%) cases (fig. 1).

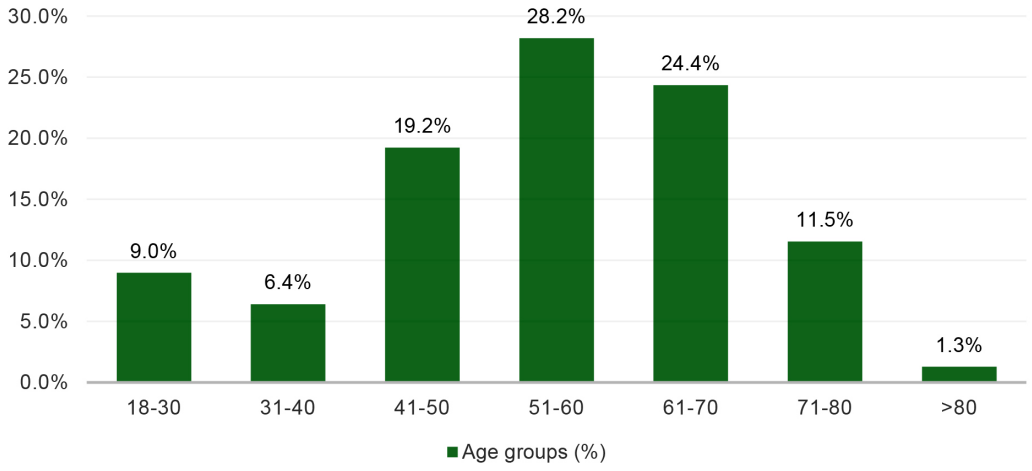


Fig. 1 Distribution of patients in the study group according to age categories.

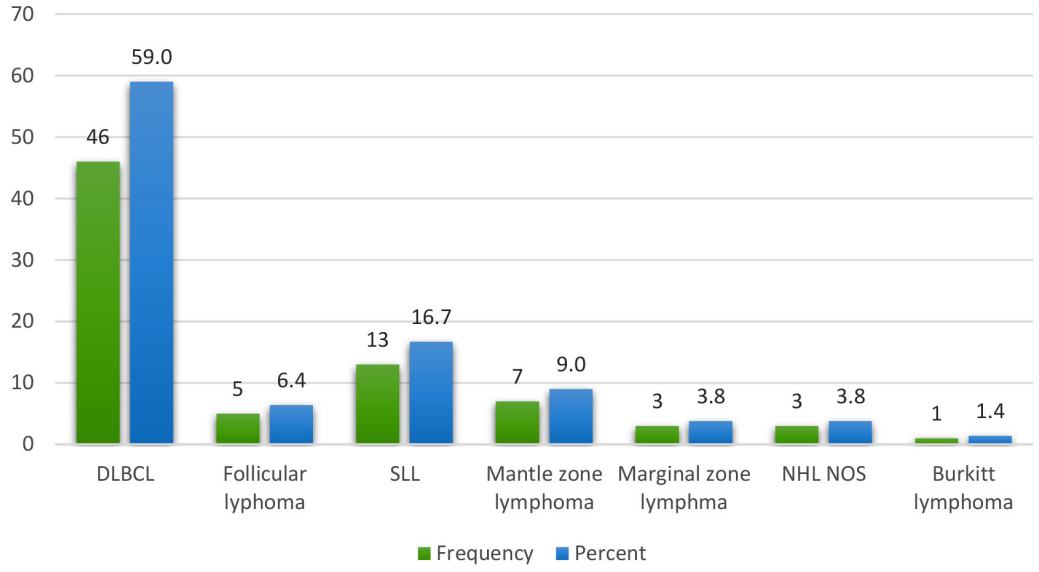


Fig. 2 Distribution of patients of the study group in regard to the type of Non-Hodgkin lymphoma.

Note: DLBCL – diffuse large B cell lymphoma; SLL – small lymphocytic lymphoma; NHL NOS – Non-Hodgkin lymphoma not otherwise specified

The analysis of the statistical distribution of the study group based on the primary area of the LN involvement showed that NHL onset occurred most frequently in peripheral lymph nodes (84.6 % of cases), followed by mediastinal lymph nodes (10.3 %), and abdominals lymph nodes (5.1%) (tab.1).

Table 1. Distribution of patients in the study group according to area of lymph nodes primary involved in the tumoral proliferation.

Area of lymph nodes primarily involved	Number of patients	Frequency (%)
Peripheral lymph nodes	66	84.6
Mediastinal lymph nodes	8	10.3
Intrabdominal lymph nodes	4	5.1
Total	78	100.0

The majority of patients in the study cohort developed aggressive forms of NHL, 57 (73.0%) patients, while indolent forms of NHL were identified in 21 (27.0%) cases. Among the aggressive forms of NHL, the most common histological subtype was diffuse large B-cell lymphoma, accounting for 46 (59.0%) of cases, followed by mantle cell lymphoma detected in 7 (9.0%) patients. NOS lymphomas and Burkitt lymphomas developed less frequently, in 3 (3.8%) and 1 (1.4%) cases, respectively. Among the indolent forms of NHL, small lymphocytic lymphoma was observed in 13 (16.7%) cases, followed by follicular lymphoma in 5 (6.4%) patients, and marginal zone lymphoma detected in 3 (3.8%) cases (fig. 2).

The subsequent phase involved assessing the performance potential of the analyzed prognostic scores (PS) to ascertain their suitability as effective predictors for NHL. To achieve this objective, Receiver Operating Characteristic (ROC) analysis was systematically applied to each of the scores. In the evaluation of prognostic scores (PS), it was observed that all scores exhibited an area under the curve (AUC) greater than 0.5, indicating a discernible discriminatory capacity.

However, not all scores demonstrated statistical significance. Specifically, the combined Hemoglobin Albumin Lymphocyte Platelets score (HALP) (AUC = 0.650; p = 0.026), International Prognostic Index (IPI) (AUC = 0,745; p = 0,0002), and Charlson Comorbidity Index (CCI) (AUC = 0.636; p = 0.043) were the only scores that yielded a p-value below the conventional significance threshold of 0.05 (fig. 3).

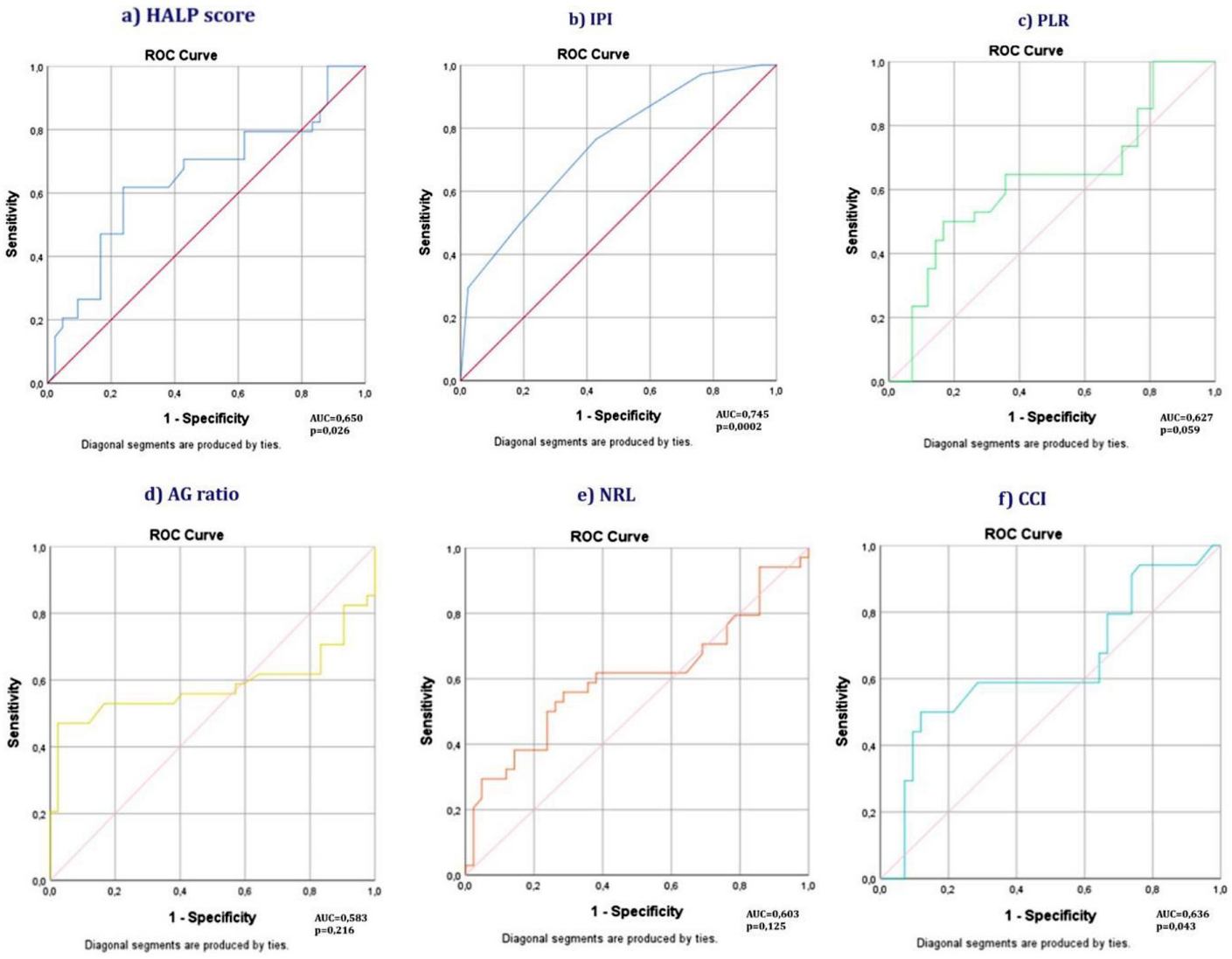
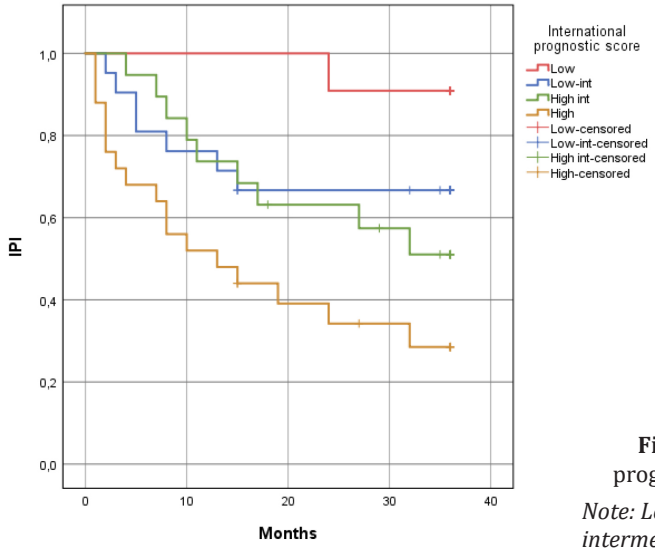


Fig. 3 The receiver operating characteristic curves (ROC) of the analyzed prognostic scores.

*Note: a) The combined score of hemoglobin, albumin, lymphocyte and platelets (HALP score); b) International prognostic index (IPI); c) Platelet to lymphocyte ratio (PLR); d) Albumin to globulin ratio (AG ratio); e) Neutrophils to lymphocyte ratio (NLR); f) Charlson comorbidity index (CCI). *AUC – area under the curve.*



In order to validate the true prognostic impact of scores with prognostic significance ($p < 0.005$), Kaplan-Meier survival curves were constructed, with the use of HALP, IPI, and CCI scores serving as discriminant variables. In order to assess Overall Survival (OS) through the lens of the IPI score, patients were methodically stratified into 4 distinct prognostic subgroups based on the cumulative score: the low-risk subgroup (0-1 points); low-intermediate risk (2 points), high-intermediate risk (3 points), and high risk (4-5 points). Kaplan-Meier survival curve analysis unveiled that

Fig. 4 Kaplan Meier overall survival curves across various international prognostic index (IPI) subgroups within the Non-Hodgkin lymphoma cohort

Note: Low - low risk group; Low-int – low-intermidiate risk group; High-int – High-intermediate risk group; High – High risk group

patients in the high-risk subgroup displayed a median OS of 13.0 ± 5.82, with a 95% Confidence Interval (CI) spanning from 1.57 to 24.42, and a statistically significant p-value of < 0.001. Conversely, for patients in the low-risk, low-intermediate risk, and high-intermediate risk subgroups, the median OS during the follow-up period was not reached (fig. 4).

To assess the OS of patients within the study cohort utilizing the Charlson Comorbidity Index (CCI), patients were divided into two categories: those with a CCI < 3 were categorized into the low CCI index group, while those with a CCI score ≥ 3 points were placed into the high CCI index subgroup. Consequently, upon analyzing OS using the Kaplan-Meier survival curve, it was noted that patients with a high CCI score had a median OS of 11 ± 2.82 months, with a 95% Confidence Interval (CI) of 5.45-16.54, p < 0.0001. Patients within the low CCI score group demonstrated significantly superior OS rates, with the median overall survival not being reached within this cohort (fig. 5).

To evaluate the OS of patients within the study cohort using the HALP score, a cutoff value of 583.5 was initially determined based on ROC curve analysis. Subsequently, patients were divided into two distinct subgroups based on this threshold: those with a HALP score below 583.5, and those with a HALP score above 583.5. Evaluation of OS utilizing Kaplan-Meier survival curves revealed that patients in the low HALP score group had a median survival of only 17 ± 5.46 months, with a 95% confidence interval (CI) of 6.29-27.70, and a p-value of 0.009. In the high HALP score group, the median survival was not reached within the follow-up period (fig. 6).

Discussion

In 1863, Rudolf Virchow, upon visualizing leukocytes within neoplastic tissue, established the pioneering connection between inflammation and cancer [22]. Persistent inflammation and immune system activation have been iden-

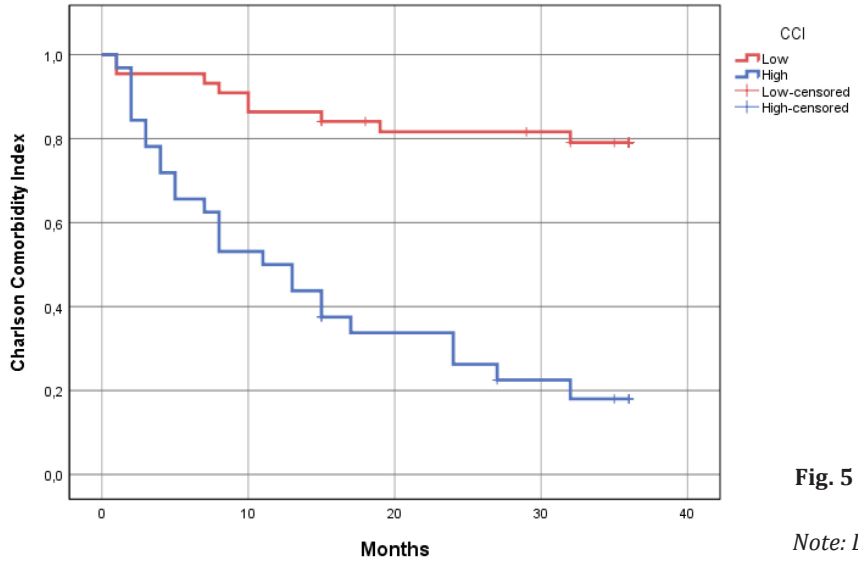


Fig. 5 Kaplan Meier assessment of Overall Survival (OS) with respect to Charlson comorbidity index (CCI).

Note: Low risk group (< 3 points); High risk group (> 3 points)

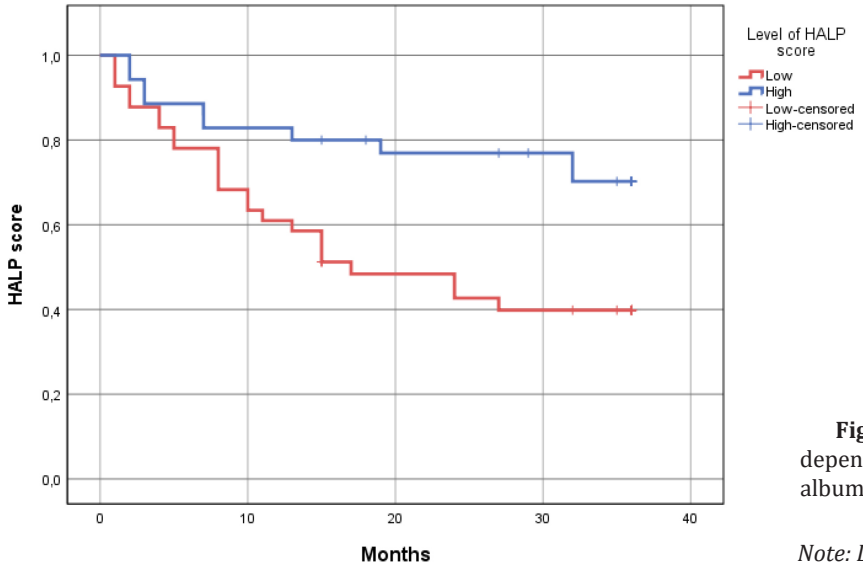


Fig. 6 Kaplan Meier estimates of overall survival (OS), depending on the level of the combined score of hemoglobin, albumin, lymphocyte and platelets (HALP score) in the study cohort of Non-Hodgkin lymphoma patients.

Note: Low - low risk group ; High - high risk group.

tified nowadays as one of the pivotal factors in the pathogenesis of NHL [23].

Immunological markers and biomarkers of cancerogenesis are often relatively inexpensive to determine and easily interpretable. Although, at present, the medical scientific community cannot modify this pathogenetic chain in the evolution of cancer, what we can certainly do is utilize and benefit from the prognostic and sometimes diagnostic role of these markers. Several, scientific works have been undertaken to assess the utility of inflammatory and nutritional markers as plausible diagnostic and prognostic indicators across a spectrum of cancers, including gastric cancer [24, 25], hepatocellular carcinoma [26-28], prostate cancer [29, 30], gynecological cancers [31], etc.

Among lymphoproliferative neoplasms, the International Prognostic Index (IPI) is the most well-known scoring system, utilizing immunoinflammatory markers, among others, to predict overall survival in patients with aggressive NHL. The IPI score was published more than thirty years ago by Shipp *et al.* [7], and despite significant advancements in clinical classification for lymphomas, including immunohistochemical and molecular testing, it is still relevant today. This enduring relevance is evidenced by its continued use in most prospective, randomized trials to stratify risk and ensure balanced group allocation [32].

The HALP score, a composite index comprising routine blood tests data such as hemoglobin, albumin, lymphocyte, and platelet counts, serves as a comprehensive tool for evaluating various aspects of patients somatic status. Originally introduced as a predictive tool for gastric cancer prognosis [17], the HALP score has garnered attention for its potential utility in prognosticating outcomes across a wide spectrum of cancer types [33-36].

Recent studies have underscored the prognostic significance of the HALP score in hematological malignancies, notably multiple myeloma and aggressive NHL. In multiple myeloma, as it is showed in the work of Solmaz *et al.* [37] for instance, lower HALP scores have been associated with shorter overall survival, highlighting its potential as a prognostic marker in this context. Similarly, in aggressive NHL, such as diffuse large B cell lymphoma, lower HALP levels have been linked to adverse clinicopathological characteristics and diminished long-term survival rates, indicative of its prognostic relevance in these settings [38, 39]. The most important evidence on the aggressive NHL comes from a 2022 report published by Vlatka *et al.* [38], on 153 newly diagnosed diffuse large B-cell lymphoma. This study found that lower HALP was shown to be associated with unfavorable clinicopathological characteristics and a predictor of long-term survival. Patients with low HALP levels were also more likely to have B symptoms ($p = 0.017$), bone marrow infiltration ($p = 0.001$), and a poorer prognosis ($p = 0.001$). Moreover, 5-year survival was considerably lower for patients with Low HALP (47.3% vs. 79.5%, $p = 0.001$). In fact, on multivariable Cox regression, revealed that patients had a greater than 2.5 increased risk of death during the 5-year period if their HALP was low ($p = 0.003$). Our data tends to align with those presented by Vlatka *et al.* [38]. Thus, patients in our study cohort from the HALP scoring group had a median survival of 17 ± 5.46 months, with a 95% CI of 6.29-27.70, $p = 0.009$. In the high HALP score group, the median survival was not reached within the follow-up period.

In 1987, Charlson *et al.* [40] established the Charlson Comorbidity Index (CCI) to evaluate clinical comorbidities, which had been frequently used as a comprehensive assessment tool for patients with chronic diseases. The Charlson Comorbidity Index is a method of categorizing comorbidities of patients based on the International Classification of Diseases (ICD) diagnosis [41]. Each comorbidity category has an associated weight (from 1 to 6), based on the adjusted risk of mortality or resource use, and the sum of all the weights results in a single comorbidity score for a patient. A score of zero indicates that no comorbidities were found. The higher the score, the more likely the predicted outcome will result in mortality or higher resource use [40].

Reports of the clinical impacts of CCI in oncology have been made for several cancer types, including renal cell carcinoma [42] and non-small cell lung cancer [43]. Recent research on the impact of comorbidities on diffuse large B cell lymphoma patient outcomes has revealed that patients with high CCI have a lower rate of overall response, greater rates of toxicity due to medication, and a higher risk of fatal outcome [44-47]. For example, Eren *et al.* [47] demonstrated on a cohort of 170 with DLBCL that the CCI has an AUC of 0.628 (95% CI: 0.506–0.749), and patients with a CCI score of ≥ 4 had shorter OS compared to those with a score of < 4. Within our study cohort, the CCI showed an AUC of 0.636, and patients with a high CCI (>3 points) score had a median OS of 11 ± 2.82 months, with a 95% CI of 5.45-16.54, $p < 0.0001$. Patients in the low CCI score (<3 points) group demonstrated significantly superior OS rates, with the median overall survival not being reached within this cohort.

Conclusions

The prognosis of Non-Hodgkin Lymphoma depends on various factors. The use of conventional scores, which incorporate baseline nutritional and immunoinflammatory indicators, can significantly reduce the financial burden on healthcare systems in developing countries. Among the prognostic scores evaluated in this study, the International Prognostic Index (IPI), the Hemoglobin, Albumin, Lymphocyte, and Platelet (HALP) score, and the Charlson Comorbidity Index (CCI) have emerged as particularly sensitive predictors for estimating the overall survival (OS) of NHL patients.

Competing interests

None declared.

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The study had no external funding.

Patient consent

Obtained.

Ethics approval

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (minutes No. 1 from 03.07.2020).

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RESEARCH ARTICLE

Protective effect of arginine glutamate (glutargin) in chronic pancreatitis induced by nitric oxide synthase blocker

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ABSTRACT

Introduction. Nitric oxide (NO) is a gaseous molecule that is a biological mediator that carries out important regulation of physiological processes necessary for the functioning of tissues. We hypothesized that nonspecific inhibition of all isoforms of NOs would cause pancreatic damage and created a model of experimental pancreatitis based on NOs inhibition. Along with the study of the mechanisms of development of chronic pancreatitis, the search for medicines to treat and inhibit the progression of this disease continues.

Purpose of the research. To study the protective effect of glutargin on the pancreas of rats with chronic pancreatitis induced by a blocker of NOs.

Material and methods. The work was carried out on 21 laboratory white male Wistar rats weighing 180-230 g. Chronic pancreatitis was induced by intraperitoneal administration of the NOs blocker - N-nitro-L-arginine (L-NNA) (Sigma-Aldrich, USA) at a dose of 40 mg/kg of body weight. Rats in group I (n = 7) were injected with L-NNA for 12 days, rats in group II (n = 7) were injected intraperitoneally with glutargin 20 mg/kg, after 20 minutes they were injected with L-NNA - within 12 days. Rats of the control group (n = 7) were injected intraperitoneally with 0.9% NaCl solution. The rats were euthanized on the 45th day and biochemical and morphological studies were carried out.

Results. After the introduction of the NOs blocker, deterioration of the general condition of the animals was determined, a sharp increase in the level of nitrites/nitrates to 80.22±19.91 μmol/l, control 32.61±4.55 μmol/l ($p < 0.05$); protein-bound hydroxyproline (PBH) to 215.21±22.01μmol/l, control 178.67±26.39 μmol/l, ($p < 0.05$); free hydroxyproline (FH) to 14.74±1.84 μmol/l, control 9.96±0.71 μmol/l, ($p < 0.05$); malondialdehyde (MDA) to 5.67±0.88 nmol/ml, control 3.62±0.13, ($p < 0.05$). Pronounced structural changes with stasis of formed blood elements in the vessels, focal accumulation of leukocytes in the parenchyma, dystrophy of acinar cells and fibrosis in the atrophy zone were determined in the pancreas of rats. Administration of glutargin contributed to the restoration of general behavioral reactions of rats, normalization of MDA – 4.81±0.15 nmol/ml, control 4.50±0.23, ($p > 0.05$); ceruloplasmin (CP) – 591.71±68.07 mg/ml, control – 663.25±34.05 mg/ml, ($p > 0.05$); PBH 183.62±5.98 μmol/l, control – 179.28±9.19 μmol/l, ($p > 0.05$); FH – 9.44±1.13 μmol/l, control – 9.96±0.71 μmol/l, ($p > 0.05$) and prevented the development of pronounced structural changes in the pancreas.

Conclusions. In chronic pancreatitis induced by a NOs blocker, glutargin can prevent chronic pancreatitis by normalizing collagen metabolism, inhibiting oxidative stress, and severity of pancreatic parenchymal damage.

Keywords: chronic pancreatitis, nitric oxide, experiment, treatment.

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Key messages

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

The incidence of chronic pancreatitis, which is characterized by fibrosis of the pancreatic parenchyma, continues to grow. The search for medicines capable of affecting the main links of the pathogene-

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sis of this disease and inhibiting its progression is current.

The research hypothesis

The medicines arginine glutamate (glutargin) in chronic pancreatitis can have a protective effect, which is associated with its antioxidant effect and the physiological effect of NO.

The novelty added by manuscript to the already published scientific literature

A model of chronic pancreatitis, which is caused by the introduction of a NO blocker, has been developed. In conditions of NOs deficiency, the protective effect of glutargin was studied, which was manifested in the inhibition of oxidative stress, the normalization of collagen metabolism, and the prevention of the development of pronounced structural changes in the pancreas. A conclusion was made about the possibility of using glutargin for the treatment of patients with chronic pancreatitis.

Introduction

Nitric oxide (NO) is a gaseous molecule that is a biological mediator that carries out important regulation of physiological processes necessary for the functioning of tissues [1-4]. NO participates in the activity of various organs and systems, contributing to both physiological and pathophysiological processes of the pancreas [3, 5, 6].

NO is produced by a family of enzymes called NO synthases (NOs), which convert L-arginine into NO and citrulline [1, 6]. The physiological effect of NO is caused by cyclic guanosine monophosphate (cGMP) and varies from modulation of the vascular system to regulation of immune processes and control of neuronal functions [7, 8]. NO plays an active role in pancreatic secretion, in the control of systemic blood pressure and active expansion of blood vessels, and is additionally involved in platelet aggregation, leukocyte activation, and their adhesion [1-3, 6].

The negative effect of NO content begins to manifest when its total concentration either sharply decreases or increases, which leads to functional and structural damage to the organ [4, 6, 9].

In conditions of pronounced oxidative stress, the physiological action of NO can be limited by the action of reactive oxygen species [1]. NO disintegrates into active forms of nitric oxide and, in combination with active forms of oxygen, can damage cells and thereby cause disease [1, 10]. Oxidative stress is one of the reasons for the development and progression of fibrosis of the gland parenchyma in chronic pancreatitis.

There is no doubt that NO is involved in the development of the pathological process. The participation of NO in the development of experimental pancreatitis is known, which is caused by the action of high doses of L-arginine (NO donor) on the pancreas [11, 12].

We hypothesized that nonspecific inhibition of all isoforms of NOs would also cause pancreatic damage and created a model of experimental pancreatitis based on NOs inhibition [4, 13]. Taking into account the fact that, along with the study of the mechanisms of development of chronic pancreatitis, the search for drugs to treat and inhibit the progression of this disease continues, we conducted an experimental research to study the therapeutic effect of arginine glutamate (the drug "Glutargin", "Zdorovya", Ukraine) in case of damage pancreas.

The drug glutargin is a salt of arginine and glutamic acid, along with a pronounced hypoammonemic effect; it has an antioxidant and antihypoxic effect. The analysis of the results obtained in the experiment when studying various aspects of the effect of glutargin on hepatocytes showed a number of positive effects of the drug: improvement of energy metabolism due to the primary accumulation of cellular energy in the form of creatine phosphate; correction of the acid-base state due to the normalization of the alkaline reserve of the blood; antioxidant and membrane-stabilizing effect due to the ability to reduce the level of lipid peroxidation products and increase the protective function of the endogenous antioxidant system, as well as stabilize hepatocyte membranes by reducing the activity of cytolytic enzymes (alanine and aspartate aminotransferases); anti-ischemic effect due to the optimization of oxygen transport and its consumption in tissues and increasing the body's resistance to hypoxia [14].

Besides, arginine in the composition of glutargin can serve as a donor of NO and thus ensure its physiological effect.

The purpose of the research is to study the protective effect of glutargin on the pancreas of rats with chronic pancreatitis induced by a NOs blocker.

Material and methods

Chronic pancreatitis was induced by intraperitoneal administration of the NOs blocker - N-nitro-L-arginine (L-NNA) (Sigma-Aldrich, USA) at a dose of 40 mg/kg of body weight. The solution was administered to animals intraperitoneally between 9:00 and 10:00 in the morning [4, 13]. Rats (n = 12) were intraperitoneally introduced with L-NNA at a dose of 40 mg/kg for 6 (n = 6) and 12 (n = 6) days. The control group (n = 12) consisted of rats that were intraperitoneally

introduced with 0.9% NaCl solution. Rats were taken out of the experiment on days 6 and 12.

The effect of glutargin was studied on 21 laboratory white male Wistar rats weighing 180-230 g, which were randomly divided into control and two research groups. 16-20 hours before the experiment, animals were subjected to food deprivation with free access to water. Rats from group I (n = 7) were injected intraperitoneally with the NOs blocker L-NNA ("Sigma-Aldrich", USA) at a dose of 40 mg/kg for 12 days, rats from group II (n = 7) were injected intraperitoneally with glutargin 20 mg/kg, after 20 minutes L-NNA was administered intraperitoneally at a dose of 40 mg/kg for 12 days. The control group (n = 7) consisted of rats injected intraperitoneally with a 0.9% NaCl solution. The rats were removed from the experiment on the 45th day.

After the animals were removed from the experiment, blood was taken to determine the levels of protein-bound hydroxyproline (PBH) and free hydroxyproline (FH) [15], hexosamines (Ha) [16], malondialdehyde (MDA) [17], ceruloplasmin (CP) using a modified method Revin [16], nitrites/nitrates [18], α-amylase according to Karavey's method [19], lipase according to Loginov's method [20], trypsin - according to Erlanger in Shaternikov's modification [16].

Rat pancreas tissue was taken for histological examination. The pancreas was isolated and immediately fixed in Bowin's environment. Microscopic tissue sections with a thickness of 3-5 μm were stained with hematoxylin-eosin and according to Mallory-Slinchenko. Microscopy was performed at a magnification of X200-400.

To study the individual and typological features of the behavior of rats, which characterize individual resistance to emotional stress, testing was conducted in an open field [21].

Animals were removed from the experiment by administering a lethal dose of ketamine hydrochloride. Research was conducted in accordance with the main provisions of the European Convention on the protection of vertebrate animals used for research and other scientific purposes [22].

Descriptive and inductive statistics were used to analyze the obtained results. In the case of quantitative data and under the condition of their normal distribution, the mean and standard error of the mean were used. The Student's t-test was used to determine the reliability of differences. In the absence of a normal distribution, the median, minimum, maximum, upper and lower quartiles were used, and the significance of differences was determined by the Mann-Whitney U-test. To describe qualitative data, we used the frequency of detection of signs (%). In this case, the χ-test was used to determine the reliability of differences between groups. The indicator $p < 0.05$ was considered statistically significant. All calculations were performed in SPSS 9.0 for Windows (or Statistica 6) [23, 24]. The work was performed at the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine.

The study was approved on September 10, 2008 by the Scientific Research Ethics Committee of the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine (minutes No. 5).

Results

To achieve the goal, we used our previously developed model of chronic pancreatitis, which occurs as a result of long-term blocking of the nitroergic regulation by intraperitoneal injection of an NOs blocker [4, 13]. When simulating NOs deficiency on the 6th day, no deterioration in the general condition of the animals was found, and on the 12th day the behavior of the rats became more passive; appetite worsened, a slight decrease (by 5.0-9.0 grams) in the body weight of each animal was established.

A slight ($p > 0.05$) decrease in the level of nitrites/nitrates to 20.76±8.36 μmol/l was noted in the blood serum of rats on the 6th day compared to the control (32.61±4.55 μmol/l), and on the 12th day – a sharp increase to 80.22±19.91 μmol/l, ($p < 0.05$).

The inhibition of NOs on the 12th day was accompanied by a significant increase in the blood serum of rats of PBH content to 215.21±22.01 μmol/l compared to the control – 178.67±26.39 μmol/l, ($p < 0.05$), and FH up to 14.74±1.84 μmol/l, compared to the control – 9.96±0.71 μmol/l, ($p < 0.05$), which indicated enhanced synthesis and breakdown collagen.

On the 12th day, the content of Ha, which are inducers of fibrosis, also increased significantly ($p < 0.001$) to 5.90±0.25 g/l, control – 4.27±0.18 g/l, which indicated change in the state of connective tissue.

The introduction of the NOs blocker caused a violation of the excretory function of the pancreas, which was accompanied by phase changes of the enzymes of protein and carbohydrate metabolism. Thus, on the 6th day, the level of α-amylase increased to 311.26±37.39 mg/s·l, ($p < 0.01$) compared to the control – 96.02±20.30 mg/s·l, and on the 12th day it slightly decreased to 205.49±31.47 mg/s·l, but remained significantly higher than the control ($p < 0.05$). The level of trypsin on the 6th day increased to 10.45±1.76 μmol/ml·min (control – 4.19±0.92 μmol/ml·min), ($p < 0.01$), and on the 12th day decreased to 5.84±2.59 μmol/ml·min and did not differ from the control ($p > 0.01$).

There was also a violation of the incretory function of the pancreas, which was evidenced by a gradual increase in the level of glucose in the blood serum of rats, which on the 12th day was 4.20±0.22 mmol/l, ($p < 0.05$) compared to the control – 3.18±0.42 mmol/l.

The level of MDA on the 6th day increased to 4.94±0.35 nmol/ml in 1.4 times compared to the control – 3.62±0.13 nmol/ml, ($p < 0.05$), on 12th day was maximally elevated - (5.67±0.88) nmol/ml, 1.6 times compared to the control ($p < 0.05$).

Morphological examination of the pancreas of rats revealed marked structural changes with stasis of formed blood elements in vessels, focal accumulation of leukocytes in the pancreatic parenchyma (Fig. 1). Dystrophy of acinar cells developed in some lobes (Fig. 2). In a number of cases, gentle fibrosis caused by the inflammatory process developed in the acinar tissue atrophy zone (Fig. 3).

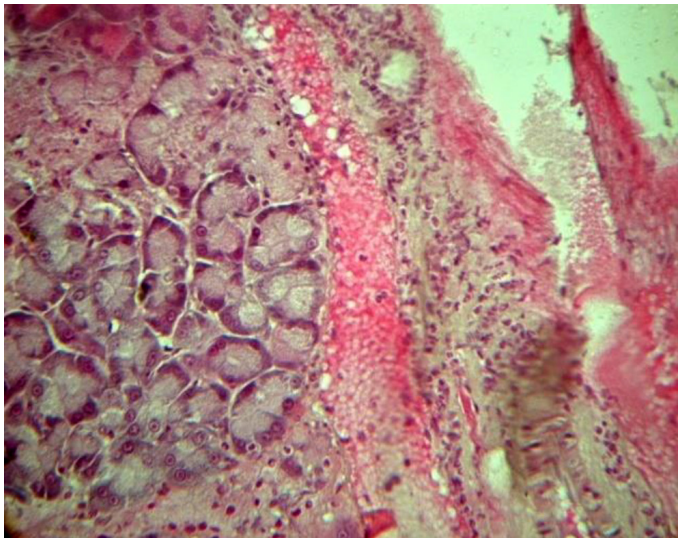

Fig. 1 Stasis in small vessels, edema of the pancreatic stroma, interlobular neutrophilic infiltration.
Staining with hematoxylin-eosin x400.

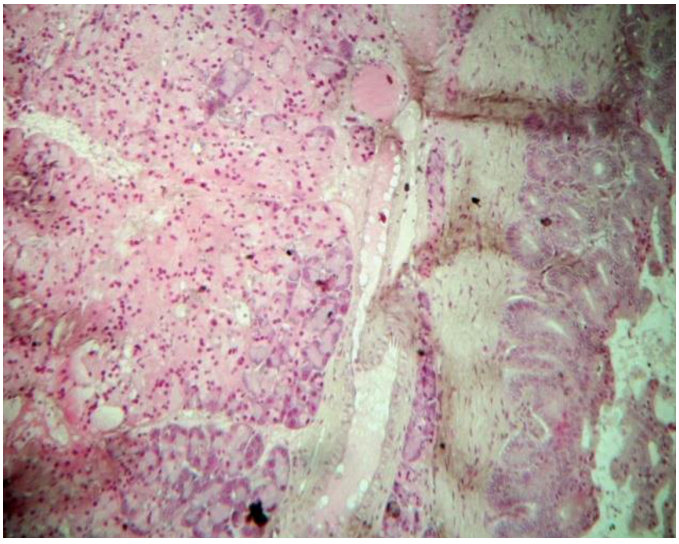

Fig. 2 Destruction of acinar tissue with its infiltration by lymphocytes and plasma cells.
Staining with hematoxylin-eosin x200.

The identified changes in total indicated that under the conditions of intraperitoneal administration of submaximal doses of a non-specific blocker of NOs, morphological changes are formed in the tissue of the pancreas of rats, which are characteristic of inflammation with chronicity of the pathological process, and the activation of the exocrine function of the pancreas is noted.

The second stage of our work was to study the effect of glutargin in pancreatitis induced by an NOs blocker.

As a result of the conducted research, changes in the behavioral reactions of rats were established. Thus, in rats of the I group, after 45 days of the experiment (which included a 12-day administration of the NOs blocker), the total motor activity decreased in relation to the control values by 46.1% ($p < 0.01$). Whereas the rats of the II group, which additionally received glutargin, the indicators of general motor activity did not have significant differences in relation to the control values (Fig. 4).

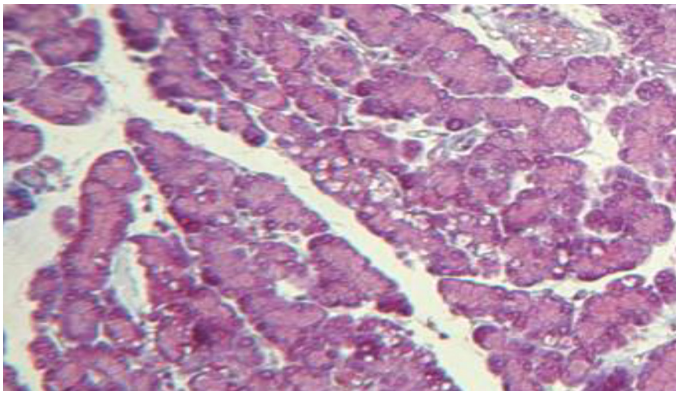

Fig. 3 Balloon dystrophy of acinar cells, gentle periductular fibrosis.
Coloring according to Mallory-Slinchenko. x200

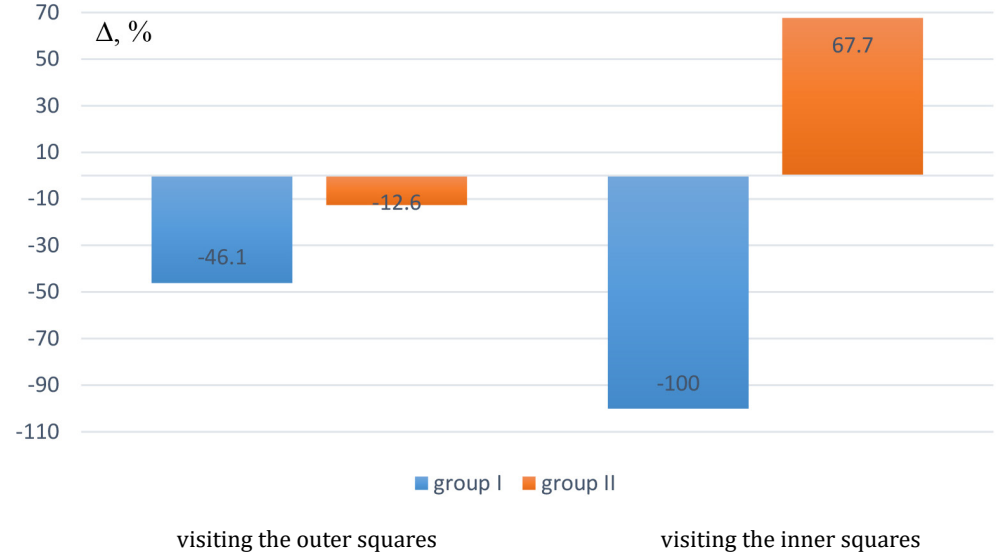

Fig. 4 Change in the number of visits by animals from outer squares during testing in the «open field» in the study of rats of groups I and II

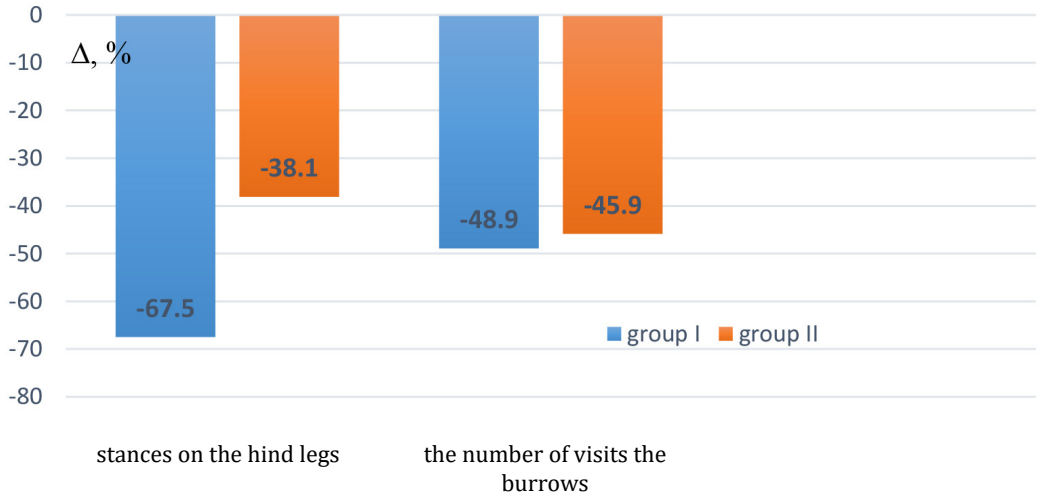

Fig. 5 Change in research activity during testing in the «open field» in rats of groups I and II

Research activity in rats of the I group was significantly reduced, both by the indicators of racks by 67.5% ($p < 0.001$) and by the indicators of visiting the burrows by 48.9% ($p < 0.01$) in relation to the control values. In the II group, there was also a decrease in research activity, both by the rate of racks by 38.1% ($p < 0.05$) and by the rate of visits the burrows by 45.9% ($p < 0.01$) (Fig. 5).

Table 1 presents the biochemical indicators of blood serum of rats of groups I and II in comparison with the control.

Table 1. Comparative characteristics of biochemical indicators of blood serum in rats (M±m)

Parameters	Control (n = 7)	I group L-NNA (n = 7)	II group glutargin+L-NNA (n = 7)
MDA, nmol/ml	4.50±0.23	4.15±0.53	4.81±0.15
CP, mg/ml	663.25±34.05	713.00±90.92	591.71±68.07
α-Amylase, mg/s·l	56.82±1.87	58.66±1.74	57.52±2.47
trypsin, μmol/ml·min	4.19±0.92	16.37±4.09*	6.95±1.10
lipase, nmol/s·l	0.87±0.086	0.79±0.09	1.26±0.07**
PBH, μmol/l	179.28±9.19	159.54±6.55	183.62±5.98
FH, μmol/l	9.96±0.71	5.81±0.64*	9.44±1.13
PBH/FH	18.0±1.2	27.46±1.02	19.45±0.53
nitrites/nitrates, μmol/l	32.61±1.63	36.46±3.87	33.59±5.84

*Note: the Student's t-test was used; * - $p < 0.05$, ** - $p < 0.01$ - compared to the values of the control group; group I - L-NNA (N-nitro-L-arginine), group II – glutargin and L-NNA, MDA - malondialdehyde, CP - ceruloplasmin, PBH - protein-bound hydroxyproline, FH – free hydroxyproline*

In rats of the group I a high level of trypsin was determined, which was 4 times higher than the indicator of the control group ($p < 0.01$) and a significantly reduced level of FH. The ratio of PBH/FH in rats of the first group increased, which indicated the predominance of collagen synthesis processes over its degradation.

Most of the indicators in animals of group II were within the physiological norm, only the level of lipase was significantly increased ($p < 0.01$). In group II, the level of PBH and FH was not significantly different from the control. Therefore, according to these indicators; no collagen metabolism disorders were detected.

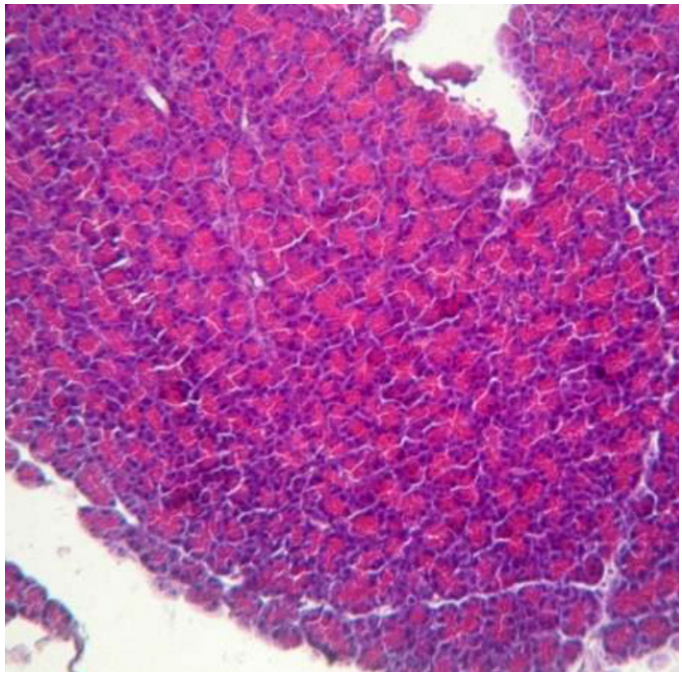

Fig. 6 Control: normal pancreatic acini with vesicular nuclei; normal intralobular pancreatic ducts.
Staining with hematoxylin and eosin. x100.

Structural changes of the pancreas were established during the morphological study of the pancreas of rats of the groups I and II. In fig. 6 the pancreas of a control group rat is presented.

Rats of group I (which received only the NOs blocker) showed pronounced degenerative changes 45 days after the start of the experiment (Fig. 7). Dyscirculatory hypoxia with foci of dystrophy and atrophy of acinar tissue developed in the pancreas of rats (Fig. 7). In the majority of acinar cells,

cytoplasmic vacuolization and a decrease in the number of zymogen granules were determined. The expansion of the interlobular ducts, expansion, and congestion of blood vessels, inflammatory infiltration, and balloon dystrophy of acinar cells were determined in the parenchyma of the pancreas.

Whereas in rats of group II (NOs blocker + glutargin) after 45 days, the morphological changes were much less pronounced (Fig. 8). Most of the acinar cells and lobules of the pancreas were almost normal and only a few acinar cells showed signs of dystrophy.

Discussion

In this study, the model of chronic pancreatitis was induced by long-term intraperitoneal administration of the NOs blocker L-NNA, which caused the activation of lipid peroxidation (MDA), the increase in the concentration of toxic products and the activation of collagen synthesis (PBH), the violation of the excretory function of the pancreas. At the same time, morphological changes were formed in the tissue of the pancreas of rats, which are characteristic of inflammation with chronicity of the pathological process, fibrosis of the parenchyma of the gland in the zones of its atrophy.

The results obtained by us are consistent with the data of Werner J *et al.* (1998), who showed that NO donors reduced the severity of inflammation, pancreatic edema, intrapancreatic trypsinogen activation, and amylase secretion, while the NO blocker nitro-L-arginine methyl ester (L-NAME) increased the severity of inflammation and simultaneously reduced pancreatic tissue oxygenation gland [1, 6].

NOs blockers have also been reported to exacerbate cerulein-induced pancreatitis by modulating intrapancreatic secretion in vivo [1, 10, 25].

Other researchers have shown that NOs blockers can have a therapeutic effect in pancreatic disease. Camargo E. A. 2014 showed that NOS blockade reduces the severity of abdominal hyperalgesia and hyperamylasemia in pancreatitis induced by phospholipase A [26]. Demir I. E. et al. [27] proposed NOS inhibition as a new strategy for the treatment of unbearable pain in chronic pancreatitis. The authors found that mice suffering from the painful form of cerulein-induced pancreatitis could get significant relief when treated with a specific NOS inhibitor (Nω-propyl-L-arginine hydrochloride at a dose of 4 mg/kg).

Many scientific works show both positive and negative effects of NO induction in pancreatitis [1-3, 25, 27].

Such diversity of scientific results reflects the multifaceted nature of NO action, the variety of experimental approaches (including dosing of NO blockers/donors) and shows the perspective of further study on the role of NO in normal and pathological pancreas.

Our study showed that glutargin in chronic pancreatitis induced by a NO_S blocker has a protective effect on the restoration of general behavioral reactions of rats, normalization of lipid peroxidation (MDA), antioxidant defense (CP) and collagen metabolism (PBH and FH) and prevents the development of pronounced structural changes in the pancreas.

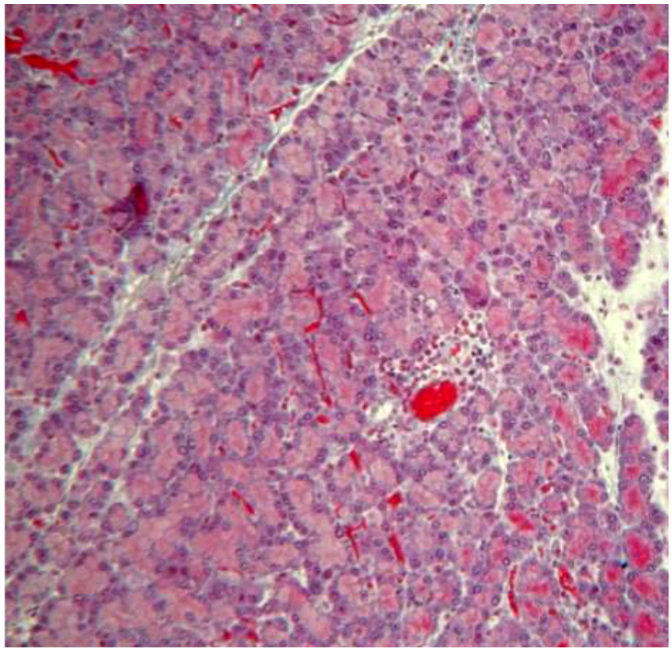

Fig. 7 Group I vacuolization of cytoplasm of acinar cells, full blood of blood vessels. Inflammatory infiltration and balloon dystrophy of acinar cells.
Staining with hematoxylin and eosin. x100.

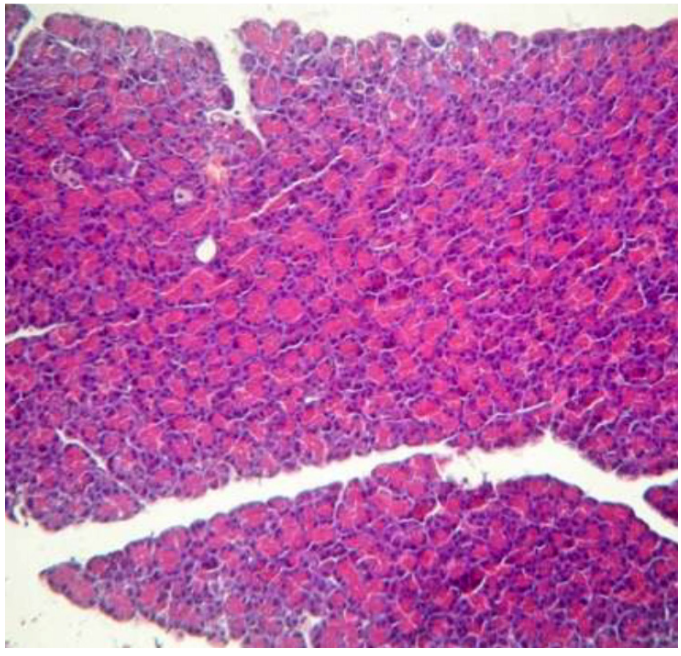

Fig. 8 Group II slightly expressed dystrophy of single acinar cells.
Staining with hematoxylin and eosin. x100.

Our data on the protective effect of glutargin coincides with the studies of V. I. Rusin *et al.* [28, 29]. The authors studied the effect of glutargin in patients with chronic pancreatitis and showed that the complex therapy of patients using glutargin contributed to the normalization of free amino acids in blood serum and was an effective tool for the correction of endothelial dysfunction.

In patients with peptic ulcer, the therapeutic effect of glutargin is realized through the limitation of oxidative stress, through the activation of glutathione peroxidase, superoxide dismutase, glutathione synthesis, which was proven in the experiment [30, 31].

Lebedeva T. (2008) found that glutargin prevents excessive activation of free radical oxidation processes and restores the activity of the antioxidant system in experimental adrenaline-induced acute myocardial damage [32].

Conclusions

To summarize our research, we can conclude that glutargin can be used in the complex treatment of patients with chronic pancreatitis as a drug that helps reduce the level of lipid peroxidation products, improves the state of the antioxidant defense system, helps normalize collagen metabolism, and prevents the development of pronounced structural changes in the pancreas glands.

Competing interests

None declared.

Authors' contributions

OK – the idea and design of the study, participated in conducting the experiment, AR – participated in the development of the study design, performed experimental studies.

Acknowledgments

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Ethics approval

The study protocol was approved by the Scientific Research Ethics Committee of the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine (minutes No. 5 from September 10, 2008)

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REVIEW ARTICLE

Inhibitory hemophilia: contemporary treatment with emicizumab. Considerations for pediatric practice

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ABSTRACT

Introduction. This article provides an overview of current knowledge and global experience regarding the use of emicizumab, with a focus on its specific considerations in pediatric practice. Emicizumab, a monoclonal antibody, operates uniquely compared to other therapies. It has been approved in Moldova since 2019 for preventing bleeding in hemophilia patients. Significant data from clinical studies and accumulated clinical practice provide answers to most questions physicians have when prescribing emicizumab. The article presents recommendations based on current information and global experience to aid decision-making in emicizumab usage. The purpose of this article is to provide information on management tactics for pediatric patients with hemophilia A receiving emicizumab.

Materials and methods. Over 40 publications were reviewed, consisting of recommendations, study results, and observations related to emicizumab use in pediatric patients with Hemophilia A.

Results. In 2017, emicizumab became the first registered non-factorial therapy for Hemophilia A. It was approved for use in treating the inhibitory form of the condition. In 2018, indications for emicizumab were expanded to include patients with the inhibitory form of hemophilia A and severe hemophilia A without inhibitors. Emicizumab is used to prevent bleeding and is not intended to stop an already occurring bleeding. If bleeding has occurred, the patient will need to be prescribed FVIII or bypassing agents. Emicizumab is administered as a loading dose of 3 mg/kg once a week for the first 4 weeks, followed by a maintenance dose of 1.5 mg/kg once a week, 3 mg/kg once every two weeks, or 6 mg/kg once every four weeks. The dose is based on the patient's body weight, which needs regular monitoring. If a dose is missed, it should be administered as soon as possible before the next scheduled dose, and the injection schedule should not be altered. Emicizumab can be used in children under one year to prevent bleeding.

Conclusions. Hemophilia, caused by a deficiency in coagulation factors VIII or IX, is a bleeding disorder. The main treatment-related complication in hemophilia patients is the development of inhibitors – alloantibodies that neutralize the procoagulant activity of infused FVIII or factor IX. The reasons why only 20%-30% of Hemophilia A patients develop inhibitors remain a challenge. Emicizumab, a bispecific monoclonal antibody, bridges the gap between activated factor IX and factor X to replace the missing activated factor VIII, thereby restoring hemostasis.

Keywords: emicizumab, hemophilia, factor VIII, factor VIII inhibitor, immune response.

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Key messages

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

Emicizumab, a type of antibody that binds to both activated FIX and FX, helps compensate for the lack of FVIII in patients with hemophilia A, restoring their ability to control bleeding. Emicizumab is currently registered and available for use in both indications in Moldova as well as in over 95 countries globally.

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The research hypothesis
Identification and analysis of recent findings in the treatment and management of inhibitors in patients with hemophilia A could provide new insights and improvements in future therapeutic approaches.

The novelty added by the manuscript to the already published scientific literature
The article presents a critical review of the existing scientific literature on inhibitory hemophilia, bringing into focus a new perspective on Emicizumab treatment. The manuscript discusses recent findings and empirical evidence, adding to existing knowledge about this complex condition. It also highlights the relevance and therapeutic impact of Emicizumab in the management of inhibitory hemophilia, providing an updated and detailed view on this topic of medical interest.

Introduction

Hemophilia A and B are genetic disorders caused by a lack of blood clotting factors. The first mentions of hemophilia date back to ancient times, and the term "hemophilia" was first documented in 1828. It is often referred to as the "royal disease" because it affected members of the European royal family. The severity of the disease is classified into three degrees depending on the activity of the factor. Approximately 900,000 people worldwide have this condition, with a significant proportion presenting with severe symptoms [1].

Hemophilia has been understood and treated more efficiently over time due to advancements in genetic and molecular biological studies. In the 20th century, treatment options improved significantly, with the development of replacement therapies greatly enhancing patient outcomes. Patients with hemophilia have recorded significant improvements in life expectancy, now averaging 63.2 years. However, those with severe forms of the disease tend to have a shorter lifespan. Despite the progress in hemophilia treatment, challenges persist, such as the burden of treatment and impaired quality of life. Gene therapy may overcome these challenges, but its introduction presents a challenge. Gene therapy involves introducing external DNA into human cells to produce the missing proteins. The main challenge is efficiently delivering genetic material to the target cells and tissues [2, 3].

The standard treatment for Hemophilia A and B has been factor replacement therapy, but it has disadvantages such as frequent intravenous administration and the development of inhibitors. As a result, alternative treatments such as extended half-life products, subcutaneous emicizumab, hemostatic rebalancing agents, and gene therapy have been developed or are being investigated for their efficacy in bleeding control [4, 5].

Despite improvements in hemophilia care, challenges such as treatment burden and impaired quality of life remain. Gene therapy has the potential to overcome these challenges. However, its introduction presents several obstacles. Gene therapy involves inserting foreign DNA into human cells to produce the missing proteins. The main challenge is the efficient delivery of genetic material to target cells and tissues. This is addressed by using viral vectors derived from mammalian viruses, where the viral genes are replaced with a therapeutic gene. Hepatic gene transfer using adeno-associated viral vectors has been successful in both preclinical and clinical studies. In hemophilia A and B, stable levels of factor VIII and factor IX, respectively, were achieved after a single treatment with adeno-associated viral vectors. Lentivirus integration is also being explored as an alternative treatment for hemophilia in children [6]. However, the efficacy of lentivirus integration in humans has yet to be determined. There are several questions about the efficacy and safety of gene therapy, including its use in patients with inhibitors, the likelihood of inhibitors occurring after therapy, and potential safety issues such as thrombosis and carcinogenic effects. It is also unclear how long the effects of gene therapy will last and whether multiple administrations will be needed [7-9].

The main objective of new drugs to treat hemophilia involves the development of recombinant preparations. Various pharmaceutical companies develop these preparations. As the production volume of recombinant blood clotting factors increases, their cost may decrease. Currently, the price of recombinant blood coagulation factor concentrate is higher than that of plasma-based concentrates [3, 10]. However, the development of long-acting preparations allowed increased intervals between administrations. This is especially beneficial for children [6]. Research is also being done to extend the half-life of these drugs. In addition, efforts are being made to develop thermostable preparations that do not require refrigeration for storage. Many current medications can already be stored at room temperature. The development of drugs that can be administered subcutaneously or orally could greatly simplify the treatment process by eliminating the need for venous access. Moreover, the use of small fragments of factor VIII obtained by recombinant technology may help to temporarily reduce inhibitory titers in patients. These fragments can neutralize the inhibitors produced by the patient's antibodies.

For example, in the treatment of hemophilia B, factor IX infusion is usually given every third day or twice a week. However, adolescents often struggle with adherence to this treatment due to the frequency of intravenous injections. In addition, prophylactic treatment in children can be difficult due to limited venous access, which may require the placement of a central catheter. While central access has its advantages, it can also lead to complications such as thrombosis and infection. The ability to undergo prophylactic treatment depends on various factors, including the patient's initial factor deficiency, the severity of bleeding symptoms, the patient's activity and age, and the ease of obtaining venous access [6].

Treatment for the inhibitory form of hemophilia B is the same as for hemophilia A, but with a lower success rate. There are potential problems such as severe allergic reactions and the development of nephrotic syndrome during the induction of immune tolerance. The most effective method for removing inhibitors from hemophilia A is to induce immune tolerance, which involves repeated infusions of factor VIII concentrate to train the immune system to accept the factor. This method has a success rate of 60-80% and allows a normal response to factor therapy and control of bleeding. However, the effectiveness of this method still needs further analysis. The success of inducing immune tolerance depends on the initial level of inhibitors, with better results observed in patients with low or transient inhibitor responses. Patients with high-responder inhibitors or the inhibitory form of hemophilia B have a less promising outlook. Treatment options to control bleeding in inhibitory forms include anti-inhibitory coagulant complex and activated eptacog alfa. A clinical trial showed similar effectiveness of these two preparations in stopping bleeding, but the choice of treatment depends on each patient [5, 10].

Despite improvements in factor therapy for hemophilia, challenges still exist, such as the risk of inhibitor formation and the need for frequent intravenous infusions, which can reduce patient adherence to treatment. Adherence rates for hemophilia treatment range from 50 to 60% [10].

Treatment of hemophilia involves the use of blood clotting factor concentrates, which have greatly improved patient outcomes. These concentrates are obtained from donor plasma or are produced using recombinant DNA technology. There are different types of recombinant FVIII preparations for the treatment of hemophilia, including those with unmodified FVIII polypeptides. The production process for recombinant preparations is long and involves various steps such as gene isolation, viral activation, stabilization, filtration, and concentration.

However, there have been various problems in applying these treatments. For example, nearly half of hemophilia patients in the US were infected with HIV and 80% were infected with hepatitis C [10]. This led to research to improve the safety of plasma concentrates. Today, there are many manufacturers of plasma concentrates that have similar efficacy but differ in purification methods and other factors.

Recombinant concentrates, which are produced using molecular technologies, are safer and cheaper to produce. They are divided into four generations based on the decrease in animal protein used in production. There is also a pig blood plasma concentrate for patients with acquired factor VIII inhibitor. Additionally, concentrates with a longer half-life have been developed to reduce the need for frequent injections.

At the current stage, new treatment methods are being developed, such as monoclonal antibodies like Emicizumab. Emicizumab is a drug used for the prophylactic treatment of patients with hemophilia A and B as well as for the inhibitory form. It is a highly effective humanized monoclonal antibody that inhibits the tissue factor pathway, allowing the generation of factor Xa and thrombin.

The medicine was registered in Moldova in October 2020 and has successfully passed phase II clinical trials. It is administered subcutaneously daily, starting with a dose of 0.15 mg/kg and increasing if necessary [11].

Materials and methods

Initially, the search was conducted using the keywords "Inhibitory Hemophilia" and "Guidelines for Contemporary Treatment in Hemophilia" through publications up to 2023 in the PubMed online database (National Library of Medicine, USA, and National Institutes of Health). After examining the titles, works representing official guidelines for the diagnosis and treatment of Hemophilia with inhibitors and severe Hemophilia without inhibitors were selected. Subsequently, a search was conducted using the keywords "Inhibitory Hemophilia Diagnosis", "Inhibitory Hemophilia Treatment", "Inhibitors in Hemophilia" in the PubMed database for publications from 2020 to 2023. The availability of medicines in Moldova was verified on the website of the Medicines and Medical Devices Agency of Moldova.

Results

Emicizumab is a medicine approved for treating patients with hemophilia A who have inhibitors to factor VIII in over 110 countries worldwide. It is also approved for treating patients without inhibitors in over 95 countries, including Moldova and the European Union. The medicine has been extensively studied in one of the largest clinical research programs for hemophilia A, with eight phase III studies conducted. A bispecific antibody binds to factors IXa and X, proteins involved in blood coagulation. Emicizumab has been approved and included in clinical guidelines for hemophilia treatment in Moldova from 2019 to 2022 [12]. Over 12,500 patients worldwide have received emicizumab in clinical studies and medical practice [1].

Clinical studies have shown promising results. The Emicizumab dosage starts with a loading dose of 3 mg/kg once a week for 4 weeks, followed by a maintenance dose of 1.5 mg/kg once a week, 3 mg/kg every 14 days, or 6 mg/kg every 28 days. Emicizumab is easy to dose and convenient for patients but cannot be used to treat acute bleeding. Thrombotic microangiopathy and thrombosis are the most serious adverse events associated with emicizumab therapy, with 4 cases of thrombotic microangiopathy and 18 cases of thrombosis reported in clinical studies [5].

Results of phase II studies showed a significant decrease in bleeding occurrences by 80-90% compared to the use of eptacog alfa therapy only when necessary [13]. Three cases of blood clot formation were observed during phase III clinical trials, leading to a temporary halt in the study pending further clarification. Additionally, other monoclonal antibody treatments targeting the tissue factor pathway inhibitor, such as PF-06741086 and MG1113, are now entering clinical trials [10].

Throughout the HAVEN clinical trial program, the efficacy of Emicizumab was demonstrated in children, adolescents, and adults with hemophilia A, with or without factor VIII inhibitors. Furthermore, inducing immune tolerance during Emicizumab prophylaxis is a feasible approach in pediatric patients with hemophilia A with inhibitors. After the 4-week loading dose period, Emicizumab concentrations are expected to remain at levels that provide protection against bleeding throughout the dosing interval, regardless of the chosen maintenance dosing regimen, i.e., weekly, every 2 weeks, or every 4 weeks. However, when used with high-dose activated prothrombin complex concentrate, Emicizumab can lead to blood clotting events. Guidelines for managing bleeding episodes in patients using Emicizumab have been issued in the UK, and a group of German specialists has gathered to provide similar guidance [14-17].

Data from HAVEN studies 1 to 4 consistently show the prevention of bleeding throughout the dosing interval, regardless of the chosen dosing regimen. These findings provide additional evidence of sustained efficacy of Emicizumab in all approved dosing regimens to reduce bleeding in individuals with hemophilia A. HAVEN 1 study found that the annual bleeding rate in patients receiving Emicizumab was 5.5 cases in total and 2.9 cases requiring treatment. Vaccination is recommended for these patients according to the national immunization schedule. Vaccination should be done on non-bleeding days and not on the same day as Emicizumab administration. Additional administration of FVIII or rFVIIa (Recombinant activated factor VII) is not necessary before vaccination. Clinical monitoring after vaccination is recommended. Currently, there are no specific studies or recommendations regarding vaccination in patients receiving Emicizumab. Laboratory tests such as aPTT and coagulation tests will be affected by Emicizumab and for 6 months after its discontinuation. Emicizumab therapy does not affect other tests, such as thrombin time. Monitoring Emicizumab concentration is not necessary to assess its efficacy or manage bleeding. Non-compliance with the dosing regimen or development of anti-drug antibodies can lead to a decrease in Emicizumab concentration. Neutralizing antibodies develop in less than 1% of cases and can lead to increased spontaneous bleeding. Consultation with expert centers is recommended if the frequency of spontaneous bleeding increases and the aPTT level is prolonged [18, 19].

Despite the available data, there are still unanswered questions about Emicizumab therapy. These questions include how Emicizumab interacts with other long-term hemostatic medications, whether patients on Emicizumab prophylaxis should continue immune tolerance induction therapy, and how to effectively manage acute bleeding and perioperative support in patients on emicizumab prophylaxis. Although Emicizumab has been proven to significantly reduce bleeding in patients with inhibitors, there is still no consensus on whether patients with inhibitors should undergo immune tolerance induction therapy [20].

Furthermore, when Emicizumab was used in combination with bypass agent prophylaxis, there was a 68% reduction in bleeding rates in the HAVEN 1 study and a 100% reduction in bleeding rates in the HAVEN 2 study. However, it is important to note that in the HAVEN 1 study, three patients developed thrombotic microangiopathy (TMA), and two patients presented with thrombosis when Emicizumab was administered together with a high or frequent dose of activated prothrombin complex concentrate (aPCC). This complication was avoided in the HAVEN 2 study by not combining Emicizumab with a high or frequent dose of aPCC. In the HAVEN 1 study, Emicizumab demonstrated an impressive reduction of 80% in all bleeding, 89% reduction in treated joint bleeds, 92% reduction in treated spontaneous bleeds, and 95% reduction in treated target joint bleeds compared to no prophylactic treatment. Similarly, the HAVEN 2 study showed a significant reduction in bleeding, with a 63% decrease in all bleeding, 94.7% reduction in treated bleeds, 94.7% reduction in treated spontaneous bleeds, 100% reduction in treated joint bleeds, and 100% reduction in treated target joint bleeds compared to absence of prophylactic treatment. The exact role of Emicizumab in combination with current factor VIII replacement therapies and emerging non-replacement therapies is yet to be determined [21-23].

A recent meta-analysis has noted that more research is needed to understand the effectiveness and safety of Emicizumab in younger age groups and those with milder forms of hemophilia A. Additionally; further studies should explore the risk of inhibitor recurrence in patients who have successfully tolerated Emicizumab and transition from other treatments. According to the opinions expressed by experts in this study, more "real-world studies" should be conducted to assess how patients perceive Emicizumab in terms of ease and tolerability to optimize personalized treatment plans [24, 25].

Clinical studies for Emicizumab involved 390 patients. These patients were enrolled in the phase III clinical trial program known as HAVEN1-4. The study program included individuals from various age groups, including pediatric patients up to 12 years old, as well as adolescents and adults aged 12 to 77 years.

HAVEN1 study focused on 109 individuals with inhibitor-type hemophilia A, of which 32 were under 18 years old. The study showed that 63% of these patients did not experience any bleeding incidents throughout the observation period. When comparing the annual frequency of bleeding, it was found that those using Emicizumab had a rate of 3.3, while those using prophylactic treatment had a significantly higher rate of 15.7. This means that Emicizumab reduced the annual bleeding frequency by an impressive 79% compared to prophylactic factor use [26-28].

HAVEN2 involved 88 children and adolescents aged 1 to 15. Among these patients, 72% underwent immune tolerance induction therapy. In the subgroup of 65 patients under 12 years old, the annual frequency of bleeding episodes was 0.3, with a 95% confidence interval ranging from 0.17 to 0.50. Additionally, a significant majority of these patients, especially 77%, did not experience any bleeding episodes requiring therapy. It is noteworthy that 91% of all reported bleeding incidents were attributed to traumatic events [29].

HAVEN4 examined individuals with severe hemophilia A, with and without an inhibitor. This included a small number of children. The study found that the median annual occurrence of joint bleeding (hemarthrosis) throughout the body was zero. However, in the specific joints targeted in the study, approximately 95-98% of patients experienced between zero and three hemarthroses over the year [30].

HAVEN 4 analyzed the use of Emicizumab as preventive treatment every 4 weeks in adults and adolescents with hemophilia A, regardless of the presence of FVIII inhibitors.

Pipe and his colleagues conducted a study involving patients with severe congenital hemophilia A or hemophilia A with FVIII inhibitors who had received treatment with FVIII concentrates or bypass agents. The study took place across multiple locations in Japan, Spain, Australia, Belgium, Poland, and the USA. Participants received subcutaneous Emicizumab every 4 weeks for at least 24 weeks. The study evaluated the pharmacokinetics and safety of Emicizumab in a run-in cohort and the prophylactic efficacy of Emicizumab in maintaining bleeding prevention in an expansion cohort. The study is ongoing and registered on ClinicalTrials.gov.

Between January 30, 2017, and February 27, 2017, a study enrolled seven patients confirming the safety and expected effects of a drug regimen. This led to the expansion of the study, enrolling an additional 41 patients. The annual rate of treated bleeding was 2.4, with over half of the patients not reporting treated bleeding, and the majority reporting from zero to three treated bleedings. The most common side effect was injection site reaction. There were no blood clotting events or development of antibodies neutralizing the drug. Overall, the drug showed effective bleeding control and could enhance care for those with hemophilia A.

In the clinical studies HAVEN 1-4, a small percentage of patients developed antibodies to Emicizumab, with a few experiencing a decrease in drug efficacy. However, these antibodies did not increase the risk of inhibitor development and did not influence the efficacy of other hemophilia treatments. The most common adverse events associated with Emicizumab were reactions at the injection site, headaches, and joint pain. Thrombotic events and thrombotic microangiopathy (TMA) were rare but serious complications requiring immediate hospitalization. Cases of TMA were more likely to occur in patients receiving high doses of activated prothrombin complex concentrate (APCC) alongside Emicizumab, while rFVIIa did not trigger TMA. Thrombotic events were also reported, but the risk was similar to that in the general male hemophilia population [25, 31].

HAVEN 5, conducted by Renchi Yang, aimed to evaluate the efficacy and safety of Emicizumab in participants from the Asia-Pacific region aged 12 and above, with severe hemophilia A without FVIII inhibitors or any severity of hemophilia A with FVIII inhibitors. Participants were randomly assigned to one of three groups: Emicizumab 1.5 mg/kg once a week, Emicizumab 6 mg/kg every 4 weeks, or no prophylaxis. The primary objective of the study was the annualized bleeding rate (ABR) for treated bleeding, compared between individuals receiving Emicizumab prophylaxis and those without prophylaxis. Secondary objectives included ABR for target joint treated bleedings. Safety was also assessed throughout the study. Based on the study results, it can be concluded that both Emicizumab dosage regimens (1.5 mg/kg once a week and 6 mg/kg every 4 weeks) demonstrated effective bleeding control in the studied population. Additionally, Emicizumab was well-tolerated, and its use as prophylaxis could improve outcomes for people with hemophilia A. The most commonly reported adverse event in the study was upper respiratory tract infection, occurring in 14 out of 56 participants (25.0%) receiving Emicizumab and in 2 out of 14 participants (14.3%) without prophylaxis. No thrombotic events, thrombotic microangiopathies, or deaths were reported during the study period [32].

HAVEN 6 evaluated the safety and effectiveness of Emicizumab prophylaxis in individuals with non-severe hemophilia A without FVIII inhibitors. The study involved 72 participants receiving Emicizumab treatment for at least one dose. Participants experienced some adverse events, but none were severe enough to discontinue treatment. The study found that Emicizumab was effective in reducing bleeding episodes, with an annual bleeding rate of 0.9 for treated bleedings. Overall, the study concluded that Emicizumab is a valuable treatment option for individuals with non-severe hemophilia A without FVIII inhibitors. New data from the HAVEN 6 study show that Emicizumab Roche is effective and safe for patients with moderate or mild hemophilia A. The study found that 66.7% of participants had no treated bleeding episodes. The updated data also confirm the favorable safety profile of Emicizumab, with no new safety signals identified. It is noteworthy that there is limited information and recommendations for the treatment of moderate or mild forms of hemophilia A, which can lead to delayed or incorrect diagnosis of bleeding episodes. Emicizumab is approved for the treatment of hemophilia A, with or without FVIII inhibitors in many countries [33-35].

The use of Emicizumab as a preventive treatment in individuals who have not been previously treated is still under discussion. It is unclear what the occurrence and effects of FVIII inhibitors are in this situation. Various efforts are being made to address this issue. A study, named HAVEN 7, is currently enrolling participants to evaluate early prevention with Emicizumab in children under 12 months with hemophilia A without inhibitors. Another study compares inhibitor data in individuals who have not received treatment before and who undergo preventive treatment either with an extended half-life recombinant FVIII concentrate or with Emicizumab. A third study also evaluates inhibitor data in individuals receiv-

ing preventive treatment with Emicizumab and a low dose of simoctocog alfa, a recombinant FVIII concentrate. Both studies are currently recruiting participants [36, 37].

The inclusion of Emicizumab in both international and national treatment guidelines for patients with hemophilia A became possible due to the positive results observed in clinical studies. In the latest edition of the World Federation of Hemophilia guideline, it emphasizes that Emicizumab prophylaxis can effectively prevent hemarthrosis, spontaneous bleeding, and interventional bleeding in individuals with severe hemophilia A without inhibitor antibodies. These findings underscore the significant potential of Emicizumab as a treatment option to improve the quality of life and reduce the risk of bleeding episodes in this patient population [38].

A study was conducted to examine bleeding patterns in 70 hemophilia A patients taking Emicizumab for over 18 months. The study found that the occurrence of spontaneous and traumatic bleeding did not significantly differ at different time points during the study. Traumatic bleeding was most commonly caused by hemarthrosis and cranial trauma, while spontaneous bleeding was most commonly caused by hemarthrosis. The study also found that the chance of bleeding during Emicizumab treatment increased with age. These findings suggest that older patients may still be exposed to the risk of bleeding despite Emicizumab therapy. This information may be useful for physicians when counseling patients and planning treatment [39].

Previous reports have discussed the efficacy, safety, and pharmacokinetics of Emicizumab, but there is limited long-term data available. The study conducted by Callaghan *et al.* (2021) examined data from 401 people with hemophilia A, who participated in four phase 3 studies to assess the long-term efficacy, safety, and pharmacokinetic profile of Emicizumab. The data showed that over an average duration of 120.4 weeks, the annual bleeding rate decreased and stabilized to less than one. Most participants did not experience bleeding episodes or had very few bleeding episodes, and bleeding in target joints significantly decreased. Emicizumab was well tolerated, with no new safety issues identified [25, 40].

A study conducted by Hankil Lee examined the cost-effectiveness of Emicizumab prophylaxis in patients with hemophilia A inhibitors and factor VIII in Korea [41]. These patients frequently experience spontaneous bleeding and require costly treatments to control bleeding. The study found that Emicizumab prophylaxis, compared to on-demand treatments, significantly reduced bleeding rates, improved quality of life, and lead to cost savings. The findings suggest that Emicizumab prophylaxis is a highly beneficial treatment option for these patients. A study conducted in February 2020 evaluated the cost-effectiveness and budget impact of using Emicizumab prophylaxis in patients with hemophilia A and inhibitors. Emicizumab was proven more efficient and economical compared to other prophylaxis methods. The use of Emicizumab also led to a significant reduction in healthcare costs. This study suggests that Emicizumab is a cost-effective and sustainable treatment option for patients with hemophilia A and inhibitors. In comparison to prophylaxis with bypassing agents, Emicizumab reduced direct and indirect costs, resulting in cost savings for the National Health System and society in Spain [42, 43].

However, another study documented the cost-effectiveness of prophylactic Emicizumab compared to prophylactic recombinant factor VIII in patients with mild or moderate hemophilia A without inhibitors in the United States. The study found that currently, Emicizumab is not cost-effective in this patient population, and its price should decrease by more than 35% to become cost-effective [44]. Similarly, a study from Canada examined the effectiveness and cost of Emicizumab for severe hemophilia A. The study found that the cost of Emicizumab was not justified based on the benefits it provided compared to other treatments. The price of Emicizumab should be reduced to make it more cost-effective. Although Emicizumab has been shown to reduce bleeding, there is not enough evidence to directly compare it with other treatments. The reimbursement demand for Emicizumab does not match the population it is intended for, adding uncertainty to its cost-effectiveness [45].

The effectiveness of Emicizumab has been compared to Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc) in many studies. rFVIIIFc has proven its efficacy and is cost-effective compared to Emicizumab, providing clinicians with a viable treatment option to improve health outcomes for adults and adolescents with hemophilia A in the UK [46]. Similar results were observed in a study conducted by Klamroth *et al.* [47]. The authors aimed to compare the effectiveness of two treatments, rFVIIIFc and Emicizumab, for patients with hemophilia A without inhibitors.

Discussions

In the last 80 years, significant progress has been made in the treatment and quality of life for hemophilia patients. The focus has shifted from simply preventing bleeding and death to improving overall independence and integration into society for patients. Although new treatment methods have been successful, the standard therapy of intravenous infusions of factor concentrates still has limitations affecting patient adherence. Inadequate disease control can lead to joint damage, especially in patients with inhibitors. However, the development of new treatments and gene therapy offers hope for further improvements, but their long-term performance and safety must be carefully evaluated.

The health of children and adolescents is a national priority due to the demographic crisis. The focus is on addressing socially significant diseases such as cancer and blood disorders, which are the second leading cause of death in children in Moldova. To ensure the best possible hematological care for children and adolescents, specific indicators measuring the quality of care in terms of structure, process, and outcomes need to be utilized. Unfortunately, these indicators have not been established or implemented nationally. Therefore, to address the challenges associated with specialized hematological care, new therapeutic technologies and research must be introduced [47].

In the last eight decades, there have been significant and monumental changes in approaches, evaluation techniques,

therapy availability, and overall quality of life for people with hemophilia. Initially, the main goal was simply to prevent patients' deaths from excessive bleeding. However, the focus has now shifted towards improving the quality of life for hemophilia patients and ensuring their complete independence and integration into society. The goal is to eliminate any exceptions from this standard and allow every child with hemophilia to live a life similar to those without this condition. Despite significant progress with the introduction of new treatment methods, it is important to note that the "gold standard" of therapy remains intravenous infusions of factor concentrates, despite its limitations that may hinder patient adherence to this form of treatment. Inadequate disease control can lead to recurrent hemarthrosis and, ultimately, the development of resistant arthropathy in the affected joints. The issue of patients with inhibitors represents a unique challenge, as existing therapy is often incredibly challenging to tolerate and does not always yield successful results. However, the emergence of new drugs with a completely new mechanism of action and more convenient administration methods, along with the ongoing development of gene therapy, offers hope for a significant improvement in the quality of life for people with hemophilia. However, it is crucial to carefully analyze the long-term efficacy and safety indicators of these new treatments.

Patients with hemophilia A with inhibitors currently have unmet needs, such as the requirement for intravenous substitution therapy and the burden of prophylactic treatment. Emicizumab is a monoclonal antibody designed to address these needs and has undergone phase III clinical trials in both adolescents and adults as well as children.

Continued real-world experience with Emicizumab has shown that it is safe and effective in treating children, adolescents, and adults with hemophilia. The launch of Emicizumab takes place in over 100 countries, including low- and middle-income countries, through the World Federation of Hemophilia's Humanitarian Aid Program. Various pharmacokinetic and dosing tools are available to customize treatment and minimize medication waste. Long-term clinical studies and real-world monitoring have further confirmed the safety and efficacy of Emicizumab in children and adolescents. Ongoing clinical studies address additional questions about the use of Emicizumab with other therapies and the recurrence of inhibitors. In conclusion, Emicizumab is widely used globally and can be customized based on individual pharmacokinetic needs, with consistent outcomes observed in different age groups.

Studies have shown significant reductions in bleeding and joint events when Emicizumab is used compared to no prophylaxis. Emicizumab has also demonstrated reductions in bleeding rates in patients on bypassing agent prophylaxis. However, in the analysis of some studies, cases of thrombotic microangiopathy and thrombosis have been reported when Emicizumab was used with a high or frequent dose of activated prothrombin complex concentrate. No patient developed these complications when the combination was avoided. The use of Emicizumab is currently being studied in patients without inhibitors and in combination with other therapies for hemophilia A, but its role with current factor VIII replacement therapies and non-replacement therapies is still uncertain.

Before using Emicizumab, healthcare professionals must have experience in treating hemophilia A and be familiar with the mechanism of action, administration, and potential side effects of the drug. Emicizumab is used to prevent bleeding, not to treat it, so patients must have other medications on hand to stop bleeding if necessary. In general, not all bleeding episodes occurring with Emicizumab prophylaxis require treatment.

Preventing hemarthrosis is an essential part of hemophilia treatment, as it can lead to joint damage and disabilities. Emicizumab has been shown to effectively protect against bleeding and reduce the frequency of hemarthrosis in patients with severe hemophilia A. It is important to initiate Emicizumab therapy under the supervision of an experienced physician, and patients should receive training in the proper injection technique. If patients have long-term venous access systems, they should be removed within one month of starting Emicizumab treatment. Patients and caregivers should be educated about the differences between Emicizumab and previous treatments and instructed in the administration of other hemophilia treatments in case of bleeding. Patients with the inhibitory form of hemophilia A should be aware of the increased risk of thrombotic events when using certain coagulation complexes with Emicizumab. For patients without inhibitors, Emicizumab therapy can be initiated without prior preparation. Patients should also be taught how to manage bleeding or trauma and should seek medical help in such cases. It is recommended that all patients receiving Emicizumab have an emergency or urgent surgical information card.

Conclusions

Alloantibodies against factor VIII develop in 25 to 50% of children with severe hemophilia A, as well as in a small percentage of children with mild or moderate hemophilia A. In patients with hemophilia B, alloantibodies appear in only 1 to 3% of individuals with the severe form.

During the initial administration of products containing FVIII, patients with hemophilia A may develop a proinflammatory immune response with the synthesis of anti-FVIII IgG1, which lacks inhibitory activity against FVIII.

Most patients with inhibitory Hemophilia A with high titers attempt inhibitor elimination using one of several immune tolerance induction (ITI) regimens. ITI has been less successful in patients with hemophilia B and inhibitors compared to those with hemophilia A.

Emicizumab is a humanized monoclonal modified immunoglobulin G4 (IgG4) antibody with a bispecific antibody structure. Emicizumab bridges activated factor IX and factor X to restore the function of missing activated factor VIII that is needed for effective hemostasis. It has no structural relationship or sequence homology to factor VIII and, as such, does not induce or enhance the development of direct inhibitors to factor VIII.

For the treatment of bleeding episodes in patients with high-titer inhibitors (≥ 5 Bethesda units), a prothrombin complex concentrate (PCC) (preferably an activated PCC [APCC]), recombinant factor VIIa, or porcine factor VIII can be used.

Competing interests

None declared.

Authors' contributions

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

Ethical statement and patient consent

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REVIEW ARTICLE

Knowledge, attitudes, and practices of the population regarding viral Hepatitis B and C worldwide: a systematic literature review

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ABSTRACT

Introduction. The term "hepatitis" implies liver damage by inflammatory cells, a condition with numerous origins. Viruses, predominantly hepatitis B and C viruses cause most cases of hepatitis, which can lead to chronic liver diseases. Assessing the general knowledge and awareness of the population regarding viral hepatitis is crucial for developing targeted and effective strategies to address these epidemics. This knowledge can help tailor interventions for different population strata, such as youth and adults, thereby enhancing the impact of prevention and care efforts.

Materials and methods. An exhaustive search of relevant literature was performed in electronic databases such as PubMed, Scopus, and Web of Science. Key terms included "viral hepatitis B," "viral hepatitis C," "knowledge," "attitudes," "practices," and "population." Articles included in the analysis were selected based on predefined inclusion and exclusion criteria.

Results. In a Nigerian hospital, 33% of healthcare workers lacked knowledge of hepatitis B, and 35% were not immunized. In an Iraqi study, 75% believed HBV is more easily transmitted than HIV, and 33.9% knew HBV could spread through toothbrushes. In India, most medical students were aware of hepatitis B (84.8%). Their knowledge about transmission through blood transfusion (81.06%) and needles (74.1%) was good, but they had poor knowledge about other modes of transmission and clinical features. A study in Tehsil Wazirabad, Gujranwala found good knowledge about hepatitis C transmission and symptoms. In Saudi Arabia, dental students revealed insufficient knowledge about hepatitis B infection. Practice levels varied, with 47.2% showing high practice and 22% low practice. Female participants exhibited higher knowledge, attitudes, and practices. In Gauteng province, South Africa, a 2015 study found that healthcare workers had inadequate knowledge of viral hepatitis. The average knowledge score was 2.0 out of 6, while practice and attitude scores were higher.

Conclusions. A significant difference in knowledge levels regarding viral hepatitis B and C was highlighted within the population. The overall level of knowledge regarding viral hepatitis B and C remains inadequate among both medical personnel and the general population. There is a growing need for education and awareness about viral hepatitis B and C.

Keywords: viral hepatitis B, viral hepatitis C, knowledge, attitudes, practices.

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Key messages

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

Current literature lacks comprehensive insights into the global variations of knowledge, attitudes, and practices among populations concerning viral Hepatitis B and C. Particularly, there is a dearth of understanding regarding the socio-cultural factors influencing awareness and behaviors related to these infections.

The research hypothesis

The study posits that a systematic review of existing literature will reveal significant disparities in knowledge levels, attitudes, and practices related to Hepatitis B and C across different regions and demographic groups worldwide. Furthermore, it hypothesizes that socio-economic factors will significantly influence the development of these differences.

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

This systematic literature review contributes novel insights by synthesizing existing research on the global landscape of knowledge, attitudes, and practices regarding Hepatitis B and C. By identifying gaps in understanding and highlighting socio-cultural influences, the study enhances our comprehension of how these factors affect public health interventions and policies worldwide.

Introduction

The term "hepatitis" implies liver damage by inflammatory cells, a condition with numerous origins. Most cases of hepatitis are caused by viruses, predominantly hepatitis B and C viruses (HBV and HCV), which can lead to chronic liver diseases. HBV has been recognized since 1965, and the first vaccines were approved in the early 1980s. HCV, previously known as non-A, non-B hepatitis, was identified only in 1989, with no vaccine available to date. These viruses are endemic in many countries worldwide; however, prevalence rates fluctuate significantly [1]. Viral hepatitis B and C pose challenges for public health systems, given the development of chronic infections, complications, and deaths. Together, hepatitis B and C account for nearly 95% of deaths caused by viral hepatitis worldwide, surpassing human immunodeficiency virus (HIV) and tuberculosis. In 2016, the World Health Organization (WHO) recognized viral hepatitis as a pandemic, focusing on reducing transmission, increasing vaccination rates, and expanding access to treatment [2]. Hepatitis B is a significant public health problem affecting nearly 10% of the global population. According to the WHO report in 2009, approximately 2 billion people are affected by HBV worldwide, with over 270 million suffering from chronic infection throughout their lives, and over one million individuals dying from cirrhosis and liver cancer each year [3-5]. Hepatitis B virus (HBV) infection is considered one of the occupational risks threatening healthcare workers. Additionally, assessing the knowledge, attitudes, and practices among these individuals can be considered one of the most crucial activities for developing strategies to prevent HBV infection [6]. The Centers for Disease Control and Prevention (CDC) reported that 3.9 million people (1.8%) are infected with HCV, and 2.7 million of these infections will become chronic [7]. The main modes of transmission of hepatitis B and C viruses are through exposure to biological fluids such as blood, semen, or vaginal secretions, sexual contact, sharing contaminated needles, razors, or toothbrushes [8-10]. Recent epidemiological studies in Hong Kong have shown that diagnosis and treatment rates within the community have significantly lacked, hovering around 50%, compared to WHO's 90% - 80% [11, 12]. Inadequate knowledge and awareness of viral hepatitis B and C in the community have been shown to contribute to these deficiencies [11-14]. Elsewhere in the world, social stigmatization resulting from poor knowledge has been reported to reduce diagnosis and treatment rates among high-risk individuals [15-17]. Knowledge about viral hepatitis B and C among healthcare workers, especially in primary healthcare and social care, has generally been poor. Although there have been no large-scale controlled studies on healthcare workers' knowledge of chronic hepatitis B and hepatitis C, knowledge has been imperfect in all surveys whose results have been published [18]. The study of knowledge, attitudes, and practices measures the key knowledge, feelings, trends, or abilities shared by a community regarding certain aspects. It has been used as a useful study tool for designing public health policies by considering the awareness, beliefs, and health-seeking behavior of at-risk populations. In Ethiopia, data on knowledge, as well as attitudes and practices regarding occupational exposure to HBV among health sciences students, are scarce, although the prevalence of infection is high in the general population [19-21]. An adequate assessment of knowledge, attitude, and practice factors, along with an understanding of the disease, are helpful in prevention and require adopting a healthy lifestyle throughout life [22-24]. Knowing the facts, having a proper attitude, and being aware can largely prevent the risk of viral hepatitis [25, 26]. For any preventive government measures or programs, community success, awareness, and participation are vital. Hence, information related to community knowledge and awareness is crucial for community prevention programs [27]. Prevention is the key to combating hepatitis B and C, which includes standard universal precautions, prophylactic vaccination, and standard treatment [28-31]. The epidemiological situation regarding viral hepatitis is determined both by the inadequacy of knowledge and by the limited access or lack of screening services [32]. Surveys on knowledge, attitudes, and practices are representative of a particular population, aiming to gather information on what is known, believed, and done regarding a specific subject, and they are the most used study tool in health behavior research [33, 34]. Knowledge is typically assessed to determine the extent to which community knowledge aligns with biomedical concepts [33, 35]. Prevention against any disease is proportional to the Knowledge, Attitude, and Practice of the population and reflects the importance given to health issues in society. Therefore, these studies play an imperative role in determining a society's ambiguities and are widely used in research evaluating population-reported assessments globally. Despite efforts by authorities to

increase knowledge and awareness of hepatitis B and C, no progress is reported [33]. Assessing the general knowledge and awareness of the population regarding viral hepatitis is crucial in developing targeted and effective strategies to address these epidemics. This knowledge can help tailor interventions for different population strata, such as youth and adults, and can enhance the impact of prevention and care efforts [36]. There are few studies on knowledge and practices related to viral hepatitis B and C among the general population in developing countries, yet such studies are crucial for the proper utilization of limited resources under poor socioeconomic and educational conditions [37]. Population health education plays a crucial role in early disease identification and initiating timely appropriate treatment [38].

Material and methods

1. Article selection and resource identification

To conduct this systematic review, an exhaustive search of relevant literature was performed in electronic databases such as PubMed, Scopus, and Web of Science. Key terms included "viral hepatitis B," "viral hepatitis C," "knowledge," "attitudes," "practices," and "population." Articles included in the analysis were selected based on predefined inclusion and exclusion criteria.

2. Inclusion and exclusion criteria

Eligible articles had to meet predefined criteria, including publication period, thematic relevance, as well as robust methodology. Articles with incomplete data or research methods deemed inappropriate for our objective were excluded.

3. Selection and data extraction process

Titles and abstracts of identified articles were evaluated. Articles deemed relevant underwent a thorough evaluation of the full text. Relevant data regarding the population's knowledge, attitudes, and practices towards viral hepatitis B and C were systematically extracted and documented.

4. Assessment of article quality

The methodological quality of each article was assessed using specific tools appropriate for each type of study included in the review. This process included evaluating the validity of the obtained results.

5. Data synthesis and analysis

The extracted data were synthesized and presented in a comprehensive manner, highlighting trends and general conclusions from the existing literature.

Results

After analyzing the available literature, a significant difference in knowledge levels regarding viral hepatitis B and C was highlighted within the population. The studies included in this analysis revealed that certain regions suffer from notable gaps in understanding the modes of transmission, symptoms, and prevention of these diseases.

Most study participants indicated that traditional sources, such as healthcare workers and awareness campaigns, serve as primary sources of information regarding viral hepatitis. However, it became evident that there is still an urgent need to improve access to information and education, especially among specific demographic groups.

According to a study conducted in China among pregnant women, 96.1% of HBsAg positive respondents knew that hepatitis B is infectious, but only 49.7% correctly identified the routes of transmission. Furthermore, 84.3% knew that hepatitis B could be prevented by vaccination, though only 58.2% mentioned that vaccination requires 3 doses. Additionally, 88.9% recognized that their children are susceptible to infection. However, 45.8% of pregnant women carrying HBV considered that cesarean section is effective in preventing mother-to-child transmission, and 23.5% mentioned that bottle-feeding is effective in preventing mother-to-child transmission. Knowledge regarding HBV and its mother-to-child transmission among pregnant women or women of childbearing age was relatively insufficient, which may contribute to the risk of HBV transmission [39].

A study conducted in the USA among persons infected with HCV and HIV/AIDS showed a low knowledge score (<50% of the total possible score) regarding HCV [40].

A study conducted in a hospital in Nigeria found that 33% of healthcare workers had poor knowledge about hepatitis B virus infection, and 35% were not immunized against HBV. Challenges identified for hepatitis control included lack of hospital policy (91.6%), weak orientation of newly hired health workers (75.9%), and low risk perception (74.6%) [41].

In a study conducted in Iraq, 75% of participants mentioned that HBV is more easily transmitted than HIV. Women had a significantly higher percentage (35.2%) believing that HIV is more easily transmitted than HBV compared to men (20.2%). Villagers also had a higher percentage (29.5%) believing that HIV is more easily transmitted than HBV compared to urban residents (22.4%). Only 33.9% of subjects knew that HBV could be transmitted through toothbrushes. Most participants without education (75.9%) believed that HBV could be transmitted through food. A moderate level of knowledge about HBV among participants was noted, suggesting the need for more studies to assess the knowledge level after the implementation of educational programs [42].

Most medical students in India (84.8%) were aware of hepatitis B infection, and 77.3% stated that it is an infectious disease. Additionally, 71.2% of students knew that hepatitis B is caused by a virus, 81.06% of students knew that it can be transmitted through blood transfusion, and 74.1% knew that it can be transmitted through contaminated needles and syringes. However, knowledge about other modes of transmission, such as sexual contact (28.8%), mother-to-child transmission (23.5%), and piercing and tattooing (31.1%) was poor. Students had poor knowledge about the clinical features of acute hepatitis B infection, with only 72.7% knowing that it can cause jaundice. Knowledge about other clinical manifestations such as fever (42.4%), loss of appetite (25.8%), and nausea and vomiting (28.0%) was also poor. There is a lack of precise knowledge and aware-

ness about hepatitis B among medical students. Reforms in the educational curriculum are necessary to promote knowledge and awareness among medical students, including knowledge about transmission, complications, and the need for health education regarding hepatitis B [43].

The study included 260 healthcare workers from two medical units in Tehsil Wazirabad, Gujranwala district. Participants had sufficient knowledge about the transmission of hepatitis C, its symptoms, and its effects on the liver. However, there were misconceptions about the transmission of hepatitis C from mother to child during childbirth, with 23% stating it is not possible. Most respondents (53.1%) stated that hepatitis C may not survive at room temperature. Respondents demonstrated sufficient knowledge about the symptoms of hepatitis C, its transmission, and its long-term effects on the liver. However, they were unaware of the transmission of hepatitis C from mother to child during childbirth and had no knowledge of updated treatment plans. The study highlights the need for improved education and awareness among healthcare providers regarding the transmission and treatment of hepatitis C [44].

A study conducted on 356 medical workers in Tehran, Iran concluded that 44.9% of participants were aware that viral hepatitis B and C can be transmitted between patients, dentists, and vice versa. Knowledge about transmission routes, prevalence, protection, and post-exposure seroconversion rates of HBV and HCV was unsatisfactory among participants. Complete vaccination against HBV was done by 88.1% of participants, and 83.8% had positive surface antibodies against hepatitis B (anti-HBs). Only 24% of surgeons often used double gloves, and 28% reported needle stick injuries. There was no significant correlation between different specialties and concern about HBV and HCV, inadequate reporting of accidental needle sticks, and correct knowledge of post-exposure management. Participants' knowledge about HBV and HCV was unsatisfactory, indicating the need for further education. Medical workers need to be well informed to improve the knowledge, attitudes, and behaviors of other healthcare workers and patients regarding HBV and HCV [45].

A study conducted at a tertiary care center in Lucknow, India, evaluated the knowledge and awareness of hepatitis B and C infection among auxiliary staff. In 2015, the awareness of transmission routes and vaccination for hepatitis B and C infection was unsatisfactory. There was a direct positive correlation between education level and awareness, indicating that participants with higher knowledge had better attitudes towards infection prevention. Most participants in both 2015 and 2017 were not fully vaccinated against hepatitis B and were unaware of the availability of post-exposure prophylaxis. The study concluded that in 2015, there was poorer knowledge and awareness among workers compared to 2017 regarding the dangers of hepatitis B, its mode of transmission, and prevention methods. The findings underscore the need to improve the level and quality of health education, enhance the accessibility and availability of vaccines, and implement effective health programs to prevent the spread of hepatitis B and C viruses [46].

A study conducted in Saudi Arabia among 890 individuals aged 20 to 50 years found that 69.3% were women, 48.3% were employed, and 77% had a university degree. Only 8% of subjects reported having contracted hepatitis B. Most participants showed a solid understanding of transmission methods, definition, symptoms, and consequences of the disease. However, due to a lack of testing or vaccination for hepatitis B infection, the practice level was diminished in 66% of subjects. Regarding hepatitis B, a notable 56% of subjects demonstrated satisfactory levels of knowledge, attitude, and practice, with commendable scores in knowledge and attitude but lower practice levels among most participants [47].

Another study conducted among medical students in Tamil Nadu, India, assessed the distribution of participants by academic year, noting that 25.4% were in the first year, 22.4% in the second year, 24.9% in the third year, and 27.3% in the fourth year of study. The findings revealed that medical students exhibited commendable performances regarding their knowledge, attitudes, and practices related to viral hepatitis prevention. Specifically, correct responses provided by participants reached 77.07% for knowledge, 77.56% for attitude, and 76.59% for practices related to viral hepatitis prevention. Regarding the vaccination status of medical students, a substantial majority of 79.5% received complete immunization against hepatitis B, while the remaining 20.5% received only partial vaccination. Based on the obtained results, it can be concluded that increasing awareness among medical students regarding hepatitis B infection and ensuring their active immunization are imperative measures to be taken to effectively control virus transmission. Additionally, it is recommended that medical students and paramedics undergo periodic retraining sessions to stay updated with current knowledge regarding universal precautions, post-exposure prophylaxis, and hospital waste management [48].

A study conducted in Australia among 194 homosexual and bisexual men reported a substantial level of understanding regarding viral hepatitis C. However, only 76% were knowledgeable about the accessibility of new treatments for HCV. A significant proportion of men, one in six, expressed uncertainty about their personal history of hepatitis C testing. This lack of certainty implies a deficiency in awareness or knowledge about their health status [49].

The study conducted in Italy in 2021-2022 aimed to analyze the level of understanding regarding hepatitis C virus infection and awareness of HCV screening tests in Italy before the initiation of awareness campaigns in 2022. The median knowledge score was 75%, while the median score for prevention and transmission knowledge was 46.2%. A total of 23.2% of participants had no knowledge of the existence of HCV screening tests. Factors such as higher education, pursuing a health-related field of study or profession, history of accidental injury, HCV infection, and active seeking of HCV information were positively associated with the Disease Knowledge Score. LGBT men had a significant-

ly lower Disease Knowledge Score. Participants affected by hepatitis C had a negative association with the Prevention and Transmission Knowledge Score. The study highlighted a concerning lack of knowledge regarding prevention and transmission, underscoring the need for targeted educational campaigns. Future research should focus on assessing the effectiveness of awareness campaigns [50].

A study conducted among dental students in Saudi Arabia in 2019 found that the level of knowledge regarding hepatitis B infection was insufficient, with only 7.6% of participants possessing a commendable level of knowledge. However, participants exhibited a positive attitude towards patients infected with hepatitis B. Nonetheless, 48.8% of participants exhibited an attitude below the average level. Regarding practice, 47.2% of participants demonstrated a high level of practice, while 22% exhibited a low level of practice. When comparing male and female participants, it was observed that female participants had higher knowledge, attitudes, and practices. The study ultimately inferred that implementing continuous education courses for health is imperative to enhance the knowledge of dental students and practitioners regarding hepatitis B infection. The study also emphasized the importance of infection control measures, including the use of personal protective equipment, to prevent cross-infection in dental clinics [51].

The study conducted in the Gauteng province, South Africa in 2015 showed that the level of general knowledge regarding viral hepatitis among healthcare workers was inadequate, as indicated by an average knowledge score of 2.0 out of 6. However, the average scores for both practice and attitude towards notification were higher, with scores of 2.9 out of 4 and 3.3 out of 5, respectively. Lack of training, limited knowledge, a complex notification process, and excessive workload were identified as the main factors contributing to insufficient knowledge regarding viral hepatitis. The study's conclusions suggest that to enhance the level of knowledge, it is imperative to provide adequate training on viral hepatitis, the notification process, and the roles and responsibilities of healthcare workers [52].

A study conducted in Northern Ethiopia among medical students showed that the majority of study participants possessed sufficient knowledge regarding risk factors, modes of transmission, and prevention of HBV infection. Approximately 83.3% of participants demonstrated a positive inclination towards adhering to infection control protocols, while 81.7% considered it of utmost importance for all healthcare professionals to receive HBV immunization [53].

The seroprevalence of HBV infection among medical and medical science students in Northeast Ethiopia was found to be 4.2% (95% CI 2.5 to 6.1%), and for hepatitis C virus (HCV) infection, it was 0.7% (95% CI 0.0 to 1.7%). Advanced age and needle stick injuries were associated with a higher risk of HBV infection. Most students (80.1%) had adequate knowledge about HBV and HCV infection, modes of transmission, and preventive measures. However, only 50.0% of students practiced safe behavior regarding the occupational risk of viral hepatitis infection. Nearly half (49.8%) of the students experienced a needle stick injury, only 53.2% reported the incident, and only 39.4% underwent screening tests for viral hepatitis. The study highlights the need for regular HBV vaccination among medical and medical science students before their clinical years. Despite having good knowledge about transmission and prevention measures, students exhibited inadequate practice regarding the occupational risk of viral hepatitis infection. Guidance on HBV and HCV transmission and prevention, with special emphasis on occupational accidents and post-exposure prophylaxis, is warranted [54].

A study conducted in Egypt, which included 308 barbers and 308 clients aged 20 to 40 years, found that nearly half of both barbers and clients had a moderate level of education, while a third of the clients had a higher level of education compared to only 7.8% of the barbers. The prevalence of HBsAg was 4.1% among both barbers and clients, with no significant difference between the two groups. The prevalence of anti-HCV antibodies was 12.5% among both barbers and clients. The prevalence of HBV and HCV infections among barbers was like that among their clients. This could be attributed to the good knowledge, positive attitudes, and good practices observed among many barbers, as well as the hygiene conditions in barber shops. The study revealed a similar rate of HBV and HCV infection among barbers and their clients compared to the national prevalence. Barbers appeared to have no workplace-related risk of acquiring viral hepatitis due to their good knowledge, positive attitudes, and good practices. The study highlights the importance of maintaining hygiene and implementing safe practices in barber shops to prevent the transmission of viral hepatitis [55].

A cross-sectional survey of barbers in Hyderabad, Pakistan, showed that 96.2% washed shaving instruments with antiseptic after each client, and 95.7% used a new blade for each client. However, only 36.6% of barbers knew that hepatitis could be transmitted through shaving instruments, indicating poor knowledge about diseases and modes of transmission. Out of 186 surveyed barbers, only 3.2% were vaccinated against HBV. Most barbers were aged between 15-30 years (69.9%) and had primary education (41.9%). The study found that barbers in Hyderabad lacked a detailed understanding of hepatitis transmission, despite numerous health education programs in the media in Pakistan. Barbers have low awareness of hepatitis and the risk of transmitting infectious agents through the reuse of razors and scissors. The study emphasizes the need for awareness strategies and regulation of barber practices to prevent the transmission of HBV and HCV [56].

Another study conducted in Mumbai on a sample of 163 pregnant women regarding knowledge, attitudes, and practices regarding HBV found that only 43 understood hepatitis B, indicating insufficient knowledge among participants. Healthcare workers were the main source of information. A substantial proportion of participants were unaware of the accessibility of the hepatitis B vaccine, modes of HBV transmission from mother to child, through contaminated blood,

and through unprotected sexual contact. Most participants erroneously stated that HBV is a bacterium, while a small number were aware that it is localized in the liver. None of the participants were informed about the National Viral Hepatitis Control Program (NVHCP) launched by the Government of India and its provisions. The study underscores the need to instill awareness about hepatitis B among pregnant women, considering that a considerable number of participants exhibited a lack of knowledge [57].

A study conducted in France found that the general population had a lower awareness of HBV transmission modes compared to HIV, with less knowledge about needle sharing during intravenous drug use and sexual intercourse as modes of transmission. Fear of both HBV and HIV was similar, with 20.3% of respondents reporting fear of both diseases. The perceived individual risk of infection was higher for HBV than for HIV, with 60.8% of respondents believing they have an equal or greater risk of being infected with HBV. However, HBV screening rates were lower than HIV screening rates, with only 27.4% of respondents reporting HBV screening in their lifetime, compared to 61.4% for HIV. Nearly half of the respondents (47%) reported HBV vaccination, but there was no significant difference in vaccination rates based on endemicity or intravenous drug use. The study highlights the need to improve knowledge, perceptions, and practices regarding HBV in the French general population, especially regarding sexual transmission, to enhance screening and vaccination practices [58].

The research conducted in Bantama, Ghana, showed that healthcare workers exhibited suboptimal knowledge, attitudes, and practices regarding hepatitis B. The mean scores for knowledge, attitude, and practice were 13.691 ± 2.81, 6.685 ± 2.28, and 2.23 ± 1.19, respectively. Age, occupation, and experience were significantly associated with average knowledge scores ($p < 0.05$). Significant positive correlations were observed between knowledge-attitude, knowledge-practice, and attitude-practice. The survey concluded that policy guidance and extensive health education campaigns are needed to improve knowledge, attitudes, and practices among healthcare workers [59].

A study among HBV-infected patients at the Hepatology Medical Center in Dhaka City, Bangladesh, reported that 19.3% of respondents were injectable drug users, among whom 27.6% shared needles. Additionally, 28% of respondents had a history of blood transfusion, 77% practiced polygamy, and only 38.8% used protection during sexual activity. Furthermore, 70.7% of respondents did not receive the HBV vaccine. There was a significant association between education and HBV vaccination [60].

The study conducted in Australia among drug users found that 64% had HCV infection, indicating a high prevalence of HCV among injecting drug users. Knowledge scores regarding HCV were moderate, with an average score of 6.5 out of 12. However, knowledge of modifiable factors affecting HCV disease progression was particularly low, highlighting the need for continuous education. Factors associated with higher knowledge scores included female gender, higher formal education, being on a current opioid substitution therapy program, and older age. The study emphasizes the need for ongoing education about HCV, especially regarding modifiable factors affecting disease progression. Improving knowledge about the long-term consequences of HCV-related liver disease and the availability of effective treatment is crucial for expanding HCV assessment and treatment among drug users [61].

The study in South Kivu, Democratic Republic of Congo, found that the overall level of knowledge about hepatitis B (HBV) and hepatitis C (HCV) among healthcare workers was low. The average proportion of correct responses regarding basic knowledge of HBV and HCV was 33.2% and 30.6%, respectively. There was no statistically significant difference in knowledge regarding age, gender, marital status, and years of professional experience among participants. However, there was a statistically significant difference in knowledge scores between professional categories, with specialists having the highest number of correct responses. The study also revealed that 42.8% of healthcare workers reported recent experiences of blood exposure accidents, with medical assistants experiencing this more frequently than doctors. Only 24.4% of participants were vaccinated against HBV, with doctors having a higher vaccination rate compared to medical assistants. The study concludes that the low level of knowledge about HBV and HCV among healthcare workers in South Kivu is a cause for concern. It highlights the need for concrete strategies to reduce the prevalence of these infections, including mandatory vaccination of all healthcare workers, improving working environments, and ongoing training on bloodborne diseases. The study underscores the importance of optimizing practitioners' knowledge to positively influence preventive attitudes toward HBV and HCV, such as vaccination and universal precautions against biological fluids [62].

The results of a study conducted among the general population in Brazil in 2016 showed that knowledge levels regarding the transmission of HBV and HCV through unprotected sexual contact were 33.1% and 34.3%, respectively. The percentage of individuals who correctly identified all modes of transmission for both HBV and HCV were substantially lower than those who accurately identified transmission routes for each specific infection. Women had a higher percentage of accurate knowledge regarding transmission routes compared to men, especially concerning dental procedures, dialysis, endoscopy, and unprotected sexual intercourse, needle sharing, as well as tattooing or piercing. The study highlighted the need to improve screening practices and knowledge about hepatitis B and C within the general population, with a specific focus on young people and those with low socioeconomic status [63].

The study conducted in Bangladesh in 2018 reported that the prevalence of hepatitis B virus infection in the adult population was 2.35%, indicating a decrease. The prevalence of hepatitis C virus infection was reported to be 0.13%. No significant relationship was found between demographic factors such as religion, locality, and occupation

of the studied population and hepatitis infection. Among the infected population, approximately 90% had an educational status below the secondary level, belonged to a low-income group, and 60% were unemployed. Most infected individuals, seeking employment, lacked adequate knowledge about the disease, modes of hepatitis virus transmission, and preventive measures, including vaccination. The study concluded that knowledge about hepatitis was poor among the infected population, emphasizing the need for awareness campaigns and preventive measures to stop the transmission of infections [64].

In South Korea, out of 1,003 study participants, 56.4% said they knew about HCV, 44.4% understood that HCV is transmissible, and 56.8% stated that HCV is curable through medication. Only 9.1% of participants disclosed that they had been tested for anti-HCV antibodies, with 11.0% obtaining positive results. Out of the 91 individuals who tested positive, a total of 8 received treatment. The most common motivations for HCV testing were health check-ups (58.5%), doctor's recommendations (11.0%), and elevated liver enzyme levels (10.7%). The study concluded that the level of knowledge regarding HCV falls below optimal levels, and the self-reported testing rate for HCV remains below 10%. However, once HCV infection is diagnosed, treatment rates appear to be high in South Korea [65].

Another study found significant gaps in physicians' knowledge about hepatitis C, especially in areas such as transmission, testing, and treatment options. The study identified several barriers to care, including limited access to testing and treatment, lack of patient education, and the stigma associated with hepatitis C. Overall, the findings highlight the need for improved physician education and increased access to testing and treatment for hepatitis C [66].

Discussions

Identified gaps in knowledge among healthcare personnel in locales such as South Kivu and Bukavu, Democratic Republic of Congo, signal crucial deficiencies in their grasp of viral hepatitis B and C. This underscores the urgent necessity for tailored educational initiatives within healthcare systems to ensure frontline workers are equipped with accurate information regarding prevention, transmission, and treatment modalities. The hurdles encountered in vaccination efforts, notably the low uptake among healthcare workers, underscore the imperative to enhance accessibility to vaccination programs and raise awareness about the advantages of vaccination. Addressing these challenges not only safeguards the health of healthcare workers but also contributes to alleviating the overall burden of viral hepatitis within communities. The findings on risk factors and practices among specific demographics, such as barbers and clients in Gharbia Governorate, Egypt, offer valuable insights into the intricacies of disease transmission within high-risk occupational settings. Patients should be informed about the modes of transmission, symptoms, and complications of viral hepatitis B and C. A more integrated and coordinated approach is necessary to improve early diagnosis and treatment of viral hepatitis. Public health policies should prioritize education and prevention of viral hepatitis through awareness campaigns. Greater collaboration between governmental and non-governmental organizations is needed to address knowledge and practice gaps related to viral hepatitis. Education about viral hepatitis should be included in school programs and public health materials. Periodic evaluation of the knowledge, attitudes, and practices of the population regarding viral hepatitis is important to adapt interventions and prevention programs. The development of adequate educational and informational resources can improve understanding and management of viral hepatitis among the general population. Greater involvement of the media in disseminating accurate and updated information about viral hepatitis is necessary. Continuous education and professional training should be priorities for doctors and healthcare workers to improve diagnosis and management of viral hepatitis.

Conclusions

The overall level of knowledge regarding viral hepatitis B and C remains inadequate among both medical personnel and the general population. There is a growing need for education and awareness about viral hepatitis B and C among healthcare workers. The results of this systematic review underscore the complexity and diversity of knowledge, attitudes, and practices within the population regarding viral hepatitis B and C. Identifying influencing factors and specific gaps can help develop appropriate strategies to increase awareness and provide education.

Competing interests

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Ethics approval

Not needed for this study.

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REVIEW ARTICLE

Anti-vascular endothelial growth factor (anti-VEGF): its function in proliferative diabetic retinopathy management

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ABSTRACT

Introduction. Among working-age adults, diabetes is a primary cause of visual impairment. Pan-retinal photocoagulation, the standard treatment for proliferative diabetic retinopathy, is effective but comes with well-established adverse effects, including limitations on the peripheral visual field. The mechanism of vascular proliferation is thought to be triggered by vascular endothelial growth factor (anti-VEGF). Anti-VEGF medications have been studied extensively in the treatment of diabetic macular edema, and the results suggest that treatment with anti-VEGF medications causes a decrease in diabetic retinopathy. Anti-VEGF therapies can be used to treat underlying proliferative diabetic retinopathy in cases of vitreous bleeding when platelet-rich plasma cannot be used, delaying, or reducing the necessity for a vitrectomy. However, the limitations of anti-VEGF therapy require careful patient selection and constant observation. Recent clinical trials and recommendations for the use of anti-VEGF in proliferative diabetic retinopathy are presented in this review.

Material and methods. The effectiveness of anti-VEGF medicines in the treatment of diabetic retinopathy was the subject of a comprehensive review of the scientific and medical literature. A structured search was performed in the PubMed, Scopus, and HINARI databases, considering relevant articles published in the last 10 years. The search terms used (in English) were: "angiogenesis inhibitors", "anti-VEGF", "pan-retinal photocoagulation", "intravitreal injection", "diabetic retinopathy". Accurate diagnosis, side effects, quality of life, and patient satisfaction were analyzed and compared for each treatment option.

Results. Anti-VEGF treatments have been demonstrated to be beneficial in reducing macular edema, enhancing visual acuity, and slowing the advancement of diabetic retinopathy. While generally safe, different anti-VEGF medicines have varied side effects profiles.

Conclusion. When choosing an anti-VEGF treatment for diabetic retinopathy, factors such as patient satisfaction, quality of life, side effects, and correct diagnosis should be taken into account. While anti-VEGF treatments show promise, further study is required to fully understand their advantages and disadvantages and to optimize their application.

Keywords: angiogenesis inhibitors, anti-VEGF, pan-retinal photocoagulation, intravitreal injection, diabetic retinopathy.

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Key messages

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

Anti-VEGF therapy has been shown to be more effective than laser photocoagulation in treating mild to severe vision impairment related to diabetic macular edema.

The research hypothesis

Although anti-VEGF therapy has been demonstrated to have better results, laser photocoagulation has been the conventional treatment for diabetic macular edema.

The novelty added by manuscript to the already published scientific literature
In high-risk proliferative diabetic retinopathy patients, the combination of anti-VEGF therapy and panretinal photocoagulation has shown significant improvements in visual acuity, central foveal thickness, and microaneurysms.

Introduction

Diabetes affects 425 million people worldwide today and is expected to affect over 690 million by the year 2045. It is a major public health concern. The Republic of Moldova's Statistical Yearbook provides data on the overall population with diabetes and those receiving insulin treatments. However, for a thorough evaluation of the effects of diabetes on the healthcare system, this data is insufficient. A survey was conducted among family physicians to gather more information about individuals with diabetes, including age, type, duration, BMI, HbA1c, total cholesterol, presence of amputations, and presence of cardiovascular pathologies. According to information submitted by 57.3% of family physicians in the Republic of Moldova, there were 60.000 patients with diabetes mellitus registered in 2019 [1]. There is no diabetes registry in the Republic of Moldova. To report diabetic patients, unified guidelines are required for determining target values, treatment features, and the presence and stage of chronic complications. The establishment of a diabetes registry will benefit healthcare providers and the overall system by enabling the collection of information needed for more effective time and resource management [1]. Among patients with diabetes, 4.4% had vision-threatening retinopathy, and 28.5% showed signs of diabetic retinopathy. Proliferative diabetic retinopathy (PDR) is characterized by the growth of neovessels, which are prone to tractional retinal detachment, hemorrhage, and the formation of vitreoretinal membranes. These vessels may also invade the anterior segment, leading to ischemia or neovascular glaucoma. PDR carries a significantly higher risk of vision loss than nonproliferative diabetic retinopathy (NPDR), and it is more common in patients with type 1 diabetes, who are typically younger [2, 3]. For many years, panretinal photocoagulation has been the standard treatment for PDR. Recent results, however, show that PDR can be treated with intravitreal injections of anti-VEGF without leading to photocoagulation of the peripheral retina. This review aims to highlight the limitations of anti-VEGF treatment, which call for prudent patient selection and monitoring, as well as to explain the positive outcomes of anti-VEGF use in PDR [4].

Materials and methods

The effectiveness of anti-VEGF medicines in the treatment of diabetic retinopathy (DR) was the subject of a comprehensive review of the scientific and medical literature. A structured search was performed in the PubMed, Scopus, and HINARI databases, considering relevant articles published in the last 10 years. The search terms used (in English) were: "angiogenesis inhibitors", "anti-VEGF", "pan-retinal photocoagulation", "intravitreal injection", "diabetic retinopathy". Accurate diagnosis, side effects, quality of life, and patient satisfaction were analyzed and compared for each treatment option. Original articles, meta-analyzes and systematic reviews were selected.

Results and discussion

After processing the information from the PubMed, Scopus and HINARI databases, according to the search criteria, 189 articles on anti-VEGF factor in diabetic retinopathy management were selected. The final bibliography contains 26 relevant sources, which were considered representative of the material published on the topic of this synthesis article. Publications that did not reflect the research topic, as well as articles that were not accessible through the HINARI database, were excluded.

Anti-VEGF treatments have been demonstrated to be beneficial in reducing macular edema, enhancing visual acuity, and slowing the advancement of diabetic retinopathy. While being generally safe, there were variations in the negative side effect profiles of the various anti-VEGF medicines.

Relative retinal ischemia creates a proangiogenic setting in proliferative diabetic retinopathy, a microvascular disease.

The pathophysiology of diabetic retinopathy (DR) involves several mediators, one of which is vascular endothelial growth factor (VEGF). Increased vascular permeability and proliferation of vascular endothelial cells are caused by aberrant VEGF synthesis and release. VEGF also plays a crucial role in the pathophysiology of diabetic macular edema (DME). Additionally, it is a key mediator in the development of retinal neovascularization, which can lead to vitreous bleeding and tractional retinal detachment.

VEGF is a major mediator of angiogenesis. Several isoforms of the VEGF family are necessary for the proper growth of the lymphatic and blood vessels. Research on molecules has demonstrated that VEGF-A stimulated angiogenesis and vascular permeability by interacting with VEGF receptor 2 on vascular endothelial cells. This interaction leads to weakening of blood arteries due to the breakdown of capillary endothelial tight-junction and the formation of endothelial cell fenestration. Additionally, VEGF-A induces the migration and proliferation of endothelial cells, which are modifications associated with early angiogenesis. Pathologic revascularization in the retina of the eye has been linked to the VEGF-A165 splice variant of VEGF-A. VEGF injections into animal eyes resulted in a feature set that includes retinal edema, intrareticular vascular proliferations, vessel tortuosity, and intrareticular bleeding, similar to those observed in diabetic or ischemic retinopathy [4].

On the other hand, VEGF suppression stopped the neovascularization of the iris. Research on humans revealed

that compared to normal eyes, eyes with PDR exhibited greater amounts of VEGF in the vitreous or fibrovascular tissues [4].

VEGF refers to a group of factors that include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and VEGF-FPGF.

The first isoform is considered one of the main pathogenic factors associated with DR. Intravitreous anti-VEGF medications became available, and they had an important impact on the course of DR and the prognosis of patients, including a notable decrease in the prevalence of legal blindness. Anti-VEGF molecules now commonly affect several VEGF isoforms and metabolic pathways. While using anti-VEGF as a first line treatment helped manage DME, there is significant disagreement regarding how to treat the proliferative type [5-7].

The use of laser techniques, delivered through various strategies (immediate, delayed, etc.), in the anti-VEGF era is still the subject of much debate in the literature. We provided a general overview of VEGF-targeting medications and their mechanisms of action to prevent the growth of retinal neovascularization in diabetic retinal disease.

Using the terms "proliferative diabetic retinopathy" and "anti-VEGF" a PubMed literature search was conducted to find studies published up until January 2024. The articles from the Retinopathy Clinical Research Network (DRCRnet) were also examined.

Vascular endothelial growth factor: methods of action and biochemical characteristics in diabetic retinopathy.

Over 100 million people worldwide suffer from diabetic retinopathy (DR), the most prevalent microangiopathic consequence of diabetes mellitus [2, 3]. In developed countries, DR is a leading cause of blindness [7]. The two traditional classifications of DR are proliferative (PDR) and non-proliferative (NPDR). NDPR is characterized by retinal hemorrhages, cotton-wool exudates, microaneurysms, intraretinal microvascular abnormalities (IRMA), peripheral and macular capillary low-perfusion, and is further divided into different stages. The onset of neovascularization marks the progression to PDR. In 5% cases, blindness is associated with neovascular glaucoma, while the majority of blindness cases result from posterior PDR problems such vitreous hemorrhage and retinal detachment [5, 6, 7].

The pathophysiology of diabetic retinal disease (DR) is primarily defined by the co-occurrence of neurovascular unit dysfunction, neoangiogenesis, inflammation, penetration of the blood retinal barrier, and capillary non-perfusion [7, 8]. Angiogenous and inflammatory mediators are produced in greater amounts because of all these abnormalities. In this case, VEGF is definitely a significant pathogenic component that describes DR and its complications. When ischemic or hypoxic stimuli are present in DR, VEGF synthesis is stimulated, leading to a number of changes at various levels. In fact, VEGF raises the phosphorylation of proteins involved in tight junctions, which in turn improves the permeability of retinal capillaries [9]. Induced changes of many intercellular molecules, including occludin, catenins, and cadherins, enhanced transcytosis, and activated NOS-mediated processes, can also lead to VEGF-related hyperpermeability [10].

The initiation and progression of a typical DR-related complication, DME, involve a complex cascade of additional processes and mediators beyond the VEGF-related phenomena, which are primarily regulated by VEGF-A isoforms [11]. Additionally, despite the paucity of research, there is mounting evidence that VEGF-B and PGF play significant roles in the pathophysiology of DR. Strong survival cues on vascular and nonvascular cells can be promoted by VEGF-B, which can also induce neovascular phenomena towards non-inflammatory mechanisms. Similarly, PGF acts as a potent pro-angiogenic mediator, with a strong correlation between the severity of DR and the likelihood that it will advance in both serum and ocular concentrations. Therefore, it is widely agreed that VEGF inhibition plays a crucial role in the therapy of DR [12].

In relation to the neovascular problem, neoangiogenesis is promoted by endothelial cell migration and proliferation, triggered by enhanced VEGF synthesis and release. In fact, VEGF molecules stimulate proliferation of endothelium by activating receptors of tyrosine kinase, VEGFR-1 and VEGFR-2, which facilitates in the progression to PDR. VEGF contributes significantly to the neovascular process associated with DR, however it is only one element in a more intricate pathogenic cascade. It has been shown that the angiopoietin system can actually stimulate and amplify the effects of VEGF neovascular stimuli in addition to regulating vascular integrity. Moreover, neuropilin-1 (NRP1) functions as a co-receptor for VEGF receptor 2 and VEGF165 receptor, further influencing the neovascular process. [7, 13].

Another system involved in DR pathogenesis and in enhancing the neovascular stimulus provided by VEGF is the renin-angiotensin system. The severity of DR, measured by the progression rate of NPDR or the transition to PDR, is closely linked to the activity of the renin-angiotensin system. Given this context, the main therapeutic target of anti-VEGF medications is the VEGF-A isoforms. [7]. It is important to note that endogenous anti-VEGF systems already exist, however they are weakened in conditions affecting the retina, such DR. The physiologic anti-VEGF mechanisms are a subject that has not yet received much attention. Limited data was derived from animal models, which reported, for instance, VEGF165b isoform's anti-VEGF action. More specifically, VEGF165b appears to impede the migration and proliferation of endothelial cells as well as angiogenic stimulation brought on by hypoxia and VEGF overexpression [7, 14].

The following molecules are currently available as anti-VEGF drugs: Bevacizumab (Avastin®, Hoffmann-La Roche); Pegaptanib (Macugen, Eyetech/Pfizer); Ranibizumab (Lucentis®, Novartis Pharmaceuticals Canada Inc.); Aflibercept (Eylea®, BAYER Pharma AG, Germany); and Brolucizumab (Beovu®, Novartis Pharmaceuticals Canada Inc.) [7].

Aflibercept. VEGF Trap, or Aflibercept (Eylea®, BAYER Pharma AG, Germany), is a 115 kDa dimeric glycoprotein. These biochemical characteristics offer relative affinity for VEGF-B and high affinity for PGF and VEGF-A isoforms. Another form of the molecule is Ziv-aflibercept (Zaltrap; Sanofi-Aventis and Regeneron Pharmaceuticals, USA), and it differs from aflibercept mainly in that it has a higher osmolarity and different excipients, but otherwise shows nearly the same biochemical profile. Despite aflibercept's potential benefits in treating macular disorders, its use is still off-label [7, 12].

Bevacizumab. The 148 kDa completely humanized immunoglobulin G1 molecule bevacizumab (Avastin®, Hoffmann-La Roche) binds to VEGF-A isoforms. The intravitreal use of this antibody for retinal illnesses is still regarded as "off-label" treatment as it was initially created for cancer treatment. The basic mechanism of action of bevacizumab is to prevent the neovascular stimulation and VEGF-induced enhanced vascular permeability. It functions as a pure anti-VEGF antibody. Moreover, bevacizumab may interact with HIF-1, obstructing its ability to stimulate the synthesis of VEGF. Bevacizumab is a cost-effective and effective treatment for retinal disorders, according to multiple studies, but its off-label designation limits its use [14-16].

Ranibizumab. Ranibizumab, also known as Lucentis® by Novartis Pharmaceuticals Canada Inc., is a 48 kDa monoclonal antibody fragment (Fab) of humanized immunoglobulin G1κ isotype that binds to various VEGF-A isoforms and blocks their interaction with VEGF receptors 1 and 2. Its affinity for other VEGF-A isoforms (VEGF165, VEGF121, and VEGF110) may have been expanded due to the absence of a fragment crystallizable (Fc) domain and its short molecule size, which may have increased the molecule's penetration into the retina and choroid [7 -11]. Because ranibizumab has a single VEGF binding site, two molecules of the antibody bind to a single VEGF dimer. This unique arrangement enables the ranibizumab/VEGF-A complex to have a higher molecular affinity for VEGF than bevacizumab and aflibercept and a higher stability energy than bevacizumab. Numerous clinical trials, such as READ-2, RESOLVE, RESTORE, RISE and RIDE, LUCIDATE, REVEAL, RELIGHT, RETAIN, and READ-3, explored the use of ranibizumab in various modalities and concentrations, as well as alone or in combination with laser. In order to compare ranibizumab to other methods of managing DR, such as corticosteroids, lasers, or other anti-VEGF molecules, the DRCR network conducted several multicenter clinical trials. These trials included Protocol S (ranibizumab vs. laser in PDR), Protocol T (ranibizumab vs. aflibercept vs. bevacizumab in DME), and Protocol I (fluocinolone acetonide vs. ranibizumab plus deferred laser).

Other notable ranibizumab clinical trials included ROTATE (ranibizumab in persistent DME after bevacizumab treatment), RELATION (ranibizumab plus laser vs. laser alone in DR), REFINE (ranibizumab vs. laser in DME), and TREX-DME (ranibizumab "treat and extend" regimen with or without laser in DME) [7, 17-22].

Brolucizumab. The purpose of brolucizumab (Beovu®, Novartis Pharmaceuticals Canada Inc.) is to reduce molecule size and improve affinity for VEGF-A isoforms in comparison to other molecules. A novel 26 kDa single-chain antibody fragment lacks the Fc portion. In comparison to other anti-VEGF molecules, brolucizumab has demonstrated non-inferiority and better penetrance inside the retina and choroid, leading to its recent approval for the treatment of neovascular age-related macular degeneration. Regarding DR, the ongoing KITE and KESTREL clinical studies have shown promising initial outcomes when it comes to the use of brolucizumab in DME when compared to aflibercept, indicating that it will soon be approved for the management of DR [7,22].

Two novel anti-VEGF compounds that are presently being researched in this area. A new generation antibody biopolymer conjugate called KSI-301 (KODIAK sciences, Palo Alto, CA) is being studied in two trials: a DAZZLE phase 2 trial (NCT04049266) and a phase 1b trial (NCT03790852). It is made up of a phosphorylcholine-based polymer that is specifically designed to prolong anti-VEGF activity and a humanized anti-VEGF monoclonal antibody. A clinical trial, Phase 2b (NCT0334582) is examining a VEGF-C/D inhibitor - OPT-302, for exudative macular degeneration. Moreover, a clinical trial, Phase 3 (NCT03610646) contrasting aflibercept with intravitreal MYL-1701P, a biosimilar recombinant fusion protein to aflibercept, is presently being conducted [7, 22-25].

PDR is a very challenging and potentially severe stage of DR. The use of panretinal photocoagulation has been the primary treatment for this complex form of DR. While it has a significant effect on the visual field, the irreversible destruction of peripheral ischemic retina is associated with a decrease in VEGF production and stabilization of the central retina. Most of the research mentioned earlier attempted to evaluate how anti-VEGF therapies affected peripheral ischemia and neovascularization regression. The key concern is whether anti-VEGF injections alone might replace panretinal photocoagulation. According to a 2014 Cochrane meta-analysis, the safety and effectiveness of anti-VEGF in PDR were found to have low levels of evidence, despite the fact that intravitreal injection use was associated with a moderate risk reduction. The viability and sustainability of DR patient care for hospitals and public health systems will be significantly impacted by the development of longer-lasting anti-VEGF treatments. This advancement is expected to shift current treatment indications away from laser approaches. Anti-VEGF molecules are the preferred treatment for the majority of DR patients, according to the EURETINA guidelines, due to their high efficacy, safety profiles, and manageability. Patients with significant cardiovascular risk may not be suitable candidates for this treatment; in these cases, alternative strategies, such as corticosteroids, should be used instead. For diabetic eyes with a very severe type of DR and for patients who are not able to comply with intravitreal therapy protocols, laser techniques remain useful [25, 26].

Conclusions

When choosing an anti-VEGF treatment for diabetic retinopathy, factors such as patient satisfaction, quality of life, side effects, and correct diagnosis should be taken into account. While anti-VEGF treatments show promise, further study is required to fully understand their advantages and disadvantages and to optimize their application.

Competing interests

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Ethics approval

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REVIEW ARTICLE

Bridging theory and practice: enhancing medical education through simulation-based training methods

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ABSTRACT

Introduction. With the complexities of contemporary healthcare systems and the paramount importance of high-quality patient care, Simulation-Based Medical Education (SBME) has emerged as a pivotal innovation in the process of training healthcare professionals. This study explores the integration of SBME in undergraduate medical education to bridge the gap between theoretical knowledge and clinical practice, thereby preparing students with the necessary competencies for effective healthcare delivery.

Material and methods. Employing a narrative review approach, this study meticulously examined relevant literature from multiple databases, including Google Scholar, PubMed, and MedEdPublish. Following objectives, we chose the sources that were best suited to explore our research questions, focusing on keywords such as „simulation", „undergraduate medical education", „simulation-based medical education", „theoretical frameworks", „procedural framework" „curriculum design", „training efficacy", and „training evaluation" with no restriction for the date of publications.

Results. The review identified foundational educational theories underpinning SBME, such as Experiential Learning Theory and Adult Learning Theory, and traced the evolution of simulation methods from simple anatomical models to sophisticated high-fidelity simulators and virtual reality technologies. Various simulation techniques, including task trainers, manikins, and standardized patients, were analyzed for their educational value. Significant benefits of SBME, such as enhanced safety, repeatability, and adaptability, were highlighted alongside challenges like high costs and limited access. Comparative analysis revealed SBME's advantages over traditional clinical education, particularly in learning efficiency and scalability.

Conclusions. SBME represents a transformative approach in undergraduate medical education, offering a dynamic and interactive learning environment that significantly enhances clinical skills, critical thinking, and confidence. Despite its challenges, the integration of simulation-based methodologies into medical curricula is essential for addressing the evolving needs of medical training and improving patient care outcomes. Future research should focus on longitudinal studies to assess the long-term impact of SBME on clinical practice and explore the integration of emerging technologies to enhance the efficacy and accessibility of simulation-based training.

Keywords: simulation-based medical education (SBME), undergraduate medical education, clinical skills development, educational technologies, healthcare training.

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Key messages

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

The manuscript identifies gaps in understanding the long-term im-

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pacts of simulation-based medical education on clinical outcomes. It calls for more research on integrating technologies like virtual reality and artificial intelligence to evaluate their efficiency and cost-effectiveness.

The research hypothesis

The article suggests that adding Simulation-Based Medical Education to medical curricula can enhance the connection between theory and practice, improving learning and patient care.

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

The article outlines frameworks merging simulation with medical education to build flexible competencies, showing their benefits for learning and patient care, and emphasizing the transformative role of technologies like virtual reality and artificial intelligence.

Introduction

Simulation-based medical education (SBME) has emerged as a cornerstone in training healthcare professionals, offering a dynamic and interactive learning environment that mirrors clinical realities without risking patient safety. It allows learners to practice procedures, make decisions, and manage patient encounters in a controlled setting, thereby enhancing their clinical skills, critical thinking, and confidence before transitioning to real-world practice. The significance of SBME lies in its ability to bridge the gap between theory and practice, providing a platform for experiential learning that is essential in preparing competent and efficient healthcare providers [1, 2].

This paper discusses the integration of simulation-based methodologies within undergraduate medical education to address the evolving needs of medical training. The complexity of modern healthcare systems and the imperative for high-quality patient care necessitate innovative educational approaches that can adequately prepare medical students for the challenges of clinical practice. Simulation-based approaches are pivotal in this regard, as they offer a versatile and effective means of teaching clinical skills, enhancing decision-making abilities, and fostering professional competencies in a safe and supportive environment [3, 4]. By using this approach, we can address current educational challenges, such as the need for safe, effective, and patient-centered training methodologies that can adapt to the rapid advancements in medical science and technology.

This study aims to facilitate the identification of optimal strategies for the integration of simulation-based training into university curricula. This work aims to improve the quality and effectiveness of medical education by creating a framework that uses effective simulation techniques, resulting in graduates with versatile and widely applicable professional skills. [5, 6]. Furthermore, it seeks to explore the various simulation techniques and methodologies, while also assessing their respective merits and drawbacks. Ultimately, the study aims to improve understanding of how to customize simulations to meet specific needs, considering available facilities and training goals. The relevance of this research lies in its potential to transform medical education by aligning teaching methods with the demands of contemporary clinical practice, ultimately improving patient care outcomes.

Material and methods

This article is a narrative review of the literature to explore crucial elements pertaining to our topic of interest – the application of simulation methods in undergraduate medical education. Our research started by identifying and choosing relevant sources of information. We employed specific keywords pertaining to the realm of medical education and meticulously examined the titles and abstracts of articles discovered in scholarly databases such as Google Scholar, PubMed, and MedEdPublish. Subsequently, we selected sources that proved to be most appropriate for addressing our research inquiries, including „simulation", „undergraduate medical education", „simulation-based medical education", „theoretical frameworks", „procedural framework", „curriculum design", „training efficacy", and „training evaluation", without imposing any restrictions on the publication date. We extracted and analyzed relevant data, identifying patterns and trends in the literature. It is noteworthy to mention that this literature review is non-systematic in nature, signifying that the selection of sources was conducted in accordance with our research objectives, but without the inclusion of a formal and rigorous selection process, as would be the case in a systematic review.

Results

Our exploration sheds light on the foundational theories underpinning medical simulation, exploring the diverse array of methods and techniques currently available and discussing their respective advantages and disadvantages in educational settings. It delves into the management of the professional training process, presenting a detailed view on procedural framework for simulation-based training. Towards the conclusion, it engages in discussions that synthesize the findings and draws conclusions, thereby providing a comprehensive overview of the state of medical simulation in professional training and education. Through this review, we aim to offer insights into how SBME can be effectively in-

tegrated and managed within medical education curricula, highlighting its potential benefits and challenges.

Theoretical framework

Simulation-based education in medical training is underpinned by several foundational theories that explain its effectiveness and importance. *Experiential Learning Theory*, posited by Kolb [7], argues that learning is a process where knowledge is created through the transformation of experience. SBME provides a direct experience which is reflective, supporting the cycle of learning from concrete experiences to reflective observation, abstract conceptualization, and active experimentation. Similarly, *Adult Learning Theory* [8], or *Andragogy*, emphasizes the importance of self-directed learning and bringing life experiences into the learning process, which is intrinsic to simulation-based education as it allows learners to engage in realistic clinical scenarios that mirror their future responsibilities.

The evolution of simulation-based methods in medical education has been significant over the decades, from simple anatomical models in the Renaissance to the sophisticated high-fidelity simulators and virtual reality technologies of today. One of the key milestones was the development of the first life-sized manikin in the 1960s, known as "Resusci Anne," for CPR training. Since then, the field has seen rapid advancements with the introduction of computer-based simulations in the 1980s, the proliferation of high-fidelity simulators in the 2000s, and the recent integration of augmented and virtual reality technologies [9]. These developments have been driven by the increasing need for safe, effective, and efficient ways to teach complex clinical skills and procedures [10].

The pedagogical foundation of simulation in education are deeply rooted in its ability to bridge theoretical knowledge and practical skills. Simulation acts as a critical bridge between classroom learning and real-world clinical practice, offering a controlled environment where students can apply theoretical knowledge to practical situations without the risk of harming patients. This pedagogical approach is supported by the *Constructivist Theory*, which posits that learners construct new knowledge based on their experiences. Simulation allows for the application of this theory by providing realistic clinical scenarios that require students to use critical thinking, clinical reasoning, and decision-making, thereby facilitating deep learning and the integration of knowledge into practice [2].

Simulation-based methods in medical education

SBME employs a variety of techniques, each designed to target specific learning objectives and competencies. Task trainers are devices that simulate specific parts of the human body, allowing students to practice procedures such as injections, suturing, or catheterization. They are beneficial for initial skill acquisition, offering repetitive practice without the need for a full-body manikin [2, 11]. High-fidelity manikins simulate full-body clinical scenarios, including vital sign changes, vocal responses, and physical findings. They are used for complex scenario-based training, such as emergency response, surgical procedures, and patient management, providing a realistic and immersive learning environment [10]. Standardized Patients (SPs) are trained actors who simulate patient scenarios in a consistent manner, allowing students to practice history-taking, physical examination, and communication skills [12]. SPs offer a unique opportunity for feedback and assessment of interpersonal skills in a clinical context [13, 14].

Simulation-based education has emerged as a pivotal tool in medical training, providing numerous benefits that enhance the learning experience. Central to its value is the provision of a risk-free environment, where learners can make mistakes and learn from them without the fear of causing harm to patients, thereby reinforcing the importance of safety in learning [3, 15]. This educational approach allows for the repeatability of procedures and scenarios, ensuring that learners can practice as many times as needed to achieve competence. Another significant advantage is the immediate feedback provided to learners, which is critical for their learning and ongoing improvement. Furthermore, the adaptability of simulation-based education means it can be customized to meet the diverse needs of learners and accommodate varying levels of complexity [16, 17].

Despite these benefits, SBME faces several limitations that challenge its widespread implementation. One of the primary concerns is the cost associated with high-fidelity simulations, such as advanced manikins and virtual reality (VR) technology, which can be prohibitive for many institutions. These simulations are also resource-intensive, requiring skilled personnel for scenario development, operation, and debriefing, which adds to the operational costs. Additionally, while high-fidelity simulations strive to replicate real-life scenarios accurately, they may still fall short in capturing the full spectrum of patient interactions and the unpredictability of real-life medical situations. Another significant issue is the limited access to these high-quality educational tools for some learners, particularly in resource-constrained environments, potentially exacerbating educational disparities [18].

Simulation-based methods and traditional clinical education approaches each have distinct advantages and roles in medical education [19]. Traditional approaches provide authentic patient interactions with real-time unpredictability, a key aspect that high-fidelity simulations strive to emulate but may not fully capture [4]. Simulation-based methods eliminate the ethical concerns and potential risks to patients that can arise with students practicing skills for the first time on actual patients [1]. Speaking about learning efficiency, simulations can be designed to target specific learning objectives and allow for immediate feedback and debriefing, which are not always feasible in a busy clinical setting. Some scholars assert that individuals who were exposed to simulation-based instruction for management of diverse medical scenarios and the execution of different diagnostic, therapeutic, and surgical processes exhibited superior acquisition compared to those who were provided with conventional education and training [20]. Traditional training approaches are constrained by the need for appro-

priate patients and clinical environments, while simulations offer the flexibility to accommodate large numbers of students through scalable, simulated scenarios of rare and critical conditions [21].

Management of the professional training process

Integrating simulation methods into the medical curriculum requires a systematic approach that encompasses curriculum design, execution, and evaluation. Designing a curriculum that incorporates simulation involves aligning simulation activities with learning objectives, ensuring that simulation experiences are integrated in the curriculum specifically where they will have the most impact. Execution requires logistical planning, including scheduling, resource allocation, and faculty training. Evaluation involves both formative and summative assessment of students, as well as evaluation of the simulation activities themselves to ensure they meet educational objectives. Effective integration also requires a feedback loop where outcomes from simulation activities inform curriculum development and refinement [2, 4].

The literature supports the effectiveness of simulation in achieving learning outcomes and improving patient safety [22]. SBME has been shown to improve knowledge, skills, and behaviors in a safe and controlled environment, leading to better preparedness for clinical practice [23-25]. Furthermore, simulation has been linked to improvements in patient safety [22], with studies demonstrating reductions in medical errors and adverse events as a result of simulation training. The deliberate practice within simulations allows for the refinement of clinical skills, critical thinking, and decision-making, which are crucial for patient care [1, 3].

Implementing simulation-based training in medical education presents several management challenges, including resource allocation, faculty development, and ensuring fidelity and realism in simulation scenarios. Solutions to these challenges include investing in technology to reduce costs, such as virtual and augmented reality simulations, and developing faculty expertise in simulation through targeted professional development programs. Additionally, creating partnerships with other institutions and sharing resources can alleviate some of the financial and logistical burdens. Ensuring fidelity in simulations involves not only the use of high-quality equipment and software but also the careful design of scenarios that accurately reflect clinical reality. Continuous evaluation and adaptation of simulation programs are essential for addressing these challenges and enhancing the effectiveness of simulation-based education [10, 26].

Procedural framework for simulation-based training

Existing frameworks for designing and implementing simulation-based training programs in medical education emphasize a structured approach to curriculum development, integrating simulation activities that align with educational objectives. One prominent model is the Kern six-step approach to curriculum development, which provides a systematic process for educational program design, including problem identification and needs assessment, setting objectives, selecting educational strategies (such as simulation), implementing these strategies, evaluation and feedback, and program refinement [27, 28]. There are several initiatives aimed at developing strategies for implementing simulation into the medical curriculum based on Kern's model. These include a stepwise model developed by Nehal N. Khamis *et al.*, which consists of problem identification and general needs assessment, targeted needs assessment, formulation of goals and objectives, selection of educational strategies, provision of individual assessment/feedback, and finally, program evaluation and implementation [29]. Similarly, a 7-step model implemented by Siyu Yan *et al.* employs a comparable approach [30]. Another framework was developed by International Nursing Association for Clinical Simulation and Learning, outlining key elements in designing and delivering simulation best practices in nursing education, including simulation design, outcomes and objectives, facilitation, debriefing, evaluation, and professional integrity [31]. The authors David Gent & Ranjev Kainth, from Faculty of Life Sciences and Medicine, King's College London, UK presents a comprehensive blueprint for designing effective simulation-based procedure training (SBPT) programs, emphasizing the integration of educational theory and practical design considerations for rarely performed medical procedures [32].

A potential enhancement in the efficiency of SBME could be achieved by implementing procedural frameworks that encompass a methodology relying on efficient techniques [33, 34]. These frameworks entail the acquisition of practical skills through a four-step process which involves demonstration, deconstruction, comprehension, and execution [35]. Additionally, the provision of feedback and the utilization of authentic medical environment are also crucial components in enhancing the effectiveness of SBME [36]. This approach aligns with experiential learning theory, emphasizing active engagement and reflection in the learning process. The use of real medical equipment and consumables further enhances the realism of the simulation, allowing students to gain hands-on experience that closely mirrors clinical practice. This methodology supports the development of transferable skills and competencies, bridging the gap between the simulated environment and real-world clinical settings.

Studies demonstrating the application and effectiveness of procedural frameworks in medical education highlight the positive impact of structured simulation-based training on learner outcomes. A systematic review by Cook *et al.* found that SBME with deliberate practice improves clinical skills, knowledge, and patient outcomes when compared to traditional clinical education [4]. Another study by McGaghie *et al.* demonstrated that using high-fidelity simulators and structured debriefing significantly enhances the acquisition and retention of clinical skills [1]. These studies underscore the value of a structured approach to simulation-based training, emphasizing the importance of realism, deliberate practice, and feedback in enhancing educational outcomes.

Discussion

The literature review highlights the significant impact of simulation-based methods on enhancing the profession-

al training process in medical education. Studies, including those by Issenberg *et al.* and Cook *et al.*, have demonstrated that simulation improves knowledge acquisition, technical skills, and professional competencies [1, 2, 4]. The integration of simulation into medical curricula supports a safe, controlled learning environment where students can practice and refine clinical skills without risk to patients [37]. These findings underscore the necessity for medical education programs to incorporate simulation-based training as a central component of curriculum design, emphasizing the development of transferable and generalizable skills that are critical for effective clinical practice [1]. While the benefits of simulation-based education are well documented, gaps remain in understanding the long-term impact of these methods on clinical practice and patient outcomes. There is a need for longitudinal studies that trace the professional development of medical students who have undergone simulation training into their clinical careers to assess the sustainability of competencies and the effect on patient care quality. Further research should also explore the integration of emerging technologies, such as virtual reality and artificial intelligence, in simulation-based training, evaluating their effectiveness in comparison to traditional methods. Additionally, studies on the cost-effectiveness of simulation-based education could provide valuable insights for institutions facing resource constraints [2, 4].

The potential of simulation-based methods to revolutionize undergraduate medical education is immense. By providing a realistic, immersive, and safe learning environment, simulation allows for the development of critical clinical skills, from technical procedures to decision-making and teamwork. The adaptability of simulation-based training to incorporate advances in medical science and technology further enhances its value, ensuring that medical education remains aligned with the demands of contemporary clinical practice. As medical education continues to evolve, the integration of simulation into curricula represents a pivotal shift towards more dynamic, effective, and patient-centered training approaches, promising to significantly improve learning outcomes and ultimately, patient care quality [1, 3].

Conclusions

Our literature review demonstrates the transformative impact of simulation-based methods on medical education. These methods not only enhance knowledge and skills acquisition but also ensure a safe environment for clinical practice without risking patient safety. The call for simulation's integration into medical curricula is clear, aiming to foster skills that are both transferable and applicable in real-world settings. However, the journey does not end here, future research directions is needed, including longitudinal studies to evaluate the long-term effects of simulation training on clinical practice, the exploration of new technologies like virtual reality, and analyses on the cost-effectiveness of such educational interventions. The promise of simulation-based education in revolutionizing medical training, by marrying technological advancements with the core demands of medical practice, heralds a new era of dynamic, effective, and patient-centered education. This evolution in teaching methodologies not only aims to enhance learning outcomes but also aspires to elevate the quality of patient care, marking a significant leap forward in the preparation of future medical professionals.

Competing interests

None declared.

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Authors' contributions

All persons listed as authors in the manuscript significantly contributed to the work. Substantial contribution to conception and design of the work (AR, IS). Drafting the article (AR, IS, IA, GR, OD, LS). Reviewing the article for important intellectual content (AR, IS, IA, GR, OD, LS). The final version of the manuscript was read and approved by all authors.

Ethics approval

Not needed for this study.

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CASE STUDY

Translabyrinthine approach in acoustic neuroma surgery – case report

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ABSTRACT

Introduction. Acoustic neuroma is a benign tumor that arises from the glial Schwann sheath junction of the vestibulocochlear nerve. It has an incidence of 1:100000 population. Despite the fact that this is a rare tumor, it accounts for approximately 6% of all intracranial tumors and 80% of tumors localized in cerebellopontine angle. Treatment of acoustic neuroma is represented by "Wait and Scan" monitoring, radiologic and surgical treatment. The latter remains the primary treatment for acoustic neuroma and consists of 3 main approaches: retrosigmoid approach, middle cranial fossa approach and translabyrinthine approach. Until now, in our country, acoustic neuroma surgery was done only by retrosigmoid approach.

Case presentation. The first translabirinthine surgery for acoustic neuroma in our country was done on 09.12.2021 on a 60-year-old patient who, during preparation for cochlear implant surgery, was accidentally diagnosed with 3rd grade right acoustic neuroma, according to Koos classification. Patient had cophosis on the right ear and moderate hearing loss in the left ear. During the surgery, a gross total resection of the tumor was accomplished. The patient was discharged from the medical institution on 20.12.2021 in a satisfactory condition. Magnetic resonance imaging performed 3 months and 1 year after the surgery showed no complications or tumor remnants.

Conclusion. The current report, which describes an accidental diagnosis of acoustic neuroma during preparation for a cochlear implantation surgery, resulted in acoustic neuroma surgery through the translabyrinthine approach. This serves as an eloquent example of why it is necessary to perform initially a magnetic resonance examination in cases of sensorineural hearing loss or tinnitus. The translabyrinthine approach in acoustic neuroma surgery allows for the removal of tumors of any size without affecting the brain, especially the cerebellum. In our case, where the patient had cophosis on the side of the tumor, this was the most appropriate surgical approach.

Keywords: acoustic neuroma, translabyrinthine approach, retrosigmoid approach, magnetic resonance imaging, hearing loss.

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Key messages

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

Acoustic neuroma is a rare brain tumor that, up to now, can only be diagnosed through magnetic resonance imaging. This article emphasizes the importance of magnetic resonance imaging in diagnosing acoustic tumors and the necessity performing it in all patients with sensorineural hearing loss.

The research hypothesis

In our case, the patient, who was prepared for cochlear implant surgery due to total hearing loss on one side, was accidentally diagnosed with an acoustic tumor during a magnetic resonance examination, and as a result underwent acoustic neuroma surgery.

The novelty added by manuscript to the already published scientific literature

The current paper describes the first translabyrinthine surgery of acoustic neuroma performed in the Republic of Moldova. Additionally, along with other studies, this paper confirms that it is essential to perform a brain magnetic resonance in patients with sensorineural hearing loss in order to diagnose or to exclude acoustic tumors.

Introduction

Acoustic neuroma (AN) which is also known as vestibular schwannoma is a benign tumor that originates from the Glial Schwann sheath junction of the VIII pair of cranial nerves and arises more frequently from the inferior vestibular nerve [1]. Although AN is a rare disorder and has an incidence of only 1.09 per 100,000 population, it represents 5-6% of all intracranial tumors and 80% of tumors found in cerebellopontine angle (CPA) [2, 3]. AN is a well-known pathology, with over 10,000 articles published in the Pubmed database and more than 2,000 articles in the last 5 years. The high interest is due to the not very elucidated evolution of AN. According to various articles, AN may remain stable, shrink in size or grow rapidly resulting in a poor outcome [4, 5]. The diagnosis of AN is made by the help of various tests: audiometry, vestibulometry, auditory evoked potentials, vestibular evoked myogenic potentials, but the gold standard in the diagnosis of this tumor is the magnetic resonance imaging (MRI) with gadolinium [6-8]. Nowadays, considering the evolution aspects of AN and the tumor size diagnosed by MRI examination, there are various treatment possibilities such as "Wait and Scan" monitoring, radiological and surgical treatment. The latter remains the gold standard in the treatment of acoustic neuroma and is done through 3 main approaches: retrosigmoid approach (RS), translabyrinthine approach (TL) and middle fosa approach. The surgical treatment of AN started with the first successful removal of this tumor by Thomas Annandale on May 3, 1895. The RS approach was further developed and extensively practiced by Harvey William Cushing who was a pioneer of neurosurgery and is considered the "father of the modern neurosurgery". He performed his first AN surgery on 12th of January 1906. The TL surgery of AN was developed by William Fouts House, who started to operate this type of tumor through TL approach on 2nd of June 1962. He is also considered to be the "father of neurootology" [9, 10]. In our country, AN surgery was done only through RS approach. A retrospective study on AN, starting from 2010 to 2019 in our country, revealed that 65 patients with histologically confirmed AN were operated in 2 high level neurosurgery departments. According to this study, most of the tumors operated on were large or giant in size, a fact that led to many postoperative complications. The late diagnosis of AN in these cases revealed a series of problems such as: insufficient study of this pathology and inadequate or insufficient use of imaging methods, especially MRI [11].

Case presentation

The 60-year-old patient was hospitalized in the ENT department of *Timofei Moșneaga* Clinical Republican Hospital with the diagnosis of bilateral sensorineural hearing loss for cochlear implantation surgery on the right ear (Fig. 1).

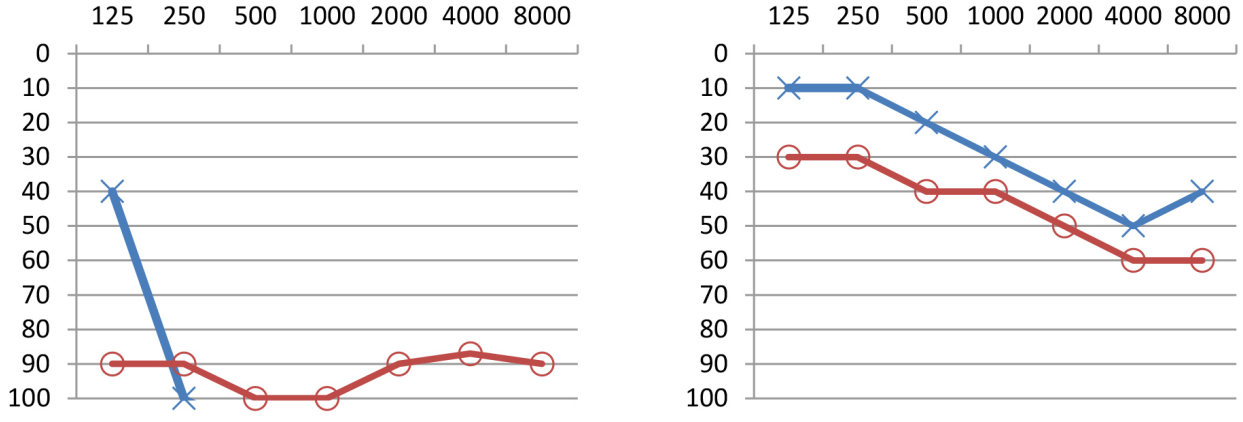


Fig. 1 Audiometry result – cophosis on the right ear and moderate sensorineural hearing loss on the left ear.

The bilateral hearing loss has been present for more than 10 years, but since 2018 a progressive hearing loss developed in the right ear. Beside the hearing loss, other symptoms were present, such as: permanent high frequency tinnitus on the right ear, which increased with tiredness; periodical vertigo; permanent moderate headache, that was influenced by emotional, physical or atmospheric pressure; unsteadiness and disturbed coordination, especially when walking in the dark. In order to perform the planned surgery (cochlear implantation surgery), patient underwent a brain MRI examination that revealed a 3rd grade CPA tumor on the right side (Fig. 2).

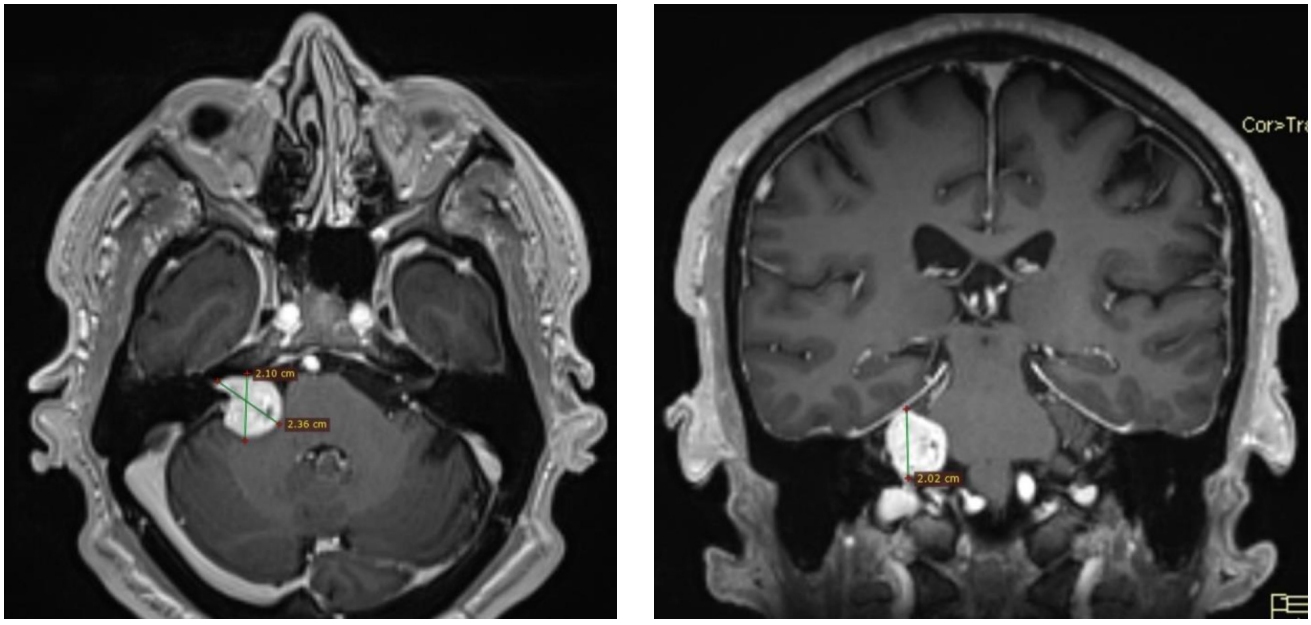
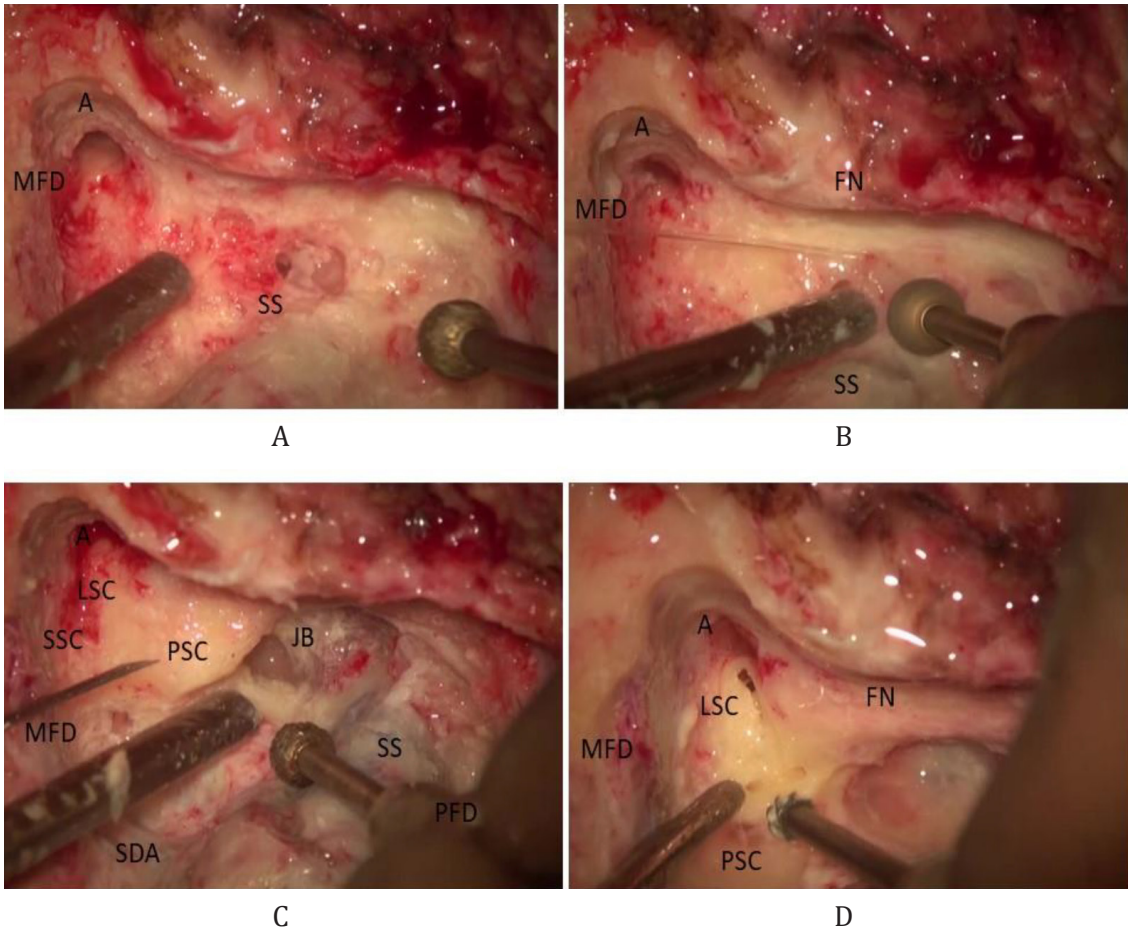


Fig. 2 Brain MRI shows an internal auditory canal and a CPA tumor on the right side.

According to the obtained results, the diagnosis of a 3rd grade right AN (by Koos classification) was established.

Afterwards, the patient was consulted by a neurosurgeon and a decision has been taken to switch from cochlear implantation surgery to vestibular schwannoma surgery through a TL approach. The surgery was performed at the *Diomid Gherman* Institute of Neurology and Neurosurgery by a team of surgeons from the above-mentioned institute, *A.I. Kolomiychenko* Institute of Otolaryngology from Kiev, Ukraine and colleagues from the *Nicolae Testemițanu* State



A B C D

Fig. 3 Microscopic imaging of the mastoidectomy:

A – opening of the antrum (A), sigmoid sinus (SS) and middle fossa dura (MFD); B – determination of the facial nerve (FN) along the mastoid segment; C – determination of the semicircular canals: lateral (LSC), posterior (PSC), superior (SSC) and determination of the jugular bulb (JB); D - opening of the lateral and posterior semicircular canals.

University of Medicine and Pharmacy and *Timofei Moşneaga* Republican Clinical Hospital.

Intraoperatively, a large mastoidectomy was performed, with opening of the middle and posterior fossa dura. The facial nerve was identified along the mastoid segment, the sigmoid sinus was uncovered down to the jugular bulb, and all three semicircular canals were determined. Subsequently, a labyrinthectomy was performed by drilling the lateral, superior and posterior semicircular canals (Fig. 3).

The internal auditory canal (IAC) was visualized and delimited from the lateral, superior, and inferior side. An incision of the dura mater was performed at the level of IAC and CPA and a total resection of the tumor was accomplished. The dura was sutured and the incus was removed. Subsequently, the middle ear has been obliterated with temporalis muscle fragments and the postoperative defect was packed with pieces of abdominal fat (Fig. 4).

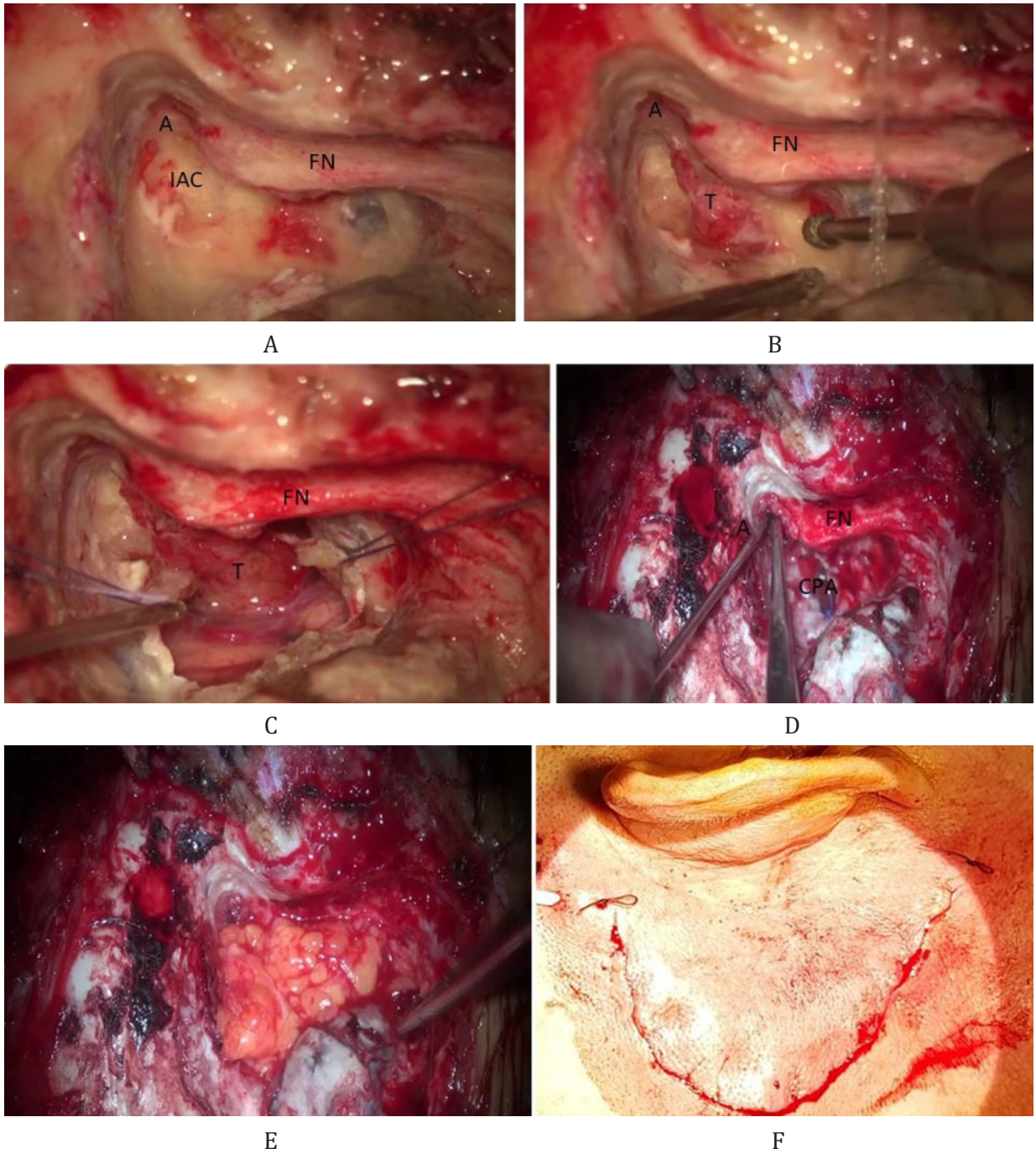
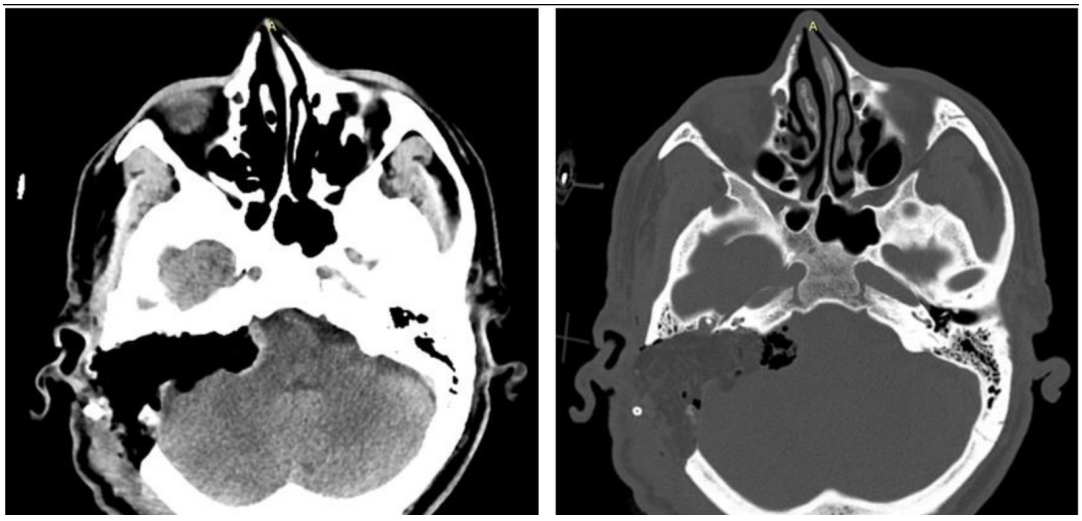


Fig. 4 Microscopic imaging of tumor resection

A – internal auditory canal (IAC); B – tumor (T) at the level of IAC; C – Tumor (T) at the level of cerebellopontine angle (CPA); D – CPA after complete removal of the tumor and obliteration of the middle ear with temporalis muscle fragments; E – packing of the postoperative defect with abdominal fat; F – aspect of intraoperative sutured incision.

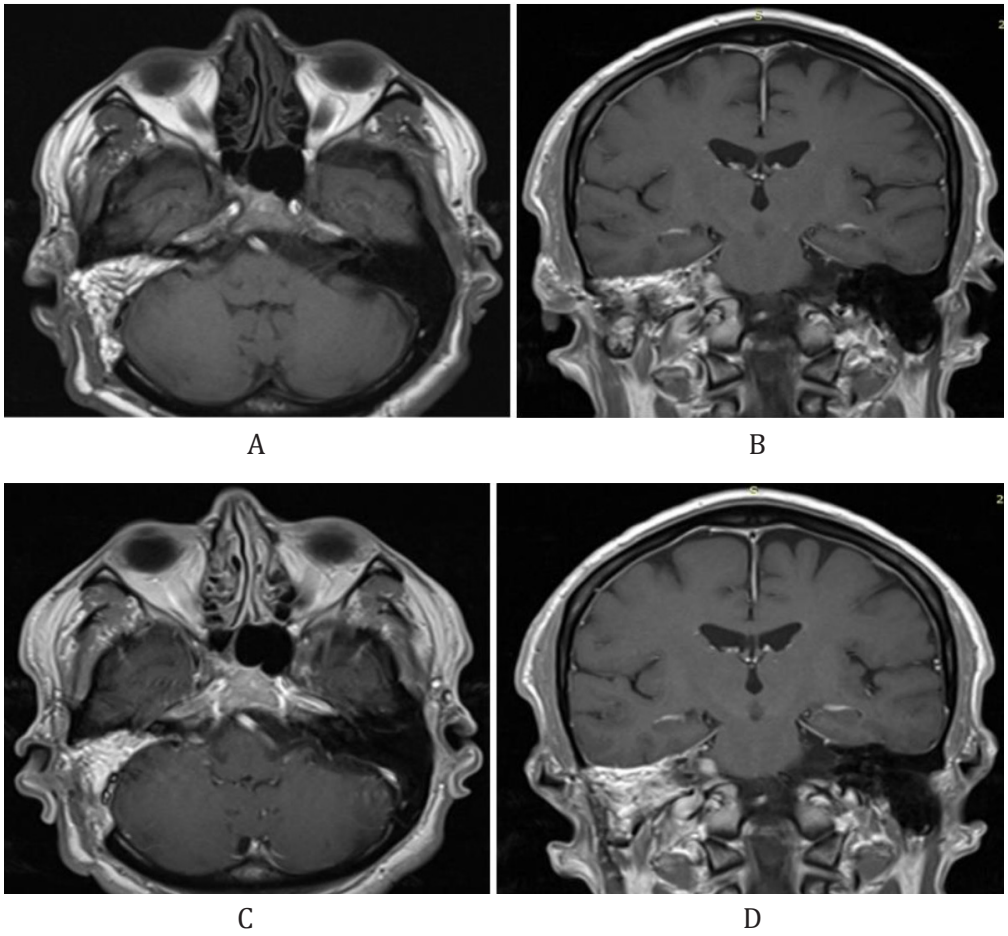


A B

Fig. 5 Postoperative computed tomography images show: A – postoperative defect; B – obliteration of the defect with fat tissue.

The surgery was performed under the permanent control of the facial nerve function with the help of intraoperative neuro-monitoring. The postoperative patient's condition was favorable without any signs of facial nerve paralysis. Postoperatively, on the second day, patient underwent a brain computed tomography that showed the volume of the performed intervention which did not reveal any complications (Fig. 5).

The patient was discharged from medical institution on 20.12.2021 in a satisfactory condition. A brain MRI with contrast was performed 3 months and one year postoperatively, both of them did not reveal any complications or signs of tumor recurrence (Fig. 6).



A B

C D

Fig. 6 MRI imaging with visualization: CPA 3 months postoperatively A – axial plane; B – coronal plane; CPA 1 year postoperatively C – axial plane; D – coronal plane.

Discussion

The role of MRI is essential in the diagnosis of AN and it is the gold standard in diagnosing this type of tumor. According to a systematic review made by Fortnum *et al.*, the incidence of AN has increased over the last 30 years due to of the widespread of MRI. This is why Celis-Aguilar *et al.* concluded that an MRI is strictly necessary in cases of unilateral sensorineural hearing loss [12]. Also, according to the protocol of the European Academy of Otology and Neuro-otology, sensorineural hearing loss of more than 20 decibels in two near frequencies or 15 decibels in frequencies between 2000 – 8000 Hz or unilateral tinnitus serve as recommendations for an MRI examination [13]. The current case revealed an acoustic tumor accidentally found on a MRI examination that was performed during preparation for a cochlear implant, in a patient who mainly complained for bilateral hearing loss and tinnitus for more than 2 years. Nowadays, the most used approaches in AN surgery remain to be the RS and TL approach. The RS approach is mainly used by neurosurgeons, has the advantage of removing CPA tumors of different dimensions, while preserving hearing. The TL approach is performed by a team an otologist and a neurosurgeon, allows for the removal of tumors of any dimension without the need for cerebellar retraction and permits identifying the facial nerve in all its segments. However, because it sacrifices hearing, it is preferably used in patients with profound or total hearing loss [14]. Many studies discuss the comparative results of both approaches and according to Cole *et al.,* the TL approach decreases the risk of facial nerve injury in comparison to RS approach [15]. Also, Pogoda *et al.*, after performing a systematic review about postoperative headache, concluded that it is more frequent in RS approach than in TL approach [16]. Obaid *et al.* concluded that both approaches have almost similar results in AN surgery, but the TL approach is associated with a less complication rate, making it preferable for patients with profound hearing loss [17]. Despite these findings, Tonn *et al.* and de Boer *et al.* stated that both RS and TL approach remain safe and efficient in AN surgery [18-19]. In addition, according to a systematic review performed by Hadjipanayis *et al.*, there is no clear superiority of one approach over the other [20].

In our case, we performed an AN surgery through TL approach because of the cophosis on the affected side and total removal of the tumor was accomplished without any postoperative complications.

Conclusion

The current report, which described an accidental diagnosis of AN, during preparation for a cochlear implantation surgery, resulted in an AN surgery through the TL approach and it serves as an eloquent example of why, in cases of sensorineural hearing loss or tinnitus, it is necessary to perform initially an MRI examination. TL approach in acoustic neuroma surgery allows removal of any size tumors without affecting the brain, especially the cerebellum. In our case, the patient had cophosis on the side of tumor, and it was the most appropriate surgical approach.

Competing interests

None declared.

Authors' contributions

Concept and design of study, acquisition of data, interpretation of data, revising the manuscript critically for important intellectual content – MB, SV, OB, GZ, VM. All authors have read and approved the final version of the manuscript.

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Patient consent

Obtained.

Ethics approval

Not needed for this study.

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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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