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ORIGINAL ARTICLE

CONTENTS

Characteristics of pro-inflammatory markers: C-reactive protein and interleukin-6 in patients with secondary bronchiectasis in chronic obstructive pulmonary disease combined with gastroesophageal reflux disease

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ABSTRACT

Aim: To assess the nature and severity of changes in inflammatory markers—C-reactive protein (CRP) and interleukin-6 (IL-6)—in patients depending on the presence of secondary bronchiectasis in the setting of COPD and GERD

Materials and Methods: 130 patients in the remission phase of COPD GOLD-3, group E, were examined by clinical, laboratory, and instrumental methods. The levels of CRP and IL-6 were measured in the peripheral blood serum. The control group - of practically healthy individuals (PHI) 15 respondents.

Results: The frequency of exacerbations in the previous year in Group I ranged from 1.7 to 2.5 and did not differ between subgroups ($p > 0.05$). In Group II - from 2.0 to 3.4 and showed difference between: subgroups Ib and IIb, subgroups IIa – IIb ($p < 0.05$). CRP levels were higher in patients with COPD + BE, showing a 47.0% increase ($p < 0.001$) compared to COPD alone. There was increase 30.7% ($p < 0.001$) in CRP levels in patients with neutrophilic-type inflammation COPD compared to eosinophilic-type. IL-6 showed an increase, ranging from 2.5 to 7 times higher ($p < 0.05$; $p < 0.001$) compared to the PHI group. The analysis of the obtained results indicates a more pronounced increase in IL-6 levels in groups with secondary BE.

Conclusions: COPD remains one of the leading causes of disability and mortality worldwide. Correlation analysis between CRP, IL-6 levels, and exacerbation frequency revealed strong positive correlations in groups with combined pathology and the neutrophilic type of inflammation in COPD ($r = +0.96$ to $r = +0.86$).

KEY WORDS: chronic obstructive pulmonary disease, secondary bronchiectasis, gastroesophageal reflux disease, CRP, IL-6, diagnosis

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INTRODUCTION

The 21st century has been marked by the rapid and progressive development of medicine as a whole and all its branches in particular, including diagnostics, prevention, treatment, and scientific research. All these advancements are aimed at achieving the crucial goal of improving patients' quality of life. This issue is especially relevant among a significant cohort of individuals with respiratory diseases.

Respiratory system disorders rank among the leading causes of morbidity and mortality worldwide. In terms of fatality rates among nosological groups, COPD is second only to cardiovascular pathologies, particularly ischemic heart disease and stroke [1-5].

According to the concept formulated by Academician Feshchenko Yu.I. (2023), timely diagnosis is a critically important component of effective management for patients with pulmonary pathology. It ensures high-quality treatment outcomes, disease control, and complication prevention. The proposed strategies not only align with the clinical guidelines developed by the World Health Organization (WHO) but also correspond to the goals of the Global Alliance against Chronic Respiratory Diseases (GARD), aimed at reducing the prevalence and severity of respiratory diseases worldwide [6].

When assessing the severity of COPD progression, we rely on data regarding the frequency of exacerbations and the intensity of clinical symptoms. However, an analysis of the obtained results indicates that the variability in the degree of emphysema severity and bronchial lumen changes can be observed even in patients with identical forced expiratory volume in one second (FEV1) values. Since a noticeable increase in FEV1 in such patients occurs later than the regression of clinical symptoms, this may delay the detection of positive disease dynamics [7-9].

Given the above, the search for and application of more sensitive biomarkers is highly relevant for assessing disease destabilization, evaluating therapy

effectiveness, enabling early diagnosis of exacerbations, and predicting potential complications. Respiratory comorbidity, especially when it involves simultaneous damage to the same organ—such as COPD and secondary bronchiectasis—is a significant factor contributing to poor prognostic outcomes [2,10,11].

Studies indicate that secondary bronchiectasis coexists with COPD in 20–60% of cases, exacerbating symptom burden, increasing hospitalization frequency, reducing therapeutic efficacy, and leading to a worse prognosis compared to single-pathology cases [2,10]. However, it is not only monosystemic but also intersystemic polymorbidity that plays a crucial role. For instance, gastrointestinal tract involvement—such as gastroesophageal reflux disease (GERD)—can act as both a predictor of respiratory disease development and a factor that worsens existing conditions. According to international studies, GERD is one of the most prevalent disorders affecting individuals in the third millennium.

The epidemiology of GERD varies significantly across different countries, ranging from 65.0% in the United Kingdom (J.R. Bennet) to 2.3% in China (M. Chen et al.). The presence of GERD in patients with COPD leads to frequent exacerbations, a shortened remission period, and a progressive decline in pulmonary function parameters [12]. The progression of chronic diseases is accompanied and exacerbated by the activation of oxidative stress, an imbalance in redox processes, and an increase in the levels of pro-inflammatory mediators and acute-phase proteins [4,13-16]. One of the most sensitive markers of the acute phase of inflammation is CRP, the level of which is proportional to the intensity of the inflammatory process [4,14]. CRP can activate a pathological cycle of mutual induction: synthesized in the liver under the influence of inflammatory response regulators – cytokines (IL-6), as a central protein of the acute phase of inflammation, it binds to phospholipids of damaged cells, activating subsequent phagocytosis, and stimulates the production of pro-inflammatory cytokines [8,17-19].

AIM

To assess the nature and severity of changes in inflammatory markers—C-reactive protein (CRP) and interleukin-6 (IL-6)—in patients depending on the presence of secondary bronchiectasis in the setting of COPD and GERD.

MATERIALS AND METHODS

The study was conducted at the Municipal Non-Profit Enterprise «Center for Infectious Diseases of the Ivano-Frankivsk Regional Council.» A survey of 130 patients under observation in the remission phase of COPD GOLD-3, group E, was carried out. All patients were divided into groups depending on the established diagnoses and the type of inflammatory response: Group I included 68 individuals with COPD, divided as follows: subgroup Ia - 44 patients with COPD and a neutrophilic type of inflammatory response, subgroup Ib - 8 patients with COPD, a neutrophilic type of inflammatory response, and secondary bronchiectasis, subgroup Ic - 16 patients with COPD and an eosinophilic type of inflammatory response; Group II included 62 individuals with COPD combined with GERD, divided as follows: subgroup IIa - 34 patients with COPD+GERD, neutrophilic type of inflammatory response, subgroup IIb - 25 patients with COPD+GERD, neutrophilic type of inflammatory response, and secondary bronchiectasis, subgroup IIc - 3 patients with COPD+GERD and an eosinophilic type of inflammatory response. The control group consisted of 15 practically healthy individuals. Patients with COPD received basic COPD therapy: tiotropium bromide 18 mcg, 1 inhalation per day, fixed combination of budesonide/formoterol 320/9 mcg, 1 inhalation twice daily. Secondary bronchiectasis was verified during previous hospitalizations for COPD exacerbation using CT scans, applying the criteria proposed by N. Aidich et al.: direct signs (broncho-arterial ratio >1, absence of bronchial tapering, visualization of peripheral bronchi within 1 cm of the costal pleura in contact with the mediastinal pleura) and indirect signs (peribronchial thickening, mucus plugging, mosaic pattern, centrilobular nodules, atelectasis/consolidation).» [14]. In Group II, the duration of GERD anamnesis was 11±2.4 years. All patients with GERD received maintenance therapy with proton pump inhibitors. In both patient groups, the concentration of C-reactive protein in the blood serum was determined using the «CRP-latex-test» reagent kit (Ukraine), and the level of IL-6 [1] using the «ELISA KIT» reagent kit (USA).

Statistical processing was performed using the statistical function package of «Microsoft Excel» programs. The reliability of the obtained indicators was confirmed by calculating the error (±m) for relative values, and the significance of the difference in data in the comparative cohorts was proven based on the calculation of Student's t-test and the determination of the accuracy of the error-free forecast (P) using the table.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

Table 1. General Characteristics of the Studied Groups

Main characteristics	Group I COPD			Group II COPD+ GERD		
Number of patients	68			62		
Sex (m, f)	41 m; 27 f			34 m; 28 f		
Age	65.3 ± 3.9			67.3± 4.2		
Frequency of exacerbations during the year in subgroups	Ia n-44	Ib n-8	Ic n-16	IIa n-34	IIb n-25	IIc n-3
	1	2	3	4	5	6
	2.2 p_{1-4}>0.05	2.5 p_{1-2}>0.05	1,7 p_{1-3}>0,05	2,7 p_{4-5} <0,05	3,4 p_{2-5} <0,01	2
Duration of history, years	COPD 18.4 ± 2.1			COPD 19.3 ± 2.9 GERD 14.5 ± 3.4		
Smoking status						
Smoker	54 (79.4%)			53 (85.5%)		
Former smoker	14 (20.6%)			9 (14.5%)		
Smoking history by sex (pack/years)	23.1±2.1 - m; 11.2 ±1.9 - f			24.1±1.8 - m; 10.1 ±2.4 - f		
Severity level for COPD	GOLD 3, Group E			GOLD 3, Group E		

Notes: n – number of patients; p - value of the difference between the data the frequency of exacerbations
Source: compiled by the authors of this study

RESULTS

The gender distribution of morbidity was characterized by a predominance of males in both patient groups: Group I consisted of 41 (60.3%) males and 27 (39.7%) females; Group II consisted of 34 (54.8%) males and 28 (41.2%) females (Table 1). The average age of patients was uniformly distributed in Groups I and II: 65.3 ± 3.9 and 67.3 ± 4.2, respectively. In Group I, the frequency of exacerbations ranged from 1.7 to 2.5 and did not significantly differ between subgroups (p > 0.05). In Group II, the fluctuations were 2.0 to 3.4 and differed significantly not only between Ib and IIb (p < 0.01) but also within the subgroup IIa – IIb (p < 0.05).

Smoking history in pack-years in each group: Group I - 23.1±2.1 males; 11.2±1.9 females; Group II - 24.1±1.8 males and 10.1±2.4.

CRP indicators is characterized by its increase in the peripheral blood serum in all groups compared to the reference values (p<0.05 - p<0.001) (Table 2). When comparing CRP levels within the COPD patient group, a statistically significant increase in this indicator was observed in patients with COPD+BE compared to COPD by 47.0% (p<0.001), and a significant increase in the indicator by 30.7% (p<0.001) between patients with COPD with eosinophilic inflammation and COPD with neutrophilic inflammation. Also, the CRP level in the COPD+GERD+BE patient group was significantly higher by 90.0% (p<0.001) compared to COPD+BE patients, and by 74.8% (p<0.001) compared to COPD+GERD patients.

The dynamics of the pro-inflammatory cytokine IL-6 (Table 2) demonstrates a significant increase of 2.5 to 7 times (p<0.05; p<0.001) compared to the control group. A significant increase in the level of the pro-inflammatory cytokine by 39.8% (p<0.05) was observed between the COPD+BE and COPD+GERD+BE subgroups, between COPD with neutrophilic inflammation and COPD+BE by 62.2% (p<0.05), and between COPD+GERD with neutrophilic inflammation and COPD+GERD+BE by 62.44% (p<0.001).

The study of the relationship between systemic immuno-inflammatory activation indicators, markers of the systemic inflammatory response of the body, and the frequency of exacerbations is important.

It was established that in patients with COPD in all subgroups, there is a strong positive correlation between the frequency of exacerbations and CRP levels (r= +0.89, p<0.001; r=+0.91, p<0.001; r=+0.78, p<0.001) (Fig. 1; Fig.2; Fig.3).

Similarly, the correlation in all subgroups of the COPD group is characterized by a similar dynamic, a strong positive correlation between the frequency of exacerbations and IL-6 levels (r= +0.86, p<0.001; r=+0.91, p<0.001; r=+0.78, p<0.001) (Fig. 4; Fig.5; Fig.6)

However, it should be noted that the lowest expression of a strong correlation was in the COPD group with eosinophilic inflammation.

The dynamics of the correlation dependence in the COPD+GERD+BE group is demonstrated in Table 3.

In the presence of COPD with neutrophilic inflammation + GERD and COPD+GERD+BE, strong positive correlations were established between the frequency of exacerbations

Table 2. Levels of CRP (mg/L) and IL-6 (pg/mL) in Peripheral Blood Serum in the Studied Groups

CRP IL-6	The Studied Groups							p1	p2	p3	p4	p5
	PHI, n=15	Group I n=68			Group II n=62							
		I a n=44	I b n=8	I c n=16	II a n=34	II b n=25	II c n=3					
		1	2	3	4	5	6					
CRP, mg/L in peripheral blood serum	1.34± 0.31	5.93±0.44 p<0.001 -Δ_{1-2} 47.0%	8.18±0.26 p<0.001 -Δ_{2-5} 90.0%	4.11±0.21 p<0.001 Δ_{1-3} 30.7%	8.89±0.33 p<0.001 -Δ_{1-4} 50.0%	15.54±0.22 p<0.001 -Δ_{4-5} 74.8%	7.87±0.17 p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
IL-6, pg/mL	3.10± 0.19	9.50± 1.97 p<0.01 -Δ_{1-2} 62.2%	15.41± 2.24 p<0.001 -Δ_{2-5} 39.8%	7.80± 2.1 p<0.05 Δ_{1-3} 17.9%	13.26± 1.96 p<0.001 -Δ_{1-4} 39.6%	21.54± 1.98 p<0.001 -Δ_{4-5} 62.44%	11.32± 2.10 p<0.01	p<0.05	p<0.05	p>0.05	p>0.05	p<0.001

Notes: Δ - absolute changes between values
p - significance of the difference between groups and PHI
p1 - significance of the difference between group I b and II b
p2 - significance of the difference between group I a and I b
p3 - significance of the difference between group I a and I c
p4 - significance of the difference between group I a and II a
p5 - significance of the difference betw
een group II a and II b
Source: compiled by the authors of this study

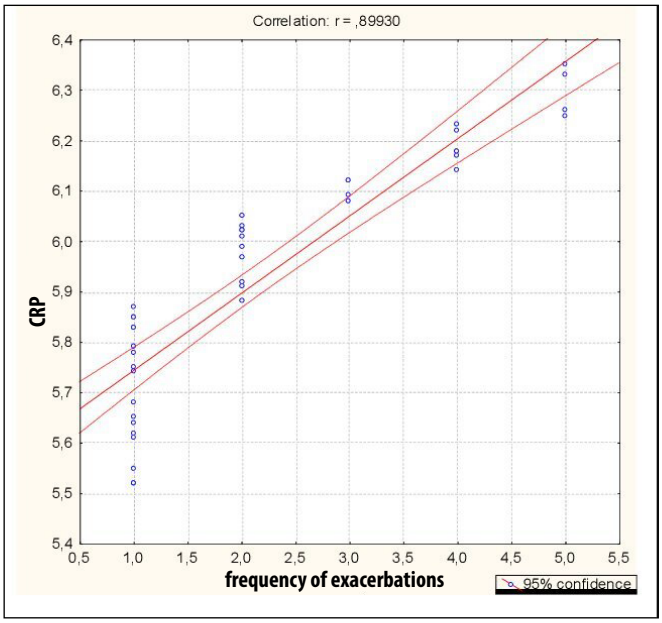

Fig. 1. Correlation between the frequency of exacerbations and CRP in patients with COPD with neutrophilic type of inflammation
Notes: 1) r – the correlation coefficient, 2) p – value
Picture taken by the authors

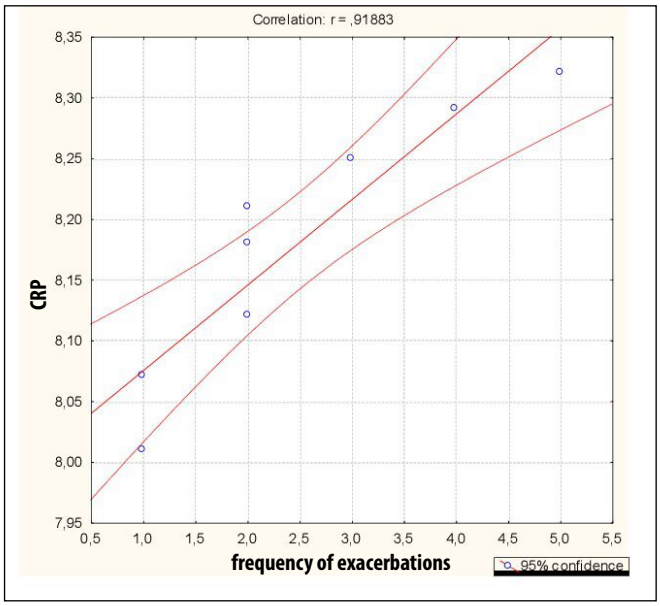

Fig. 2. Correlation between the frequency of exacerbations and CRP in patients with COPD + BE
Notes: 1) r – the correlation coefficient, 2) p – value
Picture taken by the authors

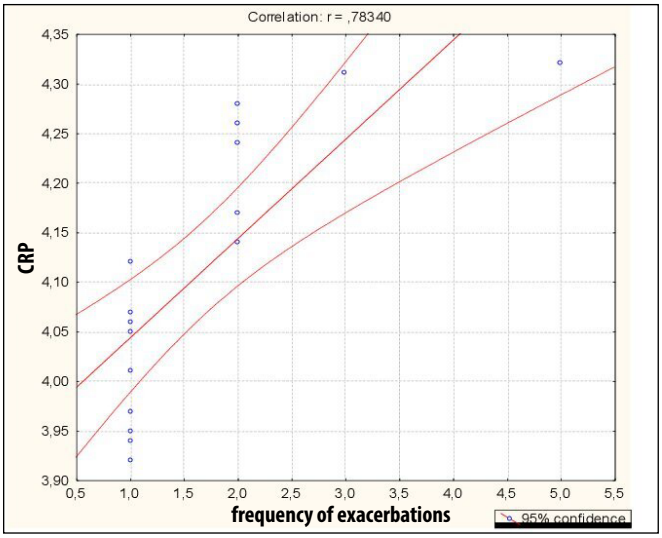

Fig. 3. Correlation between the frequency of exacerbations and CRP in patients with COPD with eosinophilc type of inflammation
Notes: 1) r – the correlation coefficient, 2) p – value
Picture taken by the authors

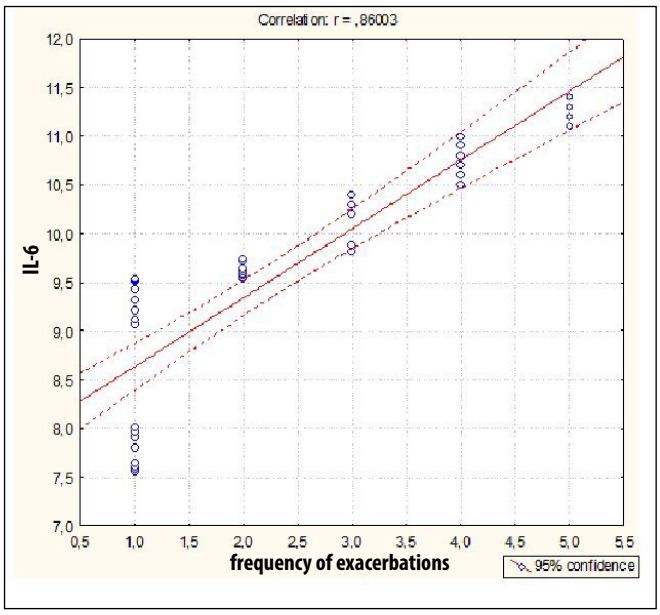

Fig. 4. Correlation between the frequency of exacerbations and IL-6 in patients with COPD with neutrophilic type of inflammation
Notes: 1) r – the correlation coefficient, 2) p – value
Picture taken by the authors

and the pro-inflammatory cytokine IL-6 (r=+0.91, +0.91) and the acute-phase inflammation marker CRP (r=+0.92, +0.96) (Table 3). In the COPD group with eosinophilic inflammation + GERD, the correlation with frequency was positive, of moderate strength (r=+0.42, +0.49).

DISCUSSION

The progressive course and tendency of respiratory diseases to develop local and systemic complications are driven by multifactorial etiology, complex pathogenesis, and dependence on the cellular type of the inflammatory process, contributing to their high prevalence. A hallmark of these conditions is the worsening severity, which increases the risk of patient disability. This phenotype is associated with an increased risk of exacerbations, severe airway obstruction, and higher mortality rates [10].

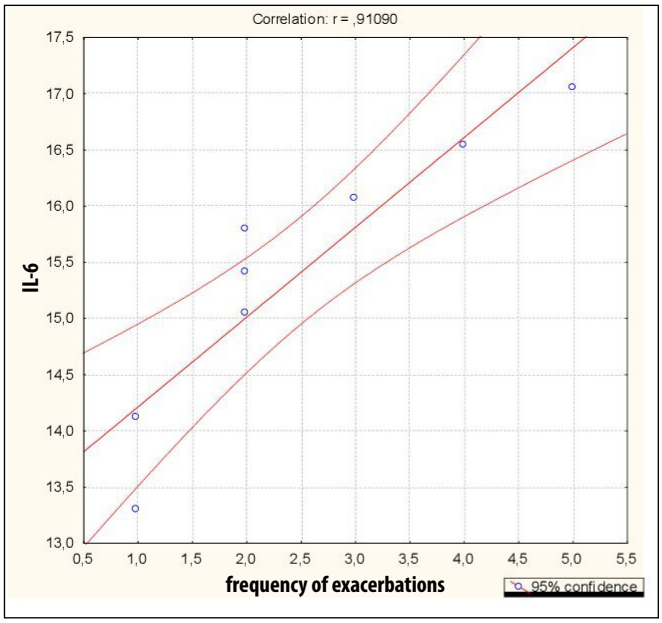

Fig. 5. Correlation between the frequency of exacerbations and IL-6 in patients with COPD+GERD+BE
Notes: 1) r – the correlation coefficient, 2) p – value
Picture taken by the authors

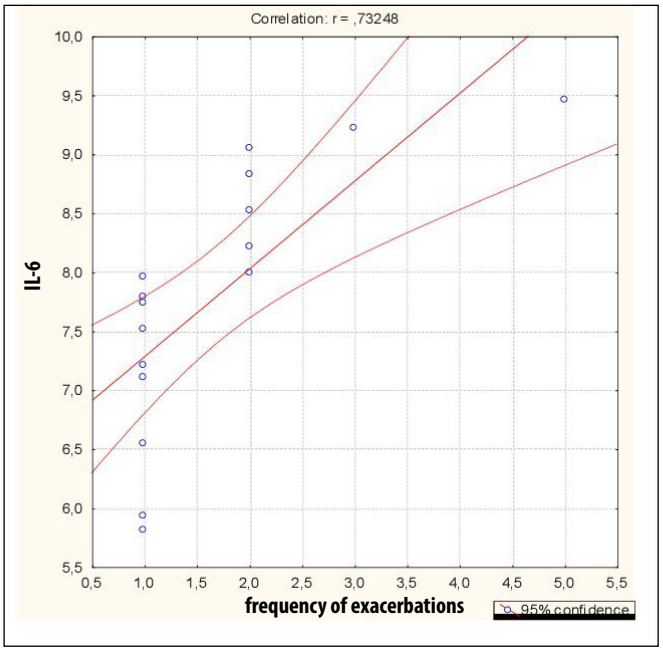

Fig. 6. Correlation between the frequency of exacerbations and IL-6 in COPD patients with eosinophilic type of inflammation + GERD
Notes: 1) r – the correlation coefficient, 2) p – value
Picture taken by the authors

Currently, chronic respiratory diseases frequently coexist with existing comorbidities, leading to a worsening of the course of each individual disease and complicating the diagnostic process, which subsequently affects the effectiveness of the dominant pathology's treatment. The combination of COPD, secondary BE and GERD is an example of interfering syntropy of diseases, in which the illnesses are not only interconnected but also contribute to the aggravation of each other's course [2,11]. The phenomenon of chronic cough is often associated with the presence of concomitant GERD. Extraesophageal manifestations of GERD are observed in 75% of patients with COPD. The presence of comorbid GERD in patients with COPD leads to a shortening of the COPD remission period, a decrease in pulmonary function indicators, which complicates the diagnostic and treatment process. Therefore, there arises a pressing need to choose an alternative comprehensive therapeutic and preventive strategy that will improve the course of diseases and enhance the quality of life of patients [11,12].

Analysis of the frequency of exacerbations during the year, before enrollment in the study, characterized the general trend described in the literature by a number of authors – an increase in morphofunctional changes from exacerbation to exacerbation, and the presence of additional factors such as obstruction, bronchiectasis, and GERD increased the number of exacerbations. The opinion regarding the role of harmful factors, particularly smoking, which is a common factor in the development and progression of both diseases and leads to more frequent and clinically severe exacerbations, remains indisputable [16]. High percentages of smoking history in pack-years in each group: Group I - 23.1±2.1 males; 11.2±1.9 females; Group II - 24.1±1.8 males and 10.1±2.4 females require careful attention and intensification of the physician's work with these patients to eliminate harmful habits. CRP indicators increased not only in compared to the reference values ($p<0.05$ - $p<0.001$), but in patients with COPD+BE compared to COPD by 47.0% ($p<0.001$), and by 30.7% ($p<0.001$) between patients with COPD with eosinophilic inflammation and COPD with neutrophilic inflammation to. Thus, we observe a more pronounced acute-phase inflammation indicator among patients with a predominant neutrophilic type of inflammation and polymorbidity.

The analysis of dynamics of the pro-inflammatory cytokine demonstrates a more pronounced increase in IL-6 in both subgroups with secondary bronchiectasis: by 39.8% ($p<0.05$) - between the COPD+BE and COPD+GERD+BE subgroups, by 62.2% ($p<0.05$) - between COPD with neutrophilic inflammation and COPD+BE, by 62.44% ($p<0.001$) - between COPD+GERD with neutrophilic inflammation and COPD+GERD+BE. Its may characterize the intensity of the inflammatory response in polymorbidity, in patients in the remission phase. Thus, we observe a strong positive correlation of systemic inflammation markers with the frequency of exacerbations in groups with combined pathology and neutrophilic inflammation IL-6 (r=+0.91, +0.91), CRP

Table 3. Correlation matrix between systemic immuno-inflammatory activity parameters, systemic inflammation marker of the body, and frequency of exacerbations in patients of Group II

Indicator	II a frequency of exacerbations	II b frequency of exacerbations	IIc frequency of exacerbations
CRP	+0.92	+0.96	+0.42
IL-6	+0.91	+0.91	+0.49

Source: compiled by the authors of this study

(r=+0.92, +0.96). In the COPD eosinophilic inflammation + GERD group, this correlation was defined as positive, of moderate strength (r=+0.42, +0.49). The obtained data suggest that polymorbidity and the predominance of neutrophilic inflammation may be predictors of a more pronounced 'smoldering inflammation' in patients in the remission phase.

Therefore, in our opinion, there is an urgent need to develop and adhere to an algorithm for managing patients with combined pathologies, which includes quality basic therapy to prevent exacerbations in general, and those leading to hospitalizations in particular.

CONCLUSIONS

1. The epidemic of respiratory and gastrointestinal diseases spreading worldwide is causing the formation of a new cluster of patients with polymorbid lesions. In this case, not only is diagnosis complicated and obstacles to treatment effectiveness arise, but there is also a need for great vigilance and caution in the patient-doctor collaboration to form a roadmap of preventive approaches to prevent exacerbations and improve the patient's quality of life. The gender distribution maintains a trend with a predominant number of males 75 (57.7%), 55 (42.3%) – females.
2. The CRP indicator is characterized by its increase in peripheral blood serum in all groups compared to the control group ($p<0.05$ - $p<0.001$). Analysis of CRP levels within the COPD patient group showed a statistically significant increase in this indicator in patients with COPD+BE compared to COPD by 47.0% ($p<0.001$), and a significant increase in the indicator by 30.7% ($p<0.001$) between patients with COPD with eosinophilic inflammation and COPD with neutrophilic inflammation. Also, the CRP level in the COPD+GERD+BE patient group was significantly higher by 90.0% ($p<0.001$) compared to COPD+BE patients, and by 74.8% ($p<0.001$) compared to COPD+GERD patients.
3. The dynamics of IL-6 are characterized by a significant increase of 2.5 to 7 times ($p<0.05$; $p<0.001$) compared to the control group. A significant increase in the level of the pro-inflammatory cytokine by 39.8% ($p<0.05$) was observed between the COPD+BE and COPD+GERD+BE groups, between COPD with neutrophilic inflammation and COPD+BE by 62.2% ($p<0.05$), and between COPD+GERD with neutrophilic inflammation and COPD+GERD+BE by 62.44% ($p<0.001$). The analysis of the obtained results demonstrates a more pronounced increase in IL-6 in both groups with secondary bronchiectasis, which may characterize the intensity of the inflammatory response in polymorbidity, in patients in the remission phase..
4. Correlation analysis between systemic immuno-inflammatory activity parameters, systemic inflammation markers of the body, and the frequency of exacerbations is characterized by strong positive correlations in groups with combined pathology and neutrophilic inflammation (r=+0.96 - r=+0.86). In the COPD group with eosinophilic inflammation + GERD, this correlation was defined as positive, of moderate strength.
5. The progression and prognostic probability of exacerbations of chronic inflammatory processes are directly related to elevated levels of CRP and IL-6, and the level significantly increases with the development of polymorbidity, for example, in COPD such as secondary BE and GERD. Thus, the determination of CRP and IL-6 can be used as a marker for predicting the probable deterioration or destabilization of COPD, as well as the development of polymorbidity.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Hydration bioanalyses of the effects of hydrogen-rich water (HRW)

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ABSTRACT

Aim: To demonstrate the effects of hydrogen-rich water (HRW) on hydration by examining hematocrit, blood viscosity, and urine osmolality.

Materials and Methods: Hydrogen Evodrop rich water (HEW), produced from a patented device with hydrogen concentration therein 900–1200 ppb or 0.9–1.2 ppm, ORP ranging from –450 to –580 mV, and pH=7.1–7.3. Blood viscosity, hematocrit and urine osmolality had been determined in 10 volunteers (five women and five men) during the 21-day period of HEW intake, under standard dietary conditions.

Results: No evidence of erythrocyte agglutination or morphological abnormalities was observed. Urine osmolality in both men and women decreased after HEW consumption, likely due to improved hydration. However, osmolality before the consumption was higher in men compared to women. Both men and women exhibited statistically significant changes in hematocrit and blood viscosity parameters after consuming HEW, which decreased. This suggests improved hydration and reduced blood viscosity.

Conclusions: These results suggest that measurable hydration effects in the human body may be achieved through regular intake of HEW. The findings of the research support the benefit of the use of HEW in promoting body hydration. The analysis suggests that hematocrit is more closely associated with other factors, such as hydration status and plasma volume following HEW consumption. HEW consumption consistently affected urine osmolality, which may reflect in overall hydration status. Overall, HEW intake produced consistent changes in key hematological and renal hydration markers, indicating favorable effects on blood fluidity and water balance.

KEY WORDS: hydrogen-rich water (HRW), hydration, hematocrit, blood viscosity, urine osmolality

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INTRODUCTION

Numerous studies and analyses have examined the factors contributing to health and longevity in the XX and XXI centuries. The key factors are heredity, physical activity, proper nutrition and hydration, psychological factors, and regular movement. Empirical data have shown that long-lived individuals and centenarians tend to reside primarily in mountainous regions. One of the co-authors, Ignatov, studied over 500 long-lived individuals, including centenarians and their siblings, in Bulgaria from 2012 to 2019. Mountain spring water was analyzed as a contributing factor. The results were published in the Journal of the Ministry of Health, Bulgaria [1,2]. The conclusion from this project is that optimal levels of calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}), manganese (Mn^{2+}), and zinc (Zn^{2+}) content in water and food are essential for the physiological balance [3]. The significance of these minerals is demonstrated in [4]. The antioxidant properties of trace elements in food and water are also analyzed [4]. Although trace elements are found in greater quantities in food than in water, researchers have also reported them in water.

It is important to note that molecular hydrogen, whether in gaseous form or dissolved in water, has no known toxic effects [5]. Despite this safety profile, its potential in medicine remained largely unexplored until 2007. That year, Oshava et al. reported that inhalation of 1–4% hydrogen gas reduced hydroxyl radicals and peroxynitrate levels [6]. This discovery represented a significant breakthrough, opening new avenues for medical applications of hydrogen.

Scientists have achieved antioxidant results with molecular hydrogen (H_2), which can be administered by

inhalation or dissolved in water [7]. Molecular hydrogen reduces oxidative stress caused by increased ROS [8]. Potential anti-inflammatory and radioprotective effects have also been observed [6,9]. The HRW has anti-inflammatory effects also [10]. A significant advantage of its application in these directions is the absence of smell, color, and taste, as well as its non-toxicity [4]. The results with molecular hydrogen were achieved on animals – anti-tumor effects of hamsters [11], anti-inflammatory intestinal influence of rats [12].

A lot of studies were performed with mice. Drinking H_2-water prevented aging-dependent memory impairment induced by oxidative stress in DAL101 mice, and improved cognitive function [13]. In a D-galactose-induced aging mouse model, H_2 (administered via inhalation, HRW, or H_2-rich saline) significantly improved aging-related biomarkers and reduced oxidative stress in liver, brain, and heart tissues [14]. HRW improved chronic inflammatory pain [15]. Repeated inhalation of hydrogen-oxygen mixed gas significantly decreased both acute and chronic mild stress-induced depressive and anxiety-like behaviors in mice [16].

Molecular hydrogen has been shown to affect metabolic diseases when used in hyperbaric therapy, raising hope for its application in clinical practice in the future, especially in patients with metabolic diseases such as atherosclerosis, diabetes mellitus, metabolic syndrome, and obesity [17].

However, hydrogen-rich water has gained wider popularity. HRW has demonstrated antioxidant [18] and anti-inflammatory effects [19]. Nakao et al. proposed obtaining HRW by placing a portable metal magnesium stick in drinking water and recommend it as a safe, easy, and effective method of providing HRW for daily consumption [18]. The oral intake of hydrogen-containing fluids, mainly through tablets that release hydrogen when dissolved in water, is a convenient and practical method, making it easy for individuals to incorporate into their daily routines. These tablets are registered as dietary supplements. Their effects on blood lipid profiles and inflammation biomarkers have been documented [20].

Hydrogen-rich water has often been generated through the chemical reaction of metallic magnesium with water, a simple process that produces molecular hydrogen *in situ*. While this approach provides a straightforward means of enrichment, recent advances have shifted attention to dedicated devices capable of continuously producing hydrogen-rich water. A hydrogen-rich water cathode chamber refers to the part of the electrolytic device that generates hydrogen gas. It is then dissolved into water to produce HRW, a consumable beverage [21].

Hydrogen-rich water has been used prophylactically in the past two decades. Animal and human studies have been conducted to demonstrate the effects of HRW. The increasing number of investigations provides more detailed analyses of HRW's effects, significantly contributing to a better understanding of its potential benefits. This rapidly expanding body of research should instill optimism about the future applications of hydrogen-rich water. The beneficial effects of HRW on human health, including physical endurance, liver function, cardiovascular health, aging retardation, and mental health, are attracting increasing interest and research. These effects have been described in the current literature [22–26].

Good hydration is an essential factor for maintaining metabolism, physiological functions, and overall health.

AIM

This study aims to demonstrate the effects of HRW on hydration by examining hematocrit, blood viscosity, and urine osmolality.

MATERIALS AND METHODS

HYDROGEN RICH-WATER

Hydrogen-rich water was produced using a patented device, the parameters of which are also published in [11,27]. In the papers [11,27] the water was defined as Hydrogen Evodrop-rich water (HEW). The hydrogen concentration therein is 900-1200 ppb or 0.9~1.2 ppm, ORP is (–450~ –580) mV, and pH=7.1~7.3.

DEVICE FOR MEASUREMENT OF VISCOSITY

IKA-Werke®GmbH&Co. KG, type: Brookfield DV2T with shear rate 94.5 s^{-1} with 5.7-inch Full-color Touch Screen Display, enhanced security, Convenient Bubble Leveling, built-in RTD Temperature Probe, DV360 Software Optional.

DEVICE FOR URINE OSMOLALITY INVESTIGATIONS

Advanced Instruments INC, type: Advanced Micro-Osmometer model 3320. Determines the osmolality of solutions using freezing point depression (FPD). An automatic single-sample instrument is designed to process a sample with one-minute test time. Feature ease-of-use, internal diagnostics, automated calibrated, on-board statistical analysis.

Table 1. Results of hematocrit before and after consumption of hydrogen-rich water (HEW)

Number men	hematocrit result (L/L) before	hematocrit result (L/L) after	norm (L/L)	Number women	hematocrit result (L/L) before	hematocrit result (L/L) after	norm (L/L)
1.	0.51	0.48	0.40–0.53	6.	0.42	0.39	0.36–0.48
2.	0.50	0.46	0.40–0.53	7.	0.42	0.40	0.36–0.48
3.	0.47	0.46	0.40–0.53	8.	0.39	0.39	0.36–0.48
4.	0.50	0.46	0.40–0.53	9.	0.40	0.39	0.36–0.48
5.	0.49	0.47	0.40–0.53	10.	0.43	0.40	0.36–0.48

Note: Reference ranges for hematocrit used in this table (men: 0.40–0.53 L/L), (women: 0.36–0.48 L/L)
Source: compiled by the authors of this study

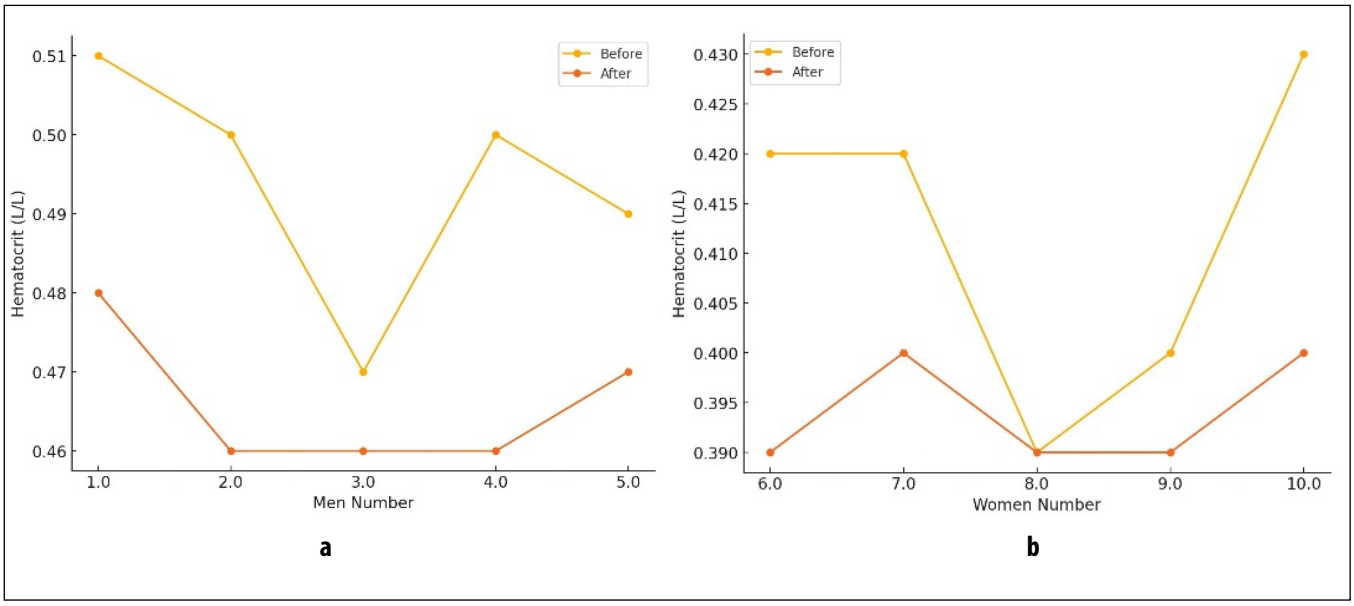

Fig. 1. Results of hematocrit before and after consumption of hydrogen-rich water (HRW): a – men; b – women
Picture taken by the authors

MICROSCOPE

The microscope for blood observation and analysis Olympus CX43 has the following parameters: head: binocular or trinocular; eyepiece: 10X/20; nosepiece: quintuple; Plan N 4x, 10x, 40x, 100 x Oil; stage: rackless XY Stage w/slide Holder; condenser: 1.25 N. A. Condenser, 7 Positions; light source: LED.

CLINICAL RESULTS OF HEMATOCRIT AND URINE

The values of hematocrit (HCT), blood viscosity and urine osmolality were determined. The research of blood and urine parameters was made in a licensed laboratory (Bodymed, Sofia, Bulgaria).

CONDITIONS FOR TESTING

The study involved 10 volunteers, five women and five men.

The present study was conducted using anonymous blood and urine samples voluntarily provided by participants. No personal information, including age or health status, was collected, and no medical or pharmacological interventions were performed. All procedures adhered to the principle of data protection and ensured minimal risk to participants, eliminating the need for bioethical committee approval. The study focused on non-invasive analysis in accordance with international standards for scientific research. The standards and parameters of the water for the tests are in accordance with the European Drinking Water Directive 2020/2184 [28].

COMPUTER PROGRAM PROCEEDING WITH PROGRAM PYTHON RELEVANCE AS A SOFTWARE DEVELOPMENT

All statistical analyses and figure generation were performed using Python programming language, utilizing libraries as Matplotlib for data visualization and statistical packages such as SciPy and pandas for data analysis.

Table 2. Results of viscosity before and after consumption of Hydrogen Evodrop-rich water (HEW) for men and women

Number men	viscosity result (mPa.s) before	viscosity result (mPa.s) after	norm (mPa.s)	Number women	viscosity result (mPa.s) before	hematocrit result (mPa.s) after	norm (mPa.s)
1.	5.85	5.42	4.0-5.5	6.	4.58	4.20	3.5-5.0
2.	5.70	5.08	4.0-5.5	7.	4.60	4.32	3.5-5.0
3.	5.26	5.13	4.0-5.5	8.	4.23	4.19	3.5-5.0
4.	5.70	5.11	4.0-5.5	9.	4.31	4.21	3.5-5.0
5.	5.55	5.27	4.0-5.5	10.	4.71	4.33	3.5-5.0

Source: compiled by the authors of this study

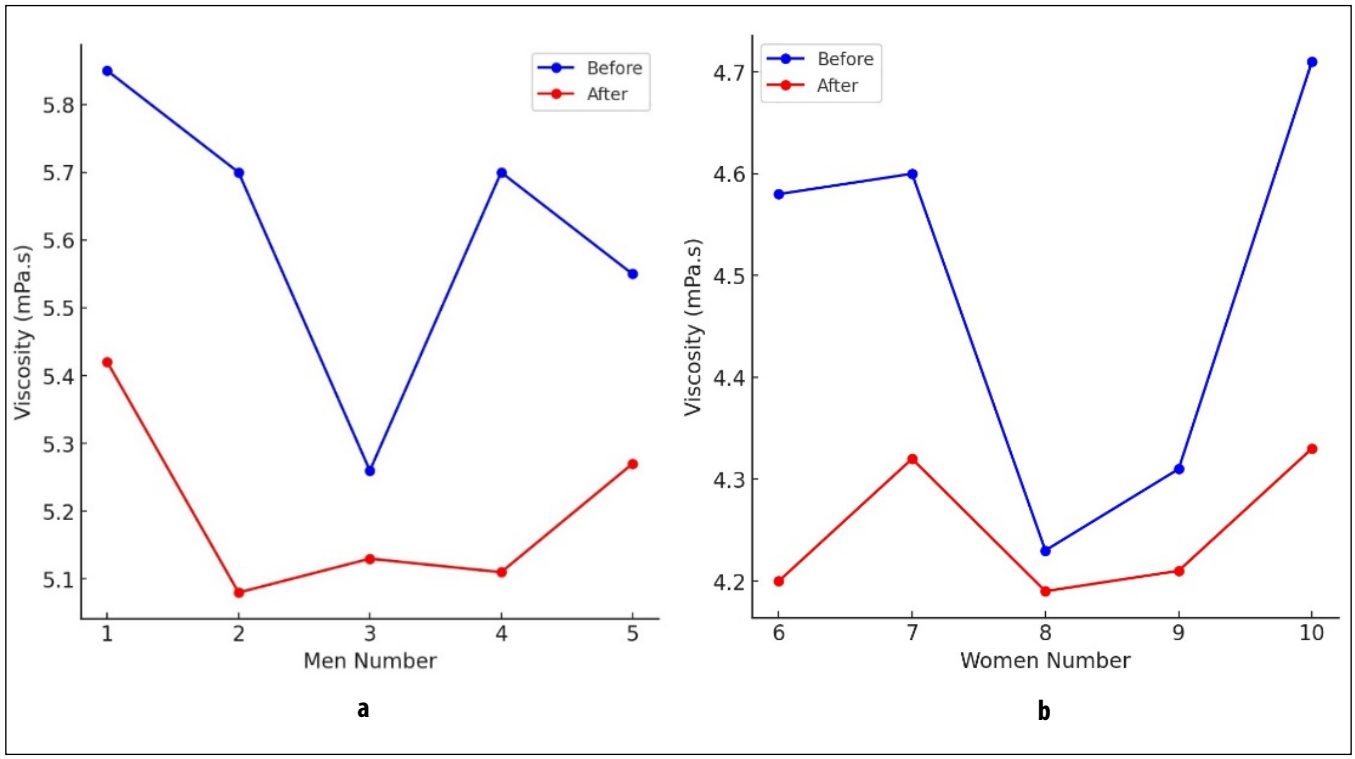

Fig. 2. Results of viscosity before and after consumption of Hydrogen Evodrop-rich water (HEW) for men (a) and women (b)
Picture taken by the authors

CONDITIONS FOR THE TESTING

The study involved 10 volunteers, five women and five men.

The present study was conducted using anonymous blood and urine samples voluntarily provided by participants. No personal information, including age or health status, was collected, and no medical or pharmacological interventions were performed. All procedures adhered to principle of data protection and ensured minimal risk to participants, eliminating the need for bioethical committee approval. The study focused on non-invasive analysis on accordance with international standards for scientific research. The standard and parameters of the water for the tests according the European Drinking Water Directive 2020/2184 with certificate No. 13100/02.02.2023 is published in [28].

ETHICS

According to the Bulgarian Law on Medicinal Products in Human Medicine and Ordinance No. 31 on Good Clinical Practice, ethical approval is mandatory only for clinical trials involving medicinal products. Drinking water is not a medicinal product. All ten participants provided informed consent before their inclusion.

RESULTS

BLOOD CLINICAL TESTING

HEMATOCRIT TEST RESULTS

Table 1 and Figs. 1a and 1b illustrate the results of hematocrit for men and women before and after consuming Hydrogen Evodrop-rich water (HEW).

Table 3. Results between osmolality of urine before and after consumption of hydrogen-rich water

Number men	osmolality result (mOsm kg-1) before	osmolality result (mOsm kg^{-1}) after	norm (mOsm kg^{-1})	Number women	osmolality result (mOsm kg^{-1}) before	osmolality result (mOsm kg-1) after	norm (mOsm kg^{-1})
1.	820	760	500-850	6.	790	710	500-850
2.	790	710	500-850	7.	770	720	500-850
3.	750	710	500-850	8.	780	720	500-850
4.	790	720	500-850	9.	760	690	500-850
5.	780	730	500-850	10.	800	730	500-850

Source: compiled by the authors of this study

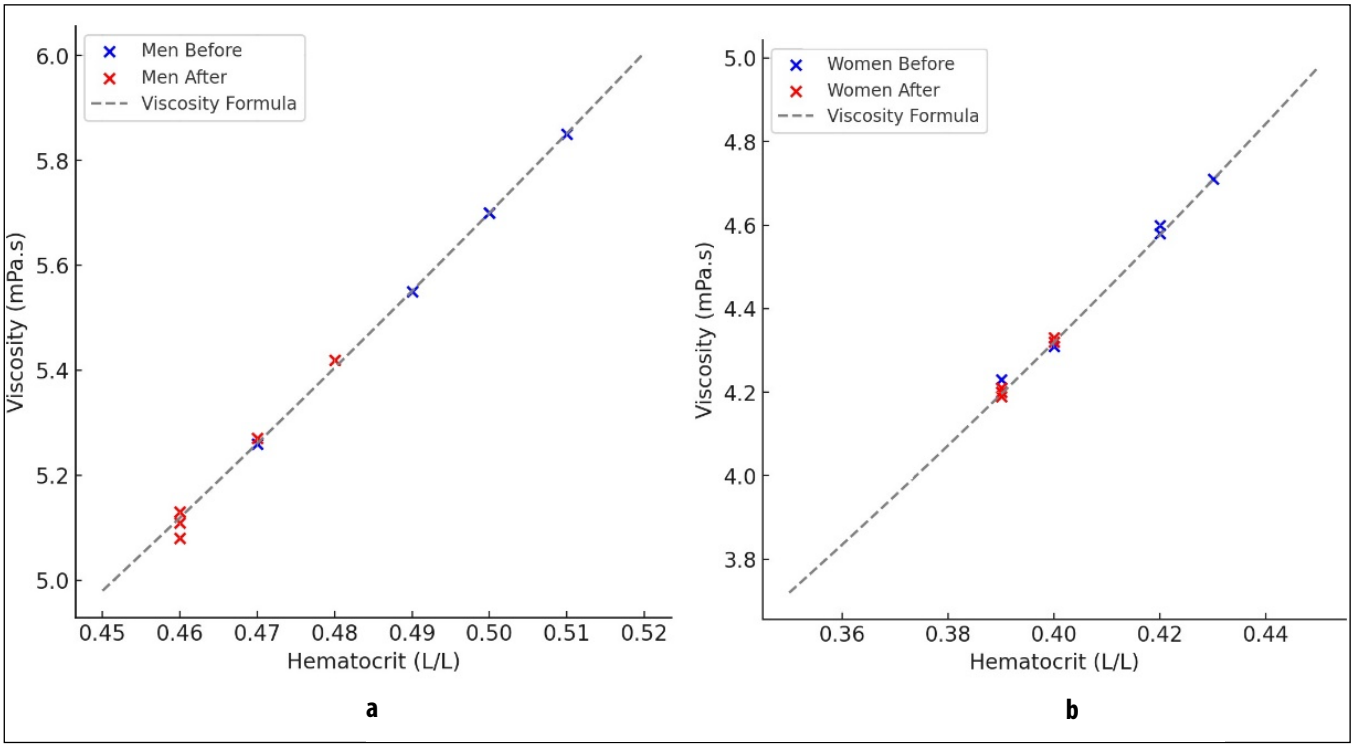

Fig. 3. Relationship between the hematocrit and blood viscosity in men (a) and women (b), both before and after consuming Hydrogen Evodrop rich-water
Picture taken by the authors

correspond to standard clinical laboratory protocols, in line with internationally accepted reference intervals as reported in *Tietz Textbook in Clinical Chemistry and Molecular Diagnostics* (6th ed., 2018) and *Henry's Clinical Diagnostic and Management by Laboratory Methods* (23rd ed., 2018)

The statistical results with the Student t-test are as next:

Men: $p<0.01$;

Women: $p<0.05$;

Paired Student's t-test showed significant differences:

Men: $t(4)=-4.84$, $p=0.0086$, mean difference=-0.028±0.013 L/L; Cohen's $dz=-2.15$

Women: $t(4)=-3.09$, $p=0.0367$, mean difference=-0.018±0.013 L/L; Cohen's $dz=-1.38$

The correlation coefficient (Men: $r=0.516$; Women: $r=0.722$) reflect the association between pre- and post-values, not effect size.

Both men and women exhibited statistically changes in hematocrit parameters after consuming HRW (Fig. 1).

BLOOD VISCOSITY TEST RESULTS

Table 2 and Fig. 2 present the viscosity results before and after consuming Hydrogen Evodrop-rich water (HEW) in men and women.

By the t-test of Student the following results were achieved:

Men; $p<0.05$:

Women: $p<0.05$;

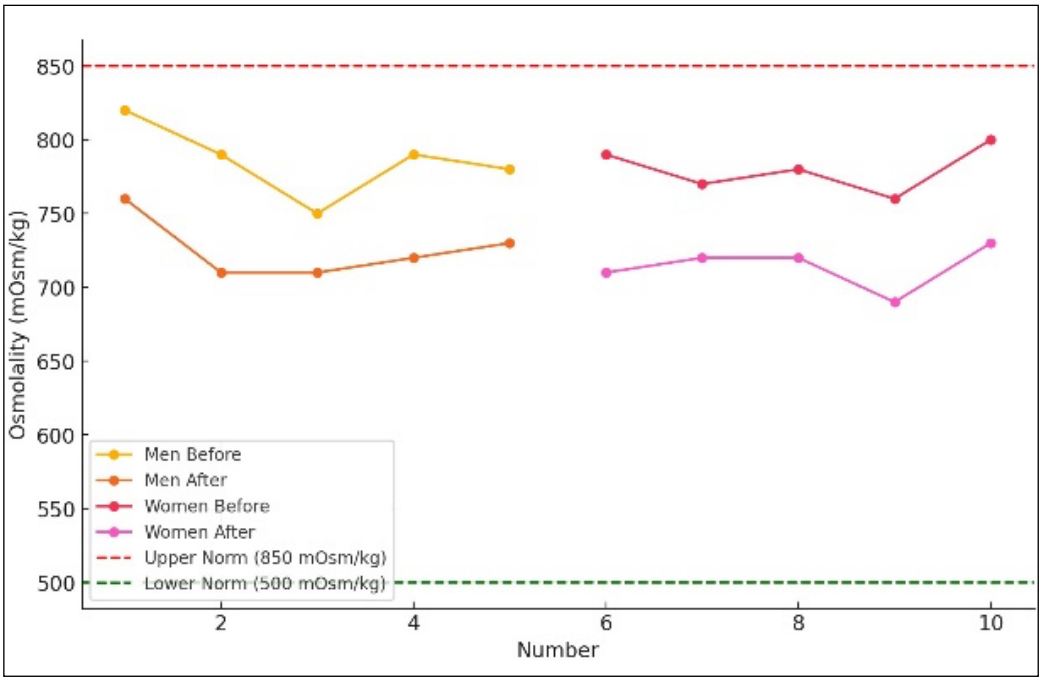

Fig. 4. Results between osmolality of urine before and after consumption of hydrogen-rich water
Picture taken by the authors

Paired Student's t-test showed significant differences:

Men: t(4)=–4.42, p=0.0115, mean difference=–0.410±0.208 mPa.s; Cohen's dz=–1.98

Women: t(4)=–3.33, p=0.0290, mean difference=–0.236±0.158 mPa.s; Cohen's dz=–1.49

The correlation coefficient (Men: r=0.43; Women: r=0.77) indicates the relationship between pre- and post-values rather than effect size.

Both men and women showed statistically changes in blood viscosity parameters after consuming HRW.

COMPARISON BETWEEN HEMATOCRIT AND BLOOD VISCOSITY

Let's compare the hematocrit and blood viscosity results. The study involved one group of 10 participants (5 men and 5 women) with pre- and post-intervention measurements.

The graphs in Figs 3a and 3b illustrate the relationship between the hematocrit and blood viscosity in men, both before and after consuming Hydrogen Evodrop-rich water (HEW).

The graphs present the relationship between hematocrit and viscosity.

There is a clear linear relationship between hematocrit and blood viscosity, visible from the figure. As the hematocrit increases, the blood viscosity increases.

The blue markers represent the values before HEW consumption, while the red markers represent the values after HRW consumption.

The red markers show that after consuming HEW, both hematocrit and blood viscosity decrease. This suggests improved hydration and a reduction in blood viscosity.

The dashed line represents the theoretical viscosity model as a function of hematocrit. The actual data points (both before and after) align closely with a linear model, indicating that the model accurately describes the relationship between these two variables.

The formulas for the linear equation, based on the data from the figure are:

For men: Viscosity=14.78xHematocrit – 1.69 (2).

For women: Viscosity=12.72xHematocrit – 0.70 (3).

The relationship between the two formulas, which describe the dependence between hematocrit and blood viscosity in men and women, can be analyzed by comparing their coefficients (slopes) and constants (intercepts):

Slope: for men (14.78) and for women (12.72)

Intercepts: (-1.69) and (-0.70)

The slope indicates how quickly blood viscosity changes with variations in hematocrit. The higher slope in (14.18) compared to women (12.61) suggests that blood viscosity increases more rapidly with increasing hematocrit in men.

The intercept represents the viscosity value when hematocrit is zero (a theoretical value). The difference in intercepts is relatively tiny but indicates that at equal hematocrit levels, blood viscosity in men would be slightly higher than in women if explored to lower hematocrit values.

OSMALALITY TESTS RESULTS

Table 3 and Fig. 4 show the results between osmolality of urine before and after consumption of hydrogen-rich water.

The graph illustrates that osmolality in both men and women decreases after the consumption of HRW, likely due to improved hydration. However, osmolality before the consumption was higher in men compared to women.

Results of t-test Student:

Men; $p<0.01$; $r=0.78$; moderate positive correlation.

Women; $p<0.001$; $r=0.73$; moderate positive correlation.

The statistical analysis confirms that HRW consumption has a significant and positive impact on hydration, as reflected by the decreased osmolality levels in both men and women. Both groups show moderate positive correlations ($r=0.78$ for men and $r=0.73$ for women), meaning that the changes in osmolality after HRW consumption are consistent and statistically significant. The slightly higher correlation in men could suggest that the effect of HRW is somewhat more pronounced in men, though the difference is relatively small.

DISCUSSION

The results obtained show that the elevated hematocrit values in men and women decreased after consuming hydrogen-rich water and approached normal values. This is an effect with a positive impact. The stronger correlation observed in women may indicate that hematocrit is more closely associated with other constant factors, such as hydration and plasma volume.

Hematocrit (HCT) is a percentage ratio of the volume of red blood cells (erythrocytes) to the total blood volume. It is a crucial indicator for evaluating the body's hydration status and other health conditions. When the body loses significant amounts of fluid or becomes dehydrated, the plasma volume decreases, increasing hematocrit. This occurs because the reduction of fluids increases the concentration of red blood cells. The movement of body water from the blood vessels to muscle tissue can cause hemoconcentration. It has been shown that an increase in HCT up to 60% can disrupt capillary blood flow, which hurts the body's capacity for physical activity [29,30]. Hematocrit is the main determinant of blood viscosity, and a doubling of HTC results in a 3- to 4-fold increase in blood viscosity. Elevated hematocrit is often a sign of dehydration and may be associated with symptoms such as dry skin, reduced urine output, and fatigue. The condition of the blood has a significant impact on the optimal physiological functions of the body. Deviations in the physical properties of blood are associated with the pathogenesis of various diseases. These deviations are also a valuable indicator of circulatory health and disease [29].

Dehydration increases the concentration of blood cells and proteins in the blood, leading in higher viscosity. Blood viscosity rises when less plasma dilutes the blood cells and other components. Elevated blood viscosity can impair average blood circulation and increase the risk of blood clot formation, which may lead to cardiovascular complications. Hyperviscosity may be due to changes in blood cells and plasma components. It can cause microvascular damage and subsequently a number of diseases.

Our analyses show gender influence on the relationship between hematocrit and viscosity. The shown formulas illustrate a stronger relationship between hematocrit and blood viscosity in men compared to women, which may be to differences in blood composition, hormonal factors, or other physiological indicators. The results also show that blood viscosity changes with variations in hematocrit. In men, blood viscosity increases more rapidly with increasing hematocrit. This may indicate that men's blood is more sensitive to changes in red blood cell concentration. The condition of the blood has a significant impact on the optimal physiological functions of the body. The physical properties of blood affect hemodynamics in health and disease, and their deviations are associated with the pathogenesis of various diseases. They are also a valuable indicator of circulatory health and disease. Aggregation of erythrocytes occurs normally in the circulation of healthy people, so it should not be considered harmful if it is within acceptable limits. However, pathological hyper aggregation is a non-specific marker in a wide range of diseases, including inflammatory disorders. Dehydration causes water-electrolyte imbalance and changes in blood volume. Adequate hydration with carbohydrate-electrolyte fluids during physical activity can prevent dehydration and delay the onset of fatigue, allowing proper biochemical and hematological responses during physical exertion.

Urine osmolality is another indicator of human health. It reflects the ratio of dissolved substances to water and can be estimated by determining the concentrations of dissolved substances and urea in it. It is used to assess kidney function and the body's hydration status. Urine osmolality is related to the body's hydration level. When a person is well-hydrated, urine osmolality is low because the kidneys excrete excess water. In cases of over hydration, urine becomes more diluted, resulting in a lower concentration of dissolved substances and lower osmolality. Conversely, during dehydration, urine osmolality increases as the body tries to conserve water. The kidneys reduce the amount of urine produced, making it more concentrated. This leads to a higher concentration of electrolytes and other dissolved substances, thereby raising urine osmolality.

Overall, our research demonstrates some of the human health benefits of consuming HRW. H_2 has been suggested in experimental and preclinical studies to have potential therapeutic effects. Consuming hydrogen-treated plant crops and hydrogen-containing beverages can offer numerous health benefits. Taking HRW has beneficial effects that enhance central nervous system functions, including mood, anxiety, and autonomic nerve function, which may help improve quality of life. The hydrogen intervention was applied with effects on rats. In addition, Toshkova et al. found experimentally in golden Syrian hamsters that hydrogen-rich EVOdrop water with an H_2 concentration of 0.9~1.2 ppm, pH=7.3 and oxidation-reduction potential ~450 mV has an antitumor effect. It has a normalizing effect on hematological parameters as well as an anticancer potential that can be used alongside conventional chemotherapeutics, even for the treatment and prevention of cancer.

Hydrogen-rich water (HRW) is enriched with molecular hydrogen (H_2). Initially, HRW was produced by dissolving magnesium in water. Currently, tablets are available as food additives that can generate hydrogen in water. Larger quantities of HRW are obtained through electrolysis devices, which produce water saturated with hydrogen. It's important to note that hydrogen gas and H_2 dissolved in water have no known toxic effects. This safety profile and hydrogen's potential in medical applications remained largely unexplored until 2007. That year, Ohsawa et al. reported that inhaling 1-4% hydrogen gas could significantly ameliorate cerebral ischemia-injury by selectively reducing hydroxyl radicals and peroxynitrite. This discovery marked a significant turning point in the field, opening up new possibilities for medical applications of hydrogen.

The medical effects were achieved with hydrogen concentration under 2.0 ppm [31]. In the hydrogen Evodrop rich water, the hydrogen concentration is 0.9-1.2 ppm, ORP is (-450~ -580) mV, and pH=7.1-7.3 [21, 22]. Numerous studies have been conducted in recent years on the bioeffects of molecular hydrogen. Its antioxidant effects have also been demonstrated [23]. Experimental studies suggest that HRW may neutralize oxidants such as hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$) inside cells. Effects on mitochondria are also described. Hydrogen quickly penetrates tissues and cells, but with its mild action, it does not disrupt metabolic redox reactions or affect signaling by reactive oxygen species, so no adverse effects have been reported. In addition, it stimulates energy metabolism. Among the possible methods of preventive and therapeutic application in humans, such as inhalation of H_2 gas, injection of saline solution with H_2, instillation of such solution into the eyes, or taking an H_2 bath, drinking H_2 water is the most accessible option with potential for clinical applications in many diseases. Importantly, research on its effects on various diseases is underway, offering hope for potential medical applications.

Dehydration increases the concentration of blood cells in the blood, leading to higher viscosity. Blood viscosity rises when less plasma is diluted with the blood cells and other components. Elevated blood viscosity can impair average blood circulation and increase the risk of blood clot formation, which may lead to cardiovascular complications. Hyperviscosity may be due to changes in the blood cells and plasma components. It can cause microvascular damage and subsequently several diseases. Prolonged hyperviscosity has been linked to reduced oxygen delivery to tissue, further aggravating cellular hypoxia. Increased shear stress on the vascular endothelium can promote endothelial dysfunction and inflammation. Clinical studies indicate that hyperviscosity is associated with hypertension, stroke, and metabolic syndrome. Even mild dehydration can significantly affect hemorheological parameters, especially elderly individuals [32].

Therefore, maintaining optimal hydration is crucial for preserving normal blood rheology and preventing viscosity-related pathologies. Furthermore, the balance of hydrogen ions (pH) in plasma playsa critical role in modulating protein charge and red blood cell interactions, thereby influencing blood viscosity [33]. Alterations in pH can exacerbate oxidative stress and vascular dysfunction, while molecular hydrogen has been reported to reduce reactive oxygen species (ROS) and protect enthelial function [34].

This dual perspective – focusing both on hydration and redox regulation highlights the importance of water balance and hydrogen-related mechanisms in maintaining healty microcirculation and preventing cardiovascular risk.

CONCLUSIONS

This study suggests that intake of hydrogen-rich water (HRW) with a concentration of molecular hydrogen 0.9–1.2 ppm, pH=7.2, and oxidation-reduction potential (-450) mV may be associated with changes in hydration-related markers such as hematocrit, blood viscosity, and urine osmolality.

The following results were achieved:

1. Hematocrit levels decreased after HRW intake in both men ($p<0.01$) and women ($p<0.05$).
2. Blood viscosity measured at shear rate of 94.5 s^{-1} was also reduced after HRW intake in both sexes ($p<0.05$).
3. Both hematocrit and viscosity changed in the same

direction, indicating a consistent effect of HRW on blood rheology.

4. These preliminary findings support the need for further studies to establish the physiological relevance of HRW consumption. Moreover, the observed improvements in rheological properties suggest that hydration-rich water (HRW) may contribute to better microcircularity function, more efficient oxygen delivery, and reduced cardiovascular strain. The alignment of decreased hematocrit and viscosity highlights a potential integrative role of HRW in modulating both plasma volume and cellular concentration.

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CONFLICT OF INTEREST

Author Fabio Huether is co-inventor of the Patent CH Patent WO2020169852A1. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL ARTICLE

CONTENTS

Six-month effects of liraglutide and dapagliflozin on lipid profile, cardiovascular risk, and NT-proBNP levels in patients with metabolic dysfunction-associated steatotic liver disease

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ABSTRACT

Aim: This study assessed and compared changes in lipid profile and cardiovascular risk in patients with metabolic dysfunction-associated steatotic liver disease after six months of liraglutide or dapagliflozin treatment. We also evaluated changes in N-terminal pro-B-type natriuretic peptide levels.

Materials and Methods: This prospective, randomized, parallel-group study included 115 adult patients with metabolic dysfunction-associated steatotic liver disease. Participants were randomized into three groups: control (n = 36, lifestyle intervention only), dapagliflozin (n = 41, 10 milligrams daily), or liraglutide (n = 38, titrated to 1.8 milligrams daily). All patients adhered to a Mediterranean diet and moderate physical activity. Lipid profile and N-terminal pro-B-type natriuretic peptide levels were measured at baseline and six months. Cardiovascular risk was assessed using five validated scales: Globorisk, Framingham Risk Score, American College of Cardiology/American Heart Association atherosclerotic cardiovascular disease Risk Calculator, Prospective Cardiovascular Münster Score, and World Health Organization cardiovascular risk charts.

Results: All groups showed significant within-group improvements in total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein cholesterol ($p < 0.001$), with liraglutide showing greater lipid improvements intergroup ($p < 0.05$). Cardiovascular risk scores decreased significantly in all groups, with no differences between them. N-terminal pro-B-type natriuretic peptide levels increased significantly in the control and liraglutide groups, but remained unchanged in the dapagliflozin group.

Conclusions: Liraglutide and dapagliflozin are effective in improving lipid profile and reducing cardiovascular risk. Liraglutide showed superior efficacy in lipid improvement. Changes in N-terminal pro-B-type natriuretic peptide require further investigation.

KEY WORDS: metabolic dysfunction-associated steatotic liver disease, cardiovascular risk, liraglutide, dapagliflozin, NT-proBNP

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most common forms of chronic liver disease, affecting over 30% of the global adult population and continuing to rise [1]. In most cases, MASLD develops in the context of obesity, insulin resistance, and type 2 diabetes mellitus, all of which are closely associated with lipid metabolism disturbances [2].

Dyslipidemia in MASLD is characterized by elevated triglyceride (TG) levels, decreased high-density lipoprotein (HDL-C), increased low-density lipoprotein (LDL-C) concentrations [3]. These changes contribute to a higher cardiovascular risk and simultaneously aggravate the progression of hepatic steatosis [4].

Current evidence indicates that cardiovascular diseases (CVDs), rather than hepatic complications, are the leading cause of mortality in patients with MASLD. This elevated risk is not only driven by associated metabolic conditions such as diabetes, hypertension, and dyslipidemia, but also by direct mechanisms linked to chronic systemic inflammation, oxidative stress, and endothelial dysfunction characteristic of MASLD [5].

Given this, the assessment of cardiovascular risk in MASLD patients becomes clinically significant. Several validated tools are available to estimate the 10-year risk of cardiovascular events, including the ASCVD Risk Calculator (ACC/AHA), Framingham Risk Score, Prospective Cardiovascular Münster (PROCAM) Score, WHO cardiovascular risk charts, and Globorisk [6-10].

Utilizing multiple models enables a more accurate stratification of cardiovascular risk. However, these tools do not consider hepatic fibrosis or steatosis, which may influence outcomes [11].

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a stable biomarker primarily used to assess cardiac wall stress and is most commonly applied in the context of heart failure. However, it is increasingly being explored in broader cardiovascular risk assessment, especially

in populations with metabolic dysfunction. This is particularly relevant in patients with metabolic disorders such as obesity, insulin resistance, and MASLD [12].

In the context of hepatic steatosis, particularly in early stages (S1–S2), a trend toward lower NT-proBNP levels has been observed. This may be related to hyperinsulinemia-mediated suppression of natriuretic peptide secretion. Such reductions may mask early signs of cardiac stress and lead to an underestimation of cardiovascular risk [13].

This reduction may complicate the detection of early stages of cardiac involvement, as traditional cardiovascular risk stratification tools do not consider hepatic steatosis as a modulating factor of NT-proBNP levels. In the context of treatment, it becomes especially important to monitor NT-proBNP changes in relation to steatosis severity, as a potential indicator of therapeutic response [14].

While lifestyle modification remains the cornerstone of MASLD management, including adherence to a Mediterranean diet and regular physical activity, these measures are often insufficient in patients with advanced metabolic derangements. Pharmacologic agents such as glucagon-like peptide-1 receptor agonists (GLP-1) and sodium-glucose cotransporter-2 inhibitors (SGLT2) have shown promising effects on body weight, glycemic control, and hepatic steatosis [15].

Liraglutide, a representative of GLP-1 receptor agonists, has shown effectiveness in reducing body weight, liver steatosis, improving glycemic profiles, and lowering alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in patients with MASLD [16].

Dapagliflozin, a representative of SGLT2 inhibitors, like liraglutide, has a positive effect on reducing blood glucose levels and decreasing liver inflammation. It is also suggested that this drug can reduce liver fat infiltration by inhibiting lipid and bile acid synthesis through suppression of LXRα-mediated pathways [17].

AIM

To assess and compare changes in lipid profiles and cardiovascular risk, based on five stratification scales, in patients with MASLD before and after 6 months of treatment with liraglutide or dapagliflozin. Additionally, to evaluate NT-proBNP levels in relation to the degree of hepatic steatosis before and after treatment.

MATERIALS AND METHODS

This prospective study was part of a dissertation project and was carried out at the clinical base of the Department of Internal Medicine №1, Bogomolets National Medical University, Kyiv, Ukraine. Ethical approval was obtained from the Bioethics Committee of the institution (Protocol №187, dated 23.09.2024).

The study followed the principles outlined in the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, and relevant Ukrainian legislation. Written informed consent was obtained from all participants.

PATIENTS

The study population included patients aged 26–67 years with a confirmed diagnosis of MASLD, based on steatometric evidence of liver steatosis and at least one cardiometabolic risk factor in accordance with the 2023 MASLD criteria [18].

Exclusion criteria included: a history of cardiovascular events, liver cirrhosis, alcoholic liver disease, viral hepatitis, oncological and hematological diseases, pregnancy and lactation.

STUDY DESIGN

This was a prospective, randomized, parallel-group study conducted in two phases of stratification. Initially, 115 patients with MASLD were enrolled and randomized into two groups. The control group (n=36) received standardized non-pharmacological treatment, including a Mediterranean diet and at least 150 minutes per week of moderate-intensity physical activity. The remaining 79 patients comprised the intervention group, receiving the same lifestyle interventions plus a pharmacological agent.

In the second step, the intervention group was further randomized into two subgroups. Group IA (n=41) received dapagliflozin at a fixed dose of 10 mg once daily for 6 months. Group IB (n=38) received liraglutide starting at 0.6 mg once daily, titrated weekly to 1.8 mg and maintained for 6 months.

Randomization was computer-generated and stratified by age to ensure balanced distribution across the main study arms and subgroups.

VISITS

During the initial visit, all patients underwent a physical examination, complaints and anamnesis were collected, instrumental assessment included liver steatometry, performed using the Soneus P7 (UltraSign, Ukraine) device located at the clinical base of the Department of Internal Medicine №1, and laboratory tests—lipid profile and NT-proBNP.

Table 1. Baseline characteristics of study participants. X±SD or Me [25%;75%]

Indicators		Control group (n = 36)	Group IA (n = 41)	Group IB (n = 38)	Significance of difference, p
Age, years		43.3 ± 11	41.7 ± 10.7	39.6 ± 11.2	0.368
Age distribution	25-34 years	8 (22.2 %)	12 (29.3 %)	10 (26.3 %)	0.972
	35-44 years	11 (30.6 %)	13 (31.7 %)	13 (34.2 %)	
	45-54 years	12 (33.3 %)	10 (24.4 %)	9 (23.7 %)	
	55-67 years	5 (13.9 %)	6 (14.6 %)	6 (15.8 %)	
Sex	Men	25 (69 %)	24 (59 %)	30 (79 %)	0.147
	Women	11 (31 %)	17 (41 %)	8 (21 %)	
Severity of steatosis distribution	S1	9 (25 %)	14 (34.2 %)	8 (21.1 %)	0.526
	S2	14 (38.9 %)	12 (29.3 %)	18 (47.4 %)	
	S3	13 (36.1 %)	15 (36.5 %)	12 (31.5 %)	
Smoking (yes, %)		12 (33.3 %)	9 (21.9 %)	11 (28.9 %)	0.529
Medication use (yes, %) *		5 (13.6 %)	6 (14.6 %)	4 (10.5 %)	0.849
Diabetes mellitus (yes, %)		23 (63.9 %)	28 (68.3 %)	23 (60.5 %)	0.77
Arterial hypertension (yes, %)		7 (19.4 %)	9 (21.9 %)	7 (18.4 %)	0.921
Other comorbidities (yes, %) **		5 (13.8 %)	8 (19.5 %)	3 (7.9%)	0.329
Systolic blood pressure (mmHg)		132.5 ± 13.6	133.7 ± 16.6	132.2 ± 15.6	0.896
Body mass index (kg/m2)		30.95 ± 3.4	31.61 ± 3.1	32.16 ± 4.4	0.371
Total cholesterol (mmol/L)		5.4 ± 1.0	5.3 ± 1.2	5.2 ± 1.3	0.844
LDL-C (mmol/L)		3.3 ± 0.7	3.1 ± 0.8	3.1 ± 0.9	0.639
HDL-C (mmol/L)		1.14 [1.05; 1.3]	1.18 [1.00; 1.34]	1.13 [1.01; 1.24]	0.699
TG (mmol/L)		2.1 [1.89; 2.47]	2.3 [2.01; 2.75]	2.4 [2.01; 2.92]	0.110
Globorisk (10-year risk, %)		25.1 [16.2; 33.9]	29.7 [19.9; 43.1]	20.2 [11.6; 29.1]	0.167
Framingham (10-year risk, %)		12.4 [6.8; 19.9]	15.2 [6.1; 23.5]	12.8 [8.9; 25.9]	0.793
ACC/AHA ASCVD (10-year risk, %)		8.2 [3.8; 11.7]	10.4 [6.2; 18.9]	5.1 [3.4; 11.2]	0.317
PROCAM (10-year risk, points)		38.1 ± 10.1	41.2 ± 11.2	38.7 ± 11.9	0.440
WHO CVD (10-year risk, %)		16 [11; 17]	17 [12; 27]	15 [9; 27]	0.322
NT-proBNP (pg/ml)		22.7 [18.6; 26.1]	32.1 [23.6; 75.5]	16.9 [13.5; 36.8]	<0.001

Note: * - medication use includes levothyroxine, sertraline or antihypertensive therapy (perindopril, enalapril + hydrochlorothiazide or valsartan); ** - other comorbidities include autoimmune thyroiditis, hypothyroidism, depressive disorder

Source: compiled by the authors of this study

After 6 months of the prescribed treatment, all aforementioned laboratory and instrumental assessments were repeated to assess the results obtained.

CARDIOVASCULAR RISK ASSESSMENT

During the first visit and after 6 months, cardiovascular risk was assessed using five validated risk scales: ASCVD Risk Calculator (ACC/AHA), Framingham Risk Score, PROCAM, WHO cardiovascular risk charts and Globorisk [6-10].

These scales were selected because they incorporate type 2 diabetes mellitus and/or multiple lipid profile indicators, making them particularly relevant for assessing cardiovascular risk in patients with MASLD.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics version 29.0. The Shapiro–Wilk test was used to assess the normality of data distribution. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data, or as median with interquartile range [Median (Q1–Q3)] for non-normally distributed data. Between-group comparisons of continuous variables were performed using the independent samples t-test (for normally distributed data) or the Wilcoxon rank-sum test (for non-normally distributed data). Comparisons among more than two groups were conducted using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, as appropriate. Post hoc analysis for multiple pairwise comparisons was performed using the Bonferroni correction. Categorical

Table 2. Intra-group changes in lipid profile, NT-proBNP, and cardiovascular risk (five scales) before and after 6-month therapy in MASLD patients. X±SD or Me [25%;75%]

Indicators	Control group (n = 36)		Group IA (n = 41)		Group IB (n = 38)		Significance of difference, p
	Before	After	Before	After	Before	After	
Total cholesterol (mmol/L)	5.4 ± 1.0	4.8 ± 0.8	5.3 ± 1.2	4.6 ±1.0	5.2 ± 1.3	4.3 ± 1.0	p1<0.001 p2<0.001 p3<0.001
LDL-C (mmol/L)	3.2 [2.8; 3.6]	2.8 [2.4; 3.1]	3.1 ± 0.8	2.7 ±0.7	3.1 ± 0.9	2.5 ± 0.7	p1<0.001 p2<0.001 p3<0.001
HDL-C (mmol/L)	1.2 ± 0.22	1.26 ±0.23	1.2 [1.0; 1.3]	1.3 [1.1; 1.5]	1.1 [1.0; 1.2]	1.4 [1.2; 1.5]	p1<0.001 p2<0.001 p3<0.001
Triglycerides (mmol/L)	2.1 [1.9; 2.5]	1.83 [1.62; 2.24]	2.3 [2.0; 2.8]	1.9 [1.6; 2.2]	2.4 [2.0; 2.9]	1.8 [1.5; 2.1]	p1<0.001 p2<0.001 p3<0.001
Globorisk (10-year risk, %)	25.1 [16.2; 33.9]	19.5 [12.4; 27.6]	29.8 [19.9; 43.1]	19.6 [16.0; 35.1]	20.2 [11.6; 29.1]	13.6 [8.8; 26.5]	p1<0.001 p2<0.001 p3<0.001
Framingham (10-year risk, %)	12.4 [6.8; 19.9]	9.4 [5.2; 15.7]	15.2 [6.1; 23.5]	9.7 [4.5; 21.3]	12.8 [8.9; 25.9]	8.7 [5.9; 19.3]	p1<0.001 p2<0.001 p3<0.001
ACC/AHA ASCVD (10-year risk, %)	8.2 [3.8; 11.7]	6.0 [2.7; 9.1]	10.4 [6.2; 18.9]	6.1 [4.4; 14.0]	5.1 [3.4; 11.2]	3.0 [2.0; 10.5]	p1<0.001 p2<0.001 p3<0.001
PROCAM (10-year risk, points)	38.1 ± 10.1	33.0 ± 10.7	41.2 ± 11.2	34.0 ± 10.4	38.7 ± 11.9	32.0 ± 10.5	p1<0.001 p2<0.001 p3<0.001
WHO CVD (10-year risk, %)	16 [11; 17]	14 [9; 16]	17 [12; 27]	14 [12; 23]	15 [9; 27]	10 [7; 18]	p1<0.001 p2<0.001 p3<0.001
NT-proBNP (pg/ml)	22.7 [18.6; 26.1]	30.7 [24.6; 34.9]	32.1 [23.6; 75.5]	38.3 [27.8; 78.4]	16.9 [13.5; 36.8]	22.8 [18.3; 46.0]	p1=0.002 p2=0.396 p3=0.022

Note: p1 - statistical significance of the difference between the control group and Group IA, p2 - statistical significance of the difference between the control group and Group IB, p3 - statistical significance of the difference between Group IA and Group IB
Source: compiled by the authors of this study

variables were compared using the chi-squared (χ^2) test. A p-value < 0.05 was considered statistically significant.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

Baseline demographic, clinical, and biochemical characteristics of the study participants are presented in Table 1. Patients were categorized into three groups: the control group (n = 36), Group IA (dapagliflozin, n = 41), and Group IB (liraglutide, n = 38). Most baseline characteristics were comparable across groups, allowing for a reliable assessment of treatment effects. However, baseline NT-proBNP levels differed significantly between groups and were therefore considered in the interpretation of outcome data.

Significant improvements in lipid profile indicators were observed in all groups after 6 months of treatment. Total cholesterol, LDL cholesterol, and triglyceride levels significantly decreased, while HDL cholesterol levels increased ($p < 0.001$ for all within-group comparisons). These findings reflect a favorable impact of all three treatment strategies—including lifestyle intervention, dapagliflozin, and liraglutide—on lipid metabolism in patients with MASLD. Detailed results are presented in Table 2.

All five cardiovascular risk scores demonstrated a statistically significant reduction after 6 months of

Table 3. Between-group comparison of lipid profile and cardiovascular risk scores after 6 months of treatment in patients with MASLD (X ± SD or Me [25%; 75%])

Indicators	Control group (n = 36)	Group IA (n = 41)	Group IB (n = 38)	Significance of difference, p
Total cholesterol (mmol/L)	4.86 ± 0.79	4.63 ±1.01	4.29 ± 1.04	p1 = 0.60 p2 = 0.04 p3 = 0.28
LDL-C (mmol/L)	2.85 ± 0.58	2.7 ± 0.71	2.45 ± 0.69	p1 = 0.62 p2 = 0.04 p3 = 0.29
HDL-C (mmol/L)	1.23 ± 0.23	1.27 ± 0.23	1.37 ± 0.21	p1 = 0.68 p2 = 0.02 p3 = 0.15
Triglycerides (mmol/L)	1.95 ± 0.44	1.90 ± 0.45	1.79 ± 0.39	p = 0.109
Globorisk (10-year risk, %)	19.5 [12.4; 27.6]	19.6 [16.0; 35.1]	13.6 [8.8; 26.5]	p = 0.106
Framingham (10-year risk, %)	9.4 [5.2; 15.7]	9.7 [4.5; 21.3]	8.7 [5.9; 19.3]	p = 0.975
ACC/AHA ASCVD (10-year risk, %)	6.0 [2.7; 9.1]	6.1 [4.4; 14.0]	3.0 [2.0; 10.5]	p = 0.24
PROCAM (10-year risk, points)	33.0 ± 10.7	34.0 ± 10.4	32.0 ± 10.5	p = 0.43
WHO CVD (10-year risk, %)	14 [9; 16]	14 [12; 23]	10 [7; 18]	p = 0.198

Note: p – statistical significance of the overall difference between the three groups; p1 – significance between control and Group IA; p2 – between control and Group IB; p3 – between Group IA and Group IB
Source: compiled by the authors of this study

treatment ($p < 0.001$ for all within-group comparisons), further supporting the positive impact of the interventions on cardiometabolic risk in patients with MASLD.

Changes in NT-proBNP levels differed across the groups. A statistically significant increase in median NT-proBNP was observed in the control group and the liraglutide group ($p < 0.05$ for both), while no significant change was detected in the dapagliflozin group.

The intergroup analysis after 6 months of treatment revealed significant improvements in lipid profile parameters in Group IB (liraglutide) compared to both the control group and Group IA (dapagliflozin) ($p < 0.05$). No statistically significant differences were observed between the control and dapagliflozin groups in terms of lipid profile ($p > 0.05$).

Despite overall reductions in cardiovascular risk across all groups, no significant differences in cardiovascular risk scores were found between the treatment groups ($p > 0.05$). Detailed comparisons are presented in Table 3.

DISCUSSION

In this prospective 6-month study, patients with MASLD underwent evaluation of lipid profile, NT-proBNP levels, and cardiovascular risk using five validated risk stratification tools (Globorisk, Framingham Risk Score, ASCVD Risk Calculator, PROCAM, and WHO CVD risk chart) [6-10].

All three treatment strategies—standardized lifestyle intervention, addition of dapagliflozin, and addition of liraglutide—were associated with significant improvements in lipid parameters and reductions in cardiovascular risk within each group [19]. These findings confirm the clinical utility of both pharmacologic agents in addressing dyslipidemia and cardiometabolic risk in MASLD patients [20].

Among the groups, the most pronounced improvement in lipid profile was observed in the liraglutide group, which showed significantly better outcomes compared to both the control and dapagliflozin groups [16]. This observation may be attributed to the known mechanisms of GLP-1 receptor agonists, including weight reduction, improved insulin sensitivity, and enhanced reverse cholesterol transport [21].

In contrast, the intergroup analysis of cardiovascular risk scores did not reveal statistically significant differences between the treatment arms. Although all groups showed significant within-group reductions, the observed similarity between treatment strategies

may reflect several factors, including the relatively short follow-up duration, modest baseline cardiovascular risk in the study population, and limited sensitivity of existing risk estimation tools to capture subtle therapeutic effects [11]. Future studies with longer observation periods and potentially more sensitive or dynamic risk assessment models may help to elucidate differential cardiovascular benefits between treatment approaches [22].

Notably, changes in NT-proBNP differed between groups. A significant increase in median NT-proBNP was observed in the control and liraglutide groups, whereas no significant change occurred in the dapagliflozin group. These divergent patterns warrant further investigation. The absence of significant NT-proBNP changes in the dapagliflozin group might reflect biological heterogeneity at baseline, given the wider distribution of initial NT-proBNP values in this group. This underscores the need for larger, more homogeneous samples to confirm potential treatment-related effects on NT-proBNP dynamics.

The lack of intergroup differences in cardiovascular risk scores and NT-proBNP changes highlights the complexity of interpreting these parameters over a relatively short treatment duration and underscores the importance of longer follow-up periods.

CONCLUSIONS

This 6-month prospective study demonstrated that both liraglutide and dapagliflozin significantly improved lipid profiles and reduced cardiovascular risk in patients with MASLD. Total cholesterol, LDL cholesterol, and triglyceride levels decreased, while HDL cholesterol increased in all groups, confirming the metabolic benefit of the interventions. Notably, intergroup analysis revealed more pronounced improvements in lipid parameters in the liraglutide group compared to both the control and dapagliflozin groups, suggesting a potential advantage of GLP-1 receptor agonists in modulating lipid metabolism.

Cardiovascular risk, assessed using five validated stratification tools, decreased consistently within all groups, although no significant differences were observed between the treatment arms.

NT-proBNP levels increased significantly in the control and liraglutide groups but remained unchanged in the dapagliflozin group; further studies are warranted to clarify the clinical relevance of this finding.

Overall, these results support the use of liraglutide and dapagliflozin as effective components of MASLD management, with liraglutide demonstrating superior efficacy in improving lipid parameters over a 6-month treatment period.

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ORIGINAL ARTICLE

CONTENTS

An assessment of the results of self-monitoring after conservative treatment of hand and wrist fractures

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ABSTRACT

Aim: The objective of this study was to evaluate the effectiveness and safety of this method after simple fractures of the hand and wrist.

Materials and Methods: 202 patients, 117 males (58%) and 85 females (42%) at mean 57 years of age, with stable, non- or minimally displaced hand and wrist bone fractures were enrolled in the study. Patients were treated conservatively by immobilization in a plaster or thermoplastic splint, or functionally, without any immobilization. After one visit to the clinic and receiving instructions on how to deal with a broken finger or hand, the patients were dismissed with recommendation to remove the plaster splint after 4-5 weeks and start using the hand. After 2 months all patients were interviewed by phone, asking about the course of treatment and satisfaction with this method of care.

Results: 179 patients (89%) were fully satisfied with the self-monitoring program, and 23 (11%) were partially satisfied. The most common problems in these patients were pain at the fracture site and limited mobility of the affected finger or wrist. No complications requiring hospitalization and surgery were found.

Conclusions: The change in the post-fracture care system from a traditional to a self-monitoring has shown great effectiveness and safety for patients. This improved the work of the hand clinic, improved patients' access, increased satisfaction and reduced costs.

KEY WORDS: fracture self-monitoring; simple hand fractures; conservative treatment; assessment of outcomes.

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INTRODUCTION

The demand for hand surgeries (both elective and outpatient) has increased significantly over the past decade, and with it, the pressure on access to the operating room and for outpatient services has increased. This is caused, among other things, by two phenomena. The first is the natural aging process of societies, which is why a larger number of older people require seeking medical attention. The second is due to cultural changes and increased demands on maintaining health and physical fitness. Patients with minor musculoskeletal, post-traumatic or post-morbid disorders, who have yet to

20 years ago, tolerated well these complaints, now they seek medical advice to return to full fitness, as they did before an injury or illness. These phenomena are compounded by the backlog in surgical treatment caused by the COVID-19 pandemic, which significantly limited access to planned medical services for 2 years. For various reasons, including economic ones, the availability of hospital treatment has become lower after the pandemic. The consequence of these trends is a change in the strategy of insurers and healthcare providers, consisting in the redesign of service delivery schemes and the implementation of new policies aimed at improving cost efficiency [1, 2]. As part of the redesign concept, it is recommended to reduce follow-up visits after hand and wrist fractures [3, 4].

In the literature, you can find information about systemic changes in outpatient care in hand surgery. They consist in limiting the frequency of follow-up visits in specialist clinics, in favour of control performed by the patient himself. This is the so-called "self-monitoring fracture care" or "virtual fracture clinic" concept, which has gained popularity during and after the COVID-19 pandemic and is well rated by patients [1-4]. This method of control after simple hand and wrist bones fractures is largely unknown in the authors' country.

AIM

The objective of this study was to evaluate the effectiveness and safety of "fracture self-monitoring" concept after simple hand and wrist fractures.

MATERIALS AND METHODS

In 2025, a total of 458 patients were treated in out-patient hand clinic due to finger, metacarpal or distal radial fractures. Of this number, 209 (46%) fractures were non- or minimally displaced and showed a stable configuration. These patients were treated conservatively either by immobilisation in the plaster splint, thermoplastic splint or brace (n=173), or functionally, without any immobilization (n=36). For the first visit to the out-patient hand clinic, patients were referred from the emergency department, where the fracture was diagnosed and primary fitted (immobilized), or from another, district hospital. After determining that fracture is non- or minimally displaced and shows stable configuration, plaster splint was corrected (if necessary) or replaced with brace, and the patient received verbal instructions for hand exercises and to use their hands in light daily activities and at work, i.e. with computer, hand writing with a pen or carrying light objects. The patients were advised to remove the splint himself/herself after 4 weeks (with finger and metacarpal fractures) or 5 weeks (with distal radial fractures). Next, the patients should start using their hands without immobilization, gradually expanding the range and load of the hand. The patients were informed that in the event of eventful course (unexpected pain, oedema, movement restrictions), they could report to the out-patient hand clinic. They were also asked to present to the clinic if any problems with use of the hand occur within a month after the removal of the immobilization. Two hundred and nine patients were recruited to participate in the study. An informed, verbal consent was obtained from each patient for the participation in the study.

FOLLOW-UP

In the next part of the study, two-months after first visit, the patients were followed-up by a phone interview. They were asked about the course of the treatment, how they coped with removal of plaster splint, whether they experienced any significant problems during this period and whether they were satisfied with this form of follow-up. A total of 202 patients, 117 men (58%) and 85 women (42%) were available. Three patients (2%) could not be contacted. A group of 202 patients (100%) is a subject of the analysis.

ETHICAL APPROVAL

The study received approval from the Bioethics Committee of the Pomeranian Medical University in Szczecin. All procedures adhered to the ethical principles of the Declaration of Helsinki, ensuring participant confidentiality and anonymity

RESULTS

Spectrum of fractures in patients enrolled in the study is summarized in Table 1. The most common were finger and II-V metacarpal fractures, making a total of 156 cases (77%). Distal radial fractures were relatively under-represented, compared to the total number of patients with these fractures presented in the clinic. The reason was the need for follow-up during and after treatment in these patients, as most fractures were displaced and required primary reduction. In this situation, follow-up during and after treatment is recommended. Flow-chart and satisfaction of patients in the study is summarized in Table 2. Of the 209 patients who were implemented in the fracture self-monitoring program, seven (3,3%) could not be contacted 2 months after fracture. Of the remaining 202 patients, eight (4%) presented to the clinic in person, during the 1-month observation period, before the telephone interview. These patients were considered not-fully satisfied with the fracture self-monitoring regimen, because the reason for their visit was a problem with the injured hand.

Of the remaining 194 patients who were contacted by a phone call at 2 months, 179 (89%) reported no problems with fracture self-care and were fully satisfied with the self-monitoring regimen. Fifteen (8%) were partially satisfied and reported various problems during post-fracture period. Including patients who reported to the clinic in person (n=8), a total of 23 patients (11% of 202) were partially satisfied with the fracture self-monitoring. Concerns of these patients are summarized in Table 3.

]The most commonly reported problem was pain at the fracture site and limitation of fractured finger or wrist movements (in the case of distal radial fractures). During a telephone or personal interview, patients were informed how to exercise to improve finger/wrist mobility. Five patients (29%) with distal radial fractures who reported limitation of wrist mobility were advised to contact their General Practitioner for referral for rehabilitation. Two patients were asked to contact the clinic in person and they had an X-ray taken that showed bone union with a slight displacement which did not require corrective treatment. In one of these patients, the wrist was slightly deformed, typical for such displacement. In 2 patients, after fractures of the proximal phalanges of fingers, significant reduction of finger mobility was found and they were also referred for rehabilitation. In one patient with a trans-articular fracture of the base of the middle phalange of the little finger, a slight subluxation was found in the proximal interphalangeal joint, but it did not require surgical correction, only more intensive rehabilitation. This patient was the second to have a slight deformity (the first was a woman after a distal radial fracture). Of the 12 patients who complained of fracture site pain, only two considered it moderately severe, but none were taking analgesic drugs. These patients were

Table 1. Spectrum of fractures in the study

Fracture type	Number of patients	[%]
Finger fracture	95	47%
Thumb fracture	7	3,5%
Thumb metacarpal fracture	10	5%
II-V metacarpal fracture	61	30%
Carpal fracture (triquetrum bone)	12	6%
Distal radial fracture	17	8,5%
Total	202	100%

Source: Own materials

Table 2. Flow-chart and satisfaction of patients in the study

Particular patients' populations	Number of patients	[%]
Number of patients implemented to the fracture self-monitoring	209	-
Patients lost from follow-up	7	3,3%
Total number of patients reviewed	202	100%
Patients contacted by telephone	194	96%
Patients presented to the clinic in person	8	4%
Patients satisfied with self-monitoring regimen	179	89%
Patients not fully satisfied with self-monitoring regimen (including those who presented to the clinic)	23	11%

Source: Own materials

Table 3. Problems identified in 23 patients who were not fully satisfied with the fracture self-monitoring regimen

Problem of patients not fully satisfied*	Number of patients	[%]
Persisting pain in the fracture site	12	52%
Reduced finger range of motion	9	39%
Reduced wrist range of motion	8	35%
Swelling around the wrist	4	17%
Deformation of the finger/wrist	2	9%
Other concerns	4	17%
Total	39	

* Total number of problems is not equal number of patients, because several patients had 2 or 3 problems at the same time
Source: Own materials

advised that if the pain does not resolve within the next month, they should report to an outpatient clinic. Among the four patients with other concerns, two reported a fracture-independent problem: trigger finger and de Quervain tenosynovitis. Other two reported formation of small lump on the palm no restricting hand function. This was probably an early form of Dupuytren's disease. These patients were advised to observe their hands and present to hand clinic in case of progression of the disease. Eight patients (4%) presented in the clinic in person due to different concerns: six due to problems with removing the stitches and 5 for the reasons mentioned in table 2. All patients who visited the clinic were given professional advice, as well as everyone who was interviewed by phone. None of the 202 patients implemented in the postoperative self-monitoring program required hospital admission due to treatment complications. 179 patients (89%) were fully satisfied, whereas 23 (11%) were partially satisfied.

DISCUSSION

The change in the concept of outpatient postoperative care after minor and medium-sized hand and wrist fractures is forced by the large number of injuries to the hand and by limited health care resources, which cause a long wait for a face-to-face follow-up visits. Experience from the Covid-19 pandemic has shown that patients can safely control themselves and start with activities (exercises) with the injured hand. All they need are instructions provided upon discharge home after primary fitting the fracture. Since the

vast majority of hand fractures heal (unite) without complications, post-fracture follow-up by a healthcare professional (nurse, doctor) is, in most cases, not necessary. Such a concept results in a significant relief of the outpatient sector, which primarily increases the availability of consultations for people who actually need it. The author's observations from the outpatient hand clinic indicates that at least 10-15% of visits after simple hand and wrist fractures are unnecessary, and patients could serve themselves. In these cases, the visit is frequently limited to a one-minute conversation. The only professional activity for which a doctor or nurse is needed is to remove plaster or thermoplastic splint from finer or wrist, which - as already mentioned - can be performed by the patient himself, and not in a specialist hand clinic. The results of our study show that minimizing follow-up visits after primary fitting of simple hand and wrist fractures is safe and convenient for both patients and the healthcare system. It is safe for doctors, because in the event of any complication during treatment, patients can present to the hand clinic, where the treatment can be modified, e.g. from conservative to surgical.

In the literature, one can find studies on the effectiveness and safety of a self-monitoring program in the treatment of simple limb fractures (called„virtual fracture clinic"VFC). This concept has been proposed as an efficacious alternative to face-to-face fracture clinics. Better usage of clinical time and resources, increased accessibility, decreased patient wait times and reduced cost have been undoubtful advantages of the virtual fracture clinic. Johnson et al. (2025) reported results of a systematic review of the literature about clinical efficacy and patient satisfaction of the VFC in the United Kingdom. The authors reviewed a total of 25 studies involving 63 thousands of patients with simple fractures treated conservatively. VFCs reported an 84% mean compliance rate with British Orthopaedic Association Standards for Trauma, compared to 6% for face-to face fracture clinics. Virtual fracture clinics make minimal diagnostic errors and report a low reattendance rate following discharge, accounting for 5%. Patients have good health outcomes, a high mean satisfaction rate of 85% and prefer this treatment model over traditional follow-up appointments [3].

Similar results are reported in an another systematic review by Davey et al. (2020). Overall, 15 studies involving 12 thousands of patients with simple fractures treated conservatively, with mean follow-up of 13 months were reviewed. In total, 66% of patients were directly discharged after primary fracture fitting, with protocol derived conservative management, with 9% using the Helpline and 16% contacting their general practitioner for advice or reassurance. A total of 1,2% of patients experienced fracture non-unions and 48 patients (0,4%) required surgical intervention. The overall patient satisfaction rate was 81%, with only 1,3% experiencing residual pain at the fracture site. The mean cost per patient for virtual fracture clinic was 71 GBP (Great Britain Pound), with a mean saving of 53 GBP when compared to traditional (face-to-face) clinic models [4].

Maunder at al. (2025) reported results of an implementation of a virtual fracture clinic in one province in Australia. The authors triaged patients to the virtual fracture clinic based on predefined criteria over a 5-week period. Primary outcomes included patient satisfaction, travel distance savings and cost savings. Out of 514 fracture cases 185 (36%) were managed through the virtual fracture clinic. Compared with the traditional face-to-face model, virtual clinic patients had shorter waiting times, and 91% of those seen in the virtual fracture clinic could be discharged without further review. Virtual clinic patients were highly satisfied, with 139 (75%) patients declaring a willingness to undergo the same treatment again. It also resulted in significant travel distance and cost savings. No patients required surgery during the follow-up period. With high patient satisfaction and no compromise in safety, this model could redefine how fracture care may be delivered in patients having problems with access to traditional acute traumatic orthopaedic care [5].

Waite et al. (2023) reported results of an another systematic review about safety and effectiveness of paediatric virtual fracture clinics. The authors reviewed 6 studies which met the inclusion criteria The results show that there was a high rate of direct discharge from the VFC leading to reduced outpatient appointments. There were limited incidences of missed fractures and the rates of re-presentation were similar to that of face-to-face orthopaedic clinics. There were significant cost savings for the hospitals and high parent satisfaction. The authors conclude that VFCs have shown to be safe and effective at managing most stable, low operative risk paediatric fractures. However, they emphasise the need of safety ensuring with a telephone helpline and an open return to fracture clinic policy [6].

Our work has some limitations: it only concerns relatively simple (although very common) hand and wrist fractures. However, it should be emphasized that these fractures account for approximately 60% of all fractures fitted in an emergency department and changes in the organization of care in this field bring visible and measurable benefits. Perhaps this model could be extended to simple hand and wrist fractures treated operatively. The authors also applied this model of care to outpatient control after common hand surgeries, such as carpal tunnel release, trigger finger release, excision of benign hand tumours and Dupuytren's disease. The implementation of postoperative self-monitoring has shown great effectiveness and safety for patients.

In summary, it can be said that the change of the post-fracture care system from the traditional face-to-face to the self-monitoring program has shown great effectiveness and safety for patients. This is another element of the optimization of organization of hand surgery services, after the

first two, which are: (1) the transfer of part of the operations from the operating theatre to the procedure room at the surgical ward, and (2) providing anaesthesia by the surgeons themselves and the cancelling anaesthesiologists assistance at operations [7, 8]. All these changes have contributed to improving the work of the surgical department, increasing the availability of patients for treatment, increasing patient satisfaction and reducing costs. The results of the current study show that the transformation of outpatient postoperative care into the post-fracture self-monitoring contributes to further improvement of standards and fits into the positive trend of changes in traditional healthcare.

CONCLUSIONS

The change in the post-fracture care system from a traditional to a self-monitoring has shown great effectiveness and safety for patients. This improved the work of the hand clinic, improved patients' access, increased satisfaction and reduced costs.

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CONFLICT OF INTEREST

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CONTENTS

Osteoblastic MG63 CELLS promoted by ATP shows apoptotic LDH release under guggulsterone exposure

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ABSTRACT

Aim: To identify the anticancer potential of guggulsterone on Mg63 cells. ATP was administered to promotes growth was given to MG-63 human osteosarcoma cells that have osteoblastic features.

Materials and Methods: Different amounts of guggulsterone were added to MG 63 cells, and their shape and rate of growth were studied. The amount of LDH in cells was measured to evaluate cell toxicity. The findings show that guggulsterone caused more changes in the shape of MG63 cells after they were exposed to it.

Results: With higher amounts of guggulsterone, cell growth slowed down significantly. The results that were seen in this study were similar to what had been seen in other studies on different cell types.

Conclusions: Based on the results, we conclude that guggulsterone plays an important part in the changes that happen to the shape of cancer cells when they grow faster.

KEY WORDS: MG63, cell lines, osteoblasts, guggulesterone, ATP, LDH, apoptosis

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INTRODUCTION

OSTEOSARCOMA AND CELLULAR RESEARCH

Osteosarcoma is the most common type of cancerous bone growth, and it tends to grow rapidly. Even though it's rare, "osteosarcoma" makes up 1% of all cancers [1]. It mostly affects kids and young people, and 5–10% of their joints are affected [2]. It's not likely that osteosarcoma will go away. Even though surgery and treatment have come a long way, only 60 to 70% of people with locally advanced disease will still be alive after 5 years. For those with a spread, that number drops to 20 to 30 percent [3]. Osteosarcoma is the second most common type of cancer in kids and teens. The outlook is not good because chemotherapy and radiation treatment did not work. To come up with successful treatment plans, it is important to understand how tumors grow and spread. Xenograft models and cancer cell lines are useful for studying these processes and testing out new ways to treat cancer. Herbal drugs have become popular recently as a way to treat a wide range of illnesses. Researchers are now interested in getting these plant parts out and figuring out how these chemicals work to treat different types of cancer and other diseases. Guggulsterone (pregna-4,17-diene-3,16-dione; C21H28O2) is a strong plant sterol that comes from the roots of many guggul trees and the myrrh tree. Scientists have found that guggulsterone can bind to and block the farnesoid X receptor (FXR), which stops the FXR gene from being expressed when an activator is present [4].

GUGGULSTERONE AS ANTICANCER THERAPY

Continouse research is carried out to look into how guggulsterone can help fight cancer. Aside from that, not much is known about how guggulsterone can be used in early and clinical studies. It will be clearer how guggulsterone can be used in humans and animals, and how well it works to treat different types of cancer, after a thorough review and meta-analysis. As of now, there hasn't been any thorough or organized study done to show how guggulsterone affects the growth and spread of cancer cells [5]. For cancer to grow, attack, and spread, the cancer cells and surrounding cells must be able to communicate with each other. Tumor cells produce growth-promoting factors, chemokines, and cytokines during cancer growth. These chemicals attract stromal

cells and let immune cells and nerves get inside. Tumor growth, spread, and metastasis rely on tumor cells and their surroundings being able to talk to each other and share information. When cancer grows, tumor cells make chemokines, cytokines, and growth-promoting factors that bring in stromal cells, immune cells, and nerves [6-8]. So, these cells also release some ECM proteins, growth factors, proteases, and basement membrane parts [9].

The TME is mostly made up of immune and inflammatory cells, fibroblasts and myofibroblasts, the extracellular matrix (ECM), and blood vessels. The molecular and cellular parts of the TME are crucial for stopping the growth, spreading, and invasion of cancer [10, 11]. The TME provides tumor cells with nutrients like glucose, glutamine, and vital amino acids, enabling them to continue growing unhindered [12, 13]. It is interesting to note that the TME had a lot more ATP than the other samples. A new study by Patrizia Pellegatti et al. uses bioluminescence imaging and pme LUC (plasma membrane luciferase) to find out how much ATP is in the body. They found that the amount of ATP in tumor tissue was hundreds of micromoles, which was more than the amount of ATP in healthy tissue, which was less than 100 nanomoles per liter [14].

There are many different sources and processes that cause eATP to build up inside the TME. The TME is made up of different levels of oxygenation caused by cellular stress, hypoxia, damage, and death, all of which produce ATP. ATP can also be released into the extracellular area by autophagy, damage to the cell membrane, and the effects of cancer drugs [15, 16]. ATP builds up in the TME with the help of cytolytic ATP release and the rapid release of ATP from cells that are activating or dying through vesicle exocytosis, transporters, and membrane-bound pathways. Some cells, like defense cells (like T lymphocytes), platelets, lymphocytes, and vascular cells, have been shown to go through exocytosis, which is the process of ATP being released from inside cells into the area outside of cells [17, 18]. ABC receptors, such as the sulfonylurea receptor (SUR), the multidrug resistance (MDR) gene product (also called P-glycoprotein), and the cystic fibrosis transmembrane conductance regulator (CFTR), move ATP from cells and do other things besides vesicle release. Purinergic messaging functions in both directions [19].

Finally, intercellular channels help release ATP. These include maximal anion channels and pore-forming channels such as connexins, pannexins, and P2X purinergic receptor 7 (P2X7R). For example, it is thought that Pannexin 1 (Panx1) and other specific pore-forming plasma proteins are the major way that ATP is released in the TME in many cells. Reports say that Panx1 creates a six-part system that lets ATP move into the space around cells in reaction to different triggers, such as low oxygen or cell death [20, 21]. Basically, Panx1 stops the negative buildup of ATP outside of cells by managing the release of ATP through a negative P2X7R-mediated route. As it turns out, Panx1 is highly linked to P2X7R, and Panx1 has a smaller affinity for ATP than P2X7R. So, as eATP rises, ATP starts to bind to P2X7R and stops Panx1 from working. For the record, ATP can be released in the TME by both stressed or dead cells and live cells in different ways [22, 23].

AIM

This study was made to look into how guggulsterone can help treat different types of cancer by using different types of cancer cell lines. The aim of this study is to identify the anticancer potential of guggulsterone on Mg63 cells and morphological changes that are induced to Mg63 cell upon exposure.

MATERIALS AND METHODS

For this study, ATCC CRL-1427TM human osteosarcoma cells MG-63 were chosen because they have osteoblastic features [24]. The cells were grown in a special medium that helped them grow bones. It had Dulbecco-Vogt's Modified Eagle's (DME) medium and Ham's F-12 (H) medium mixed together 3:1 (v/v). It also had 24.3 µg/mL adenine, 10 µg/mL human epidermal growth factor, 0.4 µg/mL hydrocortisone, 5 µg/mL bovine insulin, 5 µg/mL human transferrin, 2 x 10^{-9} M 3, 3', 5'-triiodo-L-thyronine, 100 µg/mL penicillin, 25 µg/mL gentamicin, and 10% fetal calf serum. To keep the cell growth going, the culturing was done at 37°C in a wet 5% CO_2 environment. The culture medium was changed every 24 hours until the confluence level of 80 to 90 percent was reached.

PREPARATION OF GUGGULSTERONE AND ATP

Z-Guggulsterone was bought from Steraloids, Inc. in Newport, RI. It was mixed with dimethyl sulfoxide (DMSO) to make a 10 mM stock solution and kept at -20°C. Consecutive concentrations were made to use in the study (0, 1, 5,10,25,50, 75 µM). Adenosine triphosphate purchased from Sigma Aldrich / UK was used in a concentration of 100 µM throughout the experiment as a promoter for cellular growth.

GUGGULSTERONE EFFECT ON OSTEOBLASTS GROWTH AND MORPHOLOGY

Three times as many osteoblastic cells were put into each well of a 6-well plate. The growth medium was

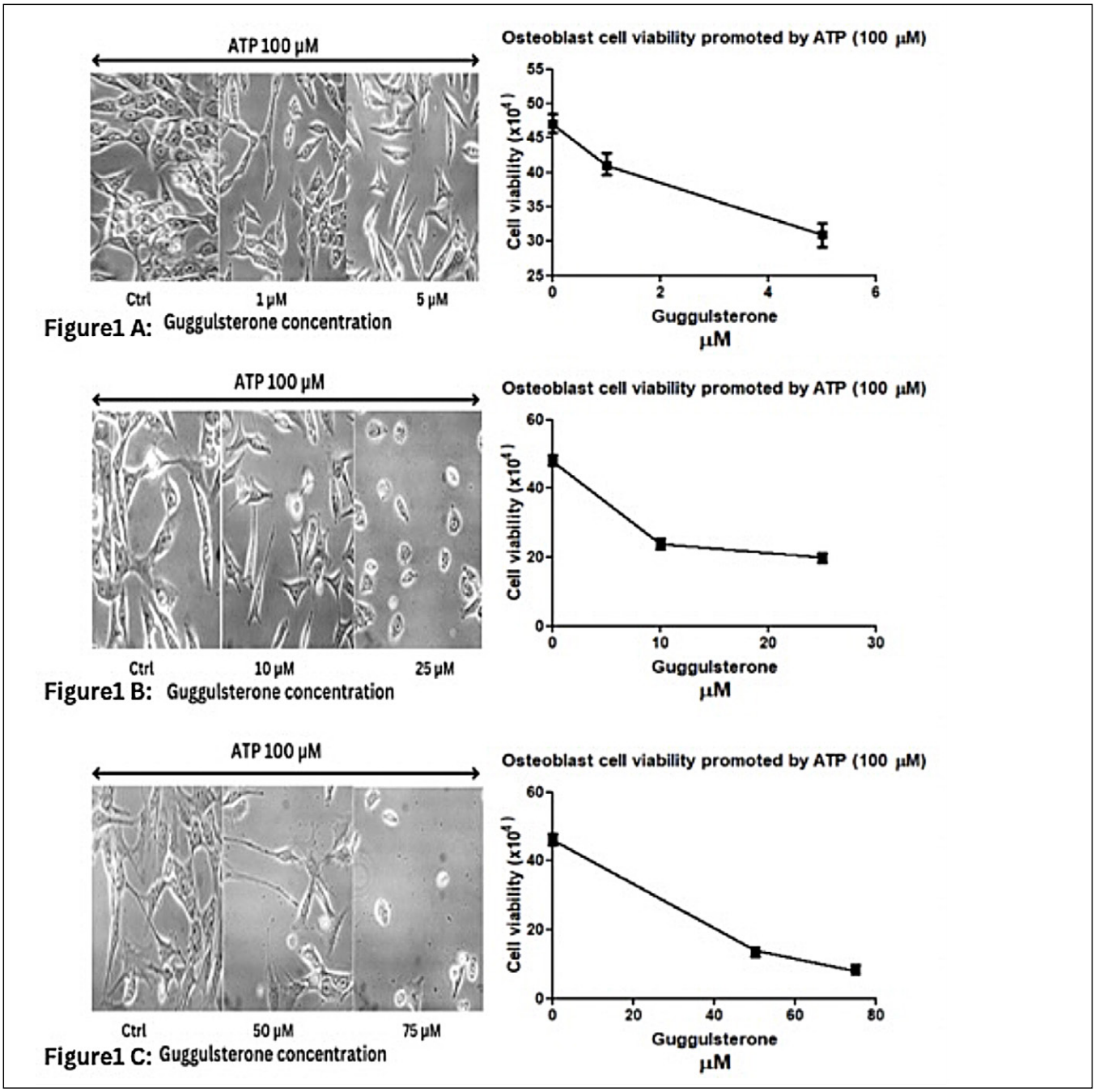

Fig. 1. Osteoblasts promoted by ATP and exposed to increased guggulsterone concentration shows round cells with reduced cell contact compared to control cells (A). Cell viability also decreases with an increase in guggulsterone concentration (A).
Osteoblasts promoted by ATP with increased guggulsterone concentration shows round cells with apoptotic signs and reduced cell contact compared to control cells (B). Cell viability also decreases with an increase in guggulsterone concertation.
Increased guggulsterone concentration shows round cells with apoptotic signs and reduced cell contact compared to control cells (C). Cell viability also decreases with an increase in guggulsterone concentration.
Source: Own materials

treated with guggulsterone at different amounts (0, 1, 5, 25 µM), and 2 ml was added to each well. The cells were kept at 37°C with 5% CO_2 for 24 hours. To sum up, trypsin was used to separate the cells from each culture well. The cells were then washed twice with culture water and used to test the survival of the cells. Each cell pellet was mixed with 1 mL of culture medium. Twenty microliters of each cell solution were mixed with twenty microliters of TB and left to sit on ice for five minutes. The mix of cells was put into a hemacytometer. An inverted optical microscope (Leica) was used to count the number of living cells (not colored blue) and dead cells (colored blue). The tests were done at least three times, each time with a copy of the original.

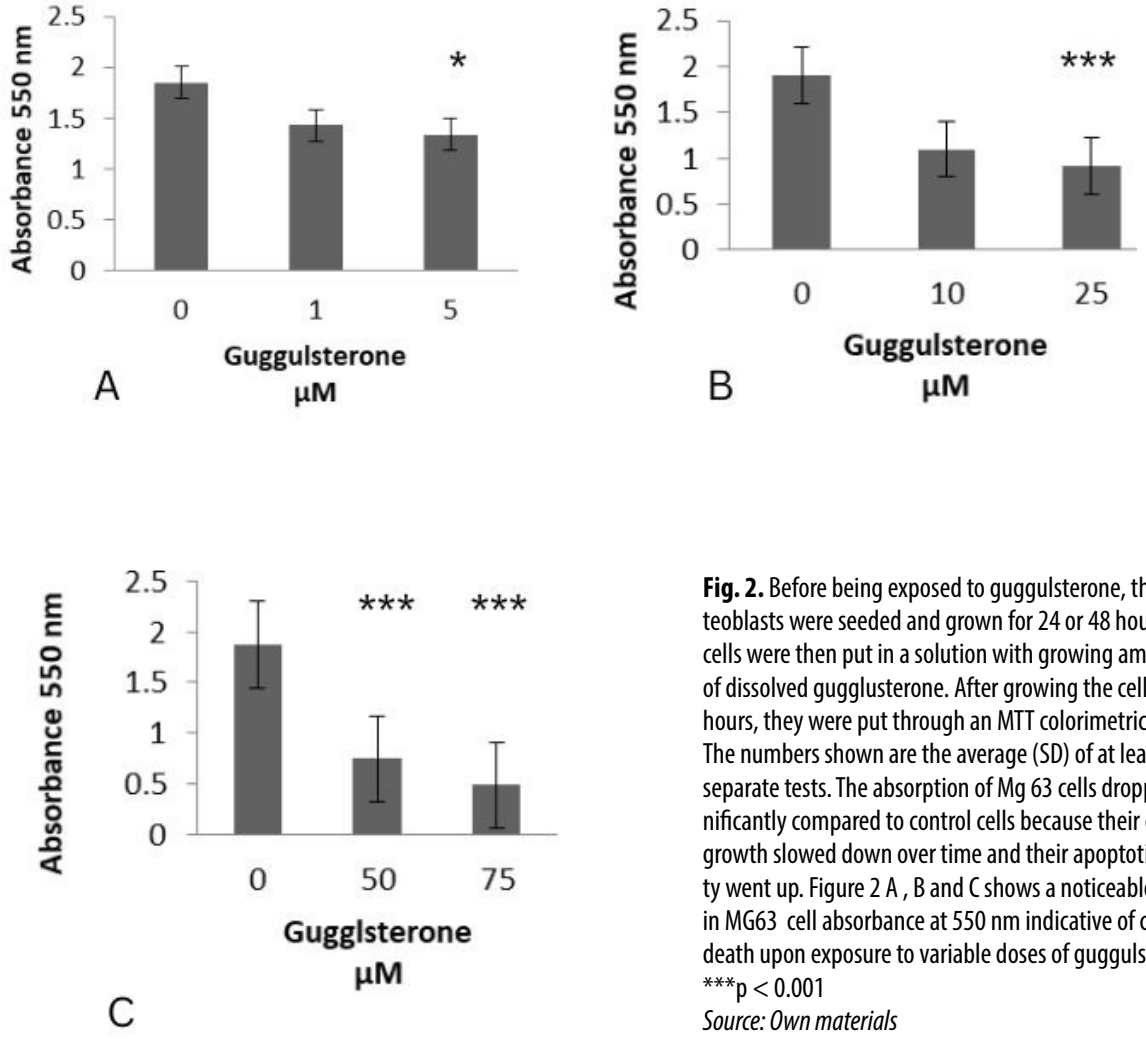

Fig. 2. Before being exposed to guggulsterone, the osteoblasts were seeded and grown for 24 or 48 hours. The cells were then put in a solution with growing amounts of dissolved gugglusterone. After growing the cells for 24 hours, they were put through an MTT colorimetric test. The numbers shown are the average (SD) of at least three separate tests. The absorption of Mg 63 cells dropped significantly compared to control cells because their cellular growth slowed down over time and their apoptotic activity went up. Figure 2 A , B and C shows a noticeable decline in MG63 cell absorbance at 550 nm indicative of cellular death upon exposure to variable doses of guggulsterone. ***$p < 0.001$

Source: Own materials

GUGGULSTERONE EFFECT ON CELLULAR LACTATE DEHYDROGENASE (LDH)

Mg63 cells were seeded into each well of a 6-well plate. The cells were then introduced to e-liquid solutions at 0%, 1%, and 5% amounts for 24 hours. After the time was up, the cell growth medium was taken out and the LDH cytotoxicity test (abcam, UK) was used to find out how harmful the cells were. In short, 50 µL of supernatant was put into a 96-well flat-bottom plate along with 50 µL of restored substrate mix. The plate was then left to sit at room temperature for 30 minutes in the dark. Lactate dehydrogenase (LDH), an enzyme that is steady and soluble and is found in all live cells, is released into the area outside of cells. 50 µL of an acid solution was added to each well to stop the process. After that, 100 µL of each reaction solution was added four times to a new 96-well flat-bottom plate, and the absorbance was measured at 490nm using an Agilent microplate spectrophotometer (Agilent, USA). Making a positive control, which means letting the cells sit in 1% Triton x 100, was done to get the most LDH release. To get the least amount of LDH production, a negative control was used, which meant that the cells were incubated without Guggulsterone. The experiment was done at least three times, each time exactly the same.

BIOSTATISTICAL ANALYSIS

The results were given as means with a standard deviation (SD). At least three times of each experiment were done. The differences between the sets of data were compared. The Shapiro-Wilk test and Levene's test of variance were used to check if the data were normal. At $p \leq 0.001$, the P value was thought to be important. A one-way analysis of variance (ANOVA) parametric test was used to find out if there was a statistically significant difference between the numbers that came from a normal distribution. A non-parametric Kruskal Wallis test was done on the data that did not have a normal distribution. Tukey and Bonferroni's change of p-value was used to compare differences between groups within and between groups after post hoc analysis. SPSS

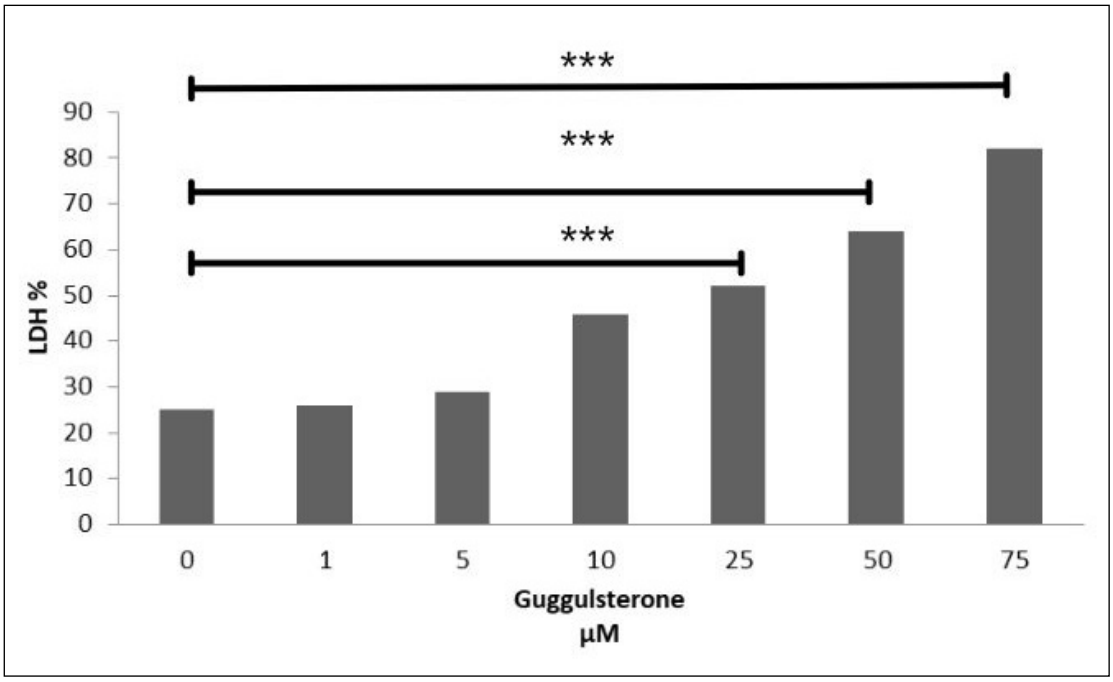

Fig. 3. The osteoblasts were seeded and cultured for 24 hrs, prior to exposure to guggulsterone. LDH levels were measured to evaluate the apoptotic activity of osteoblast cells. . The presented results are LDH level percentage after 24hrs of guggulsterone exposure. Mg 63 cells showed a significant LDH level increase *** $p < 0.01$ under 25, 50 and 75 µM of guggulsterone.
Source: Own materials

version 21.0 (IBM Corp., Armonk, NY, USA) was used for all of those statistical studies.

RESULTS AND DISCUSSION

OSTEOBLAST CELL MORPHOLOGY AND VIABILITY UPON EXPOSURE TO GUGGULSTERONE

This study showed the increasing effect of guggulsterone on actively promoted osteoblast cells. Each concentration was compared to a control medium free from gugglusterone to establish its anticancer potential. Cellular morphology was analyzed using inverted microscopy (Fig. 1). Cellular contact was decreased gradually and apoptotic changes were seen compared to control conditions. Each bar represents the mean ± SD of at least three independent experiments ***$p \leq 0.001$ when comparing the control to the other conditions. The exposure to an increasing concentration of guggulsterone showed an increased in cell roundness and overall reduction in cellular contact (Fig. 1). Osteoblast cells began losing their cell contact and change to round cell morphology after exposure to 1 µM of guggulsterone and this has increased at 5 um (Fig. 1) their viability response was also seen to decrease to $31 \pm 1.75 \times 10^4$ compared to control cells $47 \pm 1.35 \times 10^4$. Increasing the concentration of guggulsterone inflicted as shown in figure 1. Cells exposed to 10 and 50 µM of guggulsterone showed reduced cellular contact and apoptotic changes which suggests a cytotoxic effect. This is further confirmed through viability study which showed a reduction in cell viability at 10 µM of guggulsterone. Cell viability was reduced from 48 ± 1.45 x104 in control conditions to $24 \pm 1.5 \times 10^4$ at 10 µM and 20 $\pm 1.35 \times 10^4$ at 25 µM of guggulsterone.

Under increased exposure of guggulsterone, osteoblast cells showed increased cellular roundness and decreased cellular adhesion and contact compared to normally grown cells. To validate this further viability test showed a significant decrease in cellular viability. Osteoblast cells exposed to a 50 µM of guggulsterone had a lower viability 14 ± 1.45 x104 compared to control cells 46 ± 1.7 x104 while osteoblast cells were less significantly less viable 8 ± 1.45 x104 at 75 µM compared to control conditions. Overall increased osteoblast cells that have been exposed to increasing guggulsterone concentration showed a reduction in dendrites and increased floating which suggests that cellular adhesion has been lost due to weak cellular growth as well as increased apoptotic changes.

OSTEOBLAST GROWTH FOLLOWING GUGGULSTERONE EXPOSURE

An MTT assay was preformed to confirm the cellular viability and proliferative capacity in each group of osteoblast cells. The cells showed significant decreases upon

exposure to guggulsterone (Fig. 2). Guggulsterone supplemented at 1 µM resulted in a decrease in cellular absorbance 1.43 ± 0.34 in and a significant decrease ($p < 0.001$) 1.34 ± 0.21 at 5 µM when compared to control cells in the same group 1.85± 0.34 (Fig. 2A). A significant reduction in MTT metabolism in osteoblast cells is noticeably lower 1.1 ± 0.2 ($p \leq 0.001$) in cells exposed to 10 µM of guggulsterone and further stronger significant reduction ($p < 0.001$) 0.91 ± 0.18 is seen in 25 µM of guggulsterone indicating compromised cellular metabolism in comparison to control cells which had an absorbance of 1.91 ± 0.4 (Fig. 2B). Cellular proliferation and growth is confirmed by significant reduction ($p \leq 0.001$) in MTT activity through reduced cellular absorbance when cells are exposed to 50 of µM 0.75 ± 0.15. When exposed to 75 µM of guggulsterone the cells showed a very low absorbance 0.49 ± 0.11 which is significantly lower than control cells in the same group which had an absorbance of 1.87 ± 0.34 (Fig. 2C). Moreover Chen and colleagues [25] indicated that guggulsterone induces apoptosis and inhibits lysosomal-dependent migration in human bladder cancer cells. Similarly Wu et al. [26] demonstrated that Z-guggulsterone induces cell cycle arrest and apoptosis by targeting the p53/CCNB1/PLK1 pathway in triple-negative breast cancer.

EFFECT OF GUGGULSTERONE ON CELLULAR RELEASE OF LDH APOPTOTIC MARKER

Evidence from the previous experiment in the current study demonstrated the strong effect of guggulsterone on osteoblast cells. This further confirmed by LDH apoptotic assay to determine the apoptotic behavior and release of cellular LDH. The amount of LDH was measured over a period of 24 hrs post exposure to different concentrations of guggulsterone. The results in figure 3 shows the percentage of LDH released from osteoblast cells under different concentrations of guggulsterone compared to control conditions. A noticeable significant ($P \leq 0.01$) increase in LDH activity is seen at 25 µM of guggulsterone reaching up to 46% when compared to normal conditions while reaching 64% at 50 µM and 82% at 75 µM which were both significant in comparison to control conditions. There was no statistical significant in LDH release under 1, 5 and 10 µM compared to control conditions as shown in figure 3. The significant rise in LDH release indicated the potent cytotoxic activity of guggulsterone on osteoblast cells through morphological changes and growth rate reduction. A study by Wang et al. [27] demonstrated the protective effect of guggulsterone against cardiomyocyte injury induced by doxorubicin in vitro using LDH as a marker for cellular damage.

CONCLUSIONS

In conclusion, this study points out the potential cytotoxic effect of guggulsterone on osteoblast. The noted effects seen in the current study were concurrent with previous evidence shown by previous studies on various other cell lines. The effects provided an insight and solid evidence on the active role of guggulsterone in cellular morphological changes that takes place in cancerous cells during promoted growth. Guggulsterone exposure leads to a decrease in cellular contact and an increase in cell roundness in osteoblast cells. The study suggests that guggulsterone has anticancer potential by inducing apoptotic changes and reducing cellular adhesion and viability in osteoblast cells.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Effect of laser irradiation on myelo- and angioarchitecture of the distal nerve segment during reparative processes

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ABSTRACT

Aim: To investigate the effect of laser irradiation on myelinated fibers and blood vessels in the distal segment of the sciatic nerve after its transection and surgical repair.

Materials and Methods: The study was conducted on 39 male rabbits. The left sciatic nerve was transected at the mid-thigh level and repaired with epineurial sutures. The experimental animals were irradiated with a helium-neon laser with a light energy density of 2.5 mW/cm^2. The exposure duration was 5 minutes; the treatment course comprised 15 sessions; the total delivered energy was 90 J. At each time point, three animals from each group were used. All procedures involving animals were conducted in accordance with bioethical guidelines. Myelinated fibers were stained with the Kulchitsky-Pal stain, and intraneural microvessels were visualized by injection with a chloroform/ether solution of Prussian blue dye.

Results: On days 7 and 14 of the experiment, typical Wallerian degeneration developed in the distal segment of the nerve. Myelinated fibers degenerated more rapidly in irradiated animals. Three stages of Wallerian degeneration, each with distinct characteristics, were identified. On day 7, axonal fragmentation and globular fragmentation of myelin (myelin ovoids) predominated, whereas by day 15, resorption of degenerative products prevailed. From day 30 onward, reinnervation of the distal nerve segment began, with laser irradiation significantly enhancing the process. It accelerated angiogenesis, dilated and increased the number of blood vessels, and increased their total cross-sectional area, indicating improved blood supply.

Conclusions: Helium-neon laser irradiation accelerates and enhances all processes occurring in the distal segment of the nerve after neurotomy: Wallerian degeneration and reinnervation, axonal growth, myelination, and maturation; restoration of myeloarchitecture; revascularization and blood supply; and reconstruction of the nerve's angioarchitecture. Distal to the injury, reparative angiogenesis precedes the regeneration of myelinated fibers.

KEY WORDS: myelinated fibers, degeneration, regeneration, lasers

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INTRODUCTION

Traumatic peripheral nerve (PN) injuries represent a significant medical challenge. They account for 1–5% of all peacetime injuries and up to 12% of combat-related injuries [1]. During armed conflicts, extremity injuries account for 75% of all wounds, with PN involvement observed in up to 25% of these cases [2]. Extremity PN injuries are not life-threatening; however, they often result in partial or complete impairment of both daily functional and occupational capacities [3, 4]. Approximately 65–70% of patients with PN injuries ultimately develop long-term disability [5]. The full-scale invasion of the Russian Federation has significantly exacerbated this problem in Ukraine. Consequently, the search for novel approaches to stimulate the recovery of injured PNs and to improve existing strategies remains highly relevant. Currently, physiotherapeutic interventions, including low-level laser therapy, are widely employed for this purpose.

AIM

To investigate the effect of helium-neon (He–Ne) laser irradiation on the degeneration and regeneration of myelinated fibers (MFs) and vascular remodeling in the distal segment of the sciatic nerve (SN) after its transection.

MATERIALS AND METHODS

The study was conducted on 39 adult male rabbits weighing 3–4 kg. The animals were divided into three groups: intact (n = 3), control (n = 18), and experimental (n = 18).

The left SN was transected at the mid-thigh level, and the proximal and distal nerve stumps were reconnected using 2–3 epineurial sutures with atraumatic OPTIX needles. Following neurotomy and neurorrhaphy, the experimental animals were irradiated with a He–Ne laser through the skin over the posterior thigh. The exposure duration was 5 minutes, with a light energy density of 2.5 mW/cm^2. The treatment course comprised 15 sessions, with a total delivered energy of 90 J. At each time point (7, 15, 30, 90, 180, and 300 days), three animals from the control and experimental groups were assessed. Animal care, surgical procedures, laser irradiation, and euthanasia were performed in accordance with general bioethical guidelines and the established regulations on the conduct of animal experiments.

Nerve harvesting, fixation, rinsing, dehydration, embedding, preparation of paraffin sections, and MF staining with the Kulchitsky-Pal stain were performed using standard, widely accepted methods [6]. Intraneural vessels were visualized using a chloroform/ether solution of Prussian blue dye (10 g of dye, 70 ml of chloroform, and 30 ml of ether). The solution was injected into the abdominal aorta just below the diaphragm at a pressure of 120–140 mmHg. Epineurial and perineurial vessels were subsequently impregnated with silver nitrate on whole-mount preparations, as described by Kupriyanov [6].

SN sections were examined using a light microscope (Micros, MC300, Vienna, Austria) and imaged with a ToupCam 5.1M UHCCD C-Mount Sony digital camera equipped with a Toup Tek Photonics AMA075 adapter, using ToupView v.3 software. Morphometric analysis of MFs was performed using ImageJ software (version 1.47t) [7], employing a previously developed method [8].

According to our previous studies [9, 10], MFs of the SN were classified into three groups: small (1.0–4.0 μm), medium (4.1–7.0 μm), and large (>7.0 μm). These groups differed significantly in fiber, axon, and myelin sheath areas, as well as in their spatial distribution within the coordinate field. In rabbits, the number of MFs in the SN exhibits considerable individual variability. When normalized to the nerve cross-sectional area per 1 mm^2, these variations are minimal, as reflected by a low coefficient of variation (C_v = 1.25–1.82%). A statistically significant linear correlation was observed between the total number and size of MFs and the number and diameters of intraneural blood vessels. Accordingly, these vessels were classified into three groups: small (d = 1–4 μm), medium (d = 4.1–7.0 μm), and large (d > 7 μm). Given that the intraneural microcirculation consists of a collection of microvessels of varying diameters, we determined the total cross-sectional area (TCA) of intraneural blood vessels (μm^2 per 1 mm^2 of nerve).

RESULTS

DEGENERATION AND REGENERATION OF MYELINATED FIBERS IN THE DISTAL SEGMENT OF THE NERVE

Following nerve transection (neurotomy) and subsequent surgical repair (neurorrhaphy), the distal segment of the nerve underwent typical secondary (Wallerian) degeneration of MFs, followed by reinnervation (axon regrowth through the scar), myelination, maturation of the fibers (increases in axon and myelin sheath thickness), and remodeling of the vascular network.

On days 7 and 15 of the study, the number of MFs of all sizes decreased significantly (Table 1). Specifically, the number of small and medium MFs decreased compared to normal values, 3.8- and 10.9-fold, and 2.9- and 9.9-fold, respectively ($p < 0.001$), whereas the number of large MFs decreased only 1.3- and 3.17-fold ($p < 0.001$). The total number of MFs at these time points decreased 1.8- and 4.6-fold ($p < 0.001$). By day 30, active reinnervation of the distal segment was evident. On day 90, large MFs were observed, albeit in small numbers, while the numbers of small and medium MFs exceeded normal values 2.06- and 1.35-fold, respectively ($p < 0.001$). On days 180 and 300, the number of large MFs in the distal segment gradually increased; however, even on day 300, MF distribution across different diameters did not reach normal levels.

Under He–Ne laser irradiation, morphometric analysis of MFs in the SN distal segment showed that on day 7 of the experiment, the total number of MFs of all sizes was 1.2-fold lower than in the control group ($p < 0.05$) (Table 1). On day 15, the number of MFs remained 1.10-fold lower compared to the control group ($p < 0.05$). From day 30 onward, the number of regenerating MFs exceeded that of the control group, increasing 2.1-fold on day 30 ($p < 0.02$), 2.9-fold on day 90 ($p < 0.001$), 1.8-fold on day 180 ($p < 0.001$), and 1.4-fold on day 300 ($p < 0.01$).

Counting MFs in the distal nerve segment, where complete fiber breakdown occurs, based solely on fiber diameter, does not accurately reflect the actual state of Wallerian degeneration, since fibers of the same size may be at different stages of degeneration. Therefore, we quantified the number of degenerating MFs at various stages of secondary degeneration on cross-sections of the SN stained by the Kulchitsky method. Three stages of Wallerian degeneration were distinguished: Stage I – irritation of nerve fibers; Stage II – globular fragmentation of myelin (myelin ovoids) and axonal fragmentation; Stage III – granular disintegration of myelin resulting from chemical degradation and resorption of degenerative products (Fig. 1).

Table 1. Number of nerve fibers per 1 mm^2 of the distal segment cross-section in intact, control, and irradiated animals at different time points

Experimental time point, days	Number of myelinated fibers			
	Small	Medium	Large	Total
Intact animals				
	2,608 ± 98	1,143 ± 33	4,650 ± 65	8,397 ± 105
Control animals				
7	689 ± 29*	392 ± 26*	3,582 ± 110*	4,659 ± 154*
15	238 ± 16*	115 ± 5*	1,466 ± 22*	1,818 ± 31*
30	2,908 ± 188	116 ± 5*	0*	3,022 ± 191*
90	5,379 ± 380*	1,554 ± 115	769 ± 64*	4,635 ± 868*
180	6,728 ± 453*	1,597 ± 95	1,255 ± 86*	7,692 ± 341*
300	4,764 ± 343*	1,461 ± 83	2,161 ± 196*	8,402 ± 487
Irradiated animals				
7	499 ± 14 #	258 ± 7#	3,213 ± 191	3,974 ± 186#
15	225 ± 23	111 ± 10	1,319 ± 24#	1,656 ± 47#
30	5,934 ± 123 #	479 ± 54#	0	6,413 ± 126#
90	9,611 ± 301 #	2,720 ± 299#	1,051 ± 125	13,383 ± 640#
180	8,868 ± 268 #	2,274 ± 74#	2,820 ± 143	13,962 ± 197#
300	5,759 ± 122	2,646 ± 129#	3,333 ± 154#	11,800 ± 153#

Notes: *statistically significant difference compared to intact animals;
#statistically significant difference compared to control animals;
For each experimental time point, n = 3; data are presented as Mean ± SD
Source: Own materials

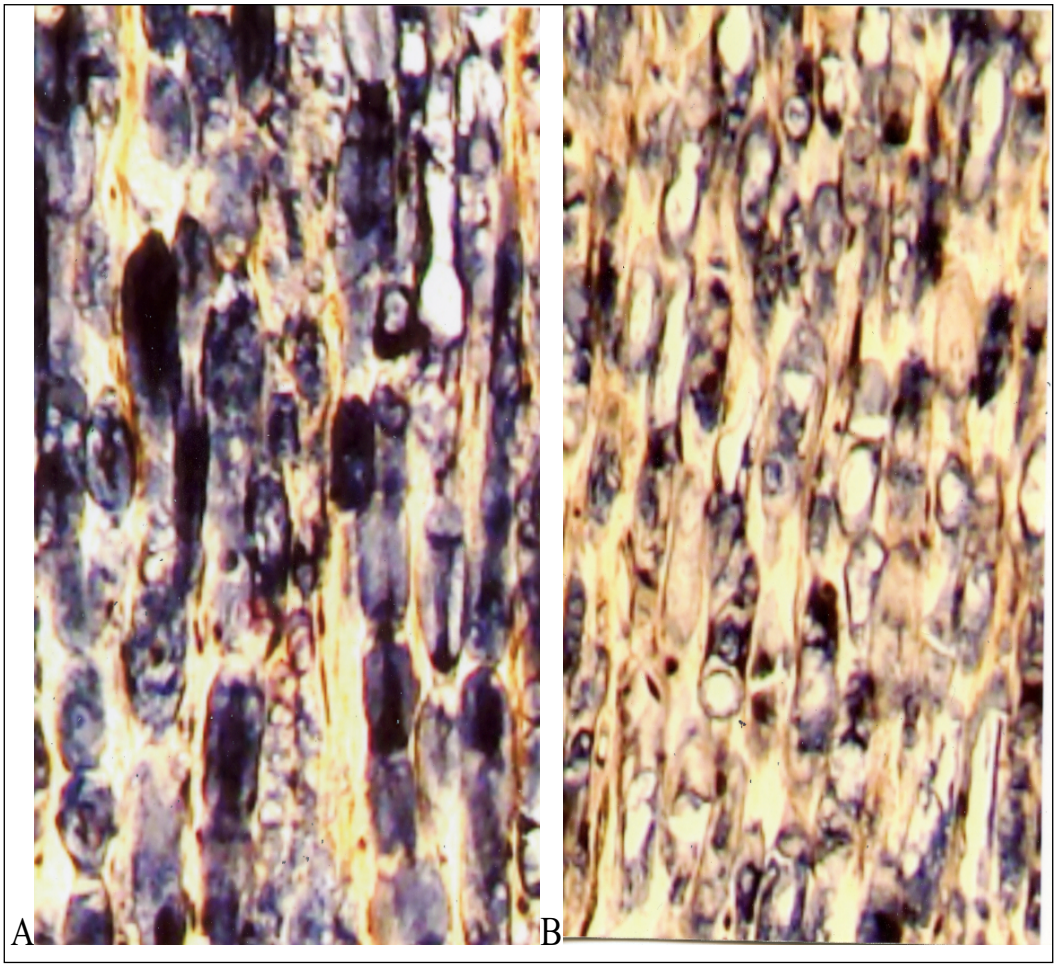

Fig. 1. A – control; B – Wallerian degeneration on day 7 of the experiment. In laser-irradiated animals, Wallerian degeneration is more pronounced, with granular disintegration of myelin and resorption of degenerative products (B), compared to control animals (A), where globular fragmentation of myelin predominates. Staining: myelinated fibers, Kulchitsky method. Magnification: ×280.
Source: Own materials

Table 2. Distribution of myelinated fibers by stages of secondary degeneration per 1 mm² of distal segment cross-section

Experimental time point, days	Number of myelinated fibers at different stages of secondary degeneration		
	Stage I	Stage II	Stage III
Control animals			
7	1,645 ± 138.81	2,288 ± 98.10	555 ± 30.60
15	218 ± 20.32	216 ± 21.55	1,084 ± 52.12
Irradiated animals			
7	1,053 ± 54.48#	1,839 ± 83.29#	945 ± 37.47#
15	46 ± 3.23#	243 ± 15.64#	1,602 ± 34.48#

Notes: #statistically significant difference compared to control animals;
For each experimental time point, n = 3; data are presented as Mean ± SD.
Source: Own materials

Table 3. Morphometric parameters of intraneural vessels in the distal segment of the sciatic nerve in the experiment

Experimental time point, days	Control animals		Irradiated animals	
	Number of vessels	Total cross-sectional area (μm²)	Number of vessels	Total cross-sectional area (μm²)
7	85 ± 3.84	2,313 ± 191.07	116 ± 5.94#	4,250 ± 237.87#
15	110 ± 1.91	3,887 ± 137.75	174 ±7.16#	10,925 ± 312.16#
30	131 ± 3.25	4,297 ± 268.23	162 ± 6.43#	9,569 ± 507.19#
90	139 ± 4.15	5,029 ± 339.91	181 ± 7.13#	6,406 ± 346.98
180	137 ± 5.61	5,454 ± 316.62	135 ± 8.99	5,106 ± 320.51
300	116 ± 2.40	3,459 ± 94.73	122 ± 3.04	3,408 ± 183.69

Notes: values are presented per 1 mm² of nerve cross-section;
#statistically significant difference compared to control animals;
For each experimental time point, n = 3; data are presented as Mean ± SD
Source: Own materials

MF irritation was manifested by increased argentophilia and edema. In cross-sections of the nerve, MFs differed only slightly from normal: the axon and myelin sheath were well visualized; the latter appeared edematous or thinned, exhibited uneven hypo- or hyperchromatic staining, and had smooth or irregular (serrated) contours. At Stage II, the axon and myelin sheath formed a fused structure that stained unevenly and had indistinct, often disrupted contours or appeared as a large vacuole surrounded by a thin myelin sheath. Stage III was characterized by myelin breakdown and resorption, resulting in pale, 'fading' MFs in nerve cross-sections. At each experimental time point, MFs at different stages of Wallerian degeneration were observed concurrently, although their relative proportions varied according to the extent of secondary nerve degeneration (Table 1, 2).

Table 1 shows that laser irradiation significantly accelerated the degeneration of small and medium MFs on day 7 compared to the control, whereas by day 15, this effect predominantly involved large fibers. In contrast, Table 2 clearly demonstrates that in the distal segment of the nerve, on day 7 after transection, processes of MF irritation, axonal fragmentation, and globular fragmentation of myelin predominated, whereas by day 15, MF resorption occurred due to chemical transformation. Furthermore, statistically significant differences were observed between irradiated and control animals in the number of MFs at different stages of secondary degeneration on days 7 and 15 of the experiment.

NEUROVASCULAR REMODELING AFTER NEUROTOMY

Dissection of the SN from surrounding tissues, followed by transection and suturing during surgery, significantly disrupts the integrity of its blood vessels, which are subsequently restored through vascular growth from the central and peripheral nerve stumps and surrounding tissues. On day 7 of the experiment, in the distal segment of the SN, a few thin vascular branches arose from the epineurium of the disrupted ends of large arteries and veins (Fig. 2).

The diameters of all components of the intraneural microcirculation approached normal values; however, the number of vessels was reduced 1.4-fold compared to intact animals ($p < 0.001$), resulting in a 1.6-fold decrease ($p < 0.001$) in the TCA of the intraneural microcirculation per 1 mm² of nerve cross-section.

Microvessels in the distal segment of the sciatic nerve were injected with a chloroform/ether solution of Prussian blue dye. Cleared specimens. Magnification: ×90.

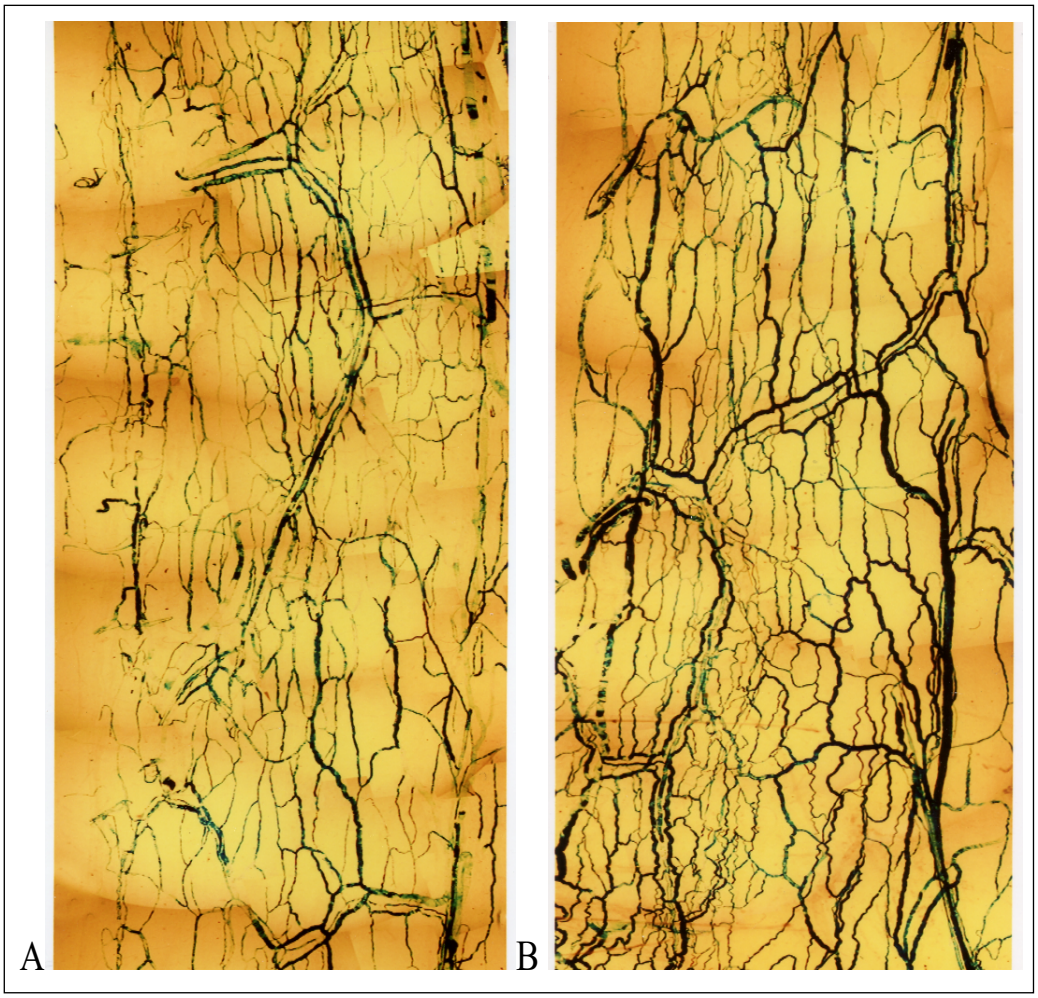

Figure 2. A – control; B – epineural vasculature on day 7 of the experiment. In control animals (A), vessels are constricted, with numerous discontinuities and avascular areas. Under helium-neon laser irradiation, microvascular density increases, and the vessels dilate.
Source: Own materials

On day 15, the number of intraneural vessels returned to the initial level, and the relative proportions of vessel groups shifted toward a higher proportion of large and medium vessels. The TCA of vessels exceeded normal values 1.2-fold ($p < 0.01$). At subsequent time points, the number of vessels remained within normal limits; however, the TCA increased 1.6–1.9-fold ($p < 0.001$). During these periods, the proportion of large vessels was highest, while the proportion of small vessels decreased slightly. By the end of the experiment, angioarchitectural parameters returned to normal values.

We found that He–Ne laser irradiation significantly affected revascularization and blood supply in the distal segment of the SN (Table 3). Notably, on day 7 after neurorrhaphy under irradiation, avascular areas were not observed in the epineural vasculature of the distal nerve segment. The number of intraneural vessels was 1.4-fold higher than in controls ($p < 0.02$). Large and medium vessels predominated over small vessels, resulting in a 1.8-fold increase in the TCA compared to non-irradiated animals ($p < 0.001$) (Table 3).

On day 15 of the experiment, epineural vascular density increased, vessels dilated, and capillary loop sizes decreased noticeably. The number of intraneural vessels increased 1.6-fold compared to controls ($p < 0.001$). Large vessels predominated over medium and small vessels, resulting in a 2.8-fold increase in the TCA ($p < 0.001$). On days 30 and 90 of the experiment, the epineural and intraneural vascular networks of the nerve remained dense. The total number of intraneural vessels at these time points exceeded that in controls 1.2- and 1.3-fold ($p < 0.05$–0.01), whereas on days 180–300, it did not differ significantly from normal. The TCA of the intraneural microcirculation increased 2.2- to 1.3-fold compared to controls on days 30 and 90 ($p < 0.001$–0.01). At subsequent time points, it did not differ from control values.

DISCUSSION

Based on counts of MFs at different stages of Wallerian degeneration on days 7 and 15 of the experiment, identified according to our findings and published data [11, 12], we found that low-level He–Ne laser irradiation accelerated and intensified Wallerian degeneration. Other researchers have reported consistent results, providing evidence that after laser irradiation, Schwann cells phagocytose myelin debris more actively and

promote macrophage recruitment from blood vessels, thereby facilitating its clearance [13–15]. This enhanced degeneration is primarily mediated by Schwann cell mitochondria, which regulate the development, maintenance, degeneration, and regeneration of PN MFs. After PN injury, mitochondrial bioenergetic dysfunction develops, leading to pain, neuropathy, and impaired MF regeneration. Laser irradiation can normalize mitochondrial and energy metabolism, either by directly affecting mitochondrial cytochromes or indirectly through water acting as a photoacceptor [14]**.** Moreover, low-level laser irradiation has been shown to reduce oxidative stress, reduce the levels of inflammatory cytokines and reactive oxygen species, and modulate immune responses [14].

By counting MFs of different sizes and the total number of MFs that regenerated through the scar into the distal segment of the SN on days 30, 90, 180, and 300 after SN neurotomy under He–Ne laser irradiation, and comparing them to control values, we concluded that laser irradiation accelerates axonal growth, myelination, and maturation (i.e., axon and myelin sheath thickening). Other researchers have reported similar findings of accelerated PN regeneration under laser irradiation. Some studies highlight increased reinnervation of the distal PN segment, others emphasize rapid axonal myelination and increased numbers of large MFs, while still others report both processes [13, 16–22].

All Schwann cell phenotypes secrete neurotrophic factors (neurotrophins), proteins that stimulate and regulate neurogenesis and axonal regeneration. Nerve growth factor (NGF) promotes neuronal growth, survival, and proliferation. Brain-derived neurotrophic factor (BDNF) supports neuronal survival and stimulates the development and differentiation of new neurons. Myelin protein zero (MPZ) maintains the integrity of the myelin sheath around the axon. Previous studies have demonstrated that laser irradiation significantly increases the levels of these neurotrophins and vascular endothelial growth factor (VEGF) [16, 23, 24]. Neurotrophins may have been among the key mediators of the laser-induced effects observed in the distal segment of the SN in our study.

Morphometric analysis of intraneural microvessels in irradiated and non-irradiated animals demonstrated that He–Ne irradiation accelerated the onset of angiogenesis in the distal segment of the SN, dilated both epineural and intraneural vessels, and increased the TCA of the microvascular bed per mm^2 of nerve tissue. Our findings are consistent with previous studies [16, 19], which reported that laser irradiation enhances microcirculation and angiogenesis in the injured PN, thereby improving blood flow and nutrient and growth factor delivery to regenerating tissue, as well as stimulating endothelial cell proliferation and migration.

CONCLUSIONS

1. The processes developing in the distal segment of the nerve under low-level laser irradiation are characterized by a shortened duration of Wallerian degeneration, an earlier onset of axonal regeneration and remyelination, accelerated MF maturation, and a more complete restoration of normal myeloarchitecture.
2. For the first time, secondary nerve degeneration was studied with consideration of the extent of MF damage. Three stages of Wallerian degeneration were identified. When comparing the effects of different factors on nerve regeneration, morphometric analysis of MFs at these stages is recommended
3. He-Ne irradiation stimulates reparative angiogenesis, dilates both epineural and intraneural vessels, and increases the number of intraneural vessels and their TCA per mm^2 of the distal nerve segment, indicating improved blood supply. It also contributes to a more complete restoration of the nerve's angioarchitecture.
4. After neurotomy and neurorrhaphy of the PN, reparative angiogenesis in the distal segment precedes MF regeneration. The number of microcapillaries and microvascular geometry were restored by days 15–30 of the experiment, whereas the number of regenerated MFs distal to the injury reached baseline levels by day 180.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Rehabilitation in women after mastectomy: Clinical aspects

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ABSTRACT

Aim: To assess the efficacy of comprehensive physiotherapy in women after mastectomy.

The paper attempts to answer the following research questions:

1. How does physiotherapy influence the size of oedema?
2. What is the correlation between physiotherapy outcomes and sociodemographic factors?
3. What is the correlation between physiotherapy outcomes and the following: BMI, time from procedure, time to the development of oedema after procedure, type of management after surgery.

Materials and Methods: A total of 32 female patients after unilateral mastectomy performed at the Holy Cross Cancer Center in Kielce underwent treatment in this study. The mean age of study patients was 68.5 years. The duration of lymphoedema rehabilitation was 2 to 4 weeks (3.6 weeks on average). Almost three-fourths (71.9%) of the patients were treated for 4 weeks. The physiotherapy included respiratory exercises, kinesiotherapy, lymphatic massage, pneumatic massage, and whirlpool baths.

Results: Significantly lower arm circumference values were achieved in the affected limb after physiotherapy as compared to the pre-treatment values.

Conclusions: 1. Breast cancer is a difficult clinical and social problem in Poland and globally.2. Implementation of an appropriate physical therapy program both before and after surgery determines the reduction of lymphedema in women undergoing surgery.

KEY WORDS: breast cancer, lymphoedema, physiotherapy

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INTRODUCTION

Globally, breast cancer is the most frequently diagnosed malignancy in women, accounting for approximately 23% of all cancer cases and being responsible for approximately 14% of the deaths. Annually, it is diagnosed in 2.3 million women globally and leads to approximately 670,000 deaths. In Poland, there are 22,000 new breast cancer cases every year. The majority of cases are diagnosed at an early stage based on a clinical examination, mammography, microscopic examination, and MRI (Fig. 1) [1-8].

Breast cancer spreads through the lymph and blood vessels. Metastases spreading through the lymphatic system first involve the regional (axillary and parasternal) lymph nodes. Interpectoral (Rotter's) lymph nodes also play an important role in this process. Spread through blood vessels leads to metastases found in almost all organs. The most common metastases include distant metastases to the bones, liver, lungs, pleura, and central nervous system [6-12].

The breast cancer incidence in Poland is alarmingly high and constitutes a serious social problem. A radical surgical procedure consisting in the removal of the breast together with the lymph nodes of the axillary fossa (lymphadenectomy) on the side of the amputated breast is one of the treatment methods used in breast cancer. These surgeries may cause short-term complications: local wound infection, skin flap necrosis, wound dehiscence, haematoma, or chylothorax. In turn, long-term complications include hyperaesthesia and paraesthesia within the area of the operated skin, neuropathies, hypertrophic surgical scar, fibrosis and contractures that limit shoulder joint mobility, atrophy of certain muscle groups, permanent shoulder girdle joint deformities, postural defects, and lymphoedema of the upper limb [4-12].

Lymphoedema is an accumulation of protein-rich fluid in the tissue space and lymph vessels. This fluid contains migrating and sedentary immune cells, metabolic products, endothelial cells, and other substances. This

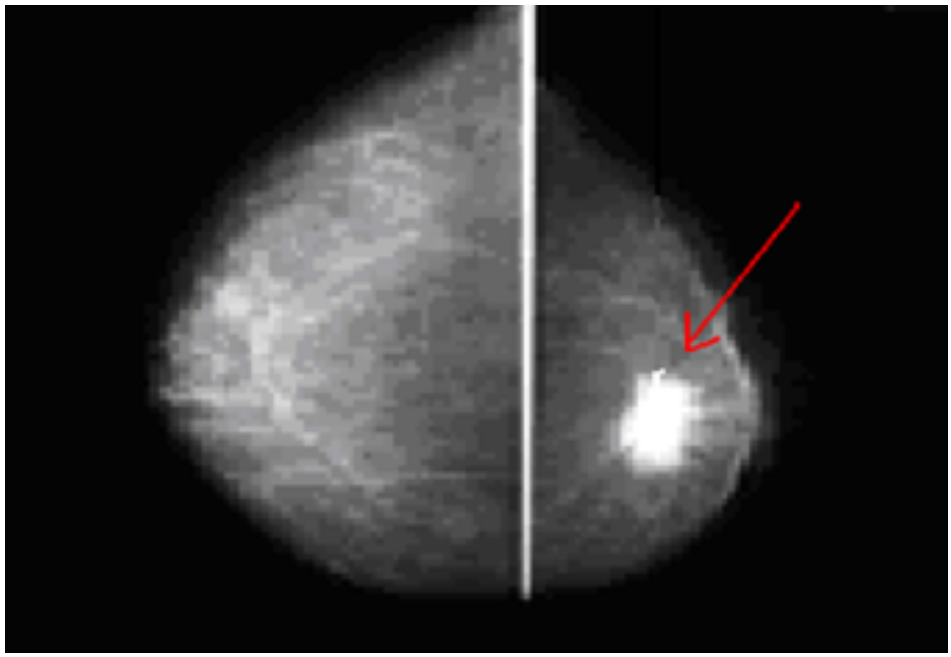

Fig. 1. Breast cancer on a mammogram
Source: Own materials

excess storage results in progressive tissue fibrosis: proliferation of keratinocytes and fibroblasts and collagen accumulation. Impaired lymph circulation contributes to the growth of skin-penetrating microorganisms and the development of inflammatory processes [5-12].

Breast cancer treatment results in an increased upper limb volume and sometimes causes considerable upper limb deformity. The limb becomes heavier, the patient develops limited joint mobility, impaired blood supply, and sometimes also neurological damage. All these changes result in an increased susceptibility to skin injuries, increased lymph infiltration, and a higher risk of complications in the form of secondary infections.

Oedema develops in one in four breast cancer patients after surgery and in one in three breast cancer patients who underwent radiation therapy after surgery, at different time points after treatment completion.

Treatment of lymphoedema consists in preventing complications and reducing limb circumference. Manual lymphatic massage is the most important therapeutic tool [8-16].

Maintaining a high quality of life in women after breast cancer treatment largely depends on an early rehabilitation programme. In order to fully restore their physical function and mental balance, patients need to systematically perform motor exercises and follow the recommendations for the activities of daily living [16-25].

Main aims of rehabilitation:

- increase the range of motion in the shoulder joint on the operated side,
- increase muscle strength of the upper limb on the operated side,
- prevent lymph retention in the limb and the area of the surgical procedure and help develop collateral circulation,
- eliminate oedema through the use of special exercises and physiotherapy procedures,
- correct postural defects that develop as a consequence of breast amputation,
- help with optimal adaptation to modified living conditions.

The first stage of rehabilitation starts before surgery. The aim of rehabilitation in this period is to prepare the patient physically and mentally for the procedure and to prevent short-term surgical sequelae. This includes training with respect to respiratory exercises and appropriate positioning of the operated limb as well as developing fundamental patient-physiotherapist cooperation, which will be beneficial after surgery. Effective cough training and elements of self-massage are already important at this stage.

After surgery, patients undergo intense respiratory exercises, exercises of all the joints, then self-assisted exercises, mobilisation, and kinesiotherapy. Limb elevation is necessary [22-25].

Rehabilitation after neoplastic disease is usually a life-long effort. Patients develop habits that need to be maintained in order to preserve physical function. Only full participation of the patient in the treatment process guarantees its long-term positive effect. Lymphatic massage should be followed by wrapping the limb in bandage or wearing appropriate compression tights to prevent recurrence. The limb should be wrapped in a tourniquet characterised by a very low elasticity, with an appropriate lower layer made of a cotton stocking,

Table 1. Circumferences and arm volumes on the side of mastectomy before treatment

Point of measurement	Mean	SD	Me	min	max
10 cm below acromion (cm)	31.2	1.0	31.0	29.1	33.0
10 cm above elbow joint (cm)	18.4	0.6	18.0	17.0	19.0
elbow (cm)	16.4	0.5	16.0	16.0	17.0
10 cm below elbow joint (cm)	25.0	0.8	25.0	24.0	26.6
3 cm above wrist (cm)	16.5	0.5	16.6	15.5	17.0
wrist (cm)	17.3	0.4	17.0	16.8	18.0
metacarpus (cm)	19.7	0.4	19.8	19.2	20.3
Volume (mL)	1823.4	46.6	1832.6	1738.8	1890.9

Source: Own materials

Table 2. Circumferences and arm volumes on the side of mastectomy after treatment in the study group

Point of measurement	Mean	SD	Me	min	max
10 cm below acromion (cm)	30.5	1.0	30.3	28.3	32.5
10 cm above elbow joint (cm)	17.7	0.8	17.3	16.3	18.5
elbow (cm)	15.9	0.8	15.5	15.0	16.9
10 cm below elbow joint (cm)	24.6	1.0	24.5	23.0	26.4
3 cm above wrist (cm)	16.0	0.8	16.0	14.0	16.9
wrist (cm)	17.2	0.5	17.2	16.5	18.0
metacarpus (cm)	19.5	0.3	19.6	19.0	20.0
Volume (mL)	1738.3	72.9	1716.0	1563.4	1830.8

Source: Own materials

cotton wool, and corrugated sponge, with the highest force tolerated by the patient.

Self-massage of the upper limb facilitates lymph and venous circulation, improves trophics, and prevents/eliminates oedema. Rehabilitation in women with impaired lymph circulation should include broadly understood motor rehabilitation. Exercises aimed at maintaining the full range of joint motion in the limbs and strengthening the abdominal and back muscles should be performed every day. Respiratory exercises play an important role [12-15].

Rehabilitation with appropriately selected exercises is very important in the treatment of lymphoedema as these reduce the volume of accumulated interstitial fluid.

Kinesio taping is an increasingly popular method of rehabilitation that consists in sticking special tape (kinesio tex) with specific properties directly onto the skin.

Pneumatic massage is used in compression therapy and is always performed immediately after manual massage. It uses single-chamber or multi-chamber sleeves that generate variable pressure up to approximately 40–60 mmHg.

Whirlpool baths may be used to improve circulation and ensure tissue flexibility. Whirlpool massage is performed according to the general methodology principles. Most physiotherapy procedures are used to reduce pain.

Comprehensive postoperative rehabilitation leads to a reduction in or elimination of lymphoedema, improved joint mobility or at least to improved nourishment of the skin of the limb and a change in the consistency of oedema. Persistent oedema and joint contractures hinder rehabilitation and lower its efficacy. Appropriate rehabilitation and education of patients help decrease the number of complications, shorten the hospital stay, and reduce treatment costs [21-25].

AIM

The aim of this study was to assess the efficacy of comprehensive physiotherapy in women after mastectomy.

The paper attempts to answer the following research questions:

1. How does physiotherapy influence the size of oedema?
2. What is the correlation between physiotherapy outcomes and sociodemographic factors?
3. What is the correlation between physiotherapy outcomes and the following: BMI, time from procedure, time to the development of oedema after procedure, type of management after surgery.

Table 3. Volume of upper limb on the side of mastectomy before and after treatment in the study group

Examination	Volume of upper limb on the side of mastectomy (mL)					Significance
	Mean	SD	Me	min	max	
Before treatment	1823.4	46.6	1832.6	1738.8	1890.9	p<0.001
After treatment	1738.3	72.9	1716.0	1563.4	1830.8	

Source: Own materials

Table 4. Treatment outcomes for upper limb oedema on the side of mastectomy in the study group

Treatment outcome	Mean	SD	Me	min	max
Oedema reduction (mL)	85.1	42.4	61.8	45.2	175.3
Oedema treatment efficacy (%)	4.7	2.4	3.4	2.4	10.1

Source: Own materials

Table 5. Effects of age of study patients and their BMI on treatment outcomes in terms of oedema reduction and treatment efficacy

	Oedema reduction [mL]		Treatment efficacy [%]	
Age [years]	r=-0.3321	p=0.063	r=-0.3421	p=0.055
BMI [kg/m^2]	r=-0.2117	p=0.245	r=-0.201	p=0.270

Source: Own materials

Table 6. Effects of level of education of study patients on treatment outcomes in terms of lymphoedema reduction

Education	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
Higher	4	107.9	53.3	105.5	45.2	175.3	p=0.208
Secondary	15	79.6	40.1	60.1	45.2	174.1	
Secondary vocational	10	74.3	37.3	52.7	45.2	147.5	
Primary	3	118.4	50.4	147.5	60.1	147.5	

Source: Own materials

MATERIALS AND METHODS

STATISTICAL METHODS

The data obtained in the study were statistically analysed.

Arm volume on the side of mastectomy before and after treatment was calculated using the following formula:

$V=1/4\pi$ (c1c2 + c2c3 + c3c4 + c4c5 + c5c6 + c6c7)

where: V: limb volume

C: limb circumferences measured in cm at 7 points:

1. below acromion 2. above elbow joint 3. elbow 4. below elbow joint
5. above wrist 6. wrist 7. metacarpus

Oedema reduction and treatment efficacy were calculated using the following formulas:

Oedema reduction = Vch2 – Vch1

Treatment efficacy = Vch2 – Vch1/Vch1 x 100%

where:

Vch2: volume of affected limb after rehabilitation

Vch1: volume of affected limb before rehabilitation

Age, BMI, limb volume, oedema reduction, and treatment efficacy were presented using distribution parameters: mean – arithmetic mean, SD – standard deviation, Me – median, or middle value, min – lowest value, max – highest value.

The statistical analysis used Student's t-test for independent groups, one-way analysis of variance, and Pearson's linear correlation.

Test results with a significance level lower than or equal to 0.05 ($p \leq 0.05$) were deemed statistically significant. The lack of statistical significance was marked with the abbreviation NS (not significant). Statistical calculations were performed with Statistica 13 PL software.

CHARACTERICS OF THE PATIENTS

A total of 32 female patients after unilateral mastectomy performed at the Holy Cross Cancer Center in Kielce underwent treatment in the study. The mean age of study patients was 68.5 years. A half of the patients were over the age of 70 years.

The BMI of study patients ranged from 20.7 kg/m^2 to 25.7 kg/m^2, with a mean BMI of 23.4 kg/m^2. The vast majority (93.8%) of study patients had a normal BMI, and two patients were overweight.

Table 7. Effects of marital status of study patients on treatment outcomes in terms of lymphoedema reduction

Marital status	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
Never married	4	59.3	28.2	45.2	45.2	101.6	p=0.363
Married	8	104.4	54.6	105.5	45.2	175.3	
Divorced	4	77.3	37.0	77.3	45.2	109.4	
Widow	16	83.9	38.7	75.4	45.2	174.1	

Source: Own materialszz

Almost half (46.9%) of study patients had secondary education, 4 (12.5%) patients had a university degree, 31.3% of study patients had vocational secondary education, and 9.4% of the women had only completed primary school.

A half of the women were widows, 25% were married, and the rest were either divorced or had never been married (12.5% each).

A total of 11 (34.4%) women were professionally active; 21 patients did not work, including 19 pensioners or individuals drawing a disability pension and 2 unemployed women.

Out of the 11 women who did work, 5 were manual workers, 4 were intellectual workers, and 2 reported their type of work as mixed.

Lymphoedema of the left arm was present in 13 (40.6%) study patients while lymphoedema of the right arm was seen in 19 (59.4%) of study patients.

All study patients underwent combination treatment after mastectomy. Postoperative chemotherapy alone was used in 14 (43.8%) study patients while chemotherapy plus radiotherapy were used in 18 (56.3%) study patients.

The mean time from the procedure was 3.6 years (between 2 and 6 years). Usually (40.6%), study patients were 3 years after the procedure.

The mean time of first onset of lymphoedema after surgery in the study group was 3.6 months. A total of 14 (43.8%) study patients developed lymphoedema in the 1st month after the procedure, another 6 (18.8%) study patients experienced it for the first time in the 2nd month after the procedure, and 12 (37.5%) study patients first developed arm oedema 3 months after the procedure.

The duration of lymphoedema rehabilitation was between 2 and 4 weeks (mean value: 3.6 weeks). Almost three-fourths (71.9%) of study patients underwent treatment for 4 weeks. The physiotherapy included respiratory exercises, kinesiotherapy, lymphatic massage, pneumatic massage, and whirlpool baths.

RESULTS

EFFECTS OF PHYSIOTHERAPY ON OEDEMA SIZE

Table 1 presents the calculated volumes of the arm with lymphoedema before treatment together with the arm circumference values that were used to calculate the volumes.

Table 2 presents the calculated volumes of the arm with lymphoedema after treatment together with the arm circumference values that were used to calculate the volumes.

After treatment, upper limb volume in the study patients was significantly lower ($p<0.001$) than before treatment. The mean volume of the treated limb was 1823.4 mL before treatment and 1738.3 mL after treatment (Table 3).

The mean lymphoedema reduction after treatment was 85.1 mL (range: 45.2 mL to 175.3 mL) (Table. 4).

Correlation between physiotherapy outcomes and sociodemographic factors

Age was inversely proportional ($r=-0.3321$) to lymphoedema reduction after treatment at a level of $p=0.063$, which was close to the significance level. Moreover, age was inversely proportional ($r=-0.3421$) to lymphoedema treatment efficacy at a level of $p=0.055$, which was close to the significance level. No correlation was found between the outcomes of lymphoedema treatment, such as oedema reduction and treatment efficacy, and the BMI value in the study patients (Table 5). Physiotherapy outcomes did not depend on the BMI. There was an inversely proportional correlation between treatment outcomes and the age of study patients, but it was not statistically significant.

The level of education of study patients did not influence lymphoedema reduction after treatment (Table 6).

The level of education of study patients did not influence the efficacy of lymphoedema treatment in the study group.

The marital status of study patients did not influence lymphoedema reduction after treatment (Table 7).

Professional activity of study patients did not influence lymphoedema reduction after treatment (Table 8).

Professional activity of study patients did not influence the efficacy of lymphoedema treatment in the study group.

CORRELATION OF PHYSIOTHERAPY OUTCOMES

BMI, time from procedure, time to the development of oedema after procedure, type of management after surgery

The side on which lymphoedema developed after mastectomy and influence on lymphoedema reduction in the study group (Table 9).

Table 8. Effects of professional activity of study patients on treatment outcomes in terms of lymphoedema reduction

Professional activity	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
Working	13	90.4	49.6	63.5	45.2	175.3	p=0.570

Source: Own materials

Table 9. Effects of the side of mastectomy in study patients on treatment outcomes in terms of lymphoedema reduction

Mastectomy side	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
Right	19	88.0	43.3	90.6	45.2	175.3	p=0.648
Left	13	80.9	42.4	60.1	45.2	174.1	

Source: Own materials

Table 10. Effects of type of treatment after mastectomy used in study patients on treatment outcomes in terms of lymphoedema reduction

Treatment after surgery	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
CHT+RT	18	90.4	46.1	77.0	45.2	175.3	p=0.430
CHT	14	78.3	37.7	60.1	45.2	147.5	

CHT: chemotherapy; RT: radiotherapy

Source: Own materials

Table 11. Effects of time from mastectomy in study patients on treatment outcomes in terms of lymphoedema reduction

Time from surgery	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
2 years	7	89.5	43.8	90.6	45.2	147.5	p=0.964
3 years	13	81.1	44.2	60.1	45.2	175.3	
4 years	5	91.2	39.9	90.6	45.2	147.5	
6 years	7	83.6	48.0	60.1	45.2	174.1	

Source: Own materials

Table 12. Effects of treatment duration in study patients on treatment outcomes in terms of lymphoedema reduction

Treatment time	n	Oedema reduction (mL)					Significance
		Mean	SD	Me	min	max	
2 weeks	3	99.4	44.3	90.6	60.1	147.5	p=0.807
3 weeks	6	79.3	51.9	54.3	45.2	175.3	
4 weeks	23	84.7	41.3	60.1	45.2	174.1	

Source: Own materials

The side on which lymphoedema developed after mastectomy in study patients did not influence the efficacy of lymphoedema treatment in the study group.

The type of combination treatment used after mastectomy in study patients did not influence lymphoedema reduction in the study group (Table 10).

Time from mastectomy in study patients did not influence lymphoedema reduction in the study group (Table 11).

The duration of treatment in study patients did not influence lymphoedema reduction in the study group (Table 12).

DISCUSSION

Breast cancer is a difficult clinical and social problem. After surgical breast cancer treatment, patients develop latent, subclinical lymphatic failure. Studies show that secondary lymphoedema occurs usually in the first year after the procedure (immediately after surgery in 30% of women and up to one year after surgery in 58% of women).

In the case of impaired lymph flow, valves begin to leak and the lymph flows backwards. Untreated oedema becomes increasingly harder, and the progressive pro-

cess of connective tissue proliferation is associated with the risk of skin sclerosis and cylindrical limb deformity. This causes aesthetic issues, often leading to stress and concomitant emotional problems and social conflicts. Moreover, the consequences of lymphoedema worsen physical activity limitations in the patients, decrease their level of independence, and cause social isolation, which includes giving up on professional activity. Mental disorders, caused by the stress associated with long-term treatment, are common. Women after mastectomy have to be aware of the factors that influence oedema severity and of the methods of management in everyday life.

In the present study, patients after treatment showed reduced upper limb circumferences on the operated side at all levels and increased ranges of motion in the joints of the limb on the operated side. The largest improvement in mobility after treatment was seen in the shoulder joint.

Physiotherapy methods used to eliminate lymphoedema should be selected on a case-by-case basis, taking into account systematic and persistent efforts from the patient. As far as oedema grades are concerned, it should be pointed out that in grade 1 lymphoedema (so-called reversible lymphoedema, where no tissue fibrosis occurs), the swelling is reduced by upper limb elevation, self-massage, and exercises. With grade 2 oedema (where considerable tissue fibrosis occurs), self-massage and limb elevation are supplementary measures.

It should also be stressed that physiotherapy methods used to treat oedema are of a long-term nature. Reducing lymphoedema has beneficial effects not only on the physical condition, but also on the mental state of the woman, allowing her to live her life by increasing her independence, helping her return to work and, consequently, improving her self-worth. Studies confirm that these methods constitute an important part of physiotherapy in patients after breast removal surgery.

CONCLUSIONS

1. Breast cancer is a difficult clinical and social problem in Poland and globally.
2. Implementation of an appropriate physical therapy program both before and after surgery determines the reduction of lymphedema in women undergoing surgery.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Effects of angiotensin converting enzyme inhibitors *versus* angiotensin receptor blockers on cognitive decline: A retrospective real-world database study

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ABSTRACT

Aim: To compare 5-year cognitive outcomes in patients with HFrEF who receive angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs).

Materials and Methods: Retrospective cohort study of: 1) 135,873 adults with HFrEF (International Classification of Diseases-10th Revision-Clinical Modification [ICD-10-CM] codes: I50.2 or I50.4) started on ACEI between Aug 1, 2019 and Aug 1, 2024; and 2) 135,873 propensity matched patients receiving ARBs during that time. Data were obtained from the TriNetX Research Network, encompassing 80 health care organizations in the United States. The primary endpoint was the composite of cognitive decline (ICD-10-CM: R41.8), dementia (ICD-10-CM: F01-F03), and Alzheimer's disease (ICD-10- CM: G30).

Results: At 5 years,17,679 patients on ACEI met the primary endpoint vs 16,345 patients on ARBs (5-year incidence: 30.71% *vs* 28.54%; HR: 1.153; 95% CI: 129-1.178; P < 0.001), with consistently higher rates of cognitive decline (24.94% *vs* 22.81%; HR: 1.146; 95% CI: 1.119-1.174; P < 0.001), dementia (15.63% *vs* 13.71%; HR:1.204; 95% CI: 1.168-1.241; P < 0.001), and Alzheimer's disease (4.15% *vs* 3.51%; HR: 1.202; 95% CI: 1.131-1.277; P < 0.001) in the ACEI cohort.

Conclusions: ACEI was associated with higher 5-year rates of neurocognitive disorders when compared to ARBs in patients with HFrEF.

KEY WORDS: ACEIs, ARBs, HFrEF, cognitive decline, dementia, neurocognitive disorders

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LIST OF ABBREVIATIONS

- ACEI – Angiotensin-Converting Enzyme Inhibitor
- ACEIs – Angiotensin-Converting Enzyme Inhibitors
- ARB – Angiotensin Receptor Blocker
- ARBs – Angiotensin Receptor Blockers
- AT2R – Angiotensin II Type 2 Receptor
- CI – Confidence Interval
- CogState – Cognitive State Test
- CPT – Current Procedural Terminology
- CV – Cardiovascular
- GDMT – Guideline-Directed Medical Therapy
- HF – Heart Failure
- HFmrEF – Heart Failure with Mildly Reduced Ejection Fraction
- HFpEF – Heart Failure with Preserved Ejection Fraction
- HFrEF – Heart Failure with Reduced Ejection Fraction
- HR – Hazard Ratio
- ICD-10-CM – International Classification of Diseases, 10th Revision, Clinical Modification
- IRB – Institutional Review Board
- LVEF – Left Ventricular Ejection Fraction
- LOINC – Logical Observation Identifiers Names and Codes
- MCI – Mild Cognitive Impairment
- NF-κB – Nuclear Factor Kappa B
- PP2A – Protein Phosphatase 2A
- RCT – Randomized Controlled Trial
- RAS – Renin–Angiotensin System
- RASB – Renin–Angiotensin System Blockade
- RECORD – Reporting of Studies Conducted Using Observational Routinely Collected Health Data
- RxNorm – Prescription Normalized Nomenclature
- STAT3 – Signal Transducer and Activator of Transcription 3

INTRODUCTION

Heart failure is a common diagnosis in routine clinical practice, where the symptoms result from a structural or functional heart disorder that reduces the ventricular

function in filling with or ejecting blood. It is classified on the basis of left ventricular ejection fraction (LVEF) into heart failure with reduced ejection fraction (HFrEF), heart failure with preserved ejection fraction (HFpEF), and heart failure with mildly reduced ejection fraction (HFmrEF). Among all these groups, HFrEF has the strongest evidence base supporting medical therapy, since the majority of clinical trials have enrolled predominantly reduced ejection fraction patients.

Cognitive impairment is a common and clinically important complication of heart failure with reduced ejection fraction (HFrEF). In addition to mild cognitive impairment, HFrEF patients also have an increased incidence of dementia, including Alzheimer's disease, compared with the general population; however, HFrEF is more strongly related to vascular and mixed dementias than to pure Alzheimer's disease. The mechanisms are multifactorial, with reduced cardiac output as a central contributor, leading to cerebral hypoperfusion, neuroinflammation, oxidative stress, and progressive neurodegeneration [1].

The renin–angiotensin system (RAS) plays a critical role in the development of heart failure, as well as in cognitive outcomes. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are key therapies in patients with HFrEF, improving survival and reducing hospitalizations [2].

Beyond cardiovascular benefits, observational studies and meta-analyses suggest that both angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are associated with a lower incidence of dementia compared with no RAS inhibition [3], [4]. However, Some studies concluded that ARBs may offer better protection, but this is not well established in the medical literature, and most available evidence comes from studies evaluating their use as antihypertensive agents in patients with hypertension rather than in heart failure patients [5].

Given the high burden of neurocognitive decline in HFrEF and the widespread use of ACEIs and ARBs as part of the Guideline Directed Medical Therapy (GDMT), understanding their comparative effects on cognition is highly relevant to clinical practice. This study therefore aims to compare the incidence of new-onset neurocognitive disorders in patients with HFrEF treated with ACEIs versus ARBs using a large, multicenter, real-world cohort.

AIM

The aim of this study is to fill the current knowledge gap by comparing the incidence of neurocognitive disorders in patients with HFrEF who receive angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs).

MATERIALS AND METHODS

DATA SOURCE AND STUDY DESIGN

This research was a retrospective, observational, propensity score matched cohort study using data from the TriNetX database. TriNetX is a global electronic health records network that provides access to anonymized patient data. In this study, data were obtained from 147 healthcare organizations in the United States, with 80 providers supplying patient data. The variables obtained from TriNetX included demographics, diagnoses, procedures, laboratory results, and medications, using standardized coding systems: ICD-10-CM and CPT for diagnoses and procedures, LOINC for laboratory values, and RxNorm for medications. A detailed list of codes and subcodes is available in the Supplemental Appendix. TriNetX, LLC holds a waiver from the Western IRB and adheres to HIPAA regulations, as only de-identified data are utilized. Data from the final search, conducted on August 10, 2025, were included in this analysis. This study was designed and reported in accordance with the RECORD guidelines to maintain quality and transparency.

STUDY POPULATION

From 80 healthcare organizations participating in TriNetX, we identified adult patients (age >18 years) with a diagnosis of HFrEF (ICD-10- CM codes I50.2 or I50.4) between Aug 1, 2019 and Aug 1, 2024, who was started on ACEI at the index visit but did not receive subsequent prescription of sacubitril/valsartan or ARB, those patients formed the parent ACEI cohort.

Patients who share the same baseline characteristics, diagnosis and healthcare encounter criteria but were treated with ARBs only across the same period of inception make up the parent ARBs cohort.

MAIN EXPOSURES

Drugs in TriNetX are recorded at the ingredient level, coded to RxNorm, and organized by Veterans Administration National Drug File therapeutic classes. For the purposes of this research, exposures of interest included ACE inhibitors (CV800), angiotensin receptor blockers (ARBs; CV805), and combination therapies for hypertension (CV400), including sacubitril as an ingredient (RxNorm code: 1656328). Supplementary Appendix contains further subcodes.

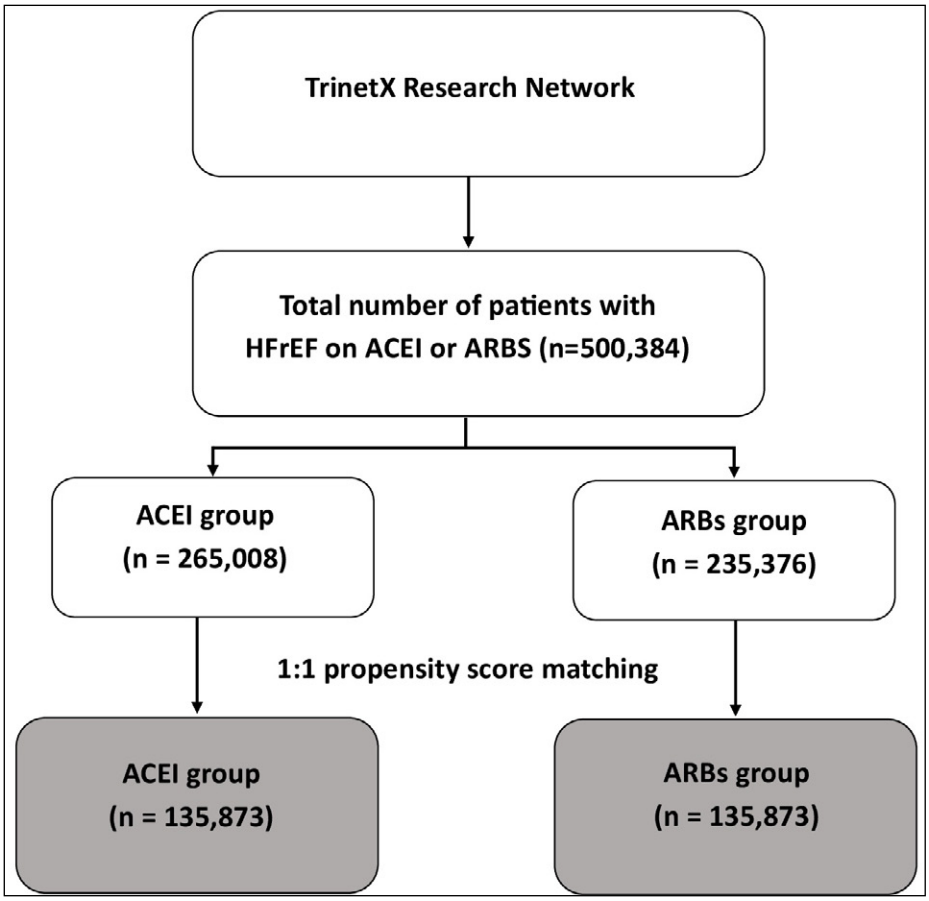

Fig. 1. Flow diagram of patient selection and cohort derivation following propensity score matching.
Source: Own materials

OUTCOMES

The primary outcome was the incidence of new neurocognitive diagnoses identified through ICD-10-CM codes, defined as the composite of cognitive decline (ICD-10-CM: R41.8), dementia (ICD-10- CM: F01-F03), and Alzheimer's disease (ICD-10-CM: G30), and these were assessed at 5 years. A 5-year follow-up window was selected to minimize censoring and maximize the follow-up. Each of the primary endpoint components were evaluated separately as a secondary endpoint (cognitive decline, dementia, and Alzheimer's disease). In subgroup analyses, we also investigated dementia subtypes, which included vascular dementia (F01), dementia in other diseases classified elsewhere (F02), and unspecified dementia (F03).

STATISTICAL ANALYSIS

Statistical analysis was performed using the TriNetX web-based platform. Propensity score matching was performed at a 1:1 ratio to create balanced cohorts, including the following characteristics: demographics, comorbidities, medications and laboratory results. Propensity scores were computed using logistic regression based on the predicted probability of a patient belonging to a certain cohort.

For each case in the smaller cohort, the system finds a match in the larger cohort using the greedy nearest neighbor approach with a caliper of 0.1 pooled standard deviations. The order of records is randomized to eliminate bias using a fixed seed during matching, allowing for reproducibility. Outcomes of interest were only compared within matched cohorts and subgroups of cohorts.

The TriNetX platform employs Kaplan–Meier survival analysis in order to estimate the incidence of the outcome of interest and compares the distribution of the eventfree curves with the log-rank test. Patients with any occurrence of any outcome of interest before the inception window were excluded from outcome analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated with Cox proportional hazards regression using the R survival package (v3.2-3). The proportional hazards assumption was tested with scaled Schoenfeld residuals. The two-tailed p-value ≤0.05 was considered statistically significant.

ETHICS APPROVAL

This study was conducted using de-identified data from the TriNetX research network. In accordance with U.S.

Table 1. Baseline characteristics of patients in ACEI and ARBs groups before and after propensity score matching (PSM)

	Before PSM			After PSM		
	Before Matching (ACEI group, n=170,555)	**Before Matching (ARBs group, n=309,793)**	**Standardized Difference**	**After Matching (ACEI group, n=138,051)**	**After Matching (ARBs group, n=138,051)**	**Standardized Difference**
Demographics						
Current Age (Mean ± SD)	72.4 ± 13.5	73.7 ± 13.1	0.103	72.1 ± 14.0	72.2 ± 13.2	0.006
Age at Index (Mean ± SD)	68.2 ± 13.9	70.0 ± 13.3	0.131	68.6±14.4	68.6±13.4	0.001
Female (%)	93,860 (35.4%)	101,666 (43.2%)	0.160	53,126(39.1%)	52,852(38.9%)	0.004
Male (%)	162,734 (61.4%)	126,770 (53.9%)	0.153	78,788(58.0%)	78,983(58.1%)	0.003
White (%)	186,005(70.2%)	146,293 (62.2%)	0.170	87,597(64.5%)	88,369 (65.0%)	0.012
Black or African American(%)	40,976(15.5%)	44,844(19.1%)	0.095	24,277(17.9%)	23,597(17.4%)	0.013
Asian(%)	5,868(2.2%)	11,249(4.8%)	0.140	4,413(3.2%)	4,462(3.3%)	0.002
Comorbid conditions						
Hypertension	186,648(70.4%)	169,194(71.9%)	0.032	83,887 (61.7%)	84,699 (62.3%)	0.012
Dyslipidemia	165,728(62.5%)	150,648(64%)	0.030	75,125(55.3%)	75,857 (55.8%)	0.011
Mental, Behavioral and Neurodevelopmental disorders	133,563(50.4%)	107,693(45.8%)	0.093	58,393(43.0%)	59,602(43.9%)	0.018
Ischemic heart diseases	142,957(53.9%)	124,370(52.8%)	0.022	65,972(48.6%)	66,853(49.2%)	0.013
Atrial fibrillation and flutter	85,889(32.4%)	77,430(32.9%)	0.010	40,883(30.1%)	41,457 (30.5%)	0.009
Diabetes mellitus	107,133(40.4%)	97,107(41.3%)	0.017	49,300(36.3%)	49,641(36.5%)	0.005
Pulmonary heart disease and diseases of pulmonary circulation	44,149(16.7%)	40,819(17.3%)	0.018	20,792(15.3%)	21,100(15.5%)	0.006
Diseases of the respiratory system	159,650(60.2%)	143,580(61%)	0.015	73,999(54.5%%)	74,735(55%)	0.011
Cerebrovascular diseases	56,291(21.2%)	50,018(21.3%)	0.938	25,235(18.6%)	25,598(18.8%)	0.007
Nicotine dependence	60,161(22.7%)	38,795 (16.5%)	0.157	23,975(17.6%)	24,515(18%)	0.010
Medication use						
Aspirin	143,585(54.2%)	122,650(52.1%)	0.042	59,460(43.8%)	60,651(44.6%)	0.018
Beta-blockers	173,250(65.4%)	152,470(64.8%)	0.013	74,208(54.6%)	75,264(55.4%)	0.016
Calcium-channel blockers	104,332(39.4%)	105,359(44.8%)	0.109	47,351(34.8%)	47,694(35.1%)	0.005
Diuretics	155,398(58.6%)	143,438(60.9%)	0.047	66,681(49.1%)	67,682(49.8%)	0.016
Antilipemic agents	157,665(59.5%)	137,695(58.5%)	0.020	65,012(47.8%)	66,257(48.8%)	0.018
Previous use of ACEI	131,785(49.7%)	48,142(20.5%)	0.644	27,393(20.2%)	28,713(21.1%)	0.024
Previous use of ARBs	15,173(5.7%)	96,003(40.8%)	0.912	15,173(11.2%)	16,673(12.3%)	0.034
Vitamins	100,836(38.1%)	93,419(39.7%)	0.034	42,963(31.6%)	43,735(32.2%)	0.012
Herbs and alternative therapy	68,584(25.9%)	65,195(27.7%)	0.041	32,552(24.0%)	32,755(24.1%)	0.003
Laboratory						
NT-proBNP, pg/mL	4332.5 ± 8401.5	4588.1 ± 8914.8	0.030	5121.0 ± 9264.4	5031.2 ± 9265.6	0.010
Total cholesterol, md/dL	157.2 ± 47.5	158.9 ± 46.6	0.038	158.5 ± 49.1	159.3 ± 48.0	0.017
Hemoglobin A1c, %	6.7 ± 1.9	6.7 ± 1.8	0.047	6.7 ± 1.9	6.6 ± 1.8	0.033
Iron, mcg/dL	61.0 ±42.5	61.1 ± 41.1	0.001	58.9 ± 42.4	60.4 ± 42.2	0.035
Ferritin, ng/mL	340.6 ± 1307.1	334.6 ± 1028.5	0.005	399.5 ± 1663.7	368.6 ±1197.8	0.021
Blood urea nitrogen, mg/dL	22.1 ± 13.1	23.6 ±14.5	0.104	22.7 ± 13.9	23.3 ±14.6	0.043
Creatinine, mg/dL	1.4 ±3.8	1.5 ± 3.5	0.043	1.5 ±4.9	1.5 ±3.4	<0.001

Source: Own materials

federal regulations, studies using only de-identified data are not considered human subjects research and are exempt from institutional review board (IRB) approval. TriNetX, LLC has received a waiver from the Western IRB and complies with the Health Insurance Portability and Accountability Act (HIPAA), with

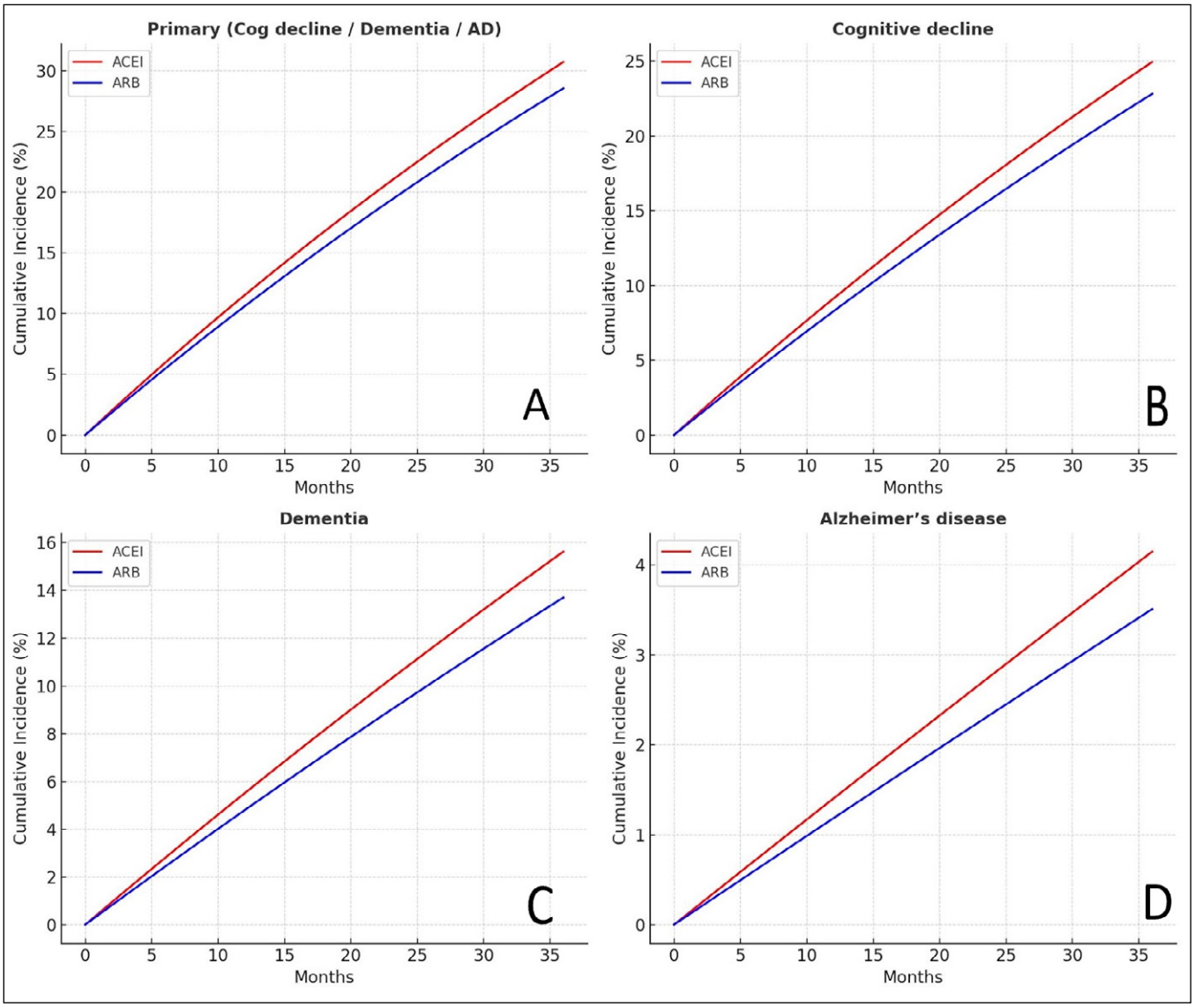

Fig. 2. Cumulative incidence of the primary and secondary outcomes: (A) the primary endpoint (cognitive decline, dementia, or Alzheimer's disease), (B) cognitive decline; (C) dementia; and (D) Alzheimer's disease, among patients with heart failure and reduced ejection fraction who started receiving ACEI *vs* propensity score-matched patients receiving ARBs with an index encounter during the same timeframe. Patients were captured from the TriNetX database

Source: Own materials

de-identification confirmed through a qualified expert determination as defined in Section §164.514(b)(1) of the HIPAA Privacy Rule.

RESULTS

BASELINE CHARACTERISTICS

Using TriNetx database we select 265,008 adults with HFrEF (I50.2 or I50.4) who started receiving ACEI between 2019 and 2024 without any subsequent exposure to ARB and 235,376 patients who received ARBs exclusively during the same period. After propensity score matching for the characteristics described in the methods, the matched cohorts comprised 135,873 patients, each summarizes the characteristics of the parent cohorts before and after the propensity score matching (Fig. 1, Table 1). Patients in the ACEI matched cohort were 68.6 ± 14.4 years of age; 58.0% were men; 64.5% were White, 17.9% Black, and 3.2% Asian; and 20.2% were previously on ACEI and 11.2% were on ARBs. The most prevalent comorbidities were hypertension (61.7%), dyslipidemia (55.3%), and respiratory disorders (54.5%), followed by ischemic heart disease (48.6%), mental and behavioral disorders (43.0%), diabetes mellitus (36.3%), and atrial fibrillation (30.1%). All characteristics of the matched ACEI cohort demonstrated standardized mean differences <0.1 *vs* the ARBs cohort.

Table 2. Primary and secondary clinical outcomes: ACEI *vs*. ARBs in HFrEF patients

	ACEI (n =135,873)		ARBs (n =135,873)			
	Events	3-y K-M Estimate	Events	3-y K-M Estimate	HR (95% CI)	P Value
Primary endpoint	17,679	30.71%	16,345	28.54%	1.153 (1.129–1.178)	<0.001
Cognitive decline	13,793	24.94%	12,750	22.81%	1.146 (1.119–1.174)	<0.001
Dementia	9,154	15.63%	8,001	13.71%	1.204 (1.168–1.241)	<0.001
Alzheimer's disease	2,269	4.15%	1,972	3.51%	1.202 (1.131–1.277)	<0.001

Source: Own materials

Table 3. Dementia subtypes: ACEI vs. ARBs in HFrEF patients

	ACEI (n =135,873)		ARBs (n =135,873)			
	Events	3-y K-M Estimate	Events	3-y K-M Estimate	HR (95% CI)	P Value
Vascular dementia	2,152	3.86%	1,925	3.41%	1.167 (1.097–1.241)	<0.001
Unspecified dementia	8,098	14.1%	6,878	12.0%	1.238 (1.199–1.278)	<0.001
Dementia in other diseases	3,193	5.56%	2,794	4.93%	1.202 (1.131–1.277)	<0.001

Source: Own materials

INCIDENCE OF NEUROCOGNITIVE DIAGNOSES

At 5 years, the primary endpoint (incident cognitive decline, dementia, or Alzheimer's disease) was met by 17,679 patients in the ACEI vs 16,345 patients in the ARBs matched cohorts. The corresponding Kaplan-Meier cumulative 5-year incidence was 30.71% *vs* 28.54%, with a HR of 1.153 (95% CI: 1.129-1.178; P < 0.001) (Fig. 2A and Table 2). The 5-year Kaplan-Meier incidence of the secondary endpoints was consistently higher in the ACEI cohort; 24.94% vs 22.81% for cognitive decline (HR: 1.146; 95% CI: 1.119-1.174; P < 0.001), 15.63% vs 13.71% for dementia (HR: 1.204; 95% CI: 1.168-1.241; P < 0.001), and 4.15% vs 3.51% for Alzheimer's disease (HR: 1.202; 95% CI: 1.131-1.277; P < 0.001), (Fig. 2B-D, Fig. 3 and Table 2). In an exploratory analysis, we examined the incidence of subtypes of dementia (vascular dementia [F01], dementia in other diseases [F02], and unspecified dementia [F03]). The results were consistent across dementia subtypes (Table 3). The 5-year Kaplan-Meier incidence of the dementia subtypes was consistently higher in the ACEI cohort; 3.86% vs 3.41%% for vascular dementia (HR: 1.167; 95% CI: 1.097-1.241; P < 0.001), 14.1% vs 12.0% for unspecified dementia (HR: 1.238; 95% CI: 1.199-1.278; P < 0.001), and 5.56% vs 4.93% for Dementia in other diseases (HR: 1.195; 95% CI: 1.135-1.257; P < 0.001) (Table 3).

DISCUSSION

ACEIs and ARBs are well-established components of guideline-directed medical therapy (GDMT) for heart failure with reduced ejection fraction (HFrEF) and are also widely prescribed as first-line agents for hypertension. Both drug classes have been associated with delayed progression of cognitive decline and a reduced risk of incident dementia; this effect is largely linked to blood pressure control [3]. In addition to this effect, recent research papers suggest that ACEIs and ARBs may also have neuroprotective benefits independent of their effect on blood pressure [3,4], with ARBs generally showed better outcomes compared with ACEIs [6].

Current evidence suggests that both ACE inhibitors and angiotensin receptor blockers may be associated with improved or preserved cognition in patients with heart failure, but ARBs may offer greater benefit in reducing progression to dementia, and definitive conclusions are limited by the quality and heterogeneity of available studies [7] .

In this large, multicenter, propensity-matched cohort study of patients with HFrEF, treatment with ARBs was associated with a significantly lower 5-year incidence of new-onset neurocognitive disorders compared with ACEIs. This protective association was consistent across the composite outcome of cognitive decline, dementia, and Alzheimer's disease.

These findings are in line with a recent randomized controlled trial by Hajjar et al. (2025) [8]. This RCT showed that the use of candesartan resulted in better neurocognitive outcome compared with lisinopril in older adults with MCI after 1-year of treatment. This effect is likely independent of the blood pressure lowering effect of candesartan.

The exact mechanism of this effect is not well established, but experimental data [9] attribute the protective effect of ARBs to the increased activation of the angiotensin II type 2 receptor (AT2R), leading to protein phosphatase 2A (PP2A) activation, stabilization of IκBα, and suppression of NF-κB and STAT3 inflammatory signaling. Thereby reducing Neuroinflammation, which is usually viewed as the preceding event in neurodegeneration.

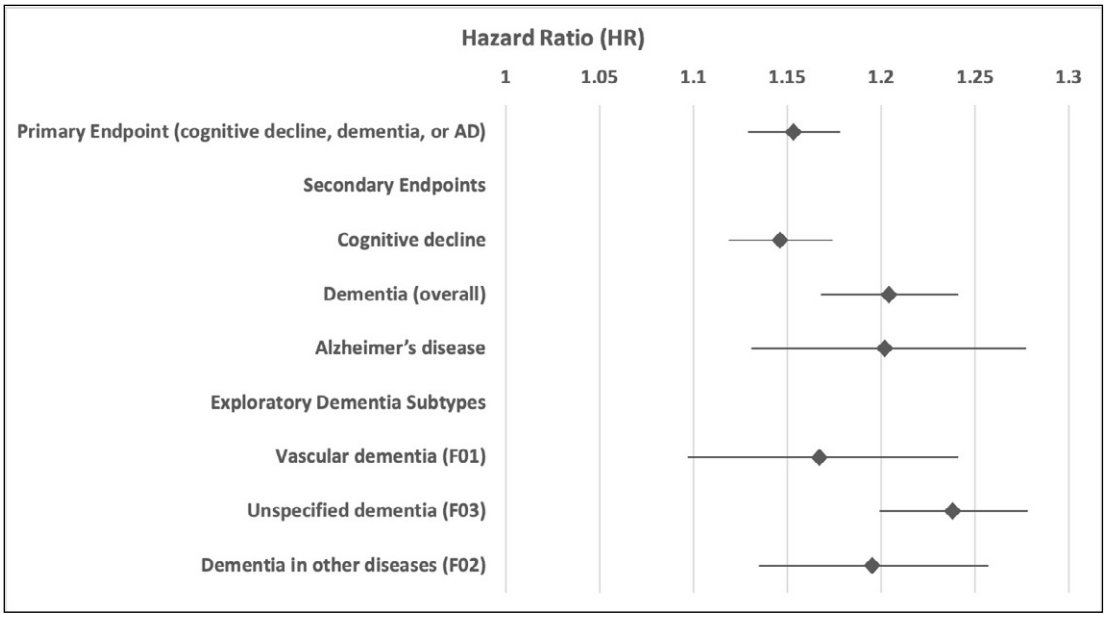

Fig. 3. Hazard ratio of the primary and secondary outcomes
Source: Own materials

However, these results contrast with earlier meta-analytic evidence by Zhuang et al. (2021) [4], which had studied the efficacy of renin–angiotensin system blockade (RASB) and the risks of cognitive decline and dementia. Their analysis showed that both ARBs and ACEIs may reduce dementia incidence, but ARBs may not confer greater benefit than ACEIs.

COGNITIVE DECLINE

The 5-year incidence of cognitive decline was higher in the ACEI cohort (24.94%) compared with the ARB cohort (22.81%). This suggests ARBs are better at maintaining global cognitive function in the long term. As expected, a network meta-analysis of 19 randomized controlled trials involving 13,734 patients demonstrated ARBs were better than ACEIs in preventing cognitive decline [10]. However, secondary analyses of three large RCTs [11, 12] did not report a distinct difference in cognitive outcomes between ACEIs and ARBs, declaring the relative cognitive advantages of these drug classes as currently inconclusive and potentially dependent on study population or study design.

DEMENTIA

Over a five-year period, the incidence of all-cause dementia was 15.63% in patients treated with ACEIs compared with 13.71% in those receiving ARBs. This difference suggests that ARBs may lower the risk of dementia relative to ACEIs. Several large cohort studies and meta-analyses have consistently reported an association between ARB therapy and a reduced incidence of dementia when compared with ACEIs [13 - 16].

Despite the consistency and biological plausibility of these findings, most of the available data come from observational studies. Direct evidence from randomized controlled trials remains limited, and therefore causality cannot be firmly established.

ALZHEIMER'S DISEASE

The 5-year incidence of Alzheimer's disease was higher in the ACEI group (4.15%) compared with the ARB group (3.51%), corresponding to a hazard ratio of 1.202 (95% CI: 1.131–1.277; $P < 0.001$). These results suggest that ARBs may provide greater protection against the development of Alzheimer's disease than ACEIs. This observation is consistent with prior studies reporting that ARBs are associated with a lower risk of Alzheimer's disease and dementia compared with ACEIs [14, 17]. The proposed mechanisms include enhanced cerebral blood flow, attenuation of neuroinflammation, and reduced amyloid-β accumulation. However, not all investigations have demonstrated this protective effect, and some studies have reported no significant differences between the two drug classes [15]. Such discrepancies may reflect differences in study design, patient populations, follow-up duration, and diagnostic criteria.

Overall, our findings add to the growing body of evidence that ARBs may offer superior neurocognitive protection compared with ACEIs in patients with HFrEF, particularly in reducing the incidence of cognitive decline, dementia, and Alzheimer's disease. Given the predominance of observational data and the limited number of head-to-head randomized controlled trials, definitive conclusions cannot yet be drawn. Future large-scale, long-term RCTs directly comparing ACEIs and ARBs with

cognitive outcomes as primary endpoints will be important to establish causality and to guide therapeutic decision-making in patients at risk of neurocognitive disorders.

STUDY LIMITATIONS

Despite the study's strengths and large real-world cohort, some limitations must be noted. New cognitive disorders were diagnosed based on diagnostic codes instead of formal cognitive tests (e.g., Mini-Cog, CogState), potentially slightly underestimating cases; however, both groups were evaluated with the same methodology, allowing for reliable comparisons to be made. HFrEF was defined by diagnostic codes rather than quantitative measurements, which could include some patients with mid-range or preserved ejection fraction, However, because the study includes a large, real-world patient population, the overall findings still likely reflect what happens in routine clinical practice, even if a few patients were misclassified. In addition, in cohorts with high mortality, as in this study, a competing-risks analysis would provide more precise estimates of absolute and relative risks for less frequent events. Due to limitations of the online analytics platform, such an analysis could not be performed. This limitation likely has minimal impact on more common outcomes, such as the primary composite endpoint and cognitive decline, but the risks for rarer outcomes, including dementia and Alzheimer's disease, may be somewhat overestimated. Furthermore, the follow-up period was short, which limited the detection of cognitive changes after the period of 3 years. Finally, as a retrospective cohort study, this analysis is inherently subject to residual confounding and selection bias despite propensity score matching. Unmeasured or unknown factors, such as socioeconomic status, lifestyle behaviors, or medication adherence, may have influenced both exposure and outcomes. Additionally, reliance on existing electronic health records limits the precision and integrity of clinical data, and causal relationships cannot be established. These limitations are inherited to observational research and should be considered when interpreting the results.

CONCLUSIONS

In this large, multicenter, propensity-matched cohort of patients with HFrEF, ACEI therapy was associated with higher 5-year rates of cognitive decline, dementia, and Alzheimer's disease compared with ARBs. ARBs demonstrated a consistent neuroprotective association across all measured cognitive endpoints, suggesting potential advantages over ACEIs in preserving cognitive function in patients with HFrEF. These findings highlight the importance of considering neurocognitive outcomes when selecting guideline-directed medical therapy for HFrEF. These observations need to be confirmed by prospective, randomized trials.

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Availability of data and material
The data supporting the findings of this study are available through the TriNetX research network but are subject to licensing restrictions. Access to TriNetX data can be obtained upon reasonable request and with permission from TriNetX, LLC.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Psychological training of future specialists in the security and defense sector in extreme conditions: Evaluation of cognitive and emotional reactions to stress and human potential development

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ABSTRACT

Aim: The aim of the study is to determine the effectiveness of psychological preparation methods for future specialists in the security and defense sector in extreme conditions, specifically evaluating emotional and cognitive reactions to stress factors, and exploring the potential of human resources in these conditions to enhance the effectiveness of professional activities

Materials and Methods: The study involved cadets from Dnipro State University of Internal Affairs, divided into two groups: Group 1 (control, n=120, mean age 18.86±1.25) received standard training, while Group 2 (experimental, n=120, mean age 18.73±1.19) underwent 10 months of specialized training for extreme conditions

Results: A special training program with crisis models, such as "Technogenic disaster in a combat zone," led to a statistically significant difference between the control and experimental groups ($p<0.05$) after using the Stress Resistance Scale.

The Cattell test (16 RF) showed that the experimental group had a higher emotional stability score compared to the control group.

Emotional stability test results revealed lower anxiety levels in the experimental group ($p<0.01$).

A framework for "Human Potential Development through Assessment and Adaptation" was developed, focusing on emotional and cognitive reactions. Practical recommendations were provided to improve training programs and enhance psychological resilience for future specialists in the security and defense sector.

Conclusions: Improving special training programs for future specialists in the security and defense sector should integrate physical, cognitive, and psychological aspects, considering individual traits. A systemic approach, including psychological preparation and stress management skills, will enhance psychological resilience, essential for success in extreme conditions.

KEY WORDS: security and defense sector, cadets, crisis situations, emotional stability, extreme conditions, cognitive reactions

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INTRODUCTION

In today's world, against the backdrop of evolving global, national, and local security threats, a high level of preparedness among specialists in the security and defense sector is one of the key conditions for effectively countering these challenges [1]. Psychological training for future specialists in the security and defense sector is a leading aspect that includes the development and improvement of stress resistance, emotional stability, and the enhancement of cognitive functions in extreme conditions [2, 3]. In this context, the evaluation of emotional and cognitive reactions to stress as an indicator of human potential development is an important component of the preparation process for future specialists in the security and defense sector [4, 5].

Considering the significant impact of stress on decision-making ability and the effective performance of service tasks in critical situations, studying cognitive and emotional reactions to stress becomes vital for developing effective, modern methods of preparing future specialists for the security and defense sector [6, 7].

Testing and training based on psychological methods not only allows for assessing the current level of readiness but also enhances the effectiveness of training programs aimed at increasing resilience to stress for future specialists.

AIM

The aim of the research is to determine effective methods for the psychological preparation of future specialists in the security and defense sector in extreme conditions, specifically evaluating emotional and cognitive reactions to stress factors, as well as exploring the potential of human resources in these conditions to enhance the effectiveness of professional activities.

The scientific novelty of the chosen topic lies in: 1) the integration of modern psychological training methods to assess and develop emotional and cognitive reactions of future specialists in the security and defense sector in extreme conditions; 2) the first-time exploration of the relationship between psychological indicators and the overall development of human potential in this context, which allows for improving preparation for stress situations; 3) the assessment of cognitive functions (attention, memory, reaction to stress) using modern testing methods and emotional self-control techniques, which allows for identifying priority contemporary approaches to preparing future specialists for effective work in conditions of uncertainty and high risk.

OBJECTIVES

1. Analysis of the main psychological aspects of preparing future specialists in the security and defense sector to work in extreme conditions.
2. Evaluation of emotional and cognitive reactions of future specialists to stress situations using specialized tests.
3. Study of the impact of key human potential indicators in the context of psychological preparation for future specialists.
4. Development of practical recommendations for improving psychological preparation to enhance the quality and effectiveness of professional activities for future specialists in the defense and security sector in extreme conditions.

MATERIAL AND METHODS

The study was conducted with the participation of cadets from Dnipro State University of Internal Affairs (future specialists in the security and defense sector). The subjects were divided into two groups: Group 1 (control, n=120) consisted of males and females aged 17-20 years (mean age 18.86±1.25 years), who were undergoing standard training without specialized training for working in extreme conditions. Group 2 (experimental, n=120), with a mean age of 18.73±1.19 years, underwent training for 10 months using a specialized training program that included crisis and combat situation simulation models, and also incorporated training for the development of self-control, stress resistance, and emotional stability. Groups 1 and 2 were formed considering sample homogeneity (age, health status, physical fitness, and psychological stability).

METHODS

Analysis of specialized scientific and methodological literature; surveys; psychological testing (Stress Resistance Scale, Cattell's Multivariate Personality Inventory (16 RF), Emotional Stability Test). The obtained results were processed using mathematical statistics methods with the Statistics 10.0 program. Calculations included the following measurements: arithmetic mean, standard deviation. For comparing independent samples, the non-parametric Mann-Whitney U test was used, as the data did not meet the normality assumption. A difference was considered statistically significant at $p<0.05$.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

The qualitative preparation of future specialists in the security and defense sector requires a special, specialized approach, as the work of this contingent is associated with extreme conditions that demand not only physical preparedness and special endurance but also a high level of psychological resilience [8, 9]. Therefore, the psychological aspect is an important component in the overall comprehensive training of such specialists. In this context, several key factors are identified that determine the effectiveness of psychological training (Fig. 1).

The training program for future specialists has been enhanced with a series of innovative, specialized crisis models in various areas. Below is an example of one such crisis simulation model, which was implemented for the experimental group under the training title "Technogenic Disaster in a Combat Zone" (Fig. 2).

Situation Type: Technogenic disaster (accident at an energy facility) during active combat operations in a territory where simultaneous rescue operations, evacuation of civilians, and ensuring the safety of infrastructure facilities must be carried out.

Duration of the scenario – four hours. The main objective of this training crisis situation is to improve the ability to overcome stress and respond effectively in extreme situations for future specialists in the security and defense sector. Overall, the experimental group

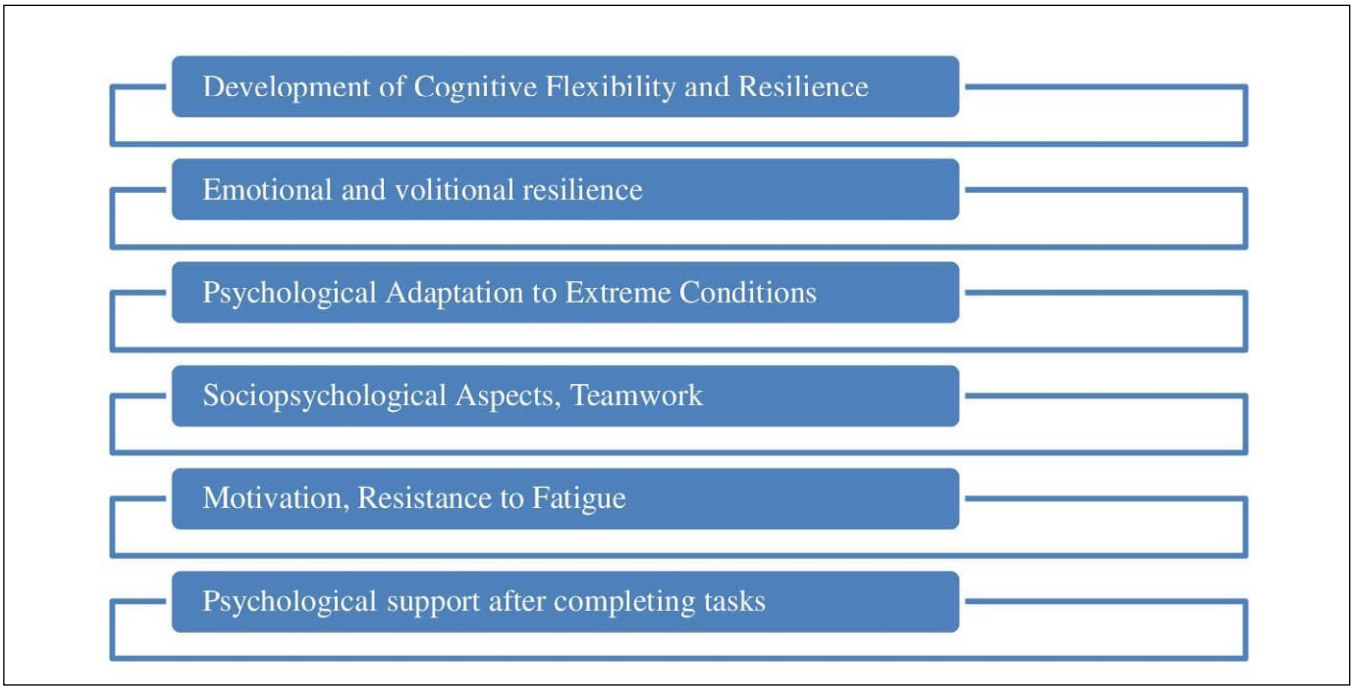

Fig. 1. Psychological aspects of training future specialists in the security and defense sector under extreme conditions
Picture taken by the authors

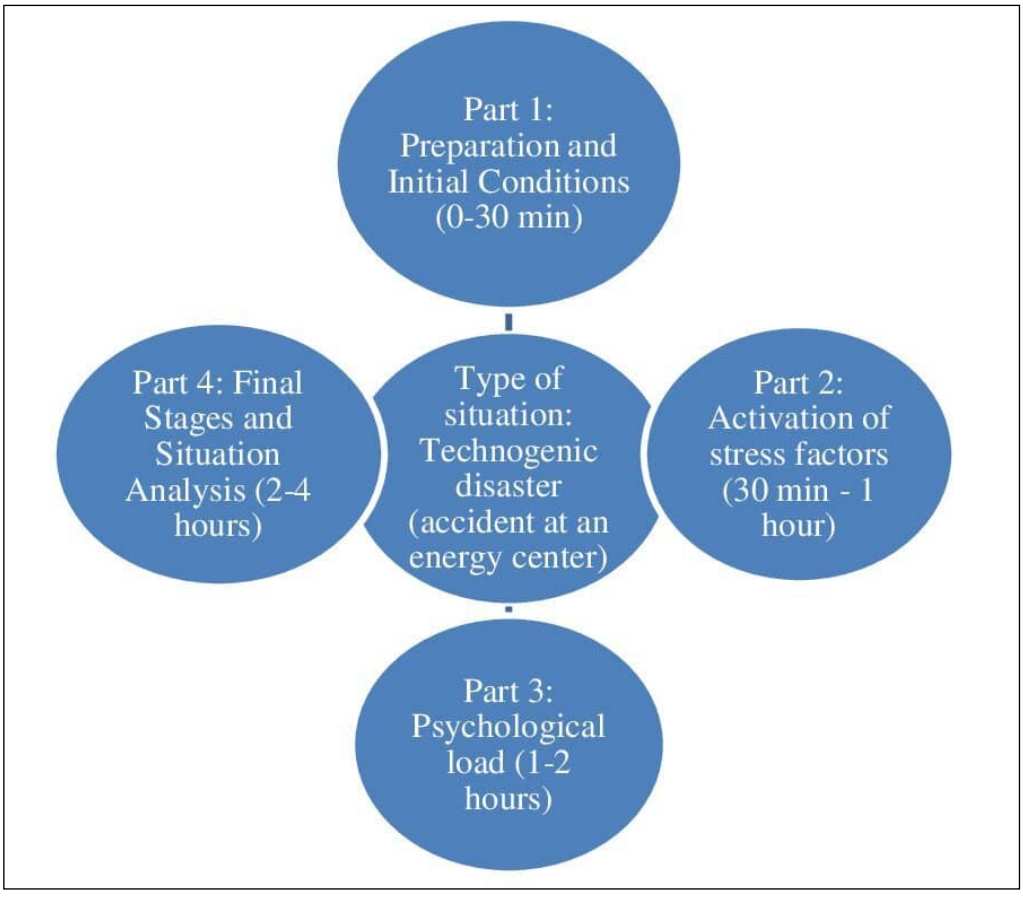

Fig. 2. Crisis model "Technogenic Disaster in a Combat Zone"
Picture taken by the authors

was trained in specialized conditions throughout the academic year (10 months).

As is well known, individuals with high stress resistance have a better ability to adapt to extreme situations. An important aspect is the ability of a person to endure high levels of stress while maintaining performance and emotional stability. Specialists with a high level of emotional stability are less prone to intense emotional reactions to stress, which allows them to maintain control over their behavior and emotions.

Table 1. Comparative characteristics of the Stress Resistance Scale test indicators for future specialists in the security and defense sector before and after the experiment

Indicators	Before implementation (X±S)		After implementation (X±S)	
	Control group (1) (n=120)	Experimental group (2) (n=120)	Control group (1) (n=120)	Experimental group (2) (n=120)
1.Stress resistance (rated on a scale of 1-10)	5.64±0.52	5.86±0.55	6.11±0.60	7.94±0.77
2. Decision-making time (seconds)	25.44±2.21	24.55±2.18	24.88±2.15	17.03±1.88
3.Team interaction effectiveness (rated on a scale of 1-10)	6.12±0.66	6.45±0.62	6.79±0.65	8.24±0.89
4.Number of mistakes during task execution (number of mistakes per 30 minutes)	3.65±0.38	3.89±0.37	3.43±0.32	1.32±0.13
5.Psychological burnout (rated on a scale of 1-10)	6.89±0.67	7.02±0.79	6.42±0.63	4.46±0.46
6.Evaluation of decisions by effectiveness level (rated on a scale of 1-10)	7.32±0.78	7.10±0.70	7.56±0.76	8.96±0.87
7.Physical endurance level (rated on a scale of 1-10)	6.62±0.69	6.96±-.66	6.54±0.65	7.58±0.76
8. Trust level within the team (rated on a scale of 1-10)	6.11±0.62	6.56±0.63	6.84±0.68	8.25±0.89
9. Psychoemotional state after training (rated on a scale of 1-10)	5.73±0.56	6.04±0.61	5.98±0.56	7.89±0.77
10. Overall task execution effectiveness (rated on a scale of 1-10)	6.32±0.64	6.74±0.67	6.87±0.65	8.74±0.88

Note: * - Statistically significant difference after the experiment compared to the control group (Group 1) according to the Mann-Whitney U test at $p < 0.05$
Source: compiled by the authors of this study

Table 2. Comparative characteristics of future specialists in the security and defense sector according to the Cattell's 16 Personality Factor Questionnaire (16 PF) – results after the experiment

Parameter (Factor)	Control group (n=120)		Experimental group (n=120)	
	X±S	Level	X±S	Level
1.Factor A (Judgment)	5.54±0.56	Average	6.98 ±0.68*	Higher
2.Factor B (Emotional Stability)	4.22±0.42	Lower	6.18 ±0.63*	Higher
3.Factor C (Principled Behavior)	6.14±0.69	Higher	7.15±0.74*	Very high
4.Factor E (Social Activity)	4.86±-.50	Average	5.91±0.56	Higher
5.Factor F (Self-Control)	5.07±0.53	Average	6.64±0.58*	Higher
6.Factor G (Intelligence)	5.98±0.58	Average	6.89±0.69*	Higher
7.Factor P (Insecurity)	7.52±0.78	Higher	5.74±0.55*	Average
8.Factor I (Emotional Sensitivity)	7.20±0.74	Higher	5.59±0.43*	Average
9.Factor L (Stress Sensitivity)	6.75±0.66	Higher	5.34±0.52	Average
10.Factor M (Optimism)	6.10±0.62	Average	6.95±0.67	Higher
11.Factor N (Dependence on Surroundings)	5.24±0.55	Average	4.38±0.43*	Lower
12.Factor O (Self-Esteem)	6.65±0.67	Higher	7.32±0.78	Very high
13.Factor Q1 (Need for Change)	5.05±0.54	Average	6.42±0.64*	Higher
14.Factor Q2 (Organizational Skills)	5.48±0.58	Average	6.12±0.65	Higher
15.Factor Q3 (Independence)	6.42±0.65	Higher	5.46±0.58*	Average
16.Factor Q4 (Tension)	6.88±0.66	Higher	5.14±0.52*	Average

Note: Statistically significant difference after the experiment between the control and experimental groups' indicators according to the Mann-Whitney U test * - <0.05
Source: compiled by the authors of this study

The main method for assessing emotional resilience and cognitive functions is psychological testing, which includes standardized techniques that allow for a qualitative assessment of an individual's overall mental readiness, emotional state, stress resistance, and cognitive abilities. Within the framework of our study, several methods were used for future specialists in the security and defense sector.

Cadets, as future specialists in the security and defense sector, were offered a stress resistance test (Stress

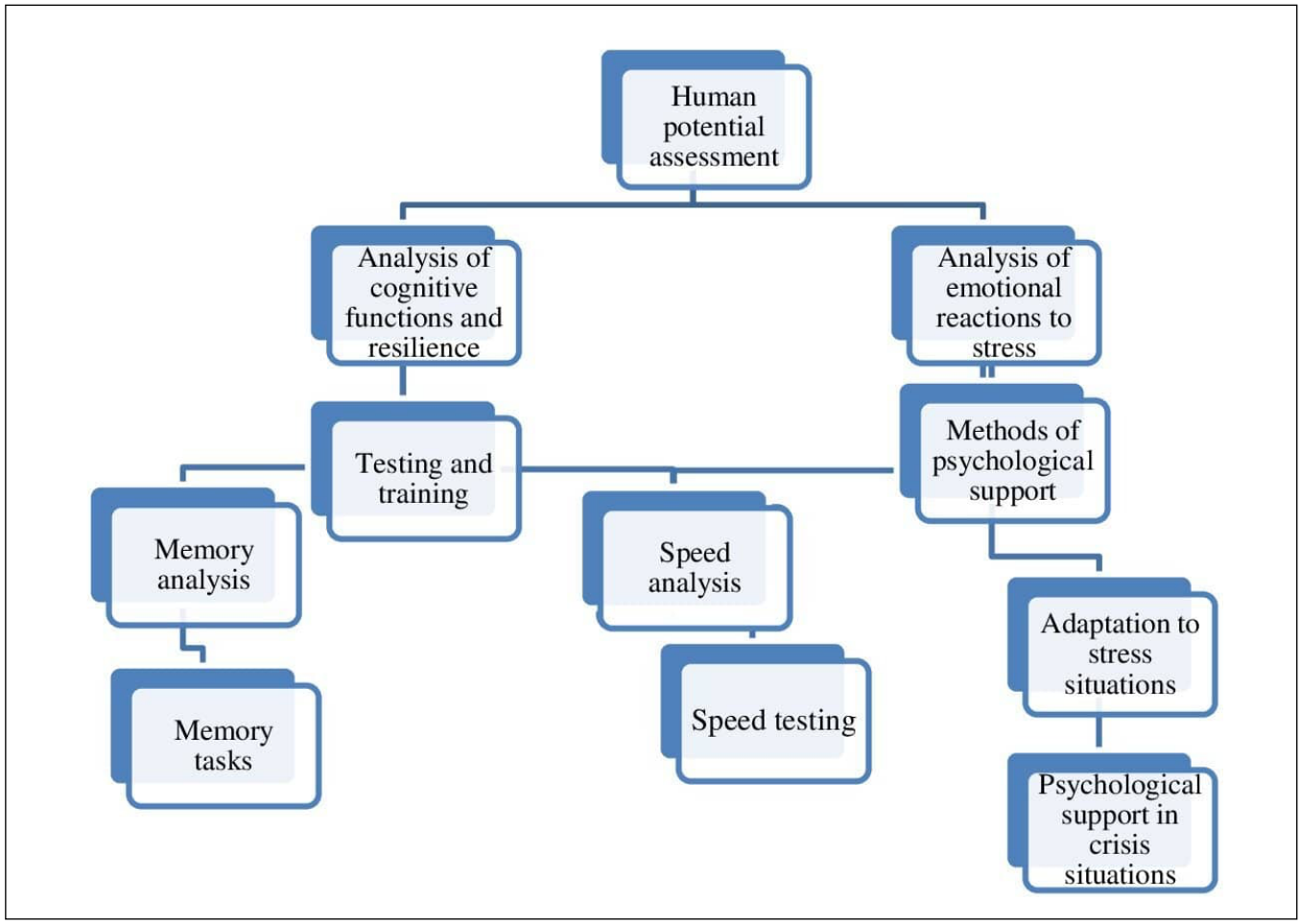

Fig. 3. Assessment of an individual`s human potential based on the quality of cognitive functions and emotional-volitional resilience
Picture taken by the authors

Resistance Scale) (Table 1) with key categories such as response time, teamwork, psychological burnout, physical endurance, and others.

The diagnosis of personal characteristics of future specialists in the security and defense sector, such as stress resistance and psychological readiness, was carried out using the Cattell's 16 Personality Factor Questionnaire (16 RF), which allows for identifying various components of personality and its reactions to stress (Table 2).

To assess the level of emotional stability in the control and experimental groups of future specialists in the security and defense sector, the Emotional Stability Test was used. The results of this test allow for determining the ability to control one's emotions and maintain calm in extreme conditions (Table 3).

The assessment of the physical and psychological aspects of training for future specialists and their impact on professional activity becomes a key factor in enhancing work efficiency in high-risk conditions.

Fig. 3 presents a model that includes the process of evaluating and developing human potential through the analysis of emotional and cognitive reactions. Memory and reaction speed training works in conjunction with psychological support and adaptation methods.

We have developed a series of practical recommendations to improve specialized training programs and enhance the psychological resilience of future specialists in the security and defense sector under extreme conditions (Table 4, Table 5).

DISCUSSION

Psychological training is a leading component in shaping the professional readiness of future specialists in the security and defense sector under extreme conditions [8, 9]. One of the general factors in the preparation of future specialists is cognitive flexibility and resilience, which is defined by the ability to quickly adapt to changing conditions and consider various decision-making options, among other things [10, 11].

Specialists in the security and defense sector must be able to rapidly adjust their behavior depending on the emerging circumstances: extreme situations can change by the minute, so they must possess the skills to regroup forces, maintain stable management, adjust

Table 3. Results of the Emotional Stability Test among future specialists in the security and defense sector after the experiment

Assessment parameter	Control group (n=120)		Experimental group (n=120)		Mann-Whitney U test
	X±S	Level of emotional stability	X±S	Level of emotional stability	
1.Anxiety level	7.42±0.90	High	5.16±0.52	Low	<0.01
2.Self-control ability	5.18±0.52	Low	7.54±0.78	High	<0.01
3.Speed of recovery after stress	6.93±0.72	Slow	4.56±0.54	Fast	<0.01
4. Stress sensitivity level	6.80±0.79	High	4.23±0.48	Low	<0.01
5.Emotional stability under stress load	5.52±0.67	Average	7.34±0.70	Higher	<0.01
6.Ability to remain calm in crisis situations	5.92±0.69	Average	6.98±0.69	Higher	<0.05
7.Reaction to external pressure	7.14±0.80	Nervous	5.56±0.62	Reserved	<0.01
8.Emotional reactivity	6.44±0.65	High	4.52±0.48	Low	<0.01
9.Ability to adapt to changes	6.43±0.59	Insufficient	4.92±0.50	Good	<0.01
10.Overall level of emotional stability	6.08±0.62	Average	6.94±0.72	Higher	<0.05

Source: compiled by the authors of this study

Table 4. Practical recommendations for improving training programs and enhancing psychological resilience for future specialists in the security and defense sector under extreme conditions

Components	Content of the program components. Examples
1. Integration of psychological training into overall preparation	
1) Combination of physical and psychological stress	Physical and psychological exercises that stimulate the ability to adapt and stress resistance in extreme situations (for example, combining physical exertion with elements of psycho-emotional stress to simultaneously improve emotional self-control and physical endurance)
2) Simulation of crisis situations and real combat operations	Development and implementation of highly realistic scenarios of terrorist attacks, combat operations, natural disasters, etc., which help future specialists prepare physically and psychologically adapt to stress factors.
2. Individualization of training based on personality type	
1) Consideration of individual characteristics	Consideration of the personality types of future specialists, psychological readiness, and levels of stress resistance (for example, for individuals with high anxiety levels, introducing more emotion management training; for highly impulsive individuals – exercises on strategic thinking and self-control).
2) Consultations, psychological selection	It is advisable to conduct this during the candidate selection stage. Regular psychological support and counseling during training and service to maintain mental stability and sustain a high level of stress resistance.
3. Training in stress management strategies	
1) Development of emotional intelligence	Training future specialists in effective emotional management strategies (meditation, breathing practices, relaxation techniques), which significantly reduces stress levels and prevents emotional instability.
2) Exercises for anxiety reduction, self-regulation	Development and implementation of training programs with a set of exercises aimed at reducing anxiety and emotional overload (self-regulation techniques to maintain clarity of thought and relative calm even in extreme circumstances).
4. Development of cognitive functions under stress conditions	
1) Intensive training on reaction speed and multi	The development of future specialists' ability to perform multiple tasks simultaneously under increased stress (simulation of real scenarios, multitasking training), which require simultaneous management and focus on several tasks, making critical decisions under limited resources and time constraints.
2) Improvement of cognitive flexibility and decision	Training using exercises focused on decision-making speed and cognitive flexibility under increased stress, to help future specialists develop the skills needed to make appropriate decisions in high uncertainty and limited information conditions.

Source: compiled by the authors of this study

Table 5. Practical recommendations for improving training programs and enhancing the psychological resilience of future specialists in the areas of team training and recreational activities

Components	Content of the program components. Examples
1. Team training, group interaction	
1) Training team interaction in stressful conditions	For the necessary and important effective teamwork in extreme conditions, it is essential to develop and implement scenarios with team interaction during physical and psychological stress. These scenarios help improve communication, mutual understanding, and reduce the level of conflict situations among team members.
2) Development and improvement of leadership qualities in stressful conditions	The development and implementation of programs that focus on the development of these qualities under stress, skills in maintaining calm and making important decisions in extreme situations, and the ability to lead a team in crisis situations.
2. The Role of Recovery and Rest	
1) Planning breaks and recovery	Incorporating sufficient rest, psychological rehabilitation exercises after stress loads and situations, into training programs for quick recovery of physical and emotional state.
2) Methods of psychological recovery	Providing qualified psycho-emotional support and the opportunity for consultations with specialists (psychologists) for effectively overcoming mental stress after undergoing high-stress training.
3. Systematic monitoring and adjustment of training programs	
1) Evaluation of the effectiveness of training programs	Systematic evaluation of the implementation and effectiveness of programs (collecting feedback from participants using data on their physical, psychophysiological, and psychoemotional state) for adjustments according to the needs and real conditions of professional work.
2) of training programs Regular improvement and feedback	Based on the collection of data on the effective completion of tasks and various stress reactions in extreme conditions, programs will be adjusted by incorporating new training methods to enhance the psychological resilience of the personnel.

Source: compiled by the authors of this study

action plans, and apply various tactical approaches [12].

Quality psychological training for the development and improvement of cognitive flexibility includes specialized exercises that help participants rapidly adjust their thinking priorities (simulations of crisis situations on various topics, where the goal is to make prompt, varied decisions).

A key component of psychological training for future security and defense sector specialists to effectively perform professional duties is emotional stability, which includes the ability to stay calm and focused, control emotions even in dangerous and stressful situations [13, 14].

Future specialists must be able to adapt to circumstances that can suddenly change, manage emotional loads without anxiety or panic. To develop and enhance emotional resilience, future specialists need training in improving self-control, stress management skills, maintaining psychological balance, and more. These skills will ensure high performance when performing professional duties in the future.

The importance of teamwork under stress is crucial in the psychological preparation of future specialists in the security and defense sector. Teamwork in high-stress conditions can be a leading and decisive factor in achieving goals and successfully completing tasks [15, 16]. In this context, psychological training includes the development and improvement of communication skills, role distribution within the group (team), the ability to respond quickly and assess the situation, with an emphasis on resolving conflicts in stressful conditions. An essential aspect of this is fostering mutual support and trust among team members.

In ensuring the high and long-term effectiveness of future security and defense sector personnel in extreme conditions, motivation plays a priority role [17]. The development and enhancement of self-support, internal motivation, and the desire to achieve positive results in challenging conditions should be considered in psychological training. Important skills that need to be taught to future specialists include proper management of fatigue and energy resources, as exhaustion while performing professional tasks can significantly reduce work efficiency and increase the risk of mistakes.

Rehabilitation aspects are among the leading elements in the psychological preparation of future specialists, including the development of psychological support programs after returning from high-stress zones to restore psychological balance and ensure mental health to prevent post-traumatic stress disorder [18].

Thus, comprehensive psychological preparation of future specialists in the security and defense sector is an important component of overall training for working in extreme conditions [19]. The development and enhancement of strategic thinking, stress resilience,

teamwork skills, and burnout prevention are fundamental factors in ensuring effective work in dangerous conditions while performing their duties. Therefore, regular training and psychological support should be an integral part of the preparation program for future specialists [20].

In our research, the control group (n=120) of future specialists underwent standard training without specialized additional exercises aimed at developing stress resilience and personal traits. The experimental group (n=120) of future security and defense specialists underwent specialized training using an experimental model for stress management, emotional self-control, and the development of psychological and psychophysiological resilience.

According to the data obtained during the experiment, it was found that the control group exhibited higher levels of anxiety, emotional sensitivity, and stress sensitivity, indicating limited ability to adapt to extreme conditions. Future specialists in the experimental group showed higher results (the majority of indicators statistically significantly improved compared to the control group at $p<0.05$) in emotional stability, self-control, cognitive functions, and optimism, demonstrating the effectiveness of training aimed at improving stress resilience and psychological readiness for working in high-stress conditions.

The results obtained in the study indicate that the experimental group, which underwent specialized training, demonstrated higher levels of self-control, emotional stability, and the ability to adapt to stressful situations, confirming the positive effect of the specialized program on the development and improvement of preparedness in extreme conditions.

The results highlight the need for and importance of special additional programs to improve emotional stability among future security and defense specialists, which will significantly enhance their ability to perform professional tasks under extreme conditions.

One of the key aspects of training future specialists is comprehensive preparation, with physical and psychological training being key elements. This ensures two important factors: readiness to perform various professional tasks and maintaining resilience during dangerous special operations or combat conditions. Given the current trends in the development of various technical tools and changing real threats, the need to improve psychological training methods aimed at effectively utilizing human potential becomes increasingly relevant [5].

The model presented in this work (Figure 2) demonstrates the evaluation of human potential based on cognitive functions and emotional-volitional resilience and visually represents the process of enhancing human capabilities through the integration of various methods for assessing emotional and cognitive reactions, specialized training, etc. The components of the model in the presented interpretation allow us to understand how various combined approaches to analysis and development can contribute to improving the overall ability of an individual to adapt to different conditions and enhance their efficiency and productivity.

The process of developing special skills within human potential occurs in three stages. The initial stage involves evaluating two main aspects: emotional and cognitive reactions of the individual. This initial assessment provides the foundation for further development, enabling an understanding of the factors on which efforts should be focused for improvement.

At the next, second stage, cognitive functions such as reaction speed and memory (e.g., mnemonic techniques or neuropsychological exercises) are trained. These are important elements in the development of human potential because they directly impact an individual's ability to adapt to various situations and achieve results in challenging extreme conditions. Such training operates in two directions: it improves cognitive abilities and promotes the development and enhancement of adaptive mechanisms in response to changing external circumstances.

A central aspect of human potential development is the stage of psychological adaptation, which includes implementing methods that reduce stress (stress and anxiety management training, mindfulness practices, which contribute to the development of operational thinking and improve emotional stability).

A critical stage in human potential development is the integration of cognitive ability training with psychological adaptation. By integrating these elements, an individual improves their cognitive functions while also effectively learning to manage emotions and purposefully adapt to new challenging situations.

The dynamic stage of assessing potential development through the analysis of emotional and cognitive reactions allows for the construction of an overall development strategy, where emotional balance and intellectual abilities play a leading role. This ensures a more stable result in human potential development, as it considers both emotional aspects and emotional abilities, which affect efficiency and productivity.

As a result, the components shown in the diagram of human potential development through adaptation and assessment represent a holistic approach, combining various methods of psychological adaptation training and cognitive abilities. This improves reaction speed, memory, increases adaptability, and enhances emotional resilience to various conditions, which are currently essential aspects for effective performance.

CONCLUSIONS

Developed cognitive functions (attention, memory, adaptability, decision-making speed, etc.) are crucial in the professional activities of future specialists in the security and defense sector under extreme conditions. The assessment of these functions through specialized methodologies is an essential component for determining their readiness to perform complex tasks in high-risk conditions.

Improving emotional-volitional stability and stress resilience through specialized training programs and psychological exercises is an important part of preparing future specialists. The results of the experimental research showed that the groups trained under the special program demonstrated better results with statistical significance at $p<0.05$ and 0.01 regarding concentration maintenance, fewer errors, and quicker recovery after stressful situations.

Taking into account the individual characteristics of the personnel (stress resilience, cognitive abilities, etc.) is essential for enhancing the effectiveness of psychological preparation. The development and implementation of individual training programs tailored to the specific needs of each specialist are necessary to achieve the best results in changing situations and high stress. Applying a comprehensive approach to psychological preparation for future specialists should include many components, namely: improving physical training, developing psychological flexibility, building individual stress management skills, and training cognitive functions and emotional stability.

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ORIGINAL ARTICLE

CONTENTS

Prognostic factors for breast cancer progression

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ABSTRACT

Aim: To investigate the clinical and laboratory features of breast cancer with the progression of the tumour process, after complex treatment, depending on the stage of the disease and the molecular type of the tumour, and to determine the prognostic value of each factor.

Materials and Methods: A retrospective analysis of 701 outpatient records of patients with breast cancer in the long term, after complex treatment, was performed.

Patients, depending on the presence of verified breast cancer progression, were divided into two groups - 472 (67.3%) patients 'without tumour progression' and 229 (32.7%) patients 'with tumour progression'.

The informativeness of the studied indicators and diagnostic coefficients was determined using the Kullback method. The results were statistically processed using Microsoft Excel spreadsheets and the PAST statistical processing software package.

Results: Breast cancer progression occurs more often at stage III of the disease, in Lum.-B, Her/2new+, and Triple-negative tumour types. The progression of breast cancer is characterized by a relatively more extended history of the disease, a larger size of the primary tumour, and a higher percentage of comorbidities. The study of the informativeness of the studied indicators and diagnostic coefficients made it possible to form a table of prognosis of possible progression of breast cancer after complex treatment.

Conclusions: The prognostic model of breast cancer progression enables to obtain the prediction of the absence of tumour progression, uncertain prognosis, and the appearance of the latter, with the sensitivity (Se=75%) and specificity (Sp=75%) of this model.

KEY WORDS: breast cancer, progression of the tumour process, breast cancer metastases

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INTRODUCTION

Despite the development of modern oncology, breast cancer (BC) remains an extremely urgent problem, as it is one of the most common forms of malignant tumours among women in the vast majority of developed countries [1].

According to the National Cancer Registry, more than 12,000 cases of breast cancer are diagnosed in Ukraine every year. Even though breast cancer is curable at an early stage in 95% of women, every fourth woman in Ukraine learns of the diagnosis at the III-IV stages, when more expensive and long-term treatment is required, and the effectiveness of the latter is significantly reduced [2].

Treatment of breast cancer is complex and involves a combination of local methods, systemic antitumour, and supportive treatment, which is carried out in different sequences and combinations, depending on the patient's condition, tumour morphology, stage of the disease, etc [3].

The effectiveness of breast cancer treatment is directly related not only to the early detection of this disease but also to the absence of progression of the tumour process, i.e. the time before metastases appear.

At present, the main prognostic guideline for the prognosis of the disease and, accordingly, the effectiveness of breast cancer treatment is the stage of the disease and the division of carcinomas into molecular types [4-6].

To date, there is a fairly large number of scientific papers in which the authors use the patient's anthropometric data, tumour size, location, presence of metastases, etc. to predict the metastatic spread of breast cancer [7-9].

However, there are no clear criteria or algorithms for predicting the progression of the tumour process, nor their prognostic value.

The present study aims to address these issues by studying the peculiarities of breast cancer progression, namely the appearance of metastases, depending on the stage of the disease, and the molecular type of the tumour, and identifying relevant prognostic and predictive factors.

AIM

The aim of the study was to investigate the clinical and laboratory features of breast cancer with the progres-

sion of the tumour process, after complex treatment, depending on the stage of the disease and the molecular type of the tumour, and to determine the prognostic value of each factor.

MATERIALS AND METHODS

A retrospective analysis of 701 outpatient records of patients with breast cancer who were treated in healthcare facilities in Ukraine from 2017 to 2021 was conducted.

Patients, depending on the presence of verified breast cancer progression after complex treatment, were divided into two groups - 472 (67.3%) patients 'without breast cancer progression' and 229 (32.7%) patients 'with breast cancer progression'.

The TNM classification of the 8th edition (2016) was used to determine the stage of breast cancer. In our studies, 54 (7.7%) patients had stage I, 227 (32.4%) had stage II A, 181 (25.8%) had stage II B, 93 (13.3%) had stage III A, 81 (11.6%) had stage III B, and 65 (9.3%) had stage III C.

Immunohistochemical classification of molecular types of breast cancer was also used []. Thus, 350 (49.9%) patients were diagnosed with Luminal-A tumour type, 127 (18.1%) with Luminal-B tumour type, 78 (11.2%) with Her/2new+ tumour type, and 146 (20.8%) with triple-negative tumour type.

The most common histological type of breast cancer was invasive ductal carcinoma (642 (91.6%)). In addition, cases of mucinous carcinoma were detected in 31 (4.4%) patients, lobular carcinoma - in 22 (3.1%) patients, and medullary carcinoma - in 6 (0.9%) patients.

The informativeness of the studied indicators and diagnostic coefficients were determined by the Kullback method.

The diagnostic coefficient was calculated by the formula:

$$DC\ (x_{ij}) = 10 \cdot \lg \frac{P(x_{ij}/G_+)}{P(x_{ij}/G_-)}$$

where G_+ - the presence of metastases in the patient, G_- - the absence of metastases, $x_{\cdot}$ - j th range of the prognostic criterion $x_i, i = \overline{1,m}$, $P(x_{ij}/G_+)$ - the conditional probability of a patient with metastases falling into the j -th range of the i-th prognostic criterion, $P(x_{ij}/G_-)$ - the conditional probability of a patient without metastases falling into the j -th range of the i-th prognostic criterion.

To determine the contribution of this prognostic criterion to the approach to the correct diagnostic threshold, the informativeness of the criteria was calculated using the formula.

$$(x_i/G_+, x_i/G_-) = \sum_{j=1}^{n_i} 10 \cdot \lg \frac{P(x_{ij}/G_+)}{P(x_{ij}/G_-)} \times 0{,}5[P(x_{ij}/G_+) - P(x_{ij}/G_-)]$$

where x_i is the prognostic criterion, n_i is the number of ranges of the prognostic criterion x_i

Statistical processing of the obtained research results was carried out on a personal computer using Microsoft Excel spreadsheets and the PAST statistical processing software package. The correctness of the data distribution in the samples was checked by applying the Shapiro-Wilk criteria. In the case of normal distribution of independent groups, the Student's t-test was used. In the case of non-normal distribution of continuous variables, the Mann-Whitney test (U-test) was used. Fisher's tests were used to assess the significance of the difference between the percentage shares of the two samples. The differences in the results obtained were considered statistically significant at $p<0.05$, which is generally accepted in biomedical research.

ETHICS

The work is written in accordance with the ethical standards of the industry.

RESULTS

The results of the study presented in Table 1 indicate a significantly lower number of people in the main group with stages I, II A, and II B of the disease and a higher number with stages III A, III B and III C.

Analyzing the results of the study, Table 2 shows a significantly lower number of patients in the main group with Lum-A tumour type and a higher number with Lum-B, Her/2new+, and Triple-A tumour types.

It should be noted that there was no significant difference in the percentage of patients with right and left breast lesions in each study group.

Analysing the results of the study of the average age of patients, it should be noted that there was no significant difference in both study groups at all stages of the disease, with the exception of stage II A, where the indicators of the comparison group significantly prevail.

Regarding the difference in the average age of individuals, depending on the molecular type of tumour, it should be noted that women with Lum.-B and Her/2new+ tumour types are significantly older.

The results of the study presented in Table 3 indicate a significant prevalence of the average duration of the disease history in women in the main group, with the exception of stage I, where this difference is not significant. In both study groups, there was a significant increase in the average duration of the disease history in parallel with the increase in stage, except that the indicators of the main group were not significant in stage II B.

Table 1. Distribution of breast cancer patients, depending on the stage of the disease, abs., %

Stage of the disease	Comparison group		Main group		Difference between both groups
	Abs.	%	Abs.	%	
I	46	9.7	8	3.5	p=0,0007
II A	182	38.6	45	19.7	p=0,0
II B	132	28.0	49	21.4	p=0,029
III A	52	11.0	41	17.9	p=0,0072
III B	39	8.3	42	18.3	p=0,0001
III C	21	4.4	44	19.2	p=0,0
Total	472	100	229	100	p=0,0

Source: compiled by the authors of this study

Table 2. Distribution of breast cancer patients, depending on the molecular type of the tumour, abs., %

Molecular type of the tumour	Comparison group		Main group		Difference between both groups
	Abs.	%	Abs.	%	
Lum. A	296	62.7	54	23.6	p=0,0
Lum. B	72	15.2	55	24.0	p=0,029
Her/2new+	31	6.6	47	20.5	p=0,0
Triple-	73	15.5	73	31.9	p=0,0
Total	472	100	229	100	p=0,0

Source: compiled by the authors of this study

Table 3. The average duration of the disease history of patients with breast cancer, depending on the stage of the disease, months (M±m)

Stage	Comparison group	Main group
I	2.5 ± 0.14 n=46	3.1 ± 0.4 n=8 p=0.175
II A	3.0 ± 0.09 n=182 p_1=0.0383	3.6 ± 0.23 n=45 p=0.0131; p_1=0.4741
II B	3.2 ± 0.09 n=132 p_1=0.0309	3.6 ± 0.16 n=49 p=0.0322; p_1=0.7668
III A	3.5 ± 0.15 n=52 p_1=0.0315	4.1 ± 0.15 n=41 p=0.0104; p_1=0.0109
III B	4.0 ± 0.17 n=39 p_1=0.0355	4.6 ± 0.17 n=42 p=0.0241; p_1=0.0375
III C	4.7 ± 0.21 n=21 p_1=0.0262	5.4 ± 0.21 n=44 p=0.0485; p_1=0.0114

Notes.
n - the number of people;
p - the difference between the two respective study groups;
p_1 - the difference about the next stage of the disease in the corresponding study group
Source: compiled by the authors of this study

When studying the average duration of the disease history, depending on the molecular type of the tumour, it should be noted that the main group is significantly more likely to have the highest rates in all types of tumours. If we compare the indicators in the average comparison groups, there is a significant difference in the Her/2new+ tumour type versus all other types. In the main group, there is a significant difference between the Her/2new+ and Triplets types, but no difference between all other types.

The results of the study presented in Table 4 indicate a predominance of malignant tumour size in patients of the main group, except that this difference is significant only at stages II A and III A of the disease.

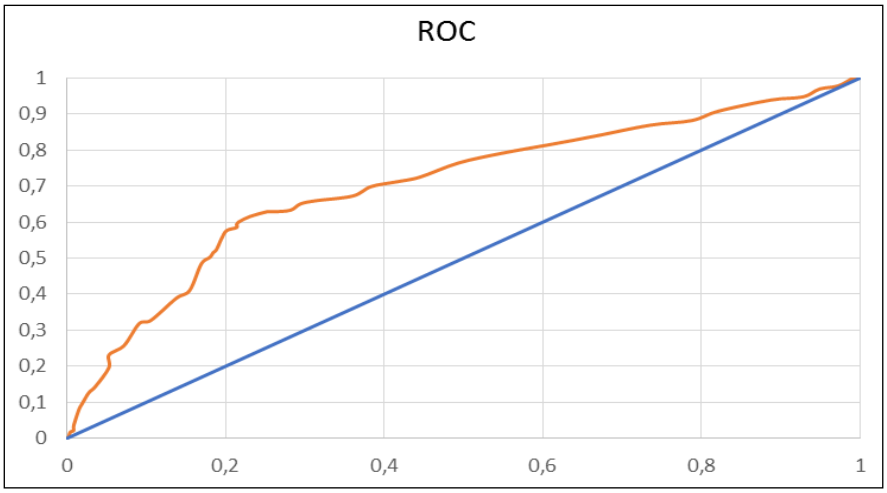

Fig. 1. ROC-curve. Area under the ROC curve AUC=0.69
Picture taken by the authors

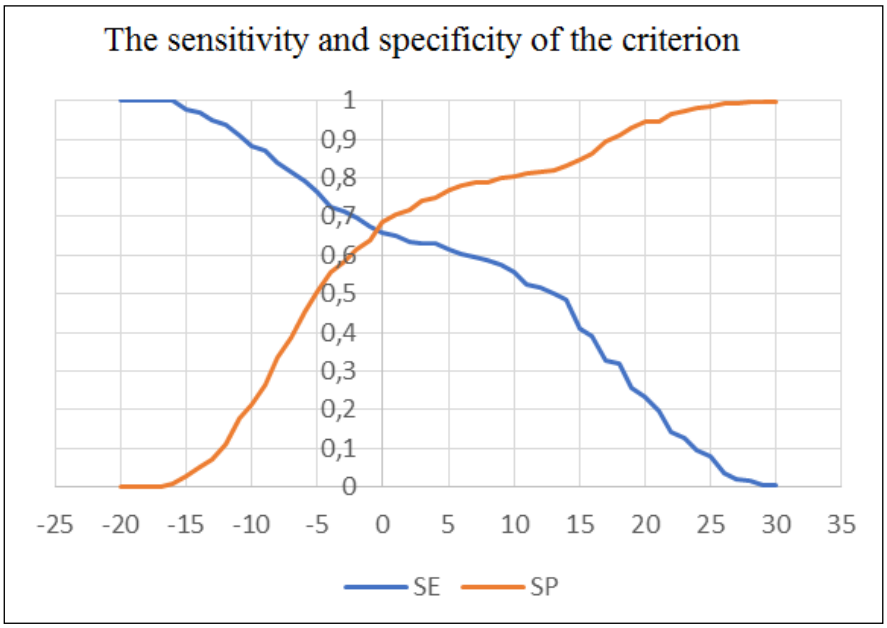

Fig. 2. Graph of sensitivity and specificity of the criterion for predicting breast cancer progression after complex treatment
Picture taken by the authors

When studying the size of the tumour, depending on its molecular type, it is necessary to note the probable predominance of the main group in all types of tumour.

When studying the frequency of concomitant pathology in patients with breast cancer, a significant difference in the total percentage of indicators between both study groups should be noted. At stages I and II of the disease, there is a significant predominance of the percentage of patients in the comparison group. At stage III of the disease, the percentage of women in the main group with concomitant pathology is significantly higher.

In patients of the main group, there is a significant prevalence of metabolic pathology in stages III A and III C of the disease. At stage III B, cardiovascular, respiratory, metabolic, and gynaecological pathology is likely to predominate in patients of the main group.

It should be noted that there is a significant predominance of the number of comparison group patients with concomitant pathology in the Lum-A tumour type, but in all other tumour types, the rates of women in the main group prevail, except for the difference in Triple tumour type. In the main group of patients with Triple tumour type, there is a significant prevalence of patients with respiratory system pathology and a significantly lower percentage of patients with metabolic diseases.

When assessing the degree of differentiation of the malignant neoplasm, it should be noted that there is no significant difference in the percentage of moderately differentiated and low-differentiated tumours between both study groups, at stages I and II A of the disease. At all other stages of the disease, the percentage of G_3 in patients of the main group and G_2 in the comparison group significantly prevails.

In the Lum-A tumour type, the percentage of G_3 tumours in women of the comparison group and G_2 tumours in the main group significantly prevails. There was no significant difference in the percentage of patients with G_2 and G_3 tumours between the two study groups. In both Her/2new+ and Triple-negative groups, there were no patients with G_2 tumours.

Table 4. Size of malignant neoplasm in breast cancer progression, depending on the stage of the disease, (M±m), cm

Stage of the disease	Comparison group	Main group
I	1.3 ± 0.06 n=46	1.6 ± 0.09 p=0.0634 n=8
II A	3.2 ± 0.08 n=182	3.5 ± 0.17 p=0.0345 n=45
II B	4.7 ± 0.15 n=132	5.0 ± 0.14 p=0.0543 n=49
III A	4.0 ± 0.26 n=52	5.1 ± 0.31 p=0.0069 n=41
III B	5.8 ± 0.26 n=39	6.3 ± 0.31 p=0.2787 n=42
III C	5.5 ± 0.34 n=21	6.2 ± 0.55 p=0.0581 n=44
Total	3.8 ± 0.09 n=472	5.1 ± 0.13 p=0.0001 n=229

Notes:
n - the number of people;
p - the difference between the two study groups
Source: compiled by the authors of this study

It should be noted that the longest time for breast cancer progression is likely to be observed in stage I of the disease and the shortest in stage III C, which is logically explained by the aggression of the tumour process. With the increase in the stage of the oncological process, there is a significant increase in the time to breast cancer progression, with the exception of stages III A and III B, where this difference is not significant.

The longest time to breast cancer progression is observed in Lum-A and Lum-B tumour types and is probably the shortest in Her/2new+. Although the time to breast cancer progression in the Triple type is shorter than in the Lum-A and Lum-B types, this difference is not significant.

In the analysis of metastatic lesions, depending on the stage of the cancer process, it is necessary to note a significantly lower number of metastases at stage I.

If we consider the spread of metastatic lesions, depending on the molecular type of the tumour, we should note a significantly higher number of metastases in the T3 tumour type compared to other types.

Patients in stage I of the disease, in both study groups, underwent only organ-preserving operations.

At stage II A of the disease, the number of organ-preserving operations performed in each study group was significantly higher.

At stages II B and III A of the disease, in the comparison group, there is no significant difference between the number of organ-preserving and radical surgical interventions, while in the main group, the number of organ-preserving operations significantly prevails.

In both study groups, organ-preserving surgical interventions were not performed at stages III B and III C of the disease.

In the Lum-A tumour type, in the comparison group, there is a significant predominance of the number of patients who underwent organ-preserving surgery, but in the main group, this difference is not significant. The number of organ-preserving and radical surgical interventions performed between the two study groups of women is not significant.

In the case of Lum-B tumours, in both study groups, there is a significant predominance of the number of patients who underwent organ-preserving operations.

In Her/2new+ and Triple-negative tumour types, in both study groups, there is a significant predominance of the number of patients who underwent radical surgery, but this difference is not significant.

Analysing the results presented in Table 5, it should be noted that all of the above indicators are arranged in the appropriate sequence, from the most informative in predicting breast cancer progression - the proliferative activity of tumour cells (Ki 67) (1.793) to the least informative - metastatic regional lymph node involvement (N3) (0.014), according to the TNM classification (2016).

For female patients, the sum of points is calculated according to this table. The higher the score, the worse the prognosis for breast cancer, i.e. the occurrence of metastatic spread of the cancer process.

The ROC curve for the proposed prognostic table is shown in Fig. 1.

To determine the threshold point for the purpose of forecasting, a graph of the sensitivity and specificity of the criterion is used, as shown in Fig. 2.

So, according to the graph, the optimal point of separation is 0 points. Patients with a score less than zero are predicted to have no metastatic spread of breast cancer, and those with a score less than zero are predicted to have metastases. The sensitivity of the test was Se=66% and the specificity was Sp=69%.

The threshold can be shifted to increase the sensitivity or specificity of the model.

The following predictive model should be considered:
- with a score of <-5 - predicting the absence of metastases;
- with a score between -5 and 5 - an uncertain prognosis;
- with a score of >5 - prediction of metastases.

For this model, Se=75%, Sp=75%.

Table 5. Predicting the metastatic spread of breast cancer

Sign (informative value)	Interval	The number of points
Ki 67 % (1.793)	[0;30]	-6
	[30;70]	3
	[70;100]	8
Duration of disease history (1.248)	0-2	3
	3-4	1
	5-6	-3
	>6	-6
PR % (0.963)	[0;25]	3
	[25;75]	0
	[75;100]	-3
SIZE P (0.884)	<2	-3
	2-5	-2
	>5	3
	Skin changes	5
ER % (0.858)	[0;25]	3
	[25;75]	0
	[75;100]	-3
Her2/new+ % (0.716)	[0;25]	3
	[25;75]	0
	[75;100]	-3
Lymph nodes (N2) (0.536)	axillary (4-9)	3
	Absent	-1
Lymph nodes (N1) (0.393)	Axillary (1-3)	2
	Absent	-2
Concomitant diseases (0.142)	Metabolic	2
	Respiratory	2
	Gynecological	0
	Cardiovascular	0
	Other	0
	Absent	-1
Histological type of tumour (0.136)	Lobular	5
	Mucinous	1
	Ductal	0
	Medullary	-1
Degree of tumour differentiation (0.112)	Low differentiation	1
	Intermediate	-2
Operation volume (0.101)	Sector	-1
	Mastectomy	1
Lymph nodes (N3) (0.014)	Axillary (10), connect	1
	Axillary (10), intrathoracic	-1

Source: compiled by the authors of this study

DISCUSSION

Summing up the results of the study, it should be noted that metastatic spread of breast cancer occurs more often in stage III of the disease, Lum.-B, Her/2new+, and Triple-neg. tumor types, which coincides with the literature data and proves a significant prevalence of the

percentage of individuals with the above-mentioned signs [7, 9].

The average age of women cannot be useful for predicting metastatic spread of breast cancer, since the difference in indicators in most cases is unreliable, and the dynamics by stage of the disease and molecular type of tumor are opposite.

In our studies, more than half of the women (53.4%) were of elderly and senile age, which explains the lack of a significant difference in age indicators and the inability to use this sign.

The average duration of the disease history is of leading importance, since with metastatic spread of breast cancer, the latter is probably longer. This feature has a logical explanation, since the longer progression of the disease before the start of complex treatment contributes to the generalization of the oncological process, which directly affects the stage of the disease and, accordingly, the metastatic spread of breast cancer, which was proven by our studies, as well as by literature data [3, 11].

In our studies, the average size of the primary malignant neoplasm in women with metastatic spread of breast cancer significantly prevails, for all molecular types of the tumor, which also has significance in the prognosis of the occurrence of this complication. This is explained by the fact that the stage of the oncological process directly depends on the size of the primary tumor, especially in breast cancer. Additionally, the prognostic value of tumor size has been repeatedly emphasized in studies by other authors [2, 3].

According to the calculations of the informativeness of the criteria for predicting metastatic spread of breast cancer, the results were obtained, namely: active proliferation marker Ki 67, duration of the disease history, expression of PR receptors, size of the primary neoplasm, expression of ER receptors, epidermal growth factor Her/2new, regional lymph nodes (N2, N1), concomitant diseases, histological type of tumor, degree of tumor differentiation, volume of the performed surgical intervention (organ-preserving or radical), as well as regional lymph nodes (N3), which have the lowest prognostic value, which is explained by the generalization of the oncological process, where treatment is the least effective [2, 10].

Thus, this expert system for predicting the metastatic spread of breast cancer allows for obtaining information with a fairly high specificity and sensitivity (Se = 69.0% and Sp = 69.0%), which in the future will enable increasing the effectiveness of its treatment.

CONCLUSIONS

1. The stage of the oncological process most informatively determines the prognosis of breast cancer after complex treatment.
2. The age of the woman, the location of the tumour in the right or left breast, and its quadrants do not affect the progression of this cancer.
3. Marker of active proliferation Ki 67, duration of disease history, expression of PR receptors, size of the primary tumour, expression of ER receptors, epidermal growth factor Her/2new, regional lymph nodes (N_2, N_1), comorbidities, the volume of surgical intervention, histological type of tumour, and degree of differentiation are prognostic factors that can be used to formulate a clear and objective prognosis of breast cancer progression after complex treatment.

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ORIGINAL ARTICLE

CONTENTS

Sex- and qualification-dependent differences in heart rate and blood pressure among swimmers

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ABSTRACT

Aim: To comparatively analyze heart rate and blood pressure in swimmers of varying skill levels and genders, utilizing contemporary scientific data alongside our own observations.

Materials and Methods: A total of 411 swimmers (270 men, 141 women) competing in 100–200 m distances were examined at the beginning of the preparatory training phase. Participants were divided into three groups: beginners, intermediate level, and high-class athletes. Blood pressure was measured using the Korotkoff method with an aneroid sphygmomanometer, and heart rate was recorded by auscultation after 5 minutes of rest.

Results: High-class athletes had lower HR and higher systolic BP compared to beginners. Male swimmers exhibited lower HR and higher BP than females, with significant sex differences in systolic BP across all qualification levels. Findings highlight the influence of sports proficiency and sex on cardiovascular parameters in swimmers.

Conclusions: Male swimmers exhibited bradycardia and hypertension more frequently, while female swimmers had higher rates of tachycardia and hypotension. Higher sports qualification in males was associated with lower heart rates and increased blood pressure, whereas female swimmers showed no HR differences across levels but had higher BP at advanced levels. Gender differences were most pronounced in high and intermediate level qualification groups, with males showing lower HR and higher BP than females.

KEY WORDS: heart rate, blood pressure, tachycardia, hypotension, hypertension, swimmers

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INTRODUCTION

The prevailing view suggests that proper training enhances cardiovascular function, typically manifesting as bradycardia and hypotension. However, empirical data and studies show these parameters don't always indicate positive adaptation; bradycardia can result from overtraining or insufficient training [1-5]. Resting heart rate (HR) is influenced by cardiac function, sinoatrial node excitability, and autonomic regulation, with non-athletic females typically having a 7–8 bpm higher HR than males [1]. HR in the general population varies with metabolic rate and activity [5]. In athletes, HR fluctuations depend on age, sex, body composition, sport, training experience, competitive level, and training phase, with lowest HR during peak conditioning [3-10]. Bradycardia in athletes often accompanies increased cardiac volume, reduced blood pressure (BP), and decreased cardiac output, suggesting efficient resting heart function [5]. Lower HR may protect against myocardial wear [3], induced by increased vagal tone from training. However, endurance athletes might not always show bradycardia due to training intensity, leading to incomplete HR recovery from fatigue. Tachycardia can result from overtraining, detraining [10], incomplete recovery, heart failure, or intoxication [5, 7].

BP in non-athletes is affected by age, sex, weight, activity, and hormones [11]. Accurate BP assessment in athletes is crucial for training eligibility and managing hypo- and hypertension [12]. Despite research, definitive conclusions on sports' effects on BP are lacking due to the diverse nature of athletic activities influencing functional state. Most athletes have normal BP [3, 13]. For speed and power athletes, systolic BP is 112.0±5.9 mmHg and diastolic BP (DBP) is 68.5±9.2 mmHg [13].

In 13-14 year old swimmers (3rd-2nd class), systolic BP is 113.9±1.87 mmHg and DBP is 68.93±2.63 mmHg [13]. BP in most athletes remains stable even at peak performance [14], though some show deviations [3, 6]. During primary training, 70.8% of athletes have normal BP, 10.1% elevated, and 19.1% hypotension [3, 6].

Hypotension prevalence is similar in athletes and non-athletes, with Lang observing a ~20 mmHg BP decrease in 63% of athletes [4]. Sports-related hypotension can be physiological or pathological, requiring exclusion of pathological causes first. Many consider sports hypotension an adaptation to exertion, influenced by age, athletic level, sport, training phase, and less so by experience and profession, being common in young, skilled athletes during main training, increasing with sports years. Hypotension incidence varies by qualification: Masters of Sport (21%), first-class (14.7%), second-class (14.9%), third-class (3.0%), and beginners (2.5%) [12]. Gender also affects prevalence [4, 6, 8], with female athletes experiencing it twice as often as non-athletes [12], and Levin reporting 26.0% in females vs. 12.8% in males [8], though Zharikov found it 1.5 times more common in men [6]. Deshin et al. [1] noted hypotension isn't always a good training marker, potentially indicating impaired circulation, overtraining, or neurocirculatory hypotension. Initially considered a hallmark of high-level training [3, 12], later analysis showed only 33.2% of hypotensive athletes exhibit it physiologically, with others possibly having infections or fatigue. Volnov [15] found SBP decreased in ~1/3 and DBP in 1/2 of athletes with increased training, generally by 10–15 mmHg. Hypotension prevalence in athletes ranges from 10% to 16% [5, 8], but some studies report higher rates (24% [16], 45-50% in highly trained athletes, including 50% in swimmers [17]). Despite this, BP below 95-100/60 mmHg is uncommon in elite athletes [5, 11]. Levin [8] found highest hypotension in gymnasts (30.0%), then track and field (25.6%), swimmers (13.0%), and soccer players (7.5%), suggesting sport-specific activity influences BP regulation.

Training regimen also affects elevated BP prevalence [18]. Serkin [19] reported 2%, Shakhlina [4] 11-14%, Volnov [15] 11.7%, and Ryzhkova [20] 17.8%. Among elite athletes, elevated BP prevalence was 5.3% [7], with highest incidence in weightlifters (21.2%), then soccer (16.6%), volleyball (15.6%), track and field (10.6%), and swimmers (9.1%) [15]; Zharikov [6] reported 6% in swimmers. Karpman [3] attributed elevated BP in athletes to multifactorial causes, including early hypertension stages, underlying diseases, or improper training leading to fatigue and overstrain. Hypertension in athletes and the general population is influenced by gender and age, being three times more common in men [12] and increasing with age [15]. Isolated BP elevations warrant attention as they may indicate vascular hyperreactivity and potential for persistent hypertension under stress [6].

Therefore, generalized HR and BP data in athletes, without considering specific factors, has limited value. Focused data on pathological hypo- and hypertension in specific groups, like 100-200m swimmers, considering skill, gender, and training phase, is needed and incorporated in our study.

AIM

The aim of this study is to comparatively analyze heart rate and blood pressure in swimmers of varying skill levels and genders, utilizing contemporary scientific data alongside our own observations.

MATERIALS AND METHODS

PARTICIPANTS

The study was conducted from 2018 to 2022 at the Zaporizhzhya Regional Medical and Physical Culture Dispensary, Zaporizhzhya, Ukraine. At the beginning of the preparatory phase of the training process, 411 athletes (270 men and 141 women), all swimmers in the 100–200 meter distance, were examined. The participants included beginners, intermediate-level, and high-class athletes (Table 1). All participants had no history of cardiovascular diseases. Informed consent was obtained from all participants (or their legal representatives) prior to their involvement in the study.

METHODS OF RESEARCH

Blood pressure was measured using the Korotkoff method with an aneroid sphygmomanometer (Romed, Netherlands) while the participant was seated. A standard cuff, appropriate for the circumference of the subject's upper arm, was used to measure blood pressure. The cuff was positioned on the upper arm of the right arm, 2–3 cm above the antecubital fossa. The diaphragm of the stethoscope was placed over the brachial artery in the antecubital fossa. The cuff was inflated rapidly to a pressure 20–30 mmHg higher than the expected systolic blood pressure (SBP), after which the pressure was decreased gradually at a rate of 2–3 mmHg per second. SBP was recorded when the first Korotkoff sounds were heard, and diastolic blood pressure (DBP) was recorded when the sounds disappeared. Measurements were repeated three times, with a 5-minute interval between each, and the lowest reading was used for analysis.

Table 1. General Characteristics of Swimmers Competing at 100-200 Meters (Mean ± SE)

Group (by sports qualification)	Subgroup (by sex)	Age, years
Beginners (3rd – 2nd class athletes)	males – 101	18.4±0.22
	females – 44	18.0±0.36
Intermediate level (1st class – Candidate for Masters of Sports)	males – 148	16.0±0.14
	females – 90	14.8±0.18
High-class (Masters of Sports – Masters of Sports, International Class)	males – 21	13.7±0.27
	females – 7	13.0±0.69

Source: compiled by the authors of this study

Heart rate was measured by auscultation in a seated position after 5 minutes of rest. The diaphragm of the stethoscope was placed at the apex of the heart. Heart sounds were auscultated for 1 minute, and the number of beats counted was recorded as the heart rate in beats per minute.

STATISTICAL ANALYSIS

Data were analyzed using the Statistica 6.0 software. The Shapiro-Wilk test was applied to assess the normality of the data distribution. Results are presented as the mean (M) ± standard error of the mean (SE). A two-sided independent t-test was used to compare differences between groups. The chi-square test was used to assess differences in categorical data. Differences were considered statistically significant at a p-value of < 0.05.

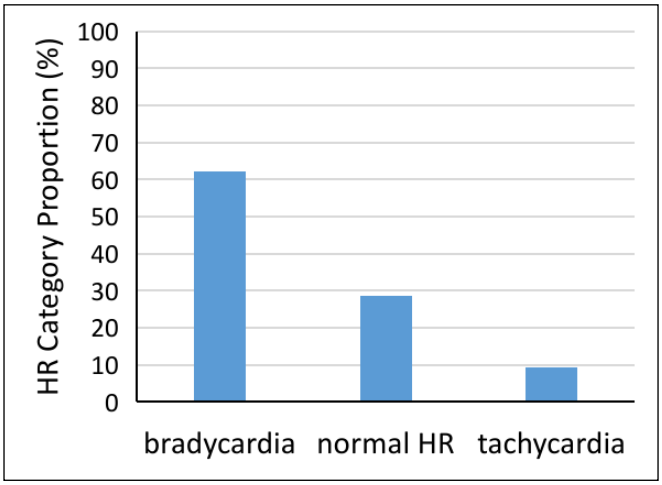

Fig. 1. Percentage ratio of male swimmers with bradycardia, normal heart rate, and tachycardia
Picture taken by the authors

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

CHARACTERISTICS OF THE STUDIED POPULATION OF MALE SWIMMERS

A total of 270 male swimmers with varying levels of sports qualifications participated in the study. The mean age of the athletes was 16.7 ± 0.14 years. The mean resting HR was 68.7 ± 0.57 bpm, the mean SBP was 118.7 ± 0.84 mmHg, and the mean DBP was 74.3 ± 0.61 mmHg.

Heart rate analysis showed that 62.2% of athletes had HR within the normal range, 28.5% had bradycardia, and 9.3% had tachycardia (Fig.1). The results of blood pressure measurements revealed that 60.4% of athletes had SBP within the normal range, 14.1% had hypotension, and 25.5% had hypertension. Regarding DBP, 72.2% of athletes had normal values, 16.3% had hypotension, and 11.5% had hypertension.

COMPARATIVE ANALYSIS OF MALE SWIMMERS BY SPORTS QUALIFICATION

Analysis of the studied indices across different qualification levels (Table 2) revealed statistically significant differences in age between all groups ($p < 0.001$). HR was significantly lower in high-class swimmers compared to intermediate level swimmers (66.4 ± 0.90 vs. 69.6 ± 0.74 bpm, $p = 0.006$), as well as compared to beginners (66.4 ± 0.90 vs. 74.0 ± 2.20 bpm, $p = 0.003$). SBP did not differ significantly between high-class and intermediate level swimmers (120.2 ± 1.11 vs. 119.3 ± 1.22 mmHg, $p = 0.546$), but was significantly higher in high-class swimmers compared to beginners (120.2 ± 1.11 vs. 107.9 ± 2.84 mmHg, $p < 0.001$). Similarly, DBP was significantly higher in high-class swimmers compared to beginners (74.8 ± 0.96 vs. 69.8 ± 1.84 mmHg, $p = 0.022$), but there were no significant differences between high-class and intermediate level swimmers ($p = 0.902$).

In the group of high-class swimmers, the highest proportion of athletes with bradycardia (36.63%) was observed compared to intermediate level (25.0%) and beginner (14.28%) swimmers. However, the differences in bradycardia prevalence between the groups were not statistically significant ($p > 0.05$, Table 1).

Table 2. Distribution of male athletes by heart rate, systolic and diastolic blood pressure depending on sports qualification (Mean ± SE)

Parameter	High-class n=101	Intermediate level n=148	Beginner n=21
Heart rate			
normal HR (61-79 bpm)	n=57 (56.4%)	n=97 (65.5%)	n=14 (66.7%)
bradycardia (≤60 bpm)	n=37 (36.7%)	n=37 (25.0%)	n=3 (14.3%)
tachycardia (≥80 bpm)	n=7 (6.9%)	n=14 (9.5%)	n=4 (19.0%)
Systolic blood pressure			
normal (101-129 mm Hg)	n=71 (70.3%)	n=82 (55.4%)	n=10 (47.6%)
hypotensive (≤ 100 mm Hg)	n=6 (5.9%)	n=24 (16.2%)	n=8 (38.1%)
hypertensive (≥130 mm Hg)	n=24 (23.8%)	n=42 (28.4%)	n=3 (14.3%)
Diastolic blood pressure			
normal (61-89 mm Hg)	n=73 (72.2%)	n=108 72.0%)	n=14 (66.7%)
hypotensive (≤ 60 mm Hg)	n=14 (13.9%)	n=23(15.5%)	n=7 (33.3%)
hypertensive (≥ 90 mm Hg)	n=14 (13.9%)	n=17(11.5%)	n=0 (0%)

Source: compiled by the authors of this study

Normal SBP values were significantly more frequent in high-class athletes (70.3%) compared to intermediate level athletes (55.4%, p = 0.05). Hypotension of SBP was more common in beginner athletes (38.1%) compared to intermediate level (16.2%) and high-class (5.94%) athletes, though these differences did not reach statistical significance (p > 0.05). No significant intergroup differences were found for DBP (p > 0.05).

CHARACTERIZATION OF THE STUDIED POPULATION OF FEMALE SWIMMERS

A total of 141 female athletes with varying sports qualifications participated in the study. The mean age of the participants was 15.7 ± 0.21 years. The mean resting HR was 69.9 ± 0.78 bpm, the mean SBP was 108.3 ± 1.07 mmHg, and the mean DBP was 71.6 ± 0.76 mmHg. HR analysis indicated that 66.7% of the athletes exhibited normal HR, 19.8% had bradycardia, and 13.5% had tachycardia (Fig. 2). Blood pressure measurements showed that 61.0% of the athletes had SBP within the normal range, 31.9% exhibited hypotension, and 7.1% had hypertension. Regarding DBP, 61.7% of the female athletes were normotensive, 32.6% had hypotension, and 5.7% had hypertension.

COMPARATIVE ANALYSIS OF INDICATORS IN FEMALE SWIMMERS BY SPORTS QUALIFICATION

Statistically significant differences in age were observed between the groups of female athletes (p < 0.05 for all comparisons). However, no significant differences in HR were detected between the groups (Table 3).

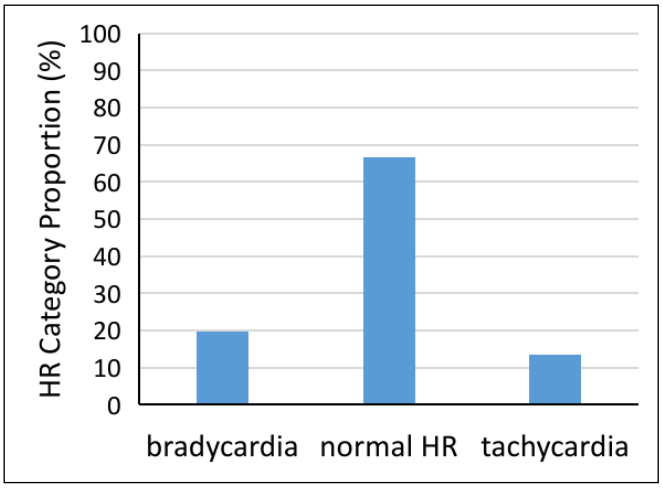

Fig. 2. Percentage ratio of female swimmers with bradycardia, normal heart rate, and tachycardia
Picture taken by the authors

There were no differences in SBP between high-class and intermediate level swimmers (p = 0.258); however, beginner swimmers had significantly lower SBP values compared to both high-class (p = 0.012) and intermediate level swimmers (p = 0.026). In terms of DBP, high-class female swimmers had significantly higher values than intermediate level swimmers (p = 0.046), while no significant differences were found when comparing high-class and beginner swimmers (p > 0.05).

Normal HR values were most frequently observed in intermediate level female athletes (67.8%), while bradycardia was more prevalent among high-class female athletes (27.3%), and tachycardia was predominantly found in beginner female athletes (42.9%). However, no statistically significant differences were identified between the groups (p > 0.05) (Table 3).

Table 3. Distribution of female athletes by heart rate, systolic and diastolic blood pressure depending on sports qualification (Mean ± SE)

Indicator	High-class n=44	Intermediate level n=90	Beginner n=7
Heart rate			
normal HR (61-79 bpm)	n=29 (65.9%)	n=61 (67.8%)	n=4 (57.1%)
bradycardia (≤60 bpm)	n=12 (27.3%)	n=16 (17.8%)	n=0 (0%)
tachycardia (≥80 bpm)	n=3 (6.8%)	13 (14.4%)	n=3 (42.9%)
Systolic blood pressure			
normal (101-129 mm Hg)	n=28 (63.6%)	n=56 (62.2%)	n=2 (28.6%)
hypotensive (≤100 mm Hg)	n=12 (27.3%)	n=28 (31.1%)	n=5 (71.4%)
hypertensive (≥130 mm Hg)	n=4 (9.1%)	n=6 (6.7%)	n=0 (0%)
Diastolic blood pressure			
normal (61-89 mm Hg)	n=32 (72.7%)	n=2 (57.8%)	n=3 (42.9%)
hypotensive (≤60 mm Hg)	n=9 (20.5%)	n=34 (37.8%)	n=3 (42.9%)
hypertensive (≥90 mm Hg)	n=3 (6.8%)	n=4 (4.4%)	n=1 (14.2%)

Source: compiled by the authors of this study

Normal SBP values were most frequently observed in high-class female athletes (63.6%). A hypotensive state of SBP was predominant in beginner female athletes (71.4%), whereas hypertensive state of SBP was absent in this group but was observed more frequently among high-class female athletes (9.1%). However, no statistically significant differences were found between the groups ($p > 0.05$).

Regarding DBP, normal values were more common in high-class female athletes (72.7%), whereas a hypotensive state was more frequently recorded in intermediate level (37.8%) and beginner (42.9%) female athletes. No statistically significant differences were observed between the groups ($p > 0.05$).

COMPARATIVE ANALYSIS OF INDICATORS IN MALE AND FEMALE SWIMMERS

HIGH-CLASS SWIMMERS

There were no significant differences in age between high-class male and female swimmers (18.4±0.22 vs. 18.0±0.36 years, p=0.340). However, male swimmers had a statistically significantly lower resting HR compared to females (66.4±0.90 vs. 70.1±1.38 bpm, p=0.026).

The proportion of athletes with bradycardia was higher in males (36.6% vs. 27.3% in females), but this difference did not reach statistical significance (p=0.552). The incidence of tachycardia was almost identical between males and females (6.9% vs. 6.8%, p=0.999).

SBP was significantly higher in male swimmers compared to females (120.2±1.11 vs. 110.7±1.76 mm Hg, p<0.001). There were no significant differences in DBP between the two groups (74.8±0.96 vs. 72.2±1.32 mm Hg, p=0.117).

INTERMEDIATE LEVEL SWIMMERS

In the group of intermediate level swimmers, males were older than females (16.0±0.14 vs. 14.8±0.18 years, p<0.001) and had statistically significantly lower resting HR (69.6±0.74 vs. 71.9±0.92 bpm, p=0.05). Intermediate level male swimmers had significantly higher SBP (119.3±1.22 vs. 108.2±1.34 mm Hg, p<0.001) and DBP (74.6±0.85 vs. 68.8±0.97 mm Hg, p<0.001) compared to female swimmers. The hypotensive state of DBP was observed in 37.8% of female swimmers and 15.5% of male swimmers (p=0.068).

BEGINNER SWIMMERS

In the group of beginner swimmers, there were no statistically significant differences in age (13.7±0.27 years in males vs. 13.0±0.69 years in females, p=0.395) or HR (74.0±2.20 vs. 78.0±3.93 bpm, p=0.395). However, SBP was significantly higher in male swimmers (107.9±2.84 vs. 95.0±4.50 mm Hg, p=0.034). There were no differences in DBP between the groups (69.8±1.84 vs. 70.0±4.36 mm Hg, p=0.961).

DISCUSSION

Early sports medicine research (1960s) on athletes' bradycardia, tachycardia, hypotension, and hypertension often lacked consideration for sport, training period, qualification, and gender. This study presents new HR data for soccer players across qualifications (3rd class to MSIC), including healthy athletes and those with bradycardia/tachycardia, alongside BP and hypo-/hypertension data [21, 22]. It also investigates HR and BP in swimmers based on qualification and distance, comparing male and female athletes.

Prior research indicates exercise lowers HR, most significantly in endurance athletes (36-66 bpm, often <50 bpm). Speed athletes show less HR reduction (48-72 bpm), as do those in static/dynamic sports (50-70 bpm in speed/strength athletes; 52.6±4.8 bpm in highly skilled speed/strength athletes [5, 7, 13]). Our study of 693 soccer players (mean age 19.9±0.18 years, 3rd class to MSIC) showed a mean HR of 67.8±0.38 bpm, with 34.49% bradycardia and 10.24% tachycardia [21]. Limited data exists on young athletes' HR [23].

In male swimmers, bradycardia was more common in high-class swimmers (36.63%) than intermediate (25.0%, p=0.048) and beginner (14.28%, p=0.047). Hypotensive systolic BP (SBP) was more frequent in beginner males (38.10%) vs. intermediate (16.22%, p=0.016) and high-class (5.94%, p<0.001). A similar trend was seen for diastolic BP (DBP) hypotension: beginner (33.3%) > intermediate (15.5%, p=0.045) > high-class (13.9%, p=0.031). No significant differences in tachycardia or hypo-/hypertensive SBP/DBP were found between male qualifications. Thus, as male swimmers' qualification increases, bradycardia becomes more frequent, while hypotensive SBP/DBP decreases.

In female swimmers, bradycardia was absent in beginners. Tachycardia was more frequent in beginner females vs. intermediate (42.9% vs. 14.4%, p=0.05) and high-class (42.9% vs. 6.8%, p=0.006). Hypotensive SBP was more prevalent in beginner females vs. intermediate (71.4% vs. 32.1%, p=0.035) and high-class (71.4% vs. 27.3%, p=0.021). Hypotensive DBP was more frequent in intermediate females than high-class (37.8% vs. 20.5%, p=0.043). No significant differences in hypertensive SBP/DBP were found in females, and hypertensive SBP was absent in beginners.

Bradycardia/tachycardia frequencies didn't significantly differ between female swimmer qualifications, except for bradycardia absence in beginners (present in 14.3% of beginner males). This suggests qualification minimally impacts HR in females. High-class females had more hypotensive SBP than high-class males (27.3% vs. 5.9%, p<0.001) and intermediate males (32.1% vs. 16.2%, p=0.004). Conversely, high-class and intermediate males had more hypertensive SBP. Intermediate females had more hypotensive DBP than intermediate males (37.8% vs. 15.5%, p<0.001). Beginner males and females showed no significant differences in hypotensive SBP/DBP, but hypertensive SBP was seen in male beginners (14.3%) only, while hypertensive DBP was seen in female beginners (14.3%) only. No significant differences in hypo-/hypertensive DBP were found in high-class males, but hypertensive DBP was seen in intermediate males.

Significant changes in training, competition, and recovery methods over the last 50-60 years necessitate considering sport, qualification, age, and gender in research. Older studies lacking these considerations provide historical context. Limited, small-sample HR studies exist for swimmers [24]. Our intermediate male swimmers (similar qualification and age to a Ukrainian group [25, 26]) showed similar HR. However, elite French male swimmers (100-400m) had lower HR than Belotserkovsky's high-class swimmers [23, 27] and our high-class male swimmers (likely due to no 400m specialists in our group). A 2006 study of elite male swimmers (50-100m) showed HR closer to the French group [25, 26]. Elite French female swimmers also had lower HR than our high-class females and those in a 2006 study (50-100m) [25-27]. These discrepancies may be due to body position during measurement (seated vs. supine) and training period phase.

Bradycardia frequency was 28.5% in our male swimmers and 19.9% in females. Tachycardia was 9.7% in males and 13.5% in females. Bradycardia was more frequent in high-class male swimmers vs. intermediate and beginner males. No significant tachycardia differences were found in male qualifications. In females, bradycardia was higher in high-class vs. intermediate (non-significant) and absent in beginners. Tachycardia was higher in beginner females vs. high-class and intermediate females. Soccer player data [22] showed more bradycardia and less tachycardia with increasing qualification. Our data suggests increasing qualification and training positively impacts the cardiovascular system in swimmers, increasing bradycardia and decreasing tachycardia.

Regarding BP, normal SBP was seen in ~60% of both genders. Hypotension was 2.27 times more frequent in females (32.6%) than males (14.1%), aligning with other findings [8, 28]. Hypertension was 3.6 times more common in males (25.6%) than females (7.1%, non-significant), also consistent with existing data [28]. Small, varied methodology BP studies in swimmers make direct comparison difficult [29-31].

Hypotension in athletes can be physiological or pathological [3, 4, 32, 33], with physiological cases being a minority [2, 8]. Our data showed statistically more frequent hypotension in female swimmers overall (31.9% vs. 14.1%, p=0.05). However, no significant gender differences in hypotension prevalence were found within qualification groups. Similarly, no significant differences in hypertension prevalence were found across male or female qualification groups, nor overall between genders (males 25.6%, females 7.1%, p=0.196).

CONCLUSIONS

1. Overall, male swimmers exhibited bradycardia 2.8 times more frequently and hypertensive states 3.6

times more frequently than female swimmers. Conversely, female swimmers experienced tachycardia 1.5 times more often ($p>0.05$) and hypotensive blood pressure states 2.3 times more often ($p=0.05$) than male swimmers.

2. Among male swimmers, those of high-class level showed lower heart rates, a higher incidence of bradycardia, and elevated systolic and diastolic blood pressure compared to male swimmers of intermediate and beginner levels. Beginner male swimmers more commonly experienced decreased systolic and diastolic blood pressure.
3. Heart rates did not differ among female swimmers of varying qualifications. High and intermediate level female swimmers had higher systolic and diastolic blood pressure and experienced diastolic hypotension more frequently than beginner female swimmers. Beginner female swimmers more often presented with tachycardia and decreased systolic blood pressure.
4. High and intermediate level male swimmers had significantly lower heart rates and higher systolic blood pressure (with intermediate level male swimmers also showing higher diastolic blood pressure), but less frequently experienced systolic hypo- and hypertension compared to female swimmers of similar qualifications. Intermediate level male swimmers had a lower incidence of diastolic hypotension than female swimmers. There were no gender differences among beginner male and female swimmers, except for systolic blood pressure, which was higher in males.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Diagnostic performance of biomarkers in colon *vs.* rectal cancer: A retrospective comparative study

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ABSTRACT

Aim: Colon cancer and rectal cancer are collectively called colorectal cancer (CRC), owing to their distinct anatomical, embryological, and functional features. The study aimed to assess the diagnostic performance of various biomarkers, including liver enzymes, lipid levels, and carcinoembryonic antigen (CEA), in differentiating between colon and rectal cancers.

Materials and Methods: The study included 70 patients with confirmed histopathology of CRC (46 patients with colon cancer (CC) and 24 patients with rectal cancer (RC), and 40 healthy control individuals. Fasting blood samples were collected to measure liver enzymes, lipid levels, and CEA levels via using enzyme-linked immunosorbent assay (ELISA) method.

Results: No statistically significant age difference ($p = 0.417$) was seen in all groups. A statistically significant differences were revealed in the distribution of body mass index (BMI) ($p = 0.006$): 31.4% of colon cancer patients were overweight, compared to 14.3% of rectal cancer patients and 11.4% categorized as obese. A statistically significant difference was also observed in the distribution of sex ($p = 0.0269$): 41.4% of colon cancer patients were male, and 24.3% were female, 24.3% of rectal cancer patients were male, and 10% were female. The findings showed that the CEA, liver enzymes, and lipid levels exhibited excellent diagnostic performance for both CC and RC.

Conclusions: These results highlight the clinical significance of these biomarkers in routine evaluations which can enhance therapeutic management and early diagnosis ultimately increasing survival and cure rates.

KEY WORDS: colon cancer (CC), rectal cancer (RC), liver enzymes, lipid profiles, CEA biomarkers

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INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignancy and the second most frequent cause of cancer-related deaths globally [1, 2]. Rectal cancer (RC) and Colon cancer (CC) are Rectal cancer and colon cancer are usually classified under colorectal cancer, based on the fact that CC and RC arise in the large intestine, which is considered a singular organ [3]. However, several biological and clinical markers show that rectal cancer differs from colon cancer. The rectum and colon differ in embryological origin, anatomy, and function. As a result, treatments for primary rectal and colon cancers vary [4, 5].

Although the incidence of CRC generally states both statistics jointly, two-thirds of CRCs are located in the colon and one-third in the rectum [6]. Out of 129,700 newly registered CRCs, 72% were diagnosed in the colon and 28% in the rectum according to the statistics of the American Cancer Society in 2015 [7].

The carcinogenic risk to develop rectal cancer by far exceeds that of the colon mucosa since the area at risk in the colon is definitely larger than that of the rectum. The rectal mucosa has an at least four times higher risk for malignant transformation than the colon mucosa [3].

However, since 2010, CRC has represented the most common type of cancer in males and the third most common type of cancer in females [8]. Across all ages in every nation, males have a 1.5-fold greater risk of developing CRC compared with females [9].

Accurate and prompt cancer diagnosis is vital for enhancing prognosis and survival rates. Biomarkers are crucial in diagnosis, helping clinicians distinguish malignant from benign conditions effectively [10, 11]. Carcinoembryonic antigen (CEA) is utilized as a tumor marker for CRC (12, 13); however, its diagnostic precision necessitates continuous assessment in conjunction with other biochemical markers, such as lipid levels and liver enzymes [14].

AIM

The purpose of this study is to assess the diagnostic value of liver enzyme lipid measurements and CEA in differentiating between healthy people and those with colon and rectal cancer. The objective is to improve the understanding of how biomarkers can be used for the early detection of cancer.

MATERIALS AND METHODS

STUDY DESIGN

The study is a retrospective comparative study designed to assess biomarker levels in 70 patients with confirmed histopathology of primary CRC, with or without metastatic disease; 46 have colon cancer, and 24 have rectal cancer, compared with 40 healthy control individuals. The study was conducted in the Oncology Teaching Hospital, Baghdad. Informed consent was obtained from all participants. The sample collection period is from December 2024 to May 2025.

DATA COLLECTION

Demographic information was collected, and body mass index (BMI) was calculated from direct measures of body weight (kg) divided by height (m^2) for all participants. Primary CRC diagnosis and clinical staging were confirmed by gastrointestinal surgeons and radiologists through a combination of clinical evaluation, imaging studies including colonoscopy, biopsy, imaging studies such as magnetic resonance imaging (MRI) and computer tomography scanning (CT), and the histopathological analysis. The anatomical location of the primary lesion was determined for colon cancer and rectum cancer.

PATIENT CLASSIFICATION

– Forty-six Colon cancer patients, 29 males and 17 females, ages 29 to 75 years.
– Rectal cancer group: 24 patients, aged 26-72, including 17 males and 7 females.
– Overall, 35 patients had metastatic disease, with metastases in the liver (25), lungs (7), or both (3).
– Forty healthy control participants, including 16 males and 24 females, aged 24-77.

INCLUSION CRITERIA

– Histologically confirmed primary colon and rectal cancers at different stages.
– No prior history of malignancy in controls.

EXCLUSION CRITERIA

– Any condition impairing liver function or lipid metabolism, Liver disease, or hematological disorders.

BLOOD SAMPLES

5 mL of fasting blood was obtained for evaluation of:
– Liver Function Tests:
- Use kinetic enzyme techniques approved by the IFCC for ALT and AST. A colorimetric assay was used to quantify ALP. Albumin and TSP were determined by bromocresol green and biuret methods, respectively [15, 16].
– Lipid Profile: Total cholesterol, triglycerides, HDL, LDL, and VLDL were measured through enzymatic colorimetric methods.
– CEA: Elabscience Human CEA ELISA kit on a HumaReader HS analyzer was used to measure CEA levels.

STATISTICAL ANALYSIS

SPSS v27 was used for data analysis. Clinical and demographic data were analyzed using descriptive statistics. Sex and the presence of metastases were assessed using chi-square tests. Biomarker values were compared using one-way ANOVA. Sensitivity, specificity, and cutoff points were calculated using ROC curve analysis. Subsequently, the area under the curve (AUC) of each biomarker was calculated to assess diagnostic accuracy. A p-value less than 0.05 was considered statistically significant when examining correlations between biomarker levels.

RESULTS

An overview of clinical and demographic traits among the study groups is given in Table 1. The study included Forty-six subjects with colon cancer, twenty-four patients with rectal cancer, and Forty healthy people as a control group. All groups had comparable mean

Table 1. Demographic and clinical features among colorectal cancer patients and healthy controls

Parameters	Participants			P value
	Colon (46)	Rectum (24)	Healthy (40)	
Age (years) (26-75 years)	55.8 ± 11.2	55.3 ± 12.4	58.8 ± 13.3	0.416
BMI- Kg/m2	26.4209 ± 3.9	27.7549 ± 3.6	28.7472 ±2.2	0.006
Normal	16 (22.9%)	6 (8.6%)	2 (5%)	
Overweight	22 (31.4%)	10 (14.3%)	25 (26.5%)	
Obese	8 (11.4%)	8 (11.4%)	13 (32.5%)	
Sex	46 (65.7%)	24 (34.3%)	(%)	
Male	29 (41.4%)	17(24.3%)	16 (40%)	0.0269
Female	17 (24.3%)	7 (10%)	24 (60%)	
	(%)	(%)		
Metastasis	24 (34.3%)	11(15.7%)		0.613
Non-Metastasis	22 (31.4%)	13 (18.6%)		

Source: Own materials

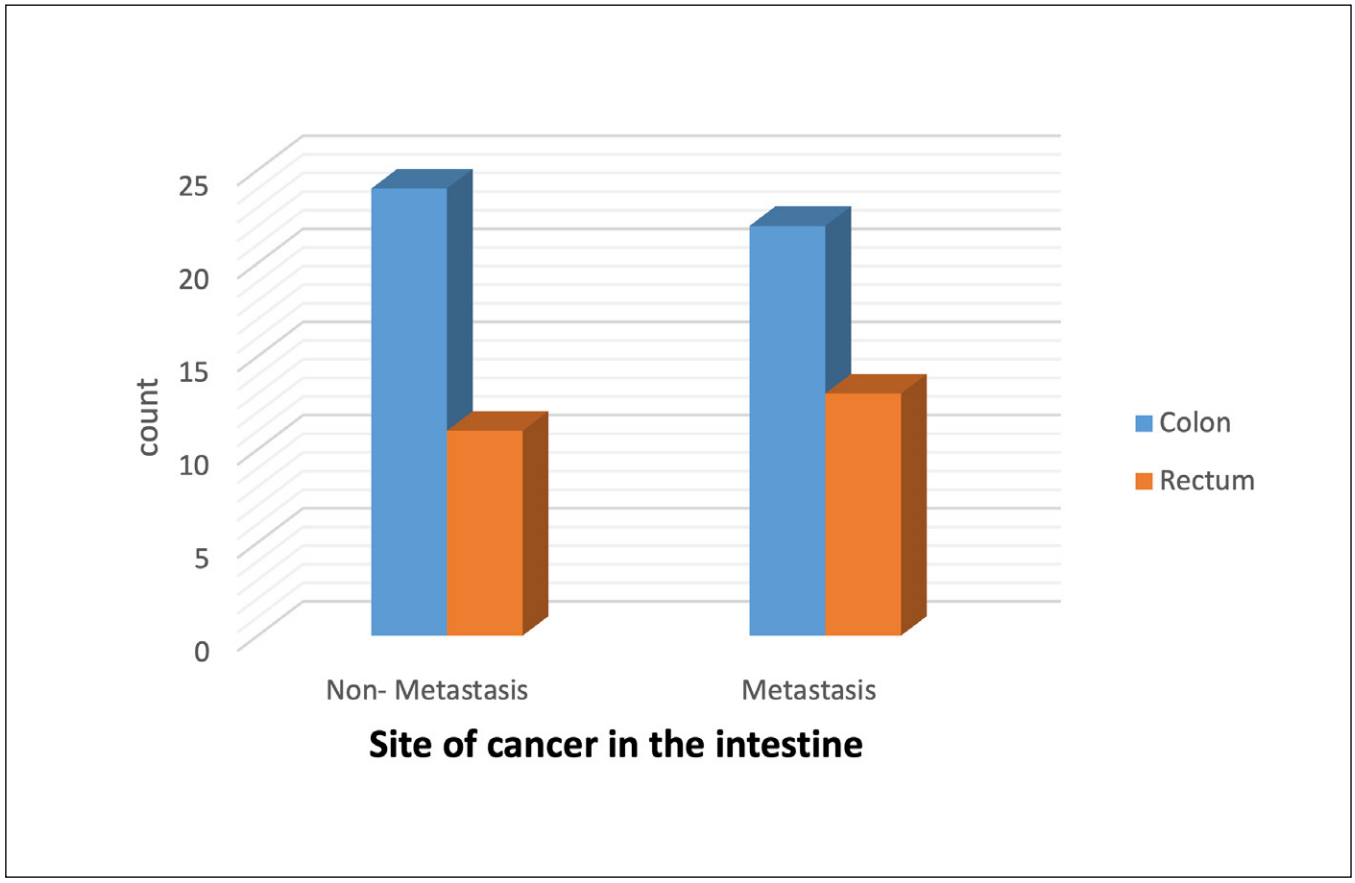

Fig. 1. Metastasis rates among colon and rectal cancer patients
Source: Own materials

ages and statistical analysis revealed no statistically significant age difference (p = 0. 417). Nonetheless, statistically significant differences were revealed in the distribution of body mass index (BMI) (p = 0.006): 31.4% of colon cancer patients were overweight, compared to 14.3% of rectal cancer patients and 11.4% categorized as obese. Additionally, a statistically significant difference was observed in the distribution of sex (p = 0.0269): 41.4% of colon cancer patients were male, and 24.3% were female, 24.3% of rectal cancer patients were male, and 10% were female. Metastases prevalence rates were not significantly different between the cancer groups (p < 0.613): 34.3% for colon cancer and 15.7% for rectal cancer, as shown in Figure 1.

Findings showed that the mean CEA level in CC patients was 3.8 ± 1.5 ng/mL; in contrast, RC patients

Table 2. Assessment of carcinoembryonic antigen (cea) levels in colorectal cancer patients and healthy controls

Parameters	Colon (46)	Rectum (24)	Healthy (40)	Significance
CEA ng/ml	3.7900 ± 1.5396	4.1118 ± 1.2905	.9094 ± .3741	0.001

Source: Own materials

Table 3. Evaluation of LFT parameters in colon and rectal cancer patients compared to healthy controls

Parameters	Participants			
	Colon (46)	Rectum (24)	Healthy (40)	P value
ALT- U/L	46.9780 ± 8.45	48.1092 ± 7.19	28.4368 ± 4.44	0.001
AST- U/L	47.6196 ± 8.98	46.5700 ± 8.59	30.1995 ± 2.08	0.006
ALP- U/L	169.8413 ± 30.08	169.4513 ± 29.45	121.5021 ± 3.96	0.001
Albumin- g/dL	4.2363 ± .35	4.2519 ± .35	3.5653 ± .04	0.001
Globulin- g/dL	1.7891 ± .72	1.7808 ± .69	.7402 ± .13	0.001
Total Protein- g/dL	6.0254 ± 1.07	6.0329 ± 1.04	4.3055 ± .14	0.001

Source: Own materials

Table 4. Evaluation of lipid profile in colon and rectal cancer patients compared to healthy controls

Parameters	Participants			
	Colon (46)	Rectum (24)	Healthy (40)	P value
Cholesterol- mg/dL	244.8811 ± 25.5	245.4729 ± 27.5	202.2463 ± 3.9	0.001
TG- mg/dL	218.4824± 20.5	216.9967 ± 23.1	184.7225 ± 5.8	0.001
HDL- mg/dL	28.4972 ± 5.5	28.2292 ± 5.3	45.5265 ± 7.2	0.001
VLDL- mg/dL	43.6965± 4.1	43.3993 ± 4.6	36.9445 ± 1.7	0.001
LDL- mg/dL	172.6874 ± 26.1	173.8444 ± 27.9	119.7752 ± 9.9	0.001

Source: Own materials

Table 5. Relationship between sex and colon *versus* rectal cancer, on metastatic and non-metastatic cases

Gender			Site of cancer in the intestine		Total	Pearson Chi-Square/ Asymp. Significance
			Colon	Rectum		
Female	Metastasis	*	9	6	15	.615
		**	8	1	9	
	Pearson Chi-Square/ Asymp. Significance			0.132		
Male	Metastasis	*	13	7	20	
		**	16	10	26	
Pearson Chi-Square/ Asymp. Significance				0.809		

Metastasis (*): non- metastasis, Metastasis (**): Metastasis out side intestine

Source: Own materials

had a slightly higher mean CEA level of 4.11 ± 1.3 ng/mL. In contrast, the mean CEA level in healthy control subjects was just 0.91 ± 0.4 ng/mL. With a p-value of 0.001, statistical analysis shows a significant difference in CEA levels across the groups, with a p-value of 0.001 (Table 2).

The findings revealed a significant difference in LFT across the groups. The cancer patients showed statistically significant raises in ALT, AST, and ALP levels, with p-values <0.01 (Table 3).

Furthermore, Lipid profiles differed significantly; as shown in Table 4, patients with colon and rectal cancer had greater levels of total cholesterol, triglycerides, LDL, and VLDL, while HDL was noticeably lower than in healthy controls.

The association between tumor metastasis and patient sex is examined in Table 5. Six females had rectal cancer with metastases and nine had colon cancer.

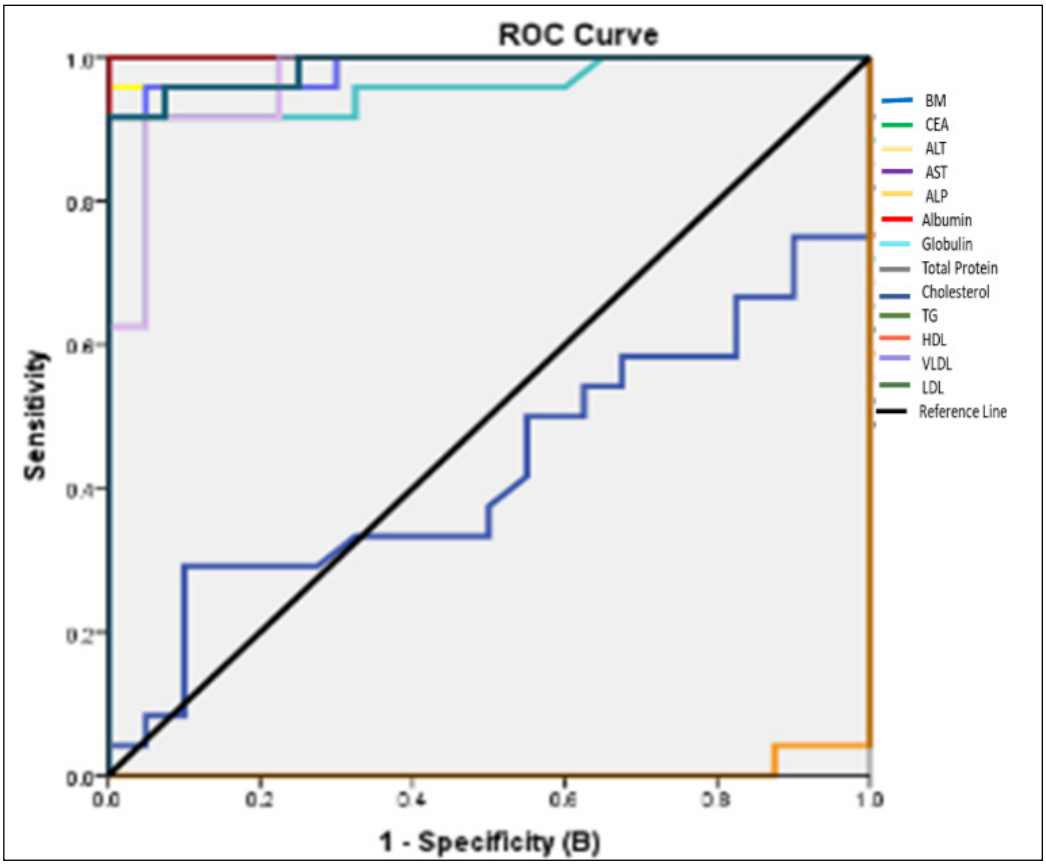

Fig. 2. ROC curves, evaluation parameters in the colon cancer (A) and rectal cancer; (B) in comparison to the healthy control group
Source: Own materials

There is no significant relationship between sex and tumor site or metastasis, according to the Pearson's chi-squared test result of roughly 0.132. Thirteen colon and seven rectal metastases were found in 20 male individuals; the chi-squared value was 0.809, indicating no significant correlation.

Table 6 assesses the diagnostic accuracy of several biochemical markers - CEA, ALT, AST, Albumin, cholesterol, triglycerides, VLDL, and LDL - in differentiating colorectal cancer patients from healthy controls. CEA performed exceptionally well, with significant asymptotic p-values (p = 0.001). The ideal cut-offs for rectal and colon cancer were 1.4840 and 1.6955, respectively, with 100% sensitivity and specificity. High diagnostic value was also demonstrated by liver marker. ALT, AST, and albumin had a significant performance for rectal and colon cancer with high sensitivity and specificity. Lipid profile, including total cholesterol, triglycerides, VLDL, and LDL, demonstrated remarkable diagnostic capacities, with high specify and sensitivities (Fig. 2).

DISCUSSION

Our results show that there was a significant difference in the BMI distribution between the study groups (p = 0. 006). Colon cancer associated with increasing BMI and Overweight. A higher body BMI has a stronger association with colon cancer compared to rectal cancer, primarily because the metabolic impact of visceral fat has a more significant effect on the proximal colon due to elevated insulin levels, persistent inflammation, and altered adipokines. On the other hand, rectal cancer generally shows weaker or inconsistent connections with these risk factors. This is probably due to the fact that alterations in insulin resistance, metabolic pathway, and cumulative exposure to an obesogenic environment might encourage the procancerous pathophysiological processes [17]. However, another study showed that overweight and obese individuals had 18% and 32% increased risk of colon cancer compared and rectal cancer, respectively [18].

Furthermore, our study revealed significant variations in sex among the study groups (p = 0.0269). Literature reported that men tend to have a greater overall incidence of both colon and rectal cancer than women, with higher risk for rectal cancer. However, although rectal cancer is more frequently reported in men, women are more prone to developing colon cancer. Additionally, males were often exhibiting higher prevalence and mortality rates than females (19). Men are

Table 6. ROC: assessment of parameters in colon and rectal cancer groups compared to the healthy control group

Parameters		Area under the curve (AUC)	Asymptotic sig.	CUT-OFF point	Sensitivity	Specificity
CEA	Colon	1.000	0.001	1.6955	1.000	1.000
	rectum	1.000	0.001	1.4840	1.000	1.000
ALT	Colon	.990	0.001	33.0050	0.957	1.000
	rectum	1.000	0.001	33.7850	1.000	1.000
AST	Colon	.984	0.001	32.4800	.978	1.000
	rectum	1.000	0.001	33.0550	1.000	1.000
ALP	Colon	.949	0.001	128.3700	.935	1.000
	rectum	.991	0.001	129.8650	.958	1.000
Albumin	Colon	.997	.003	3.6500	.957	1.000
	rectum	1.000	0.001	3.6700	1.000	1.000
Globulin	Colon	.908	.038	.9500	.870	1.000
	rectum	.960	0.001	1.0100	.917	1.000
Total Protein	Colon	.943	.030	4.7200	.870	1.000
	rectum	.988	0.001	4.5850	.958	1.000
Cholesterol	Colon	.973	.022	209.7600	.957	1.000
	rectum	.985	0.001	211.7100	.917	1.000
TG	Colon	.959	.024	192.2450	.957	0.950
	rectum	.967	0.001	193.1450	.917	0.950
VLDL	Colon	.959	.024	38.4490	.957	0.950
	rectum	.967	0.001	38.6290	.917	0.950
LDL	Colon	.985	.015	134.6060	.978	1.000
	rectum	.986	0.001	135.5720	.917	1.000

Source: Own materials

more prone to colon and rectal cancers due to their higher rates of smoking, alcohol consumption, and accumulation of visceral fat. Additionally, molecular factors, gut microbes, or changes in sex hormones could be the cause of these variations.

However, our findings showed significant variations in CEA levels among the cancer groups with high sensitivity and specificity (100%). Distinct biological behavior, cancer stages, and tumor aggressiveness may be associated with varying CEA levels. Furthermore, our results corroborate previous findings indicating varied CEA levels in CRC [20]. Although CEA is not a disease-specific marker, our results may be useful in predicting of establishing and staging of lesion particularly in rectal cancer patients. However, further studies were needed to support this suggestion.

Additionally, our findings showed that ALT, AST, and albumin levels varied significantly among cancer groups, with p-values <0.01. Elevations of these enzymes may indicate liver involvement. Furthermore, the study observed that notable diagnostic potential has established by ALT, AST, and albumin for colon and rectal cancers with high sensitivity and specificity. Hepatic enzymes can offer important insights into the progression of cancer and are known to be involved in the disease. The specificity and accuracy of ALT and CEA levels in predicting liver metastases in CRC patients have been shown in recent studies which may improve the early detection and treatment of such problems [21]. Furthermore, studies have indicated that liver enzymes are crucial biomarkers in the treatment of colorectal cancer, serving as key indicators of cancer metastasis to the liver. Regarding long-term risks, research suggests that elevated blood levels of liver enzymes are more strongly associated with the development of colon cancer than with rectal cancer [22].

Otherwise, other studies have found that hepatic enzymes are not a substitute for imaging studies in detecting liver metastases, as their levels can be normal even with a small metastatic burden [23].

Furthermore, diagnostic evaluation found that elevated levels of total cholesterol, triglycerides LDL and VLDL have been associated with colon and rectal cancer. However, based on recent meta-analyses and studies, dyslipidemia associations with CRC show distinct differences between colon and rectum cancer, with higher triglyceride levels being more associated with colon cancer and higher total cholesterol with rectal cancer [24]. These

variations could result from dietary variations altered metabolic environments or the impact of gut microbes.

CONCLUSIONS

By illustrating the variations in the tumor-marker profiles of RC and CC this study highlights the distinctions between rectal and colon cancers. As evidenced by the elevated CEA levels and altered liver and lipid profiles the hosts reaction to these tumors may vary throughout the body. Comprehending these variations is crucial for develop individualized treatment plans and diagnostic techniques that can ultimately improve prognosis and survival rates.

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ORIGINAL ARTICLE

CONTENTS

Adaptation of the small intestinal mucosa after single anastomosis gastric bypass

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ABSTRACT

Aim: The aim of our study was to perform morphological and morphometric analysis of biopsy specimens of the common and biliopancreatic loops after gastric bypass with a single anastomosis 3, 12, 24 months after surgery, which included measurement of villi length, ratio of villi length to the thickness of the lamina propria layer containing crypts, estimation of the number and distribution of goblet cells, quantification of number crypts and Paneth cells and comparing the changes in the biliopancreatic and common loops.

Materials and Methods: This study included 36 patients who underwent bariatric surgery due to morbid obesity. Patients underwent one of the following procedures: long-loop gastric bypass with one anastomosis, distal gastric bypass with one anastomosis, or mini-gastric bypass. Patients underwent EGDS with mucosal biopsy from the common and biliopancreatic loop at 3, 12, 24 months after gastric bypass with one anastomosis, followed by morphologic and morphometric study of biopsy specimens, which was part of our study.

Results: 2 years follow up show statistically significant differences in villus length were observed between the common and biliopancreatic limbs, with the length being greater in the common limb (0.390 ± 0.199 mm) than in the biliopancreatic limb (0.377 ± 0.184 mm) ($p < 0.05$). These changes may indicate hypertrophy of the villi in the efferent limb to increase the absorptive surface area. The thickness of the basal layer was greater in the biliopancreatic limb than in the common limb, measuring 0.196 ± 0.068 mm versus 0.167 ± 0.043 mm, respectively ($p< 0.05$). Regulatory functions of Paneth cells were preserved in both groups.

Conclusions: Adaptation of the small intestinal mucosa occurs after gastric bypass with one anastomosis, and these changes are more pronounced in the common loop of the small intestine. The regulatory functions of Paneth cells and their number involve both the common loop and the biliopancreatic region.

KEY WORDS: Obesity, one anastomosis gastric bypass, intestinal adaptation, small intestine villi, morphometry

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INTRODUCTION

Physiological adaptation in the small intestine is a continuous process of self-renewal through crypt cell proliferation, migration, and differentiation into specialized mucosal cells (such as enterocytes, enteroendocrine cells, goblet cells, or Paneth cells) [1]. Postresectional adaptation of the intestine is a natural compensatory process that leads to structural and functional changes in the intestine, improving the absorption of nutrients and fluids in the remaining functional section of the gut.

Structural adaptation of the intestine includes morphological and morphometric changes in the intestinal wall, such as mucosal hyperplasia, angiogenesis, elongation, and dilation of the remaining intestine [2]. Functional adaptation involves accelerated differentiation of crypt cells, delayed transit time, and increased expression of transporter proteins and exchangers involved in the absorption of nutrients, electrolytes, and water.

In adults, data on adaptive changes are rare, and the mechanisms underlying intestinal adaptation are not fully understood. Factors influencing the degree of intestinal adaptation after resection include the site and extent of resection, stimulation of the intestinal lumen by enteral nutrients, and intestinotrophic factors. Two intestinotrophic growth factors - teduglutide, a glucagon-like peptide II analog, and recombinant growth hormone (somatotropin) - have now been approved for clinical use in patients with short bowel syndrome. Both Of these medications enhance fluid absorption and reduce the need for parenteral nutrition and/or intravenous fluid administration. It is believed that intestinal adaptation in humans is limited to the first 1–2 years after resection.

Enterocyte apoptosis typically occurs in the remaining intestine due to increased crypt cell proliferation and is not considered to be inloved in adaptation mechanisms [3]. Therefore, most of the literature on adaptive and

Table 1. Characteristics of the study group

Variable	Results (n=36)
Sex	M: 15(41.7%); F: 21(58.3%)
Age (year)	47,9±10,6
Body mass index (kg/m^2)	54.07 ± 8.8
%EWL (1, 2 years follow up)	1 year - 65± 2,6; 2 years- 67±1,1

Source: compiled by the authors of this study

Table 2. Comorbid diseases in general group (n=36)

Comorbidity	Patients with comorbidities n (%)
Type II diabetes mellitus	5(13.9)
Prediabetes	7(19.4)
Hyperlipidemia	25(69.4)
Hypertension	26(72.2)
Sleep apnea	12(33.3)
Osteoarthritis	16(44.4)

Source: compiled by the authors of this study

morphometric changes in the small intestinal mucosa has focused on treating short bowel syndrome.

Data on potential adaptive changes in the intestine after gastric bypass surgery primarily involve studies on glucose metabolism [4], experimental research on intestinal adaptation after gastric bypass in rats [5], and comparisons with massive resection influenced by glutamine [6]. We found studies examining lipogenesis in the jejunum to be more pronounced in patients with remission of diabetes after Roux-en-Y gastric bypass surgery. Another study that investigated the processes of intestinal adaptation was devoted to the study of intestinal permeability in which lactulose, mannitol ratio and lactulose excretion rate were evaluated. Increased mucosal permeability, according to the authors, was due to hyperplasia of the small intestinal mucosa in patients after bypass surgery [7,8].

However, no studies have been found specifically examining the morphological and morphometric changes in the small intestinal mucosa after single anastomosis gastric bypass in humans.

AIM

The aim of our study was to perform morphological and morphometric analysis of biopsy specimens of the common and biliopancreatic loops after gastric bypass with a single anastomosis 3, 12, 24 months after surgery, which included measurement of villi length, ratio of villi length to the thickness of the lamina propria layer containing crypts, estimation of the number and distribution of goblet cells, quantification of number crypts and Paneth cells and comparing the changes in the biliopancreatic and common loops.

MATERIALS AND METHODS

PATIENTS

The study included 36 patients who underwent bariatric surgery at the Thoraco-Abdominal Surgery Department of the State Institution "National Institute of Surgery and Transplantation named after O.O. Shalimov" of the National Academy of Medical Sciences of Ukraine due to morbid obesity. These patients received either long-loop gastric bypass with one anastomosis with a 200-cm biliopancreatic loop, distal gastric bypass with one anastomosis with a 250-cm common loop., or mini-gastric bypass with a 200-cm biliopancreatic loop between 2016 and 2022.

Inclusion criteria were patients who underwent long-loop gastric bypass with one anastomosis, distal gastric bypass with one anastomosis, mini-gastric bypass followed by endoscopic examination with biopsy at 3, 12, 24 months after surgery. Patients who had peptic ulcer disease were excluded from the study. Patients who had peptic ulcer disease were excluded from the study.

Characteristics of the study group presented in Table 1, Table 2.

Comorbid diseases are presented in Table 2.

A BRIEF DESCRIPTION OF THE SURGICAL TECHNIQUE OF GASTRIC BYPASS

The MGB technique was performed according to the standard method R. Rutledge with a 200-cm long biliopancreatic loop.

Long-loop gastric bypass with one anastomosis involves horizontal gastric transection at the border of the antrum and body using 2-3 non-articulating 60 mm blue loads (Covidien Endo GIA). After the gas-

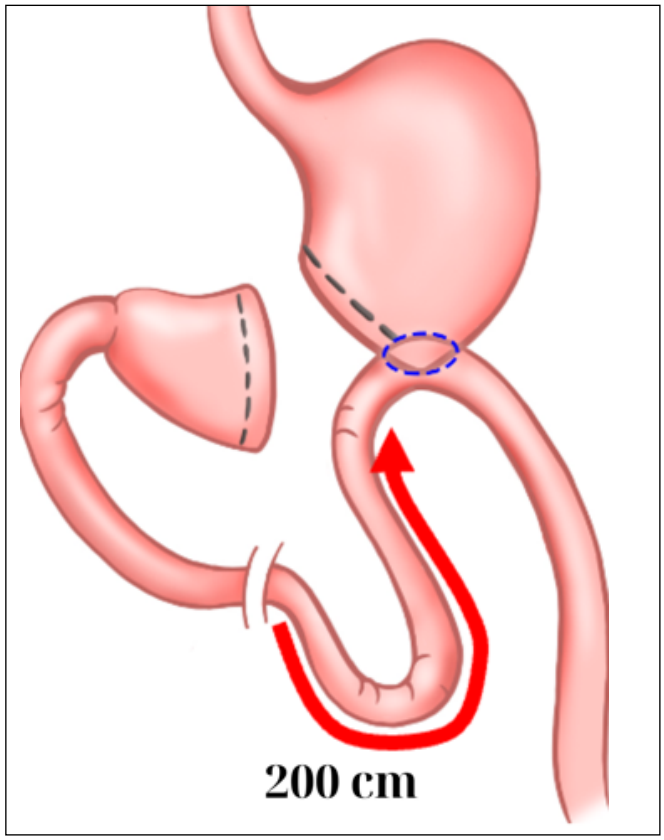

Fig. 1. Scheme of long - loop gastric bypass with one anastomosis
Source: compiled by the authors of this study

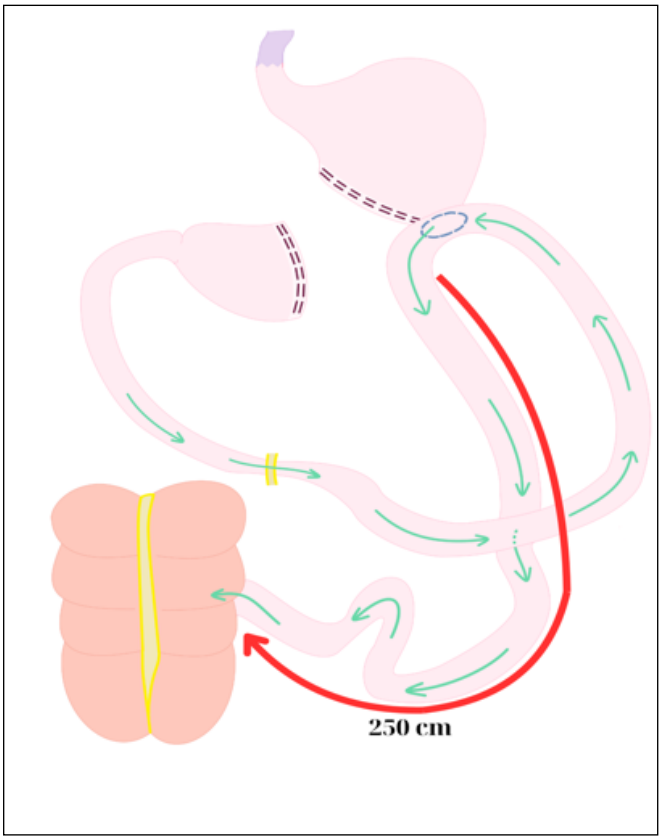

Fig. 2. Scheme of distal gastric bypass with one anastomosis
Source: compiled by the authors of this study

tric transection, 200 cm of the biliopancreatic loop is measured distal to the ligament of Treitz (Fig. 1). The gastrojejunostomy is formed using a single 45 mm non-articulating load through the angle of the staple suture along the posterior wall of the stomach at an angle of 90° to the line of horizontal gastric transection. Final closure of the gastrojejunostomy is hand sewn.

The distal gastric bypass with one anastomosis involves a similar gastric reservoir as the long-loop gastric bypass with one anastomosis. After transection of the stomach, 250 cm of the common loop is measured proximally from the ileocecal valve (Fig. 2). Gastrojejunostomy technique, closure of the gastrojejunostomy was done using a similar technique.

In all cases, the total measurement of the small intestine was not performed.

ENDOSCOPIC TECHNIQUE

Examinations were conducted using an Olympus GIF-EZ 1500 device. After endoscopic inspection of the esophagus and gastric stump, the mucosa of the biliopancreatic and common limbs was visually assessed 35–40 cm distal to the gastroenterostomy site, followed by forceps biopsy of the mucosa, with 1–2 samples taken from each section of the small intestine (Fig. 3, Fig. 4). The samples were placed in 10% formalin solution within 10 minutes and sent to the pathology department for further analysis. The endoscopic examination was performed by one person.

MORPHOLOGICAL STUDIES

Preparation for histological examination was carried out using standard methodology: after fixation of tissue fragments in neutral buffered 10% formalin, they were processed according to generally accepted histological techniques to obtain paraffin blocks. From the obtained paraffin blocks, 5 µm thick tissue sections were prepared and stained with hematoxylin-eosin according to standard procedures.

Two slides containing 2 to 6 sections were prepared for more detailed microscopic examination of the material. For description and morphometry, one section was selected that was free of technical defects and contained the largest area of the muscularis mucosa and the overlying propria and villi in maximum quantity. Histological examination was performed using an Olympus BX41 microscope, and morphometric analysis was conducted with an Olympus EP50 camera connected to the microscope and EPview software (Fig.5).

During the histological examination, a detailed description of the biopsy specimens of the mucous membrane of the biliopancreatic and common loop was performed.

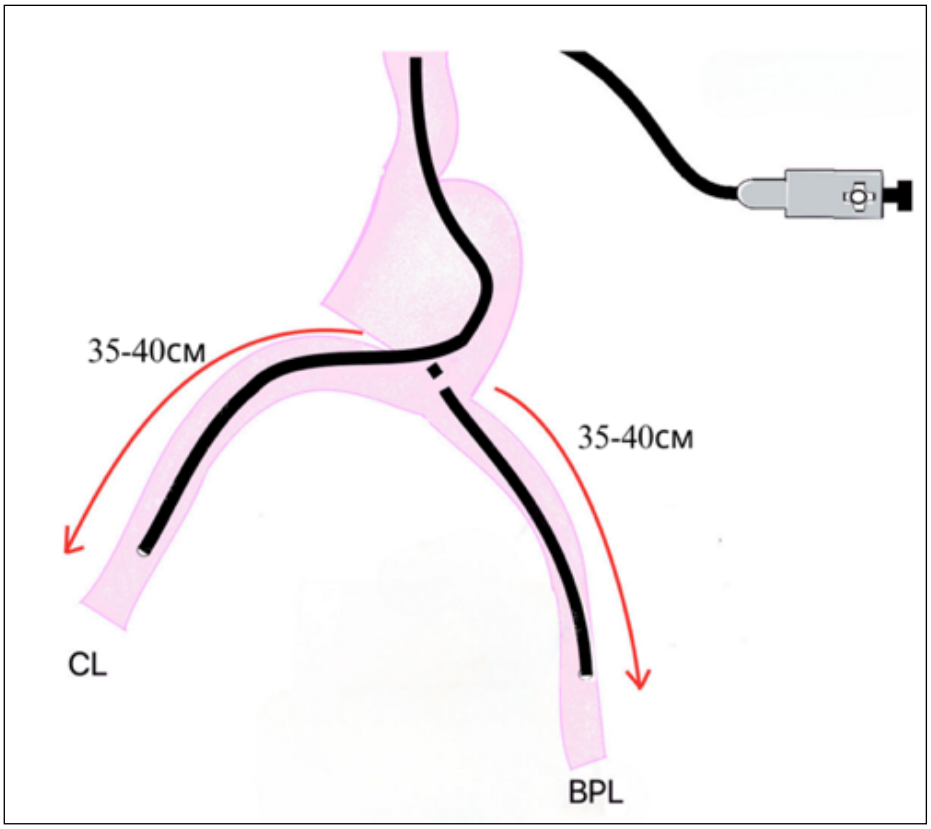

Fig. 3. Schematic representation of endoscopic forceps biopsy of the mucosa from the biliopancreatic limb (BPL) and common limb (CL) of the small intestine
Source: compiled by the authors of this study

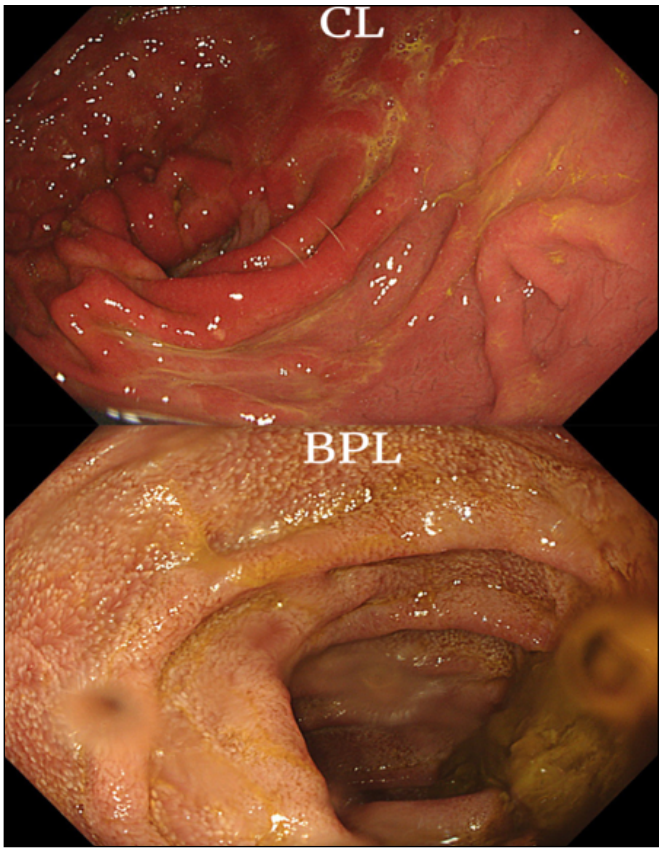

Fig. 4. Endoscopic view of the common limb (CL) and biliopancreatic limb (BPL) in a patient 3 years after single anastomosis gastric bypass surgery. Thickened folds of the mucosa are observed in the common limb
Picture taken by the authors

Morphometric analysis included measuring the length of the villi, the ratio of the length of the villi to the thickness of the layer of the lamina propria containing the crypts, assessing the number and distribution of goblet cells, and counting the number of crypts and Paneth cells.

ETHICS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (Ethics Committee of National Institute of Surgery and Transplantology named after A.A. Shalimov of the National Academy of Medical Sciences of Ukraine №16/01/2016).

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Informed consent was obtained from all individual participants included in the study.

RESULTS

LENGTH OF VILLI AND THICKNESS OF THE BASAL LAYER

The main function of intestinal villi in the small intestine is to directly and indirectly increase the absorption area,

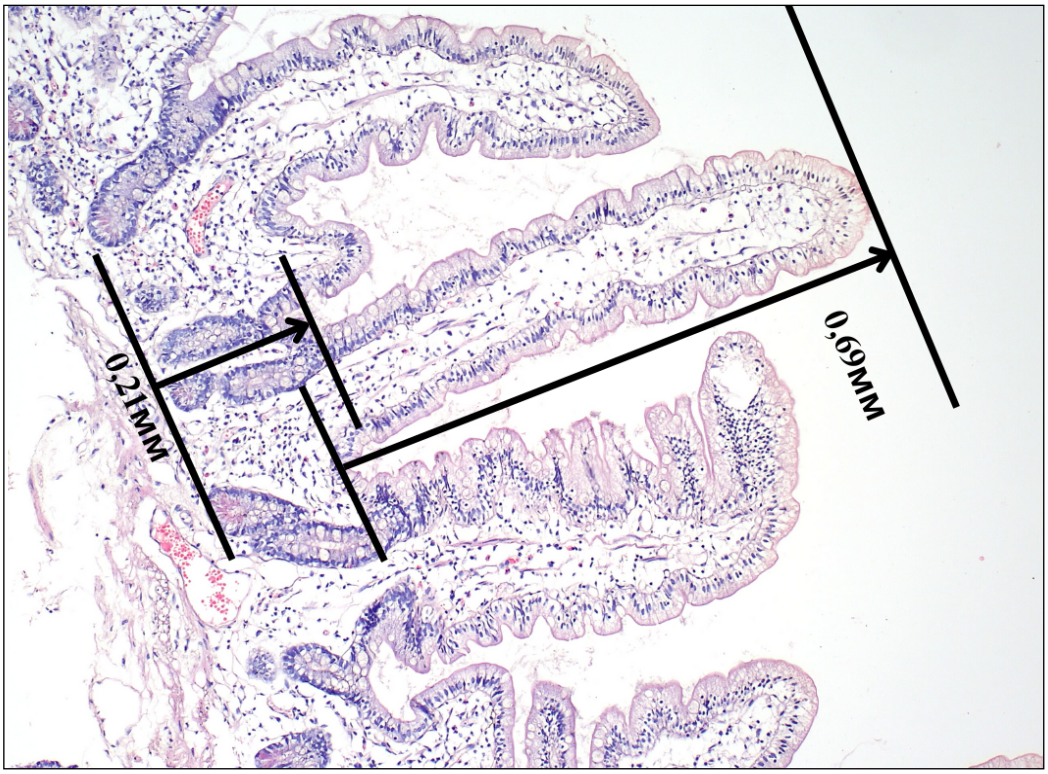

Fig. 5. Morphometry. Measurements of basal layer thickness (0.21 mm) and intestinal villus height (0.69 mm) were performed. Common loop with uneven villus expansion in the patient 14 months after mini-gastric bypass surgery. Magnification, 100X. Hematoxylin-eosin staging
Picture taken by the authors

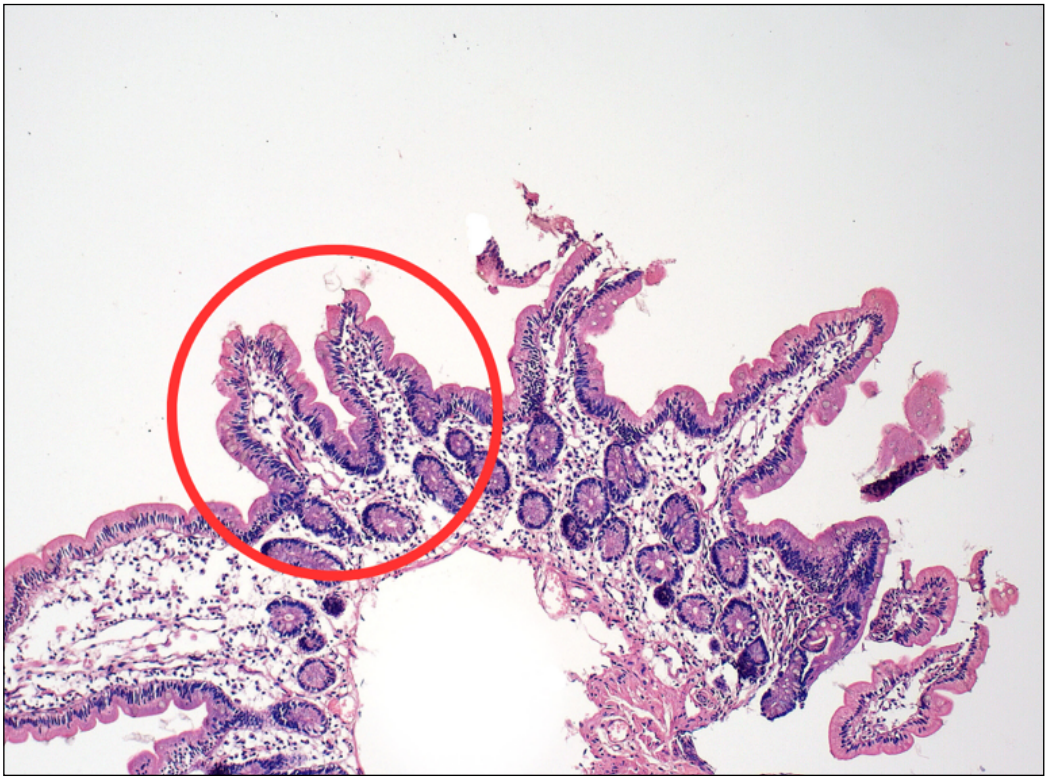

Fig. 6. A section of the mucous membrane of the biliopancreatic loop with flattening of the villi. Magnification, 100. Hematoxylin-eosin staining
Picture taken by the authors

thereby shortening the distance for nutrient molecules to reach the bloodstream and lymphatic vessels. **:** At 3 months follow up, no morphological or morphometric changes were observed in the biopsies from the biliopancreatic or common limbs. The first morphological and morphometric changes in the small intestinal mucosa were noted at 12 months after surgery. The results show the following changes in the mucosal biopsy after 2 years follow up. A total of 504 villi were studied in 36 patients. For all 36 patients, the average length of the villi in the biliopancreatic and common loops was calculated (see table). Subsequently, these indicators were compared, and statistically significant differences in the length of villi and the thickness of the basal layer

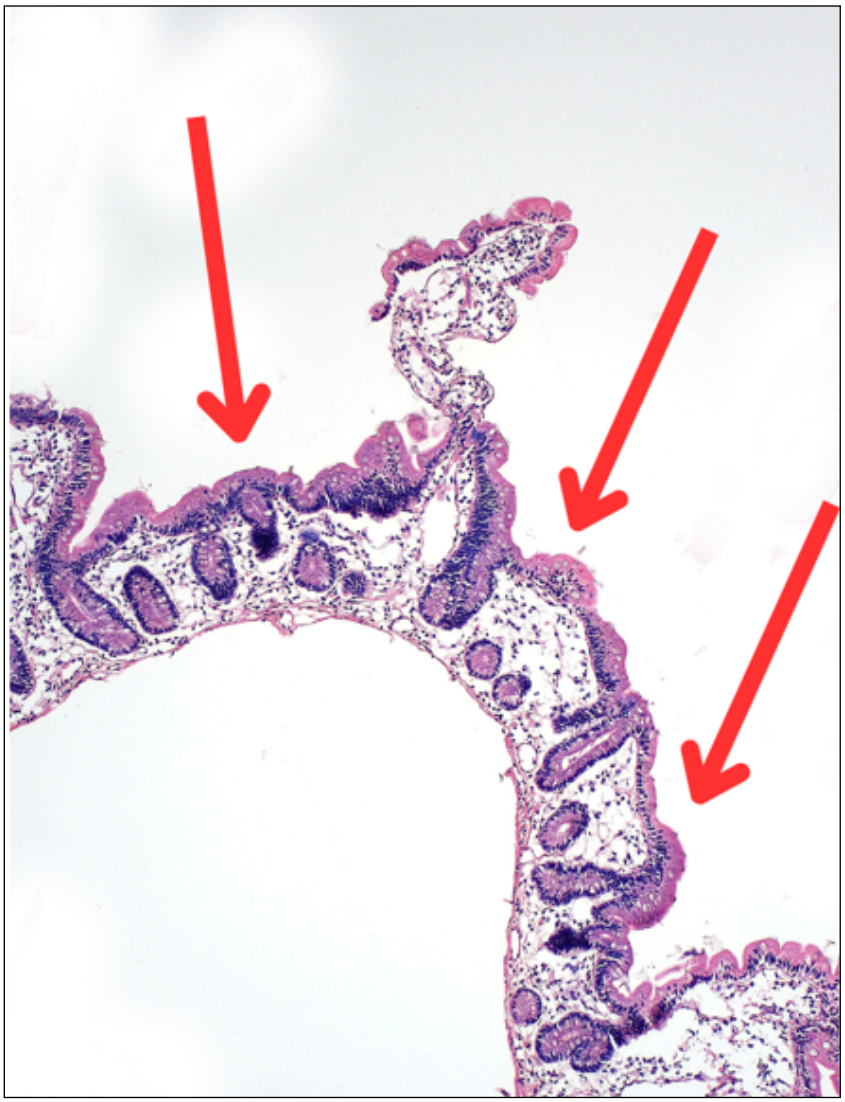

Fig. 7. Mucosal area with shortened villi. Magnification, 100X. Hematoxylin-eosin staining
Picture taken by the authors

between the common and biliopancreatic loops were analyzed using the non-parametric Mann-Whitney U-test. (Table 3).

A statistically significant ($p < 0.05$) difference in villi length was noted between the common and biliopancreatic loops of the small intestine, with the villi being longer in the common loop (0.390 ± 0.199 mm) compared to the biliopancreatic loop (0.377 ± 0.184 mm). These changes suggest villi hypertrophy in the efferent loop of the intestine to enhance the absorption area. This is also indicated by the prevalence of tortuous, unevenly expanded, and branched villi over shortened, deformed, and fused ones, which may correspond to structural intestinal adaptation (Fig. 6, Fig. 7).

The underlying cause of these changes is the reduction in the functional area of the small intestine, where absorption occurs—the area of the common loop—during surgery, as well as the body's need to compensate for postoperative malabsorption, which is the goal of the surgical intervention. In contrast, in the biliopancreatic loop, there is no need for absorption processes due to the absence of chyme, leading to villi atrophy. These results fully align with these hypotheses.

Statistically significant ($p < 0.05$) differences were also found in the average thickness of the basal layer between the common and biliopancreatic loops, with greater thickness observed in the biliopancreatic loop (0.196 ± 0.068 mm) compared to the common loop (0.167 ± 0.043 mm).

QUANTIFICATION OF CRYPTS AND PANETH CELLS

Paneth cells and crypts perform two primary functions: producing α-defensins and lysozyme to protect the body from microorganisms in the intestinal lumen, and regulating apoptosis and necroptosis in the intestinal mucosa. Crypts were identified as any closed tubular structures within the thickness of the basal layer of the mucosa, except for those where tangential sections only revealed cell nuclei.

The following results were obtained from observing the number of crypts and Paneth cells (Table 4).

Table 3. Villus length, basal layer thickness of the biliopancreatic (BPL) and common loop (CL) layers and the mean ratio were determined

Average pile length, mm			Average thickness of the basal layer, mm			Correlation		
CL	BPL	Mann-Whitney U test.	CL	BPL	Mann-Whitney U test.	CL	BPL	Mann-Whitney U test.
0.390± 0.199	0.377± 0.184	p<0.05*	0.167± 0.043	0.196± 0.068	p<0.05*	1.927± 1.30	2.342± 1.07	p<0.05*

* - Nonparametric Mann-Whitney test

Source: compiled by the authors of this study

Table 4. Average values of the total number of crypts and crypts containing Paneth cells

Biliopancreatic Loop		Common Loop		p
Crypts total, pcs.	Crypts containing Paneth cells, pcs.	Crypts total, pcs.	Crypts containing Paneth cells, pcs.	
54.2±14.8	19.7±7.2	52.8±13.8	18.2±7.1	>0.05 *

* - Nonparametric Mann-Whitney U test

Source: compiled by the authors of this study

Table 5. Results for goblet cell count and their distribution in the epithelial lining

Loop Type	Patients with Discrete Goblet Cell Distribution	Goblet Cell Percentage in Epithelium	Patients with Physiological Goblet Cell Distribution	Goblet Cell Percentage in Epithelium
Biliopancreatic	18 (50%)	10%	18 (50%)	25.9%
Common	0 (0%)	–	36 (100%)	18.2%

Source: compiled by the authors of this study

The average total number of crypts in the biliopancreatic loop was slightly higher than in the common loop (54.2 ± 14.8 vs. 52.8 ± 13.8, respectively), but the Mann-Whitney U-test depicted no statistically significant differences ($p > 0.05$). Therefore, the number of crypts in the biliopancreatic and common loops can be considered comparable.

Similarly, the number of crypts containing Paneth cells was slightly higher in the biliopancreatic loop compared to the common loop (19.7 ± 7.2 vs. 18.2 ± 7.1, respectively). However, no statistically significant differences were found using the Mann-Whitney U-test ($p > 0.05$). Thus, the number of crypts containing Paneth cells can also be considered comparable. These findings suggest that the regulatory functions of Paneth cells are preserved regardless of the type of single-anastomosis gastric bypass performed.

ASSESSMENT OF GOBLET CELL COUNT AND DISTRIBUTION

Goblet cells exhibit physiological characteristics in their distribution within the small intestine: their count decreases from the base to the tip of the villus and increases from the duodenum to the ileum.

In the small intestine, goblet cells perform mucin secretion through two mechanisms: regulated vesicular secretion and compound exocytosis.

1. Regulated secretion involves individual secretory vesicles merging with the plasma membrane to release their contents.
2. Compound exocytosis entails intracellular vesicle fusion followed by the "bursting" of the entire cell and the release of its contents into the intestinal lumen.

Given the migration of cells from crypts to the villus tip, fewer mature goblet cells (those that have not yet "burst") are found at the tip, while younger cells are concentrated at the base.

The observations yielded the following results for goblet cell count and their distribution in the epithelial lining (Table 5).

Interestingly, the distribution of goblet cells in the mucosa of the biliopancreatic and common loops differed significantly. In the biliopancreatic loop mucosa, half of the patients exhibited discrete goblet cell distribution, while the other half showed physiological distribution. For discrete distribution, goblet cells accounted for 10% of all epithelial cells on average, while physiological distribution yielded an average of 25.9%.

In the common loop mucosa, all 36 patients demonstrated preserved physiological goblet cell distribution, with goblet cells accounting for an average of 18.2% of all epithelial cells. These findings may reflect adaptation to environmental factors, such as altered microbiota or local influences affecting mucin secretion. A higher concentration of bile and pancreatic juices, undiluted by chyme, may negatively impact the epithelial lining, necessitating compensation through increased mucin production for protective functions.

The differing goblet cell distribution patterns and counts may be associated with the predominance of different mucin secretion mechanisms. However, these findings require further investigation to understand the regulatory mechanisms of goblet cell function fully.

DISCUSSION

The mucosa of the biliopancreatic limb, excluded from the digestive process, is constantly exposed to digestive juices, including secretions from the bypassed gastric reservoir, the pancreas, and bile. Unlike those in the common limb, luminal and membrane digestion and absorption of micronutrients and nutrients do not occur in the biliopancreatic limb, leading to impaired nutrition of the enterocytes themselves.

Considering these processes, the following adaptive changes in the mucosa of the biliopancreatic limb of the small intestine can be predicted [10]:

1) The villi were flattened, and the mucosal surface was smoothed to reduce the contact area with irritants;

2) An increase in the number of goblet cells producing mucins to enhance the protective function against digestive juices;

3) The number of Paneth cells in the crypts decreases as the need for α-defensins and lysozyme production decrease. However, since these cells still play a role in modulating apoptosis and necroptosis, this change is less likely.

4) Increased chronic inflammatory infiltration, mucosal erosion, and focal fibrosis in areas of continuous damage to the intestinal wall.

The common limb of the small intestine retains all of its functions -digestion, absorption, motility, and evacuation - and is in contact with digestive juices mixed with chyme. However, due to the reduced surface area of the small intestine following gastric bypass surgery, the following changes can be predicted:

1) The enlargement of villi, branching, and deformation occur, with uneven widening to increase the surface area available or contact with chyme;

2) Hypertrophy of the epithelium, especially enterocytes, to enhance digestive activity and absorption;

3) The enlargement of lymphatic capillaries in the lamina propria occurs as a result of increased absorption.

For a more objective interpretation of the morphological changes in the small intestine, the following microscopic and morphometric criteria were considered [11]:

1) Villi are elongated and finger-shaped;

2) The normal villus structure included at least four elongated, finger-shaped villi in succession;

3) The ratio of villus length to the thickness of the lamina propria containing crypts should be 3-5:1;

4) The surface is covered with microvilli that visually form an eosinophilic brush border, allowing for the identification of enterocyte damage during erosion;

5) The number of intraepithelial lymphocytes (IELs) is 1 for every 5 enterocytes;

6) The number of goblet cells decreases from the base to the tip of the villus, with a general increase from the duodenum to the ileum;

7) Paneth cells are located at the basal region of the crypts (lower 25%), and their number increases from the duodenum to the ileum;

8) The lamina propria contains loose connective tissue with minimal lymphoplasmacytic infiltration and occasional eosinophils.

Compared to the large number of animal studies investigating intestinal adaptation after gastric resection or gastric bypass surgery, the number of human studies is relatively small. The reasons for this discrepancy are the invasiveness of many procedures and the need to assess structural and functional adaptation [9]. For example, histologic analysis of the small intestine requires a biopsy, which can be particularly difficult for patients who have undergone multiple abdominal surgeries.

In our study, we compared the morphological changes in two types of intestinal loops-common and biliopancreatic - after single-anastomosis gastric bypass surgery, with a special focus on changes in villus length, basal layer thickness, and the number of crypts and Paneth cells. Our findings reveal that certain differences between these two types of loops may indicate adaptive changes in the intestine in response to gastric bypass surgery with a single anastomosis, which in turn may explain the stabilization of excess weight loss after 6-12 months of the postoperative period, "unsatisfactory" loss of excess weight, weight gain 2-3 years after surgery, or even regression of obesity and metabolic disorders in the longer-term postoperative period.

CONCLUSIONS

After 2 years follow-up the average length of the villi in the common loop was longer than that in the biliopancreatic loop which may indicate structural adaptation of the intestine, which increases the absorption area in the common loop. The thickness of the basal layer also tend increase in the biliopancreatic loop. Preservation of the number of Paneth cells in both loops indicates the constancy of their functions, regardless of morphological changes and changes in the anatomy of the gastrointestinal tract.

To further deepen our understanding of these processes, additional studies with a larger number of samples and the use of modern methods of morphological analysis are needed.

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CONFLICT OF INTEREST

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CONTENTS

Assessment of local immunity markers in patients with chronic rhinosinusitis and biofilms in the upper airway mucosa

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ABSTRACT

Aim: To comprehensively evaluate local immune markers in patients with chronic rhinosinusitis (CRS) with and without biofilms in the upper airway mucosa, and to determine pathogenetically significant immune alterations associated with biofilm presence that may contribute to chronic inflammation and reduced treatment effectiveness.

Materials and Methods: Oropharyngeal secretion was analyzed in 20 CRS patients (with and without biofilms) and 8 healthy controls. Levels of interleukin-1β, α-interferon, secretory IgA, immune complexes, and cellular composition were evaluated. Biofilms were detected via SYTO9/propidium iodide fluorescent staining.

Results: CRS patients with biofilms showed significantly reduced α-interferon levels and increased concentrations of immune complexes. IL-1β and sIgA levels did not differ between CRS subgroups. All CRS patients exhibited reduced epithelial cell counts and increased neutrophil percentages.

Conclusions: The study demonstrates that low α-interferon levels and high immune complex concentrations in patients with CRS and biofilms represent pathogenetically significant immune alterations. These findings highlight the need for further investigation of local and systemic immune mechanisms that support biofilm persistence and may open perspectives for more effective therapeutic strategies.

KEY WORDS: mucosal immunity, rhinosinusitis, biofilms, chronic, interferon-alpha, immune complexes

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INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common inflammatory diseases of the upper respiratory tract and significantly affects patients' quality of life, causing persistent symptoms such as nasal congestion, facial pain, hyposmia, and nasal discharge. Despite extensive research efforts, the pathogenesis of CRS remains complex and multifactorial, which limits the effectiveness of standard treatment approaches [1]. One of the key factors believed to contribute to CRS pathogenesis is local immune deficiency, which promotes biofilm formation and the chronicity of inflammation in the mucosal lining.

Biofilms are structured microbial communities in which bacteria adhere to surfaces—such as the epithelium of the nasal cavity or paranasal sinuses—and are embedded in an extracellular matrix. This matrix, composed of polysaccharides, proteins, and nucleic acids, serves a protective function, making the microorganisms significantly less susceptible to antibiotics and immune responses. Biofilm formation is a stepwise process involving initial bacterial adhesion, active proliferation, the development of a stable polysaccharide matrix, and the potential dispersion of individual cells or clusters capable of colonizing new mucosal areas. The complex composition of the biofilm matrix provides unique properties, including a high degree of intra- and interspecies communication, metabolic flexibility, and the ability to adapt to environmental stressors [2, 3]

The presence of biofilms in the nasal cavity and paranasal sinuses is associated with persistent or recurrent forms of CRS and often explains the poor efficacy of antibiotic therapy. The protective matrix of biofilms hinders the penetration of medications into bacterial colonies and reduces their susceptibility to phagocytosis and other immune mechanisms. Chronic inflammation is sustained by ongoing interactions between biofilm-associated cells and the immune system, leading to epithelial barrier damage, activation of pro-inflammatory cytokines (IL-6, IL-8, TNF-α), and suppression of regenerative processes [1].

Biofilms are capable of disrupting the function of the ciliated epithelium, which plays a key role in mucociliary clearance, and they promote the accumulation of pathogenic and opportunistic microorganisms, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [2] In CRS with biofilms, host defense mechanisms are frequently impaired. For example, biofilms can alter the expression of antimicrobial peptides such as lysozyme, lactoferrin, and β-defensins, thereby reducing the mucosa's ability to neutralize pathogens [4, 5]. In addition, the production of interferons—key mediators of antiviral defense and regulators of the inflammatory response—is suppressed. Reduced levels of these molecules in saliva may weaken antiviral immunity and increase susceptibility to secondary infections [6, 7]. Although some studies have addressed the role and mechanisms of biofilm influence on local and systemic immunity, further investigation is warranted.

AIM

To comprehensively evaluate local immune markers in patients with chronic rhinosinusitis (CRS) with and without biofilms in the upper airway mucosa, and to determine pathogenetically significant immune alterations associated with biofilm presence that may contribute to chronic inflammation and reduced treatment effectiveness.

MATERIALS AND METHODS

The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Kolomiychenko Otolaryngology Institute of National Academy of Medical Sciences of Ukraine (Approval No. 0412/2023, Date: 04/12/2023). Written informed consent was obtained from all participants prior to inclusion in the study.

A total of 20 patients with chronic rhinosinusitis (with and without biofilms) and 8 practically healthy individuals (control group) were immunologically examined. The material used for the study was oropharyngeal secretion (OPS), which is considered an indicator not only of local but also of general mucosal immunity. Samples of OPS were collected at a fixed time in the morning, prior to tooth brushing. The samples were then centrifuged using a refrigerated centrifuge (800R, Turkey) at 4°C and 100 g for 15 minutes. The liquid phase was separated, transferred into Eppendorf tubes, and stored at -25°C for up to 2 months.

The presence of biofilms in mucosal samples of the upper respiratory tract was assessed by fluorescent staining using dyes specific to components of the extracellular matrix [8]. A combination of SYTO9 and propidium iodide stains (LIVE/DEAD™ BacLight™ Bacterial Viability Kit, Thermo Fisher Scientific, USA) was used to differentiate between viable and non-viable bacteria within the biofilm [8].

Tissue samples were initially fixed in 4% paraformaldehyde for 30 minutes, then rinsed with phosphate-buffered saline (PBS, pH 7.4), and incubated with the working solution of dyes in the dark for 15 minutes at room temperature. The stained specimens were then analyzed using a fluorescence microscope (Axio Imager.A2, Zeiss, Germany) equipped with appropriate filters for green (SYTO9) and red (propidium iodide) fluorescence channels. Images were captured with a digital camera, and quantitative assessment of biofilm density was performed using ImageJ software (NIH, USA). The presence of a biofilm was defined by the visualization of structured bacterial microcolonies embedded in a dense matrix, with both viable and damaged cells visible in distinct fluorescence spectra.

The levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) (LABOR Diagnostik Nord, Germany), early interferon-α (FineTest, China), and secretory immunoglobulin A in two forms—secretory (sIgA) and monomeric (mIgA) (Hema Medica, Ukraine)—were measured in the oropharyngeal secretion.

In addition, the content of immune complexes in the oropharyngeal secretion was determined using a precipitation assay with 3.75% polyethylene glycol. Cellular components of various histogenetic origins were quantified in the sediment of the OPS using smear preparation and hematoxylin-eosin staining, in accordance with standard recommendations.

The analyses were performed using the enzyme-linked immunosorbent assay (ELISA) method with a StatFax 2100 reader (USA). Statistical analysis of the obtained data was carried out using the one-sided non-parametric Mann–Whitney U test. Calculations were performed using open-access software packages WINPEPI and Biostat, following established guidelines from the literature [9]. Results were presented as arithmetic means (M) and interquartile ranges (Q25–Q75). In cases of limited data volume, ranges (min–max) were used instead of quartiles. The number of observations was denoted as *n*. Differences were considered statistically significant at $p < 0.05$.

RESULTS

A comparative assessment of inflammatory, antiviral, humoral, and cellular parameters in oropharyngeal secretion was performed across three groups: CRS

Table 1. Levels of interleukin-1β (pg/mL) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+) and without biofilms (BF−) compared to control group (C)

Parameter:	BF+	BF-	C (Control)
n	10	10	8
Mean (M)	58.1	128.4	36.5
Median	40.7	135.5	22.4
Interquartile range (Q25—Q75)	17.0—85.0	80-155.2	17.8-71.2
P-value (vs. control)	0.06	< 0.05	Reference level

Source: compiled by the authors of this study

Table 2. Levels of α-interferon (pg/mL) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+), without biofilms (BF−), and in the control group (C)

Parameter:	BF+	BF-	C (Control)
n	10	10	8
Mean (M)	0.125	7.5	5.3
Median	0	3,8	3,9
Interquartile range (Q25—Q75)	0—0.4	2.25—14.8	1.4—6.8
P-value (vs. control)	<0.02	>0.05	Reference level

Source: compiled by the authors of this study

Table 3. Levels of secretory immunoglobulin A (g/L) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+), without biofilms (BF−), and in the control group (C)

Parameter:	BF+	BF-	C (Control)
N	10	10	8
Mean (M)	0.40	0.25	0.45
Median	0.30	0.28	0.35
Interquartile range (Q25—Q75)	0.24—0.49	0.22—0.40	0.25—0.55
P-value (vs. control)	>0.05	>0.05	Reference level

Source: compiled by the authors of this study

Table 4. Levels of immune complexes (arbitrary optical density units) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+), without biofilms (BF−), and in the control group (C)

Parameter:	BF+	BF-	C (Control)
N	10	10	8
Mean (M)	63.6	39.2	24.0
Median	60.0	20.0	36.6
Interquatile range (Q25—Q75)	48.5—70.9	12.5—75.5	19.0—43.0
P-value (vs. control)	= 0.0048	>0.05	Reference level

Source: compiled by the authors of this study

patients with biofilms (BF+), CRS patients without biofilms (BF−), and healthy controls (C). The analysis focused on differences in median values, variability within groups, and distributional patterns of the examined indicators.

The concentration of IL-1β was elevated in both CRS groups compared with healthy individuals (Table 1). The BF− group demonstrated the highest values, with a median of 135.5 pg/mL and a broad interquartile range (80.0–155.2 pg/mL), indicating marked heterogeneity of inflammatory activation. The BF+ group showed a more moderate increase (median 40.7 pg/mL, IQR 17.0–85.0 pg/mL), yet most individual values remained above those of controls. Although the BF+ group did not reach the threshold of statistical significance when compared with the control group ($p = 0.06$), the consistent upward trend suggests a clinically relevant elevation. Direct comparison of BF+ and BF− groups revealed that IL-1β levels were generally higher in CRS patients without biofilms.

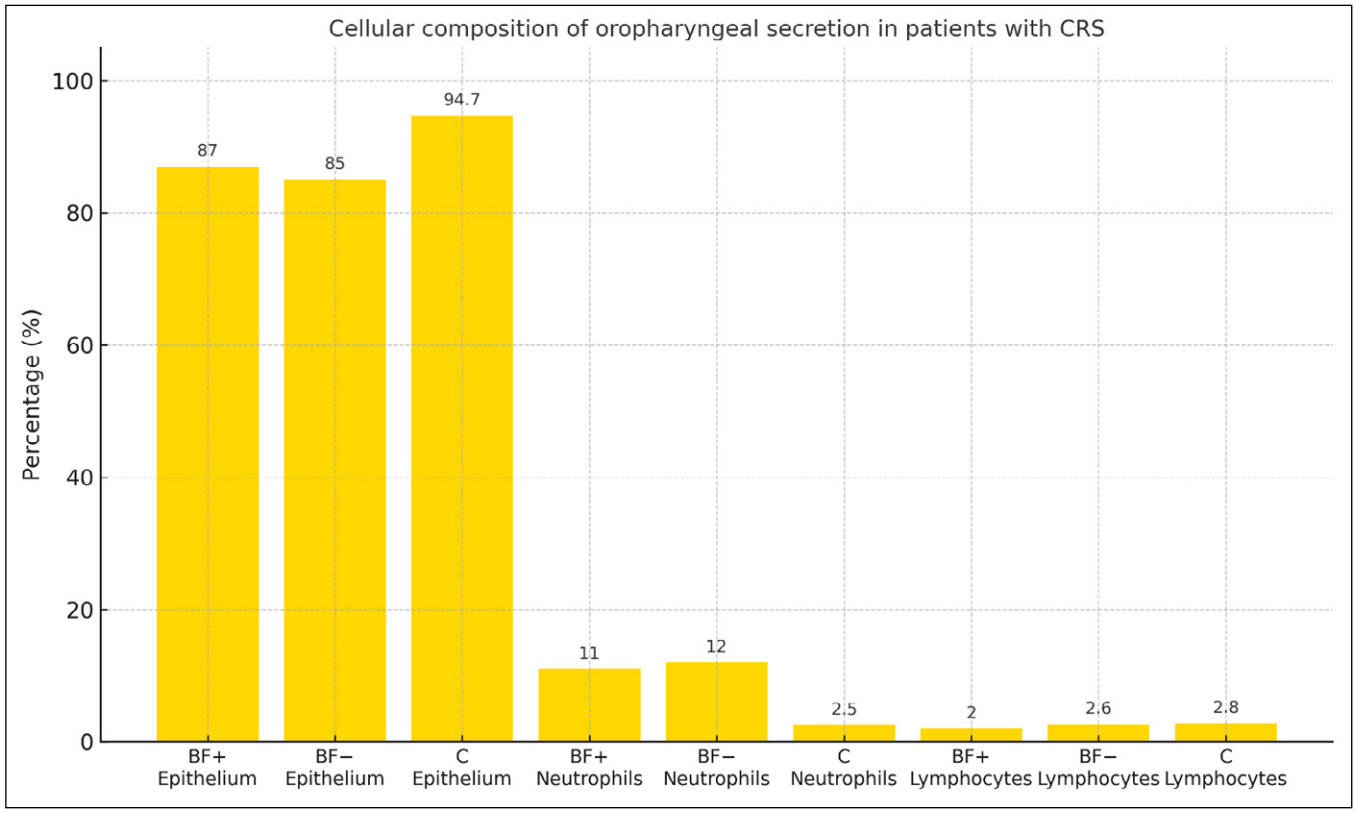

Fig. 1. Cellular composition (in percentage) of oropharyngeal secretion in patients with chronic rhinosinusitis with (BF+) and without (BF–) biofilms.
Note: Statistically significant differences in segmented neutrophils for both BF+ and BF– groups ($p < 0.05$).
Picture taken by the authors

A pronounced difference in α-interferon levels was observed across the groups (Table 2). Nearly all BF+ patients exhibited extremely low or undetectable concentrations (median 0 pg/mL, IQR 0–0.4 pg/mL), with minimal variability within the group. In contrast, the BF– group showed substantially higher and more dispersed values (median 3.8 pg/mL; IQR 2.25–14.8 pg/mL), including several measurements comparable to the control range. Healthy individuals demonstrated expected physiological levels of α-interferon (median 3.9 pg/mL, IQR 1.4–6.8 pg/mL). The suppression of α-interferon in the BF+ group was statistically significant ($p < 0.02$), forming a distinct pattern not observed in BF– patients.

No statistically significant differences in sIgA were recorded among the three groups (Table 3). Nevertheless, a tendency toward reduced sIgA was observed in the BF– group (median 0.28 g/L), while the BF+ group demonstrated values closer to those of healthy individuals (median 0.30 g/L vs 0.35 g/L in controls). The distributions of sIgA overlapped broadly between all groups, and the interquartile ranges were comparable. Thus, despite a downward trend in some CRS patients, sIgA levels did not show diagnostic or discriminatory value in the present cohort.

The analysis of circulating immune complexes (ICs) demonstrated clearer group differentiation (Table 4). The BF+ group displayed consistently elevated IC concentrations, with median values (60.0 optical units) more than twice as high as in the control group (36.6 optical units). The interquartile range in BF+ was narrow (48.5–70.9), reflecting a stable pattern of elevated ICs among all participants in this group. In contrast, the BF– group showed substantial variability (IQR 12.5–75.5), with individual values spanning both below and above the ranges of BF+ and control subjects. This heterogeneity prevented statistical significance. The BF+ group differed from controls at $p = 0.0048$, indicating a robust upward shift in IC levels in the presence of biofilms.

Analysis of the cellular profiles revealed characteristic shifts in the CRS groups (Fig. 1). Both BF+ and BF– patients demonstrated a marked increase in segmented neutrophils compared with healthy controls, and this elevation reached statistical significance ($p < 0.05$). The proportion of epithelial cells showed a downward trend in both CRS groups, though the variability within the control samples limited statistical separation. Lymphocyte counts remained largely comparable across all groups and showed no significant trends. Importantly, the cellular distribution in BF+ and BF– patients was similar, indicating that biofilm presence had no distinct impact on the cytological composition of oropharyngeal secretion.

DISCUSSION

Despite intensive research efforts, the pathogenesis of chronic rhinosinusitis (CRS) remains complex and multifactorial, which limits the effectiveness of standard treatment approaches. One of the important pathogenic factors in CRS is believed to be the presence of biofilms in the mucosa of the upper respiratory tract, which may be associated with the development of local immune deficiency. Numerous studies of oropharyngeal mucosal immunity have indicated that the main components involved in protection against microbial and viral agents include immunoglobulins, interferons, phagocytes, and various groups of defensins [10]. Among the tested parameters, the most pronounced deviations from healthy controls were observed in the levels of interferon-α. The reduced concentration of this cytokine in the oropharyngeal secretion of CRS patients with biofilms may indicate a weakened antiviral defense, particularly during the early stages of infection.

Another test that showed statistically significant deviations from the control group was the level of immune complexes, which was 2.5 times higher in the BF+ group. Elevated levels of immune complexes in saliva may, on the one hand, indicate activation of humoral immune responses, but on the other hand, immune complexes can act as immunopathological factors capable of causing tissue damage under certain conditions.

Regarding the level of the pro-inflammatory cytokine IL-1 in oropharyngeal secretion, it was found to be elevated in both groups—BF+ and BF−—with very similar values. This suggests that the increased IL-1 level is likely due to the prolonged inflammatory process present in both patient groups, making it impossible to determine the specific role of biofilms in this context. A similar conclusion applies to the cellular composition of the secretion: the relative proportion of inflammatory cells—segmented neutrophils—was nearly identical in both study groups and exceeded that of the control group. The relative number of epithelial cells showed a clear downward trend, but the difference compared to the control was not statistically significant. The level of secretory immunoglobulin A was reduced only in the BF− group and did not demonstrate meaningful diagnostic value.

Thus, the conducted study indicates that in cases of CRS associated with biofilm formation, certain factors may be considered pathogenetically significant—namely, a low level of α-interferon and a high concentration of immune complexes in oropharyngeal secretion. Further research into the local and systemic immune mechanisms that contribute to the formation and persistence of biofilms in CRS appears to be warranted.

CONCLUSIONS

A low level of α-interferon is a pathogenetically significant factor in chronic rhinosinusitis with biofilm presence. A high concentration of immune complexes in patients with CRS and biofilms represents an immunopathological mechanism contributing to the persistence of inflammation. The levels of interleukin-1β, the cellular composition of oropharyngeal secretion, and the concentration of secretory immunoglobulin A are characteristic of CRS without biofilm structures in the upper respiratory mucosa.

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CONTENTS

Psychosocial factors in the development of inclusivity and accessibility in the student environment under crisis conditions

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ABSTRACT

Aim: Identifying key psychosocial factors that contribute to the development of inclusivity and accessibility among students in contemporary crisis conditions, as well as assessing their impact on readiness for social and professional interaction in an inclusive environment.

Materials and Methods: The materials of the research were based on the results of an anonymous online survey of students from Ukrainian higher education institutions, conducted in the summer semester of 2025 using Google Forms. The questionnaire included blocks of questions aimed at identifying the psychosocial factors influencing the development of inclusivity and accessibility. Descriptive statistics, elements of comparative and correlational analysis, as well as qualitative processing of open-ended responses were used for data analysis, which allowed for a comprehensive assessment of the relationship between students› psychosocial resources and their readiness for inclusive and barrier-free interaction.

Results: The development of inclusivity and accessibility among students is formed through a complex interaction of psychosocial factors: personality traits, social environment, interaction experience, and value orientations. Effective development of readiness for inclusive behavior requires a systematic approach that combines theoretical training, practical tasks, and socio-psychological support.

Conclusions: The research findings indicate that the development of inclusivity and accessibility in the student environment is determined by the interaction of personal resources (empathy, tolerance, communication skills), the social environment, participation in communities, family values, the educational context, and experience of living in crisis conditions. Crises can create barriers but at the same time stimulate social empathy, solidarity, and collective support. Effective formation of an inclusive culture requires a comprehensive approach that combines personal development, social integration, and structural changes in the educational environment.

KEY WORDS: inclusivity, accessibility, psychosocial factors, students, crisis situations, social support, resilience, emotional well-being, professional training, higher education

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INTRODUCTION

The modern academic community of Ukraine is influenced by numerous crisis factors, including prolonged war, social instability, internal student mobility, and digital threats. These challenges create a significant psychosocial burden, requiring a high level of adaptability from students and the implementation of systemic mechanisms of support and development by higher education institutions. Under such conditions, inclusivity and accessibility become especially important, as they ensure equal access to education, promote social integration and professional development of young people, and help form a resilient and adaptive educational environment.

Despite the considerable attention given to issues of inclusive education, contemporary research insufficiently addresses the psychosocial mechanisms of forming inclusivity in the student environment, especially under crisis conditions. In particular, a limited number of studies focus on examining the impact of crisis circumstances on the development of value orientations, empathy, tolerance, and readiness for non-discriminatory interaction among students.

MATERIALS AND METHODS

The research materials were based on the results of an online survey of students from Ukrainian higher education institutions conducted in 2025. The survey was created using Google Forms and distributed through university student groups, academic communication channels, and social networks. Participation in the

study was voluntary and anonymous, which ensured openness of responses and compliance with ethical standards. The total sample consisted of more than 150 participants, making it possible to consider the results representative of the typical socio-demographic characteristics of contemporary Ukrainian students.

The research instruments were aimed at identifying psychosocial factors that influence the level of inclusivity and accessibility in the student environment. The questionnaire included blocks of questions covering the level of social support, sense of belonging to the academic community, emotional state, stress-coping strategies, experience of interaction with vulnerable groups, attitudes toward inclusive practices, and self-assessed readiness for barrier-free interaction under crisis challenges. A separate section focused on assessing the impact of military events, internal migration, changes in learning formats, and digital risks on students' psychological well-being.

The methodological framework included the use of descriptive statistics, comparative analysis, and elements of correlational analysis to identify relationships between psychosocial variables. A combined approach was applied, integrating quantitative measures (rating scales, Likert scales) and qualitative open-ended questions, which allowed for deeper interpretation of students' individual experiences and their attitudes toward inclusive practices in crisis conditions. Methods of logical-analytical generalization and content analysis of open-ended responses made it possible to identify key psychosocial trends affecting inclusivity and accessibility. Additionally, the obtained data were compared with the results of contemporary international studies, which made it possible to identify both universal factors and those specific to the Ukrainian context.

The ethical principles of the study complied with recommendations for conducting social and humanitarian surveys in crisis conditions, in particular by ensuring confidentiality of responses, voluntary participation, the possibility to withdraw at any stage, and the absence of risks for respondents.

AIM

The aim of this study is to identify the key psychosocial factors that contribute to the development of inclusivity and accessibility among students in contemporary crisis conditions, as well as to assess their impact on readiness for social and professional interaction in an inclusive environment.

RESULTS

The issue of developing inclusivity and accessibility in the student environment becomes especially relevant in the context of contemporary crises, including military actions, forced displacement, the COVID-19 pandemic, and prolonged educational transformations. The combination of psychosocial factors, institutional characteristics, and students' individual resources determines their ability to maintain educational engagement, adaptability, and a sense of safety. Contemporary research indicates that inclusive educational systems become a key mechanism for reducing students' vulnerability and strengthening their psychosocial resources in crisis conditions. UNESCO reports emphasize that accessibility of educational services, inclusive policies, and support for high-risk groups are critically important for ensuring sustainable learning during emergencies [1]. Similar conclusions are presented in practical documents of UNICEF/EiE Hub, which stress the necessity of universal design for learning, psychosocial support, and the creation of adaptive educational environments capable of responding quickly to crises [2].

Ukrainian studies confirm the findings of international reviews. In the works of H. Bilavych et al. [3; 4], it is emphasized that the development of inclusive education in Ukraine is taking place amid a significant increase in the needs of students with special educational and psychosocial needs. The authors highlight the importance of intersectoral cooperation, staff training, improvement of communication accessibility, and the integration of technologies that enhance the learning autonomy of students from vulnerable groups.

Reviews of research on education in emergencies (for example, Burde et al., 2017 [5]) indicate that crises sharply intensify social inequality, increase levels of psychological distress, and deepen barriers to access to education. Disruptions to the learning process, breakdown of social ties, and uncertainty lead to decreased student motivation, reduced cognitive resources, and a growing need for social and emotional support. Similar findings are reported by J. Forsberg et al. (2023) [6], showing that school and university interventions focused on psychosocial support significantly reduce anxiety levels, enhance a sense of community, and facilitate young people's return to educational pathways. Importantly, these models assign a leading role to educators and educational institutions in restoring social integration.

Ukrainian authors (S. Bogdanov, I. Pinchuk) [7] proposed a multi-level model of psychosocial support in educational institutions that combines preventive, counseling, and crisis interventions. The model confirms the importance of early identification of students' needs, mobilization of community resources, and the creation of a safe environment in which inclusivity is regarded as an element of psychological safety.

Recent studies focusing on the Ukrainian context show a sharp deterioration in students' mental health

during the war. Among them are the works of S. Hozak [8], I. Pinchuk et al. [9], and T. Kuprii et al. [10], which document significant increases in anxiety levels, emotional exhaustion, cognitive difficulties, and feelings of social isolation. At the same time, studies by M. Błaszczyk [11] and S. Londar et al. [12] demonstrate that universities are developing new models of institutional resilience that include digital support, flexible learning formats, psychological service units, and the development of student communities.

International studies on student well-being in crisis situations [13; 14; 15; 16], among others) indicate the presence of a range of psychosocial factors that directly influence the development of inclusivity and accessibility in the educational environment. Among these, social support—received by students from peers, instructors, and institutional services—plays a key role, as does a sense of belonging to the academic community. Emotional stability, levels of anxiety and depressive symptoms, and the stress-coping strategies chosen by students are also important. Additionally, the level of digital accessibility and the availability of necessary technical resources that ensure effective participation in the learning process, the quality of communication with the university, and the ability for self-regulation and maintenance of academic motivation are significant. All these factors are interconnected and together create the conditions necessary to ensure accessibility and support for students in crisis situations.

Publications by INEE/JEiE (2024–2025) [17] and numerous studies on education in emergencies emphasize the role of universities as centers of psychosocial support and safety. Institutions that provide access to psychological services, create opportunities for student initiatives, and support academic communities demonstrate a higher level of adaptive resilience.

The literature review shows that psychosocial factors are central determinants of inclusivity and accessibility in the student environment under crisis conditions. War, pandemics, and other traumatic events not only increase young people's psychological vulnerability but also exacerbate existing barriers to access to education. The most critical factors include social support, emotional well-being, a sense of belonging to the academic community, access to resources, and the quality of institutional communication.

The concept of inclusivity in the student environment means not only creating conditions for the presence of representatives of various social, physical, or cognitive groups, but also ensuring their full participation in the academic, social, and cultural life of the institution. This implies equal opportunities for learning, social interaction, and development, based on respect for diversity, empathy, tolerance, and social justice (L. Zadorozhna, [18]). In the context of the university community, inclusivity refers to the readiness of students, faculty, and administration to provide support and engage in interaction regardless of physical or social differences. This approach corresponds to the understanding of inclusive education as a practical embodiment of social justice.

At the same time, accessibility (barrier-free environment) functions as a socio-pedagogical category that encompasses physical, informational, communicative, and social accessibility, as well as the elimination of architectural, infrastructural, social, and psychological barriers. Accessibility is not only about adapting the environment but also about creating a culture of tolerance, respect, and willingness to support anyone in need. In educational institutions, accessibility means not only access to facilities, but also adaptation of the learning process, information resources, communication methods, and social support.

Psychosocial factors in the context of inclusivity and accessibility are understood as a set of individual, interpersonal, social, and contextual components that influence behavior, values, attitudes, and readiness for inclusive interaction.

Individual components include personal qualities such as empathy, tolerance, and communication competence, as well as prior experience interacting with different categories of people. Empirical studies confirm that a high level of communication competence correlates with empathetic abilities and prosocial behavior among students. Analysis of contemporary practices indicates that empathy significantly contributes to the development of inclusive behavior in the learning environment (O. Chaikovska, L. Melnyk, L. Kuzo [19]). Interpersonal and social components include the influence of the social environment, particularly family, friends, colleagues, and instructors, as well as family values that shape students' attitudes toward diversity and their readiness for inclusive interaction. Research shows that support from the social environment and mutual assistance are key factors in the formation of inclusive behavior, especially within the university setting [20].

Contextual and institutional components encompass the educational, cultural, and institutional environment, including the policies and practices of higher education institutions, the availability of adaptation mechanisms and resources, as well as social and crisis conditions that may affect students. The study N. Dub "Inclusion in Higher Education: Key Ideas, Challenges and Barriers" [21] demonstrates that the absence or presence of support systems and inclusive practices in higher education institutions largely determines the level of

inclusivity and accessibility in the student environment.

Individual, interpersonal, and contextual components function interactively to ensure the development of inclusive behavior. The presence of empathy, communication skills, and tolerance among students, combined with a supportive social environment and inclusive university policies, creates favorable conditions for socially responsible interaction, readiness to support vulnerable groups, and adaptation to diverse social situations. University practices that foster the development of empathy and communication competence simultaneously cultivate a culture of support and responsibility, which contributes to the resilience of an inclusive environment.

Thus, psychosocial factors determine the extent to which students are prepared for barrier-free interaction, support, and social participation. The complex interplay of personal qualities, social environment, and institutional context is a necessary condition for fostering an inclusive culture in higher education and ensuring the sustainability of barrier-free practices, particularly under crisis conditions.

Crisis situations—such as war, social instability, displacement, economic difficulties, pandemics, and digital risks—significantly alter students' living conditions and require them to adapt and mobilize internal resources. In this context, psychosocial consequences may manifest as anxiety, insecurity, isolation, or loss of motivation and social support. At the same time, crises can stimulate solidarity, empathy, mutual assistance, social mobility, and active participation in community life, creating conditions conducive to the development of inclusivity.

In student communities under conditions of instability, the ability for collective support, building social connections, adaptability, and the development of resilience becomes particularly important. Interaction among students with diverse experiences, social statuses, backgrounds, and needs can serve as a source of social support resources and foster the development of an inclusive culture.

Based on current scientific approaches [6; 9; 11; 13], a research model was developed, which assumes the following relationship: psychosocial factors (personal traits, social environment, family values, prior experience, educational context) lead to mechanisms of social support, interaction, solidarity, and adaptation (emotional, informational, and communicative support; social connections; participation in communities; volunteering), which in turn result in readiness for inclusive interaction, barrier-free thinking, social resilience, tolerance, empathy, and active participation in social life.

In the empirical study of psychosocial factors influencing the development of inclusivity and accessibility in the student environment under crisis conditions, 152 students at the bachelor's and master's levels from eight Ukrainian higher education institutions participated: Carpathian National University named after Vasyl Stefanyk, Ivano-Frankivsk National Medical University, Ivano-Frankivsk National Technical University of Oil and Gas, King Danylo University, Ivan Franko National University of Lviv, Lviv Polytechnic National University, Ukrainian Catholic University, and Kherson State University. The representativeness of the study was ensured by a wide range of respondents' fields of study, including preschool education (A2), primary education (A3), secondary education (A4), vocational education (A5), special education (A6), physical culture and sports (A7), psychology (C4), management (D3), public administration (D4), law (D8), ecology (E2), medicine (I2), therapy and rehabilitation (I7), social work and counseling (I10), and child and youth services (I11). This diverse composition of respondents allowed the study to include both students who are just beginning their professional training and those who already have a developed vision of their professional activity.

Respondents evaluated the areas where they considered accessibility to be most relevant. The largest number of students, as shown in Figure 1, indicated public spaces, education, and transportation (105 respondents in each category), accounting for 69.1% of the total. The high selection of these areas reflects students' awareness of the social and practical impact of accessibility on their participation in the educational process and social functioning. The significance of the informational space was noted by 88 respondents (57.9%), and social services by 86 (56.6%), highlighting the importance of digital accessibility and the availability of support from social institutions. The option "Other / difficult to answer" was chosen by 23 students (15.1%), which may indicate insufficient awareness of specific or less obvious areas of accessibility.

Analysis of these data demonstrates that students' perception of accessibility encompasses not only physical access but also social and informational integration, emphasizing the psychosocial aspect of social inclusion.

Regarding practical experience in interacting with individuals who require additional support, 28.3% of respondents reported regular interaction with such groups, 50% indicated occasional experience, and 21.7% had no such experience. These data suggest that while most students have basic experience interacting with vulnerable groups, systematic engagement remains limited. From a psychosocial perspective, this underscores the importance of integrating practical skills for inclusive interaction into educational programs

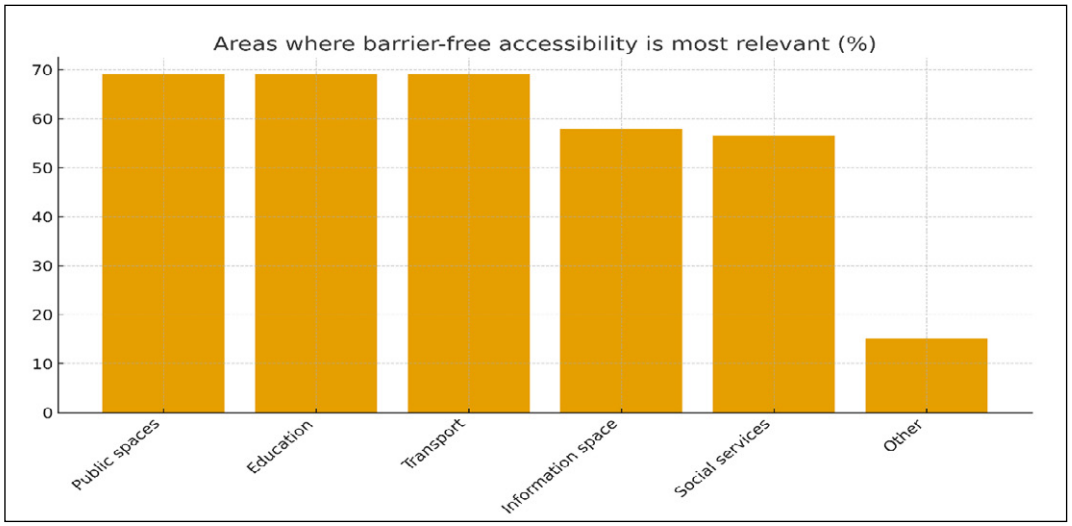

Fig. 1. Areas where barrier-free accessibility is most relevant (%)
Source: compiled by the authors of this study

to develop stable social and emotional competencies in future professionals.

The level of students' confidence (Figure 2) in interacting with vulnerable groups was assessed on a five-point scale. Among respondents, 38.2% selected level 3, another 38.2% chose level 4, 12.5% indicated the maximum level of 5, and 11.2% reported a low level of confidence (1–2 points).

These results indicate a moderate to high level of students' readiness for socially responsible interaction, while simultaneously pointing to the presence of psychological barriers and limitations due to insufficient practical experience.

The most frequently identified factors contributing to confidence (Figure 3) were personal experience and communication skills – 106 responses (69.7%), knowledge of inclusivity principles – 79 (52%), support from their environment – 53 (34.9%), and fear of "saying something wrong" – 44 (28.9%). Only 14 students (9.2%) were unable to identify a specific factor.

This demonstrates that the development of communication competence and practical experience is critically important for increasing readiness for barrier-free interaction.

Regarding value orientations, 76.3% of students (116 individuals) fully agreed that inclusivity is an important value in modern society, 18.4% (28 individuals) partially agreed, 2.6% (4 individuals) disagreed, and another 2.6% indicated difficulty answering. The statement that barrier-free access is an essential condition for social resilience in crisis situations received an average score of 4.24 out of 5: 2% of respondents rated it 1 point, 1.3% – 2 points, 17.1% – 3 points, 29.6% – 4 points, and 50% – the maximum 5 points. This indicates a high level of awareness of the role of barrier-free access in ensuring social stability and emphasizes the importance of value-based attitudes as a psychosocial factor of resilience.

The assessment of contemporary youth's openness to inclusivity and barrier-free practices showed an average score of 3.68. Among respondents, 37.5% chose 3 points, 31.6% – 4 points, 23% – 5 points, and only 7.9% rated openness at 1–2 points. This reflects a moderately high level of awareness and readiness for social inclusion among young people while highlighting the need for systematic development of socio-psychological competencies and value orientations through educational and practical programs.

Students also identified factors that shape youth readiness for inclusive interaction. The most frequently mentioned were personal traits (empathy, tolerance) – 122 responses (80.3%), social environment – 111 (73%), and family values – 102 (67.1%). Less prominent but still significant were experience in crisis situations – 71 responses (46.7%) and educational context – 74 responses (48.7%). Only 5.3% of students were unable to identify a specific factor. These data confirm that the development of readiness for inclusive behavior is a multi-level process, where internal psychological characteristics combine with social support and educational factors.

Regarding factors that strengthen barrier-free thinking, respondents noted awareness of diversity – 100 responses (65.8%), understanding of human rights – 99 (65.1%), experience in supporting others – 94 (61.8%), communication skills – 82 (53.9%), and volunteering or civic engagement – 77 (50.7%). Only 10 students (6.6%) could not identify a specific factor.

These results demonstrate that both knowledge and practical experience, combined with social skills, are key psychosocial determinants in forming an open, barrier-free worldview.

The findings indicate that the development of inclusiveness and barrier-free behavior among students is the result of a complex interaction of various psycho-

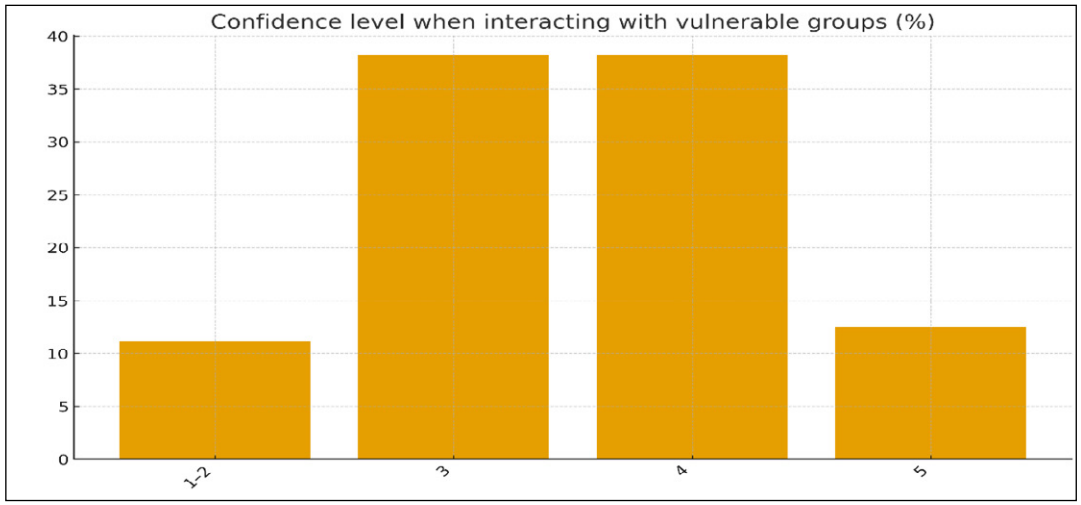

Fig. 2. Confidence level when interacting with vulnerable groups (%)
Source: compiled by the authors of this study

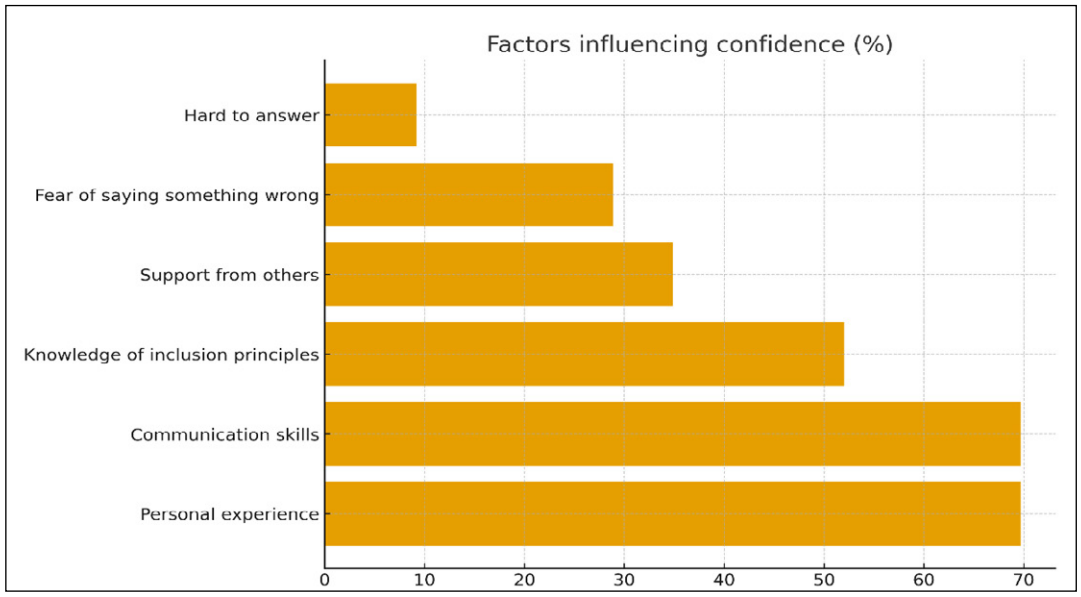

Fig. 3. Factors influencing confidence (%)
Source: compiled by the authors of this study

social factors. This process is not limited to personal traits or isolated external influences. It is shaped at the intersection of individual characteristics, social environment, experience in different forms of interaction, and value orientations, which are gradually built through education and practice.

Together, this creates a multi-level system within which students acquire the ability for inclusive behavior and barrier-free thinking. For these qualities to truly develop, theoretical knowledge or situational practical tasks alone are not sufficient. Effectiveness is achieved when the educational process is structured as an integrated model, combining academic preparation, practical interaction in real or simulated situations, and the presence of socio-psychological support. Such a systemic approach not only expands understanding of inclusiveness but also fosters an internal reorientation of students toward the values of accessibility, mutual respect, and non-discrimination.

DISCUSSION

The results of the study confirm that psychosocial factors significantly determine students' readiness for inclusive interaction and the development of barrier-free thinking. Personal resources such as empathy and tolerance, the social environment, and the educational context are key determinants of youth behavior.

Crisis conditions, such as war, social instability, and displacement, modify the mechanisms of inclusiveness formation. On one hand, they create additional barriers, including anxiety, uncertainty, and the risk of social isolation. On the other hand, crises stimulate solidarity, collective support, and active engagement in volunteer work and community initiatives. The study's findings show that students with experience participating in crisis situations demonstrate greater confidence in interacting with vulnerable groups, highlighting the role of crises as a catalyst for the development of social empathy and inclusive behavior. Student communities play a critically important

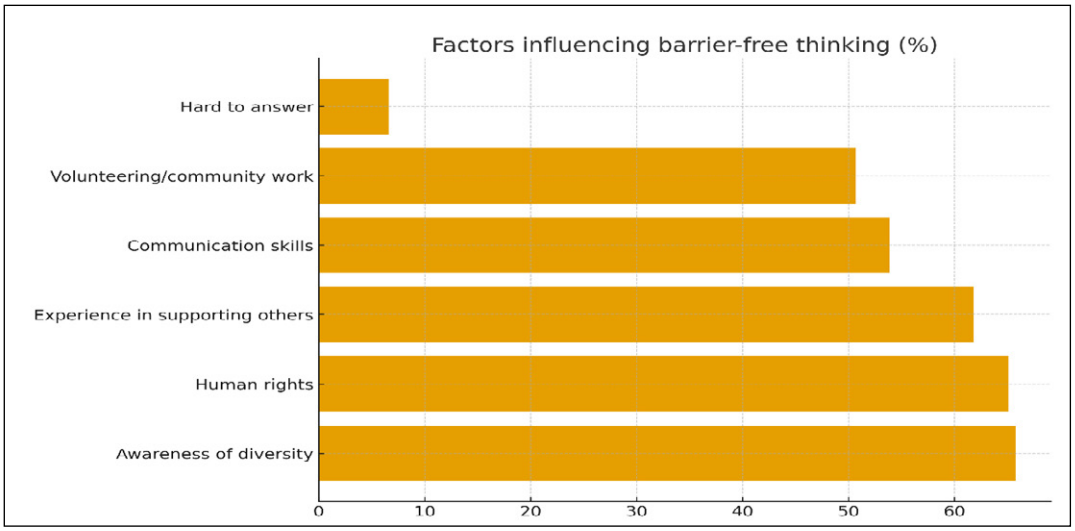

Fig. 4. Factors influencing barrier-free thinking (%)
Source: compiled by the authors of this study

role in social integration. Interaction within support groups, participation in collective activities, and projects contribute to the development of barrier-free thinking and tolerance. This emphasizes the importance of strategically involving students in socially oriented initiatives, creating conditions for interpersonal support, and teaching skills for interacting with diverse population groups.

The contribution of the study lies in the systematic identification of psychosocial determinants of inclusiveness in the student environment under crisis conditions. The practical novelty of the research consists in identifying key factors that universities can use to develop a barrier-free culture, while the theoretical generalization lies in the formation of a logical model "factors → mechanisms → outcomes," relevant for further empirical and cross-cultural studies.

The study results have important practical implications and can be used to promote an inclusive and barrier-free culture within student communities. The practical significance lies in the need for a systemic approach to student preparation that combines the development of personal skills, social support, and the creation of an accessible educational environment. The design of educational programs aimed at developing empathy, tolerance, and communication skills should integrate theoretical knowledge about inclusiveness with practical cases and role-playing exercises simulating real-life interactions with vulnerable groups. An essential element is the development of social support systems in educational institutions through mentorship, collective activities, and mentoring programs that enhance emotional safety and group cohesion among students. Adapting educational and informational resources to ensure physical and digital accessibility, as well as involving students in volunteer and community initiatives, fosters barrier-free thinking and the development of socially responsible behavior under crisis conditions.

At the level of educational policy, the study results emphasize the need to create strategies that ensure equal access to educational resources and services for all students, regardless of physical, social, or psychological limitations. Integrating psychosocial support into educational programs provides adequate conditions for the adaptation and development of students in vulnerable or crisis situations. Establishing student support services and inter-university platforms for experience exchange promotes university cooperation and the dissemination of best practices in inclusion and barrier-free education. It is also important to monitor and evaluate the effectiveness of educational and social programs, which allows for strategy adjustments and the development of new practices based on empirical evidence.

CONCLUSIONS

A synthesis of the results indicates that psychosocial factors exert a significant influence on the development of inclusivity and barrier-free environments within the student community. Personal resources—such as empathy, tolerance, and communicative skills—alongside the social environment, participation in student communities, family values, the educational context, and the experience of navigating crisis conditions, shape practical skills and confidence in inclusive behavior. While crises create additional barriers, they simultaneously act as a catalyst for social empathy, solidarity, and collective support. The findings emphasize the necessity of a comprehensive approach to developing an inclusive culture in higher education, one that integrates students' personal development, the creation of conditions for social integration, and structural changes within the educational environment. These conclusions hold practical value for universities, student support services, and educational policymakers, while also opening new perspectives for further research aimed at refining the psychosocial mechanisms involved in fostering accessibility and inclusivity.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Clinical, endoscopic, morphological and microbiological characteristics of diverticular disease in patients with metabolic disorders

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ABSTRACT

Aim: The aim of the study was to identify the clinical course and endoscopic activity features in patients with uncomplicated diverticular disease (UDD) associated with diabetes mellitus (DM), overweight, and obesity and perform an analysis of the gut microbiome and morphological characteristics of the colonic mucosa in such patients.

Materials and Methods: 259 patients with UDD, hospitalized in the Department of Gastroenterology of Feofaniya Clinical Hospital of the State Administration of Affairs during the period of 2020-2024, were included in the study. Among all patients with DD included in the study, 43 had no metabolic disorders (MD) (DM, overweight, obesity), while 216 presented with varying degrees of MD. All patients underwent total colonoscopy with assessment of endoscopic activity of diverticular inflammation using the Diverticular Inflammation and Complication Assessment (DICA) score. The clinical course of DD was evaluated according to the classification proposed by the German Society of Gastroenterology, Digestive and Metabolic Diseases and the German Society of General and Visceral Surgery in 2021. The microbiome analysis was conducted only in 172 patients who had the financial means to undergo this test. During endoscopic examination of the colon in all patients, mucosal biopsies were obtained from the diverticular orifice. Histological, histochemical, and immunohistochemical methods were used. The obtained digital indicators in the groups were analyzed using statistical methods.

Results: This study evaluated clinical, endoscopic, morphological, and microbiological characteristics of DD in patients with metabolic disorders. A recurrent course of diverticular inflammation was observed more frequently in patients with metabolic dysfunctions. Endoscopic assessment of inflammatory activity correlated with histological changes of colon mucosa and alterations in mucin expression (MUC2 and MUC4). Microbiological analysis revealed a reduction in butyrate-producing flora (*Akkermansia muciniphila*, *Faecalibacterium prausnitzii*) with predominance of *Bacteroidetes* in metabolic disorder patients.

Conclusions: The clinical, morphological, microbiological and endoscopic features of DD identified by the authors highlight pathophysiological links between metabolic dysfunction (DM, overweight, obesity) and DD progression.

KEY WORDS: diverticular disease, clinical and endoscopis features, diabetes mellitus, obesity, gut microbiota, colon mucosa morphology

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INTRODUCTION

Diverticulosis and DD are common gastrointestinal conditions worldwide, with an increasing prevalence and a significant financial burden on healthcare systems in every country. It is estimated that one in four to five individuals will develop diverticulosis or diverticulitis during their lifetime [1]. Traditionally, DD was considered a disease of the elderly; however, over the past decades, its incidence has risen among younger individuals. Contributing factors of DD include population aging, Westernized diet, sedentary lifestyle, overweight and obesity, as well as the widespread use of screening colonoscopy. Certain comorbidities, particularly DM, may promote diverticula formation and influence the inflammatory activity and clinical course of DD.

Epidemiological data on the prevalence of DD in patients with DM remain inconsistent. Danish cohort study demonstrated a slightly higher incidence of DD among patients with type 2 diabetes (0.76 vs. 0.54 events per 1000 person-years) compared to non-diabetic individuals, with the risk increasing with longer disease duration. However, after adjusting for body mass index (BMI), DM was paradoxically associated with a significantly lower risk of DD, suggesting that patients with lower BMI carry a reduced risk. One possible explanation is lifestyle modification required in diabetes management [2], as well as metformin use, which has been shown to reduce the risk of acute diverticulitis [3].

The association between DD and metabolic disorders is further supported by genetic studies. Genome-wide

association studies suggest a causal relationship between type 2 diabetes, higher BMI, smoking, and increased risk of DD [4]. Mendelian randomization analyses demonstrated that genetic predisposition to type 2 DM is associated with higher risk of DD [5].

DM clearly induces physiological changes in the colonic wall that favors diverticula formation. Most importantly, colonic motility is impaired [6]. Autonomic diabetic neuropathy leads to delayed colonic transit and increased retrograde movements, promoting stasis and elevated intraluminal pressure [7]. Furthermore, accumulation of advanced glycation end-products in extracellular matrix proteins, such as collagen and elastin, increases tissue stiffness, reduces elasticity, and disrupts cell-matrix interactions [8]. Both increased intraluminal pressure and these structural wall alterations are central to diverticula formation.

DM also worsens the course of acute diverticulitis. Patients with DM exhibit more advanced inflammation according to Hinchey classification and higher complication rates. They are more frequently subjected to surgery (46.9% vs. 15.5% in non-diabetics) and have longer hospital stays [9]. Interestingly, DM increases the risk of diverticular bleeding in acute diverticulitis but does not significantly influence the risk of abscess formation, bowel obstruction, or colectomy. Moreover, complicated diabetes is associated with longer hospitalizations and higher healthcare expenditures related to diverticular disease [10].

The mechanisms underlying the more severe clinical course of DD in the context of metabolic disorders are multifactorial. Experimental animal studies demonstrated that DM disrupts intestinal barrier function, leading to reduced mucus layer thickness, impaired tight junction integrity, and development of "leaky gut." These changes are accompanied by low-grade chronic inflammation, severe dysbiosis with predominance of proinflammatory Proteobacteria, and Paneth cell depletion [11]. A defective mucosal barrier allows translocation of bacterial wall components, such as lipopolysaccharides, which activate Toll-like receptor 4. This receptor triggers downstream signaling cascades that stimulate production of proinflammatory cytokines, including TNF-α, IL-6, IL-8, and IL-12, resulting in both local and systemic inflammation [12].

Most available scientific publications have focused on the clinical course of acute and complicated diverticulitis in diabetic patients.

AIM

The aim of the study was to identify the clinical course and endoscopic activity features in patients with UDD associated with DM, overweight, and obesity and perform an analysis of the gut microbiome and morphological characteristics of the colonic mucosa in such patients.

MATERIALS AND METHODS

259 patients with UDD, hospitalized in the Department of Gastroenterology of Feofaniya Clinical Hospital of the State Administration of Affairs during the period of 2020-2024, were included in the study. The cohort comprised 101 men and 158 women. All patients had BMI calculated, and all underwent total colonoscopy with assessment of endoscopic activity of diverticular inflammation using the DICA score.

The clinical course of DD was evaluated according to the classification proposed by the German Society of Gastroenterology, Digestive and Metabolic Diseases and the German Society of General and Visceral Surgery in 2021 [13]. According to this classification, patients were divided into the following groups: diverticulosis as an incidental finding during screening colonoscopy; acute uncomplicated diverticulitis (without pericolic phlegmonous reaction); chronic forms of DD: symptomatic uncomplicated DD and recurrent course (recurrent inflammation within one year).

The microbiome analysis was conducted only in 172 patients who had the financial means to undergo this test. The intestinal microbiome was assessed by qRT-PCR using primers targeting the 15S rRNA gene. Quantitative determination of bacterial taxa was performed by qPCR with primers specific for *Firmicutes*, *Bacteroidetes*, *Akkermansia muciniphila*, and *Faecalibacterium prausnitzii*, as well as universal bacterial primers (Table 1).

Clinical specimens were processed with a lysis buffer in the presence of silica particles as sorbent. This procedure resulted in the disruption of cell membranes, viral envelopes, and other biopolymeric complexes, leading to DNA release. In the presence of the lysis buffer, the liberated DNA bound to silica particles, while other components of the lysed clinical material remained in the solution and were removed by centrifugation and subsequent washing. The addition of elution buffer caused the transfer of DNA from the silica surface into the solution, which was separated from the sorbent particles by centrifugation. As a result, highly purified DNA free of PCR inhibitors was obtained, ensuring high analytical sensitivity of the amplification reaction.

During endoscopic examination of the colon, mucosal biopsies were obtained from the diverticular orifice. Biopsy samples were fixed in 10% neutral buffered formalin (pH 7.4) for 24-48 hours. After fixation, tissue processing was performed using an *Excelsior AS* processor (Thermo Fisher Scientific, UK), followed by paraffin embedding with the *HistoStar* embedding system

Table 1. Characteristics of bacterial primers

Target flora	Primer sequences
Bacteroidetes	798ebF AAACTCAAAKGAATTGACGG (Forward) cfb967R GGTAAGGTTCCTCGCGTAT (Reverse)
Firmicutes	928F-Firm TGAAACTYAAAGGAATTGACG (Forward) 1040FirmR ACCATGCACCACCTGTC (Reverse)
Akkermansia muciniphila	CAGCACGTGAAGGTGGGGAC (Forward) CCTTGCGGTTGGCTTCAGAT (Reverse)
Faecalibacterium prausnitzii	GGAGGAAGAAGGTCTTCGG (Forward) AATTCCGCCTACCTCTGCACT (Reverse)
Universal	926F AAACTCAAAKGAATTGACGG (Forward) 1062R CTCACRRCACGAGCTGAC (Reverse)

Source: compiled by the authors of this study

Table 2. Clinical features of DD in the examined patients

	DD	%	DD-MD	%	DD+MD	%
Acute diverticulitis	23	8.9	10	23.3	13	6.0
Recurrent diverticulitis	55	21.2	3	7.0	52	24.1
Diverticulosis	47	18.2	17	39.5	40	18.5
Symptomatic uncomplicated DD	134	51.7	13	30.2	111	51.4
Total	259	100	43	100	216	100

Source: compiled by the authors of this study

(Thermo Fisher Scientific, UK). Serial histological sections of 2–3 μm thickness were prepared and stained with hematoxylin and eosin. Periodic acid-Schiff (PAS) reaction was also carried out.

Immunohistochemical analysis was performed on adhesive slides (*Super Frost Plus*, Menzel, Germany). For antigen epitope retrieval, citrate buffer (pH 6) and EDTA buffer (pH 8) were used. The HRP UltraVision Quanto detection system and DAB Quanto chromogen (Thermo Fisher Scientific, USA) were applied. Immunohistochemistry was performed with mouse monoclonal antibodies against MUC2 (clone Ccp58, Master Diagnostica, Spain) and MUC4 (clone 8G7, Master Diagnostica, Spain).

The slides were examined with a *ZEISS Primostar 3* microscope (Carl Zeiss, Germany) equipped with an integrated digital color camera, and a *BRESSER Science TFM-301 Trino* microscope with a *BRESSER Full HD* camera (Bresser GmbH, Germany).

Statistical analysis was performed using *STATISTICA 12* (StatSoft Inc.). Data accumulation, correction, and systematization, as well as visualization of results, were performed with *Microsoft Excel 2015*. Descriptive statistics and variation analysis were applied. Pearson's Chi-square (χ^2) test was used to evaluate differences in categorical variables. For small sample sizes (≤5), Fisher's exact test was applied. A significance threshold of $p<0.05$ was considered statistically significant, with a confidence level not lower than 95%.

RESULTS

Among 259 patients with DD included in the study, 43 (16.6%) had no MD (DD-MD), while 216 (83.4%) presented with varying degrees of MD (DD+MD) (58 (26.9%) patients – DM, 158 (73.1%) patients – overweight or different grades of obesity. Patients with DD were predominantly individuals over 60 years old with a mean age of 65.6±1.45 years. In all groups the number of women was greater ($p<0.05$) than the number of men (Fig. 1).

In all groups diverticula were predominantly localized in the left colon (Fig. 2). Single and multiple diverticula were detected in patients of all groups. The number of cases with single and multiple diverticula did not differ ($p>0.05$) in all groups (Fig. 3).

In patients with DD and MD, a recurrent course of diverticular inflammation was significantly more common – 52 (24.1%) vs. 3 (7.0%) in patients without MD ($p<0.05$). Symptomatic uncomplicated DD was also more frequently diagnosed in patients with metabolic disturbances – 111 (51.4%) vs. 13 (30.2%) ($p<0.05$). At the same time, acute diverticulitis was identified in 10 (23.3%) patients with normal BMI and without DM, compared to 13 (6.0%) in the comparison group ($p<0.05$) (Table 2).

Patients with DD demonstrated disturbances in the population of butyrate-producing bacteria. A normal level of *Akkermansia muciniphila* was found in 19.4% (n=29) of patients with MD and in 45.5% (n=10) of patients with normal BMI ($p<0.05$). A re-

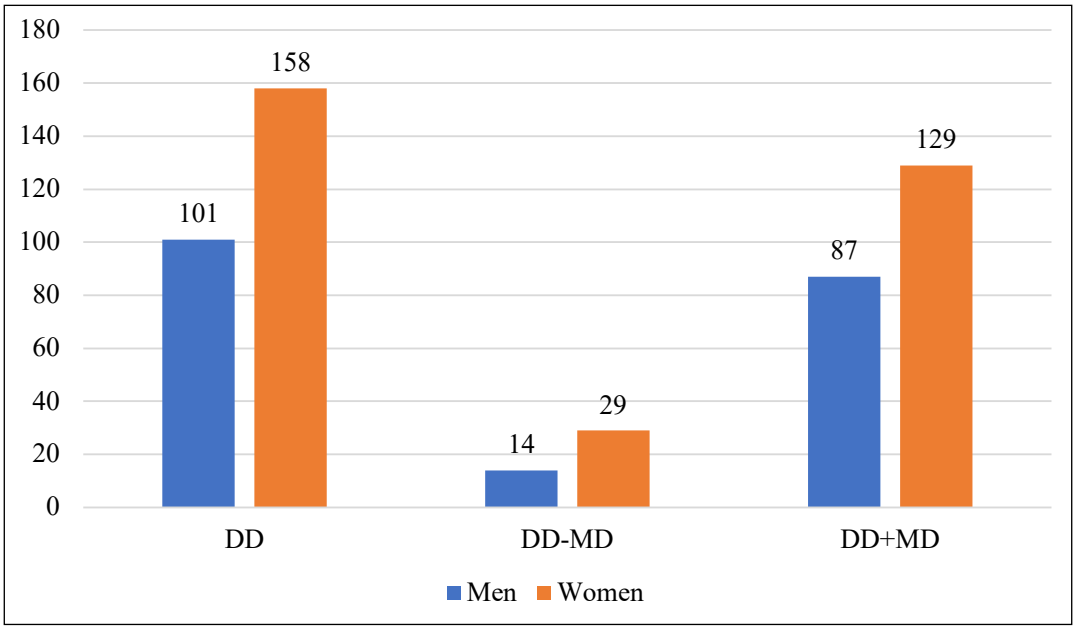

Fig. 1. Gender characteristics of patients with DD
Source: compiled by the authors of this study

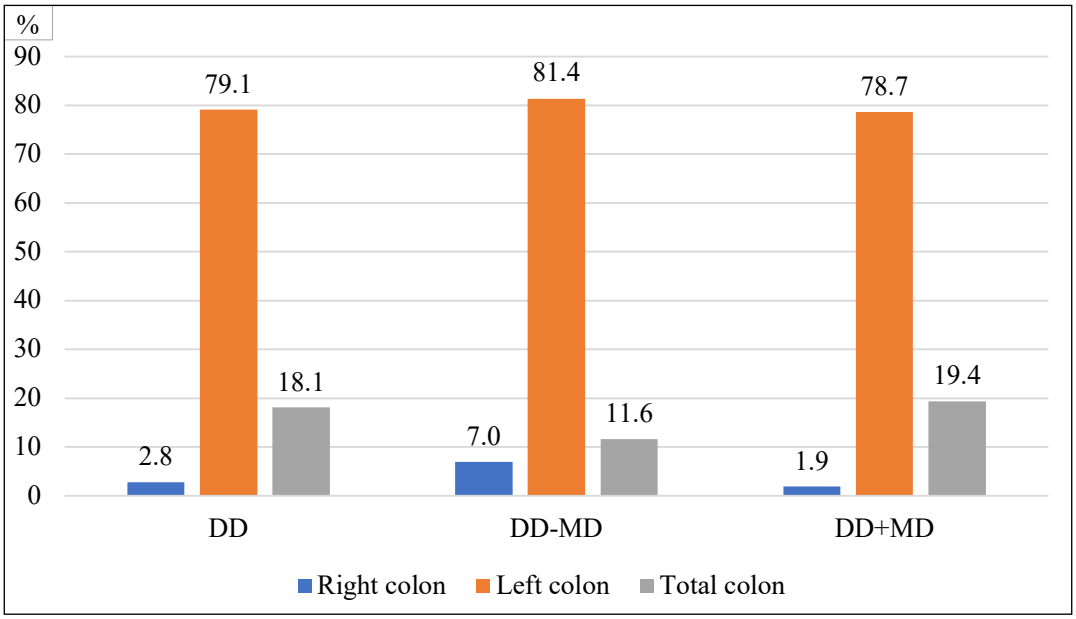

Fig. 2. Localization of diverticula in patients with DD
Source: compiled by the authors of this study

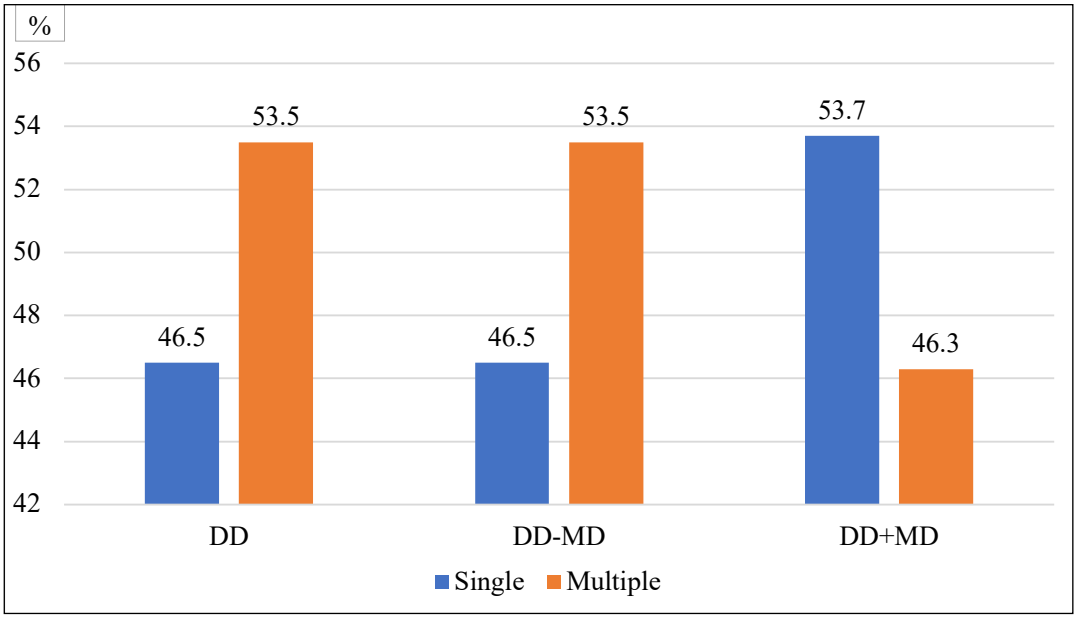

Fig. 3. Number of diverticula in patients with DD
Source: compiled by the authors of this study

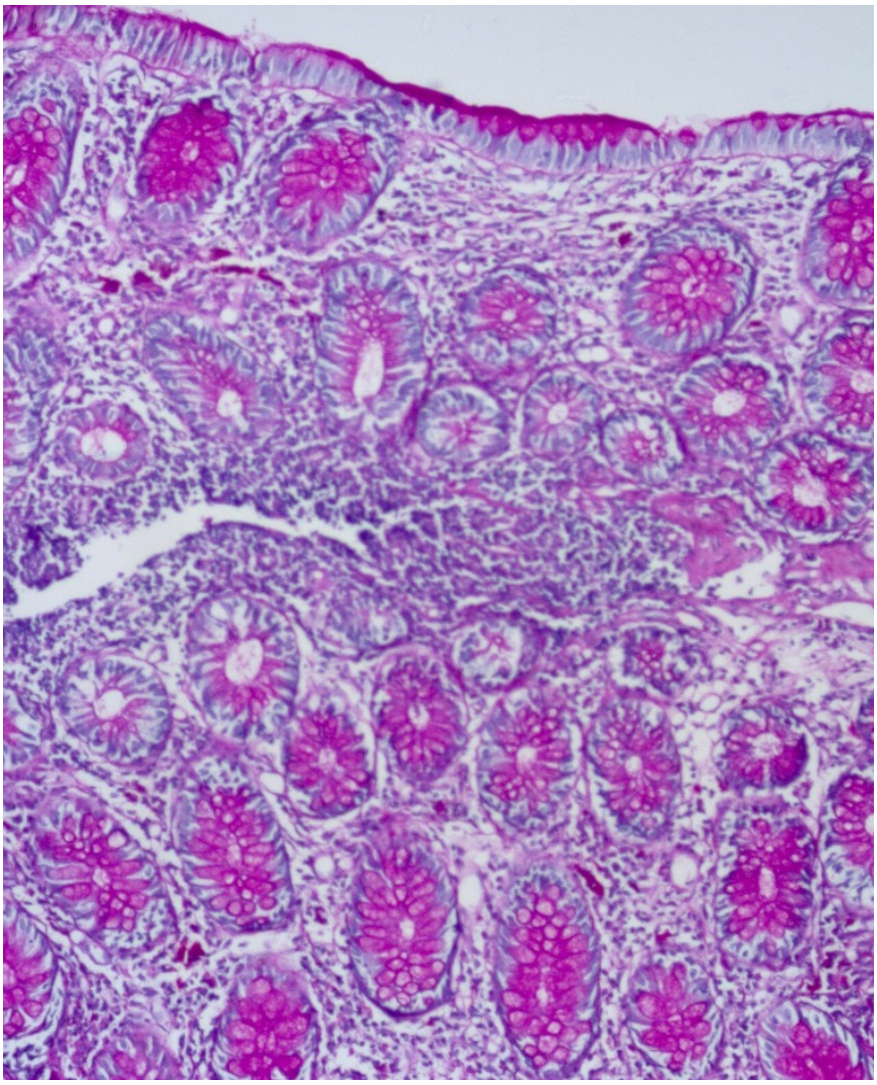

Fig. 4. Decreased PAS reaction intensity in a patient with DD, DM and obesity. PAS reaction, ×100
Picture taken by the authors

duction of *Akkermansia muciniphila* (<10^{10} CFU/g) or its absence was detected in 39.3% (n=59) and 41.3% (n=62) of patients with DM and high BMI, respectively, and in 31,8% (n=7) and 22.7% (n=5) of patients without MD.

A more pronounced deficiency of *Faecalibacterium prausnitzii* (<10^{10} CFU/g) was observed in patients with diverticular inflammation and MD compared to the control group: 41.3% (n=62) vs. 22.7% (n=5).

The *Firmicutes/Bacteroidetes* ratio <1.0 was found in 44.5% (n=10) of patients with DD without metabolic disturbances and in 74.0% (n=111) of patients with MD (p<0.05).

It should be noted that the intensity of colonic mucosal inflammation differed among DD patients of different groups. The mean DICA endoscopic activity score of diverticular inflammation in patients with MD was 1.23±0.23, while in patients with DD without metabolic disturbances it was 0.97±0.35. This correlated with a greater inflammatory intensity on histological examination. Hematoxylin and eosin staining of colonic biopsies in patients with DD and metabolic dysfunction more frequently revealed a denser inflammatory cell infiltrate with neutrophils compared to patients with isolated DD.

A decrease in butyrate-producing microflora in patients with diverticular inflammation caused the alteration of the morphofunctional state of the colonic mucosal barrier. These changes manifested by a reduction in mucus layer thickness, decreased number of goblet cells and their vacuole size, reduced mucus production capacity, as well as alteration in the mucin profile (changes in MUC2 and MUC4 expression). Analysis of PAS reaction demonstrated a decrease in its intensity in DD patients with type 2 diabetes, particularly in combination with obesity (Fig. 4).

Furthermore, analysis of mucin expression in the colonic mucosa revealed that patients with symptomatic uncomplicated DD and MD demonstrated reduced and heterogeneous expression of MUC2 and MUC4. In contrast, in DD patients without metabolic disturbances, alteration in mucin expression were minimal (Fig. 5, 6).

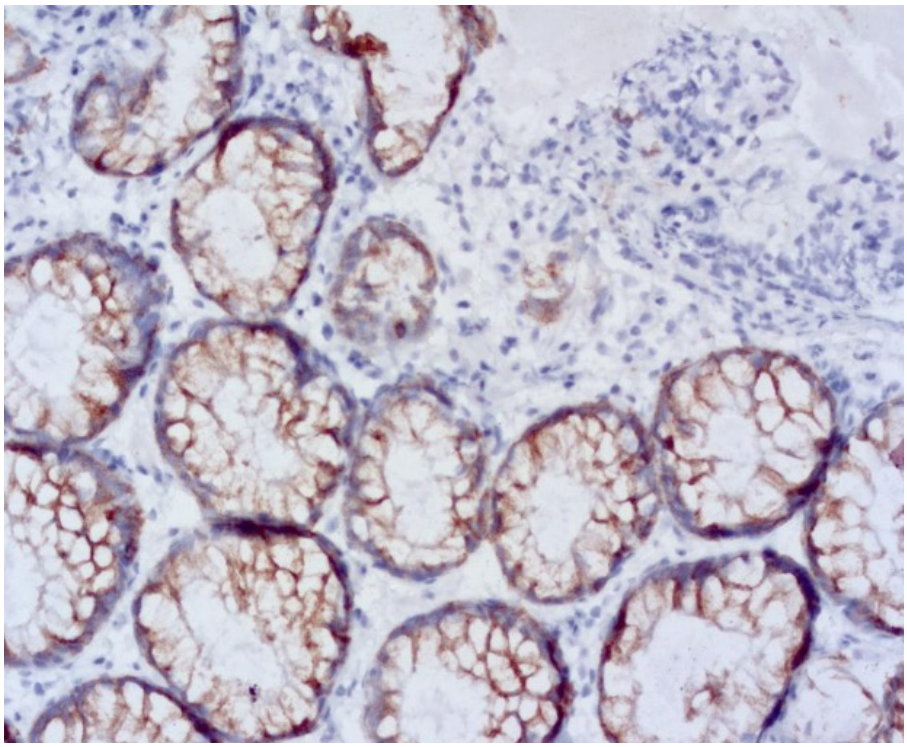

Fig. 5. Reduced and heterogeneous expression of MUC2 in the colonic mucosa of a patient with DD and MD. Immunohistochemical reaction with monoclonal antibody against MUC2, ×200
Picture taken by the authors

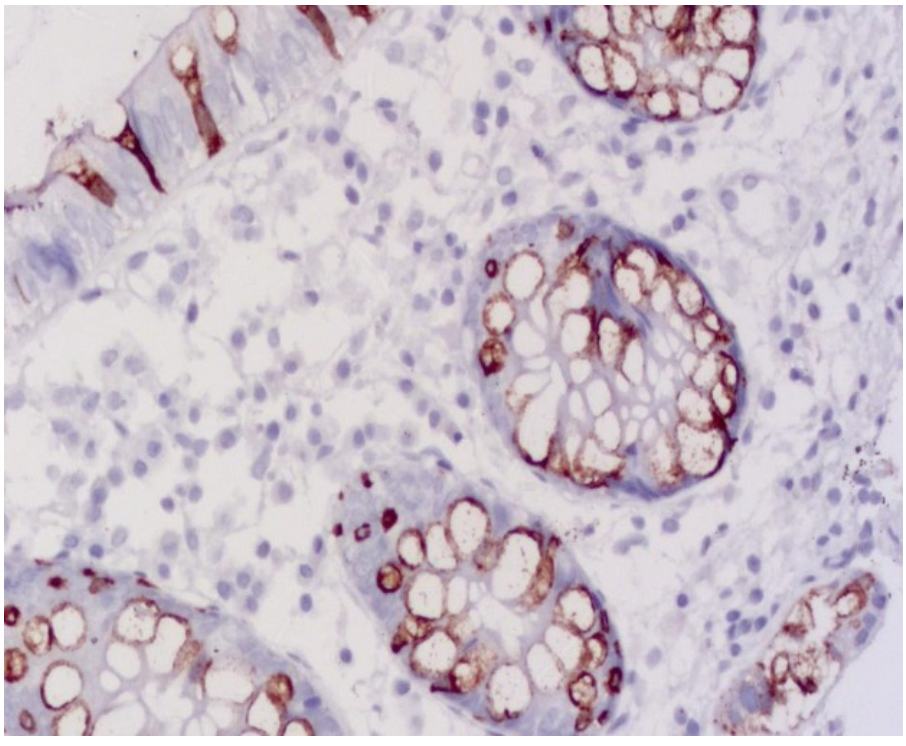

Fig. 6. Reduced and heterogeneous expression of MUC4 in the colonic mucosa of a patient with DD and MD. Immunohistochemical reaction with monoclonal antibody against MUC4, ×200
Picture taken by the authors

DISCUSSION

In patients with DD and MD, distinct clinical, endoscopic, morphological, and microbiological features of the disease course were identified. A recurrent course of diverticular inflammation was more frequently observed in DD patients with metabolic disturbances [14].

Inflammatory activity in patients with diverticula can be assessed endoscopically. Although the endoscopic index of diverticular inflammation is rarely used in routine practice, it is valuable in predicting the risk of recurrence of DD exacerbations [15, 16]. In our study, patients with MD demonstrated higher endoscopic activity of diverticular inflammation compared with those with isolated DD. Increased endoscopic inflammation correlated with more pronounced histological inflammatory activity in patients with DD and metabolic disturbances, as well as altered mucus production and modification of the colonic mucus composition, includ-

ing decreased expression of MUC2 and MUC4. A higher DICA score in these patients may explain the recurrent course of DD in the setting of metabolic dysfunction.

The population of butyrate-producing bacteria in DD patients with metabolic disturbances reflected a general trend towards reduction, consistent with inflammatory conditions of the colon and metabolic dysfunction. A more pronounced deficiency of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* in DD patients with metabolic disturbances indicates a deeper impairment of the morphofunctional state of the colonic mucosal barrier. Reduced numbers of mucus-producing bacteria result in thinning of the mucus layer, decreased size and number of goblet cells with reduced mucus secretion capacity, and altered mucin profile (decreased MUC2 and MUC4 expression) [16-18].

The *Firmicutes/Bacteroidetes* ratio <1.0, reflecting predominance of *Bacteroidetes*, was detected more often in patients with DD and MD compared to patients without MD. Excessive abundance of *Bacteroidetes* has been associated with intestinal inflammation and its severity [14]. By contrast, *Firmicutes* enrichment is more characteristic for obesity and metabolic syndrome. Interestingly, in our cohort, *Bacteroidetes* predominance was observed among overweight and obese patients, supporting the concept of more active diverticular inflammation in this group.

CONCLUSIONS

A recurrent course of diverticular inflammation is more frequent in patients with MD. The level of butyrate-producing bacteria is significantly reduced in DD patients with metabolic disturbances, reflecting impaired morphofunctional status of the colonic mucosa. In patients with DD and MD, *Bacteroidetes* predominate, indicating higher inflammatory activity in the colon. A higher endoscopic index of diverticular inflammation, correlating with the intensity of histological changes and mucus production alterations (decreased MUC2 and MUC4 expression) may underlie the recurrent course of DD in patients with metabolic dysfunction.

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CONFLICT OF INTERESTS

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Paradigmatic divergences between Polish special education and contemporary psychiatry in the care of individuals with intellectual disability

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ABSTRACT

After the fall of communism, Polish psychiatry and special education underwent profound transformations, adopting different paradigmatic assumptions in their approach to intellectual disability. The aim of this study is to compare the theoretical foundations of both disciplines and analyze the effects of these discrepancies. Contemporary psychiatry and clinical psychology are based on the biopsychosocial paradigm, integrating biological factors with the environmental model and quantitative research, which ensures their presence in international scientific discourse. Polish special education, on the other hand, has adopted a humanistic paradigm, favoring qualitative methodologies and rejecting the biomedical perspective, which results in its methodological isolation and the neglect of the medical aspects of disability. Despite the converging practical goals of community psychiatry and pedagogy, there is a fundamental divergence between the disciplines that hinders cooperation. In conclusion, there is a need to establish a dialogue and develop a common conceptual framework that combines the medical basis of neurodevelopmental disorders with the humanistic dimension of support.

KEY WORDS: psychiatry, special pedagogy, paradigms, disability

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INTRODUCTION

Intellectual disability constitutes an area of scholarly and professional interest for representatives of multiple disciplines, including primarily psychiatry, clinical psychology, and special education, and to a lesser extent professionals from other fields such as physicians engaged in somatic medicine (specialists in pediatrics, internal medicine, and surgery, as well as their respective subspecialties), social workers, occupational therapists, nurses, medical caregivers, and numerous other allied health and support professions [1]. The perspectives of psychiatry, clinical psychology, and special education in the field of intellectual disability should be mutually complementary, forming a coherent continuum both in the domain of social practice and at the level of underlying paradigmatic frameworks [2]. However, contemporary Polish special education for individuals with intellectual disability is currently grounded in paradigmatic assumptions that differ fundamentally from those adopted in psychiatry and clinical psychology [3–5]. In particular, Polish special education has become substantially embedded in postmodern modes of thought, displaying notable affinities with the currents of antipsychiatry [4, 5]. In contrast, contemporary Polish psychiatry and clinical psychology are constructed within the biopsychosocial paradigm, which assumes the equal importance of biological, psychological, and social factors in understanding the etiology, course, and consequences of mental disorders [6, 7].

AIM

The aim of this article is to compare the theoretical foundations of contemporary Polish special education for individuals with intellectual disability with the conceptual framework of modern psychiatry, and to a lesser extent, clinical psychology.

REVIEW AND DISCUSSION

CONTEMPORARY PSYCHIATRIC AND CLINICAL PSYCHOLOGICAL PERSPECTIVES ON INTELLECTUAL DISABILITY

Contemporary psychiatry and clinical psychology conceptualize intellectual disability as a neurodevelopmental disorder characterized by an intelligence quotient that is at least two standard deviations below the population mean, accompanied by significant limitations in adaptive functioning [8]. Intellectual disability is formally recognized in all major diagnostic classification systems, including the ICD-10, DSM-5, and ICD-11 (where it is classified as disorders of intellectual development) [9]. According to the biopsychosocial paradigm that underpins modern medicine, every mental disorder emerges from the complex interaction of biological, psychological, environmental, and social factors [7]. Only an integrative consideration of these dimensions provides a comprehensive and scientifically valid understanding of the phenomenon of intellectual disability. The assessment of intellectual functioning and adaptive abilities is conducted using standardized psychometric instruments designed to identify an individual's capacities and resources rather than merely their deficits. Across all three classification systems, intellectual disability is stratified into mild, moderate, severe, and profound levels of impairment [9]. Polish psychiatry has historically remained largely independent of Soviet ideological influence, and the scientific contributions of Polish psychiatrists have consistently been represented in international scholarly literature [10, 11]. In the 1960s and 1970s, Western psychiatry experienced the emergence of the antipsychiatry movement, influenced by postmodern philosophical currents and challenging traditional conceptions of mental illness [12]. This movement contributed to a profound transformation in psychiatric practice, including a reduction in long-term hospitalization within total institutions such as psychiatric hospitals, in favor of community-based models of care emphasizing treatment and support within the patient's social environment [12]. The resulting concept of community psychiatry represents a Hegelian synthesis of twentieth-century biological psychiatry with the critical postulates of antipsychiatry [13]. Contemporary psychiatric reform in Poland is progressively oriented toward the community psychiatry model, which empirical evidence identifies as the most effective and efficient framework for supporting individuals with mental disorders [14]. Parallel to the development of community psychiatry, the concept of neurodiversity has emerged, framing individuals with intellectual disability as a minority group characterized by atypical neurodevelopment relative to the majority population [15]. The neurodiversity framework encompasses a range of phenomena included in psychiatric classifications, such as intellectual disability, autism spectrum disorder, attention-deficit/hyperactivity disorder, and numerous related conditions. In addition, it extends to traits such as left-handedness, non-heteronormative sexual orientation, and gender non-binary identity - phenomena that are no longer classified as psychiatric disorders and are now regarded as variations within the broad spectrum of human normativity [16,17]. In summary, within the biopsychosocial paradigm, intellectual disability is understood as a neurodevelopmental phenomenon formally recognized in psychiatric classification systems, which under specific social conditions may become functionally disabling for the individual. This functional impairment necessitates appropriate environmental support and, in certain cases, targeted medical intervention.

PARADIGMS OF CONTEMPORARY POLISH SPECIAL EDUCATION

Special education for individuals with intellectual disability (formerly referred to as oligophrenopedagogy) has undergone continuous evolution with respect to its paradigmatic foundations, applied terminology, teleological assumptions, and forms of education, rehabilitation, and revalidation [4,5]. Within this field, ongoing efforts are devoted to the identification of new research domains and the development of pedagogical practices that are optimally suited to the needs of individuals with intellectual disability. During the communist period in Poland, the dominant paradigm in special education was dialectical materialism in its Soviet interpretation. The political and social transformation of 1989 brought about a fundamental change in the conceptualization of intellectual disability [3, 18]. The abandonment of dialectical materialism was followed by a phase of methodologically fragmented empirical research conducted on small samples within a positivist framework. This form of academically isolated "micro-research," largely devoid of coherent theoretical grounding, generated significant tension within the community of special education scholars [3,18].

The introduction of postmodern educational theory into Polish pedagogy in 1993 by Tomasz Szkudlarek marked a decisive turning point. As Polish special education increasingly adopted postmodern epistemological assumptions, it shifted away from quantitative methodologies toward qualitative research grounded in social constructionism [19, 20]. These transformations were systematically synthesized by Amadeusz Krause - drawing on analogous analyses within German-language scholarship - through the formulation of the so-called humanistic paradigm of special education, composed of four interrelated microparadigms [3-5]. The paradigmatic transformation was further reinforced by the activities of non-governmental organizations and public discourse, including emerging online forums [21]. Equally important was the growing role of persons with disabilities and their families, who began establishing foundations and associations exerting substantial influence on public opinion. Parallel processes of social self-organization occurred among previously marginalized groups, including women, gay, bisexual, lesbian, and transgender individuals, who publicly asserted their normality, autonomy, dignity, and claims to rights, recognition, and respect. These movements developed spontaneously, largely independent of grand ideological frameworks, established academic authorities, or traditional institutions of social legitimacy. Postmodern philosophy proved particularly adept at capturing this emergent social transformation by responding to the needs of newly forming identity groups. Changes in Polish special education extended beyond paradigmatic assumptions [21]. They encompassed substantial modifications in professional terminology (including the renaming of the subdiscipline itself), a broadening of the scope of pedagogical influence, the inclusion of individuals with severe and profound intellectual disability in educational and rehabilitative interventions, and a gradual transition from segregated schooling toward integrative and, increasingly, inclusive educational models [4, 5]. It was further recognized that in many cases the development of intellectual disability could potentially be prevented, or its impact substantially mitigated, provided that appropriate developmental conditions and supportive social environments were established [4, 5]. Within the humanistic paradigm of special education, four principal microparadigms are distinguished: the social model of disability, the normalization paradigm, the emancipatory paradigm, and the qualitative (interpretative) paradigm [3-5]. The social paradigm conceptualizes disability as a socially constructed condition arising from adverse social circumstances, thereby transforming individual impairment into a social problem [3-5]. The normalization paradigm emphasizes the necessity of adapting the environment of the individual with intellectual disability, rather than coercively adapting the individual to the standards of the able-bodied majority [22,23]. The emancipatory paradigm advocates the genuine emancipation of persons with intellectual disability [3–5], while simultaneously raising critical questions regarding the appropriate scope of autonomy and self-determination that can realistically be exercised by individuals with varying levels of cognitive functioning [22, 23]. The contemporary conceptualization of intellectual disability within this framework is inherently dynamic and affirms the fundamental right of each individual to self-determination [4, 5]. However, persons with moderate to profound intellectual disability are frequently deprived of meaningful opportunities for autonomous decision-making and rendered excessively dependent on others, a process that may lead to objectification and social marginalization [4, 5]. Numerous studies have demonstrated that well-intentioned caregiving practices may inadvertently inhibit personal development. Emancipation thus applies simultaneously to individuals with intellectual disability as a social group and to each person as a unique individual [4, 5]. The interpretative (qualitative) paradigm reflects a departure from quantitative methodologies in special education research in favor of qualitative approaches. Within the humanistic paradigm, biomedical research in special education has been criticized as positivist, non-hermeneutic, and insufficiently contributory to the understanding of disability as a social phenomenon [3]. The emergence of the humanistic paradigm in Polish special education - including special education for individuals with intellectual disability - represented a transformative development in the discipline [4, 5, 21]. It catalyzed significant improvements in the care and support of persons with intellectual disability, stimulated new research trajectories, and fostered innovative practical solutions. The contemporary departure from positivist discourse constitutes a central feature of current pedagogical transformation and a rejection of the previously dominant tradition of fragmented empirical "micro-research." This turn toward postmodernity also represents an intellectual resistance to the historically submissive and, at times, professionally compromising compliance of pedagogy with the grand ideologies of the twentieth century [3]. Ideological systems such as dialectical materialism, national socialism, and certain forms of confessional pedagogy sought to subordinate educational discourse to their utopian visions of society, often invoking the rhetoric of scientific method to legitimize their claims [3].

CRITIQUE OF THE HUMANISTIC PARADIGM OF SPECIAL EDUCATION FROM THE PERSPECTIVE OF CONTEMPORARY PSYCHIATRY

The positive changes that occurred in Polish special education for individuals with intellectual disability following the collapse of the communist system and the adoption of the humanistic paradigm as the dominant framework are not free from significant limitations and negative consequences. The most important of these may be summarized as follows.

MARGINALIZATION OF THE BIOMEDICAL PARADIGM AND METHODOLOGICAL ISOLATION

The rejection of the biomedical paradigm and the near-exclusive preference for qualitative research in special education, at the expense of quantitative methods, has led to a growing divergence between medicine and special education [24]. Special educators have insufficiently recognized that contemporary medicine differs fundamentally from twentieth-century medicine, in which disability was conceptualized primarily in terms of defect and disease [13]. Modern community psychiatry has moved away from this defectological mode of thinking by adopting the biopsychosocial paradigm in the understanding of intellectual disability. Quantitative research remains the cornerstone of medical sciences and dominates international high-impact scientific literature [25, 26]. In contrast, Polish special educators—who focus almost exclusively on paradigms, discourses, contexts, and qualitative inquiry—rarely publish in internationally indexed journals [25, 26]. As a result, their scholarly output remains largely unknown beyond national borders, conferring a local and peripheral character on Polish special education for individuals with intellectual disability [25, 26]. Moreover, special educators seldom participate in psychiatric and psychological conferences, while psychiatrists rarely attend events organized by pedagogical communities. Despite frequent declarations of interdisciplinarity and transdisciplinarity within special education, there is in practice a clear deficit of genuine scientific cooperation between Polish psychiatry and Polish special education for individuals with intellectual disability. The field thus remains enclosed within a hermetic, domestically oriented academic circle, and its achievements are poorly recognized within the international scholarly community.

DENIAL OF THE MEDICAL DIMENSION OF DISABILITY

The denial of disability as a medical phenomenon constitutes, in effect, a rejection of the very essence of special education itself [27, 28]. Disability is a conceptual category that emerges at the intersection of social sciences and medical sciences, and special education is "special" precisely because it addresses individuals with medical conditions [27, 28]. The rejection of this dimension therefore undermines the fundamental identity of the discipline. Disability develops as a social construct superimposed upon the individual's primary medical condition—in this case, the neurodevelopmental disorder of intellectual disability [27, 28]. For persons with disabilities, medical problems invariably represent the central dimension of their life situation. Failure to acknowledge the interdependence between medical and social aspects introduces a partially illusory distortion of social reality [27, 28]. Podgórska-Jachnik aptly captured this problem through the metaphor of the "pursuit of the disappearing subject": if the goal of pedagogy is the complete normalization of the environment and the full social participation of the individual, the individual paradoxically ceases to remain the subject of special educational concern.

DEFICIT OF PRACTICAL COMPETENCE IN PROFESSIONAL TRAINING

Pedagogical universities educating special educators - including professionals prepared to work with individuals with intellectual disability - place excessive emphasis on paradigms, discourses, contexts, and narratives [29,30]. While academic education necessarily requires solid theoretical foundations, these cannot replace the practical skills essential for effective work with persons with disabilities in real social environments [30]. Special educators require concrete professional competencies and social skills enabling them to respond effectively to the complex needs of individuals with disabilities. Yet methodological manuals are frequently dismissed by educators themselves as instruments of "coercive normalization." This raises a fundamental question: if not methodology, then what should replace it? Paradigms, discourses, and contexts cannot substitute for professional practical competence [30].

CONCLUSIONS

Polish psychiatry and Polish special education conceptualize intellectual disability in fundamentally different ways. In our view, there is a critical absence of a broad and sustained forum for intellectual exchange between these two scholarly communities. Although the objectives of community psychiatry and contemporary Polish special education for individuals with intellectual disability largely converge, they are grounded in divergent

paradigmatic assumptions. It would therefore be highly desirable for representatives of both subdisciplines to develop a shared conceptual framework encompassing the full spectrum of intellectual disability - from its medical foundations to its humanistic dimensions. We further express the hope that the scholarly contributions of Polish special educators working in the divergence from psychiatric perspectives, their work constitutes a valuable contribution that merits presentation to the global community of specialists.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

CONTENTS

Complex diagnostic approach in early manifestation of Crohn's disease in children

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ABSTRACT

Aim: To summarize current approaches to diagnosing and evaluating clinical features and disease course of Crohn's disease, and to provide competency-based practical recommendations for diagnostic assessment in children with early-onset disease.

Materials and Methods: Literature analysis was conducted using electronic databases (PubMed, Medline, the Cochrane Library) and the author's clinical cases of Crohn's disease in 7 children aged 1 year 10 months to 4.5 years. The diagnostic algorithm in young children included general clinical examination and laboratory and instrumental methods, including genetic testing and serological markers. Morphological verification of the diagnosis by histological examination of biopsy specimens is mandatory.

Conclusions: Delayed diagnosis of Crohn's disease in young children results from nonspecific symptoms, variable early manifestations, and prominent extraintestinal features. Early-onset disease poses a major diagnostic challenge and requires increased awareness of extraintestinal manifestations, perianal lesions, and growth failure. Serological tests serve as supportive tools and do not exclude the diagnosis when negative, particularly in children under 6 years. Genetic screening to rule out primary immunodeficiencies is an essential part of the diagnostic workup in this age group.

KEY WORDS: Crohn's disease, young children, clinical manifestations, diagnostic evaluation

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INTRODUCTION

Crohn's disease (CD) is a chronic transmural granulomatous inflammatory disease of the gastrointestinal tract (GIT) of multifactorial etiology, characterized by segmental involvement of various parts of the digestive tract. The disease may affect any segment from the oral cavity to the anal canal, leading to both local and systemic complications [1-3]. Particular attention of the global scientific community is focused on the phenotype with very early onset (Very Early-Onset Inflammatory Bowel Disease, VEO-IBD) occurring in children under 6 years of age. Within this group, infantile-onset disease (debut within the first 2 years of life) and neonatal-onset disease (debut within the first 28 days of life) are distinguished [4-7]. The incidence of CD in young children is estimated at 0.5–1.5 cases per 100,000 pediatric population, with a global trend toward increasing prevalence [8=10]. A distinctive feature of early-onset CD is not only the severity of gastrointestinal involvement but also a strong association with monogenic immune defects, positioning this pathology at the intersection of gastroenterology, immunology, and genetics [11-16]. Recently, an increasing proportion of CD has been observed within the structure of chronic inflammatory bowel diseases in early childhood [17].

At present, diagnosis of CD in young children remains challenging. An atypical clinical presentation, predominance of extraintestinal manifestations and growth retardation, and an aggressive disease phenotype often lead to delayed diagnosis [7, 18-20].

Delayed diagnosis increases the risk of severe disease course and development of serious complications [21]. *However, despite growing interest, the number of studies addressing this issue remains limited.*

Understanding the pathogenesis of CD in early childhood is essential for appropriate clinical management. Compared with older children, early-onset CD is characterized by a stronger genetic contribution, pronounced dysbiosis, and impaired immune regulation. Monogenic mutations affecting epithelial barrier function and immune response are frequently identified in children

under 6 years of age. Early-life dysbiosis is marked by reduced microbiome diversity. Impaired autophagy and phagocytic defects lead to ineffective pathogen elimination, resulting in chronic uncontrolled inflammation.

AIM

The aim of this study is to systematize current diagnostic approaches, assess clinical manifestations and features of the clinical course of CD, and provide practical recommendations using a competency-based approach for diagnostic evaluation in cases of early disease manifestation in children.

MATERIALS AND METHODS

A literature review was conducted using the electronic databases PubMed, Medline, and the Cochrane Library, as well as the author's own clinical cases of CD in young children. The clinical manifestations, disease course, and diagnostic findings were analyzed in 7 children aged from 1 year 10 months to 4.5 years, including 5 boys (71.43%) and 2 girls (28.57%). The study included patients with early-onset Crohn's disease who were monitored and treated in pediatric surgical departments in Kyiv and Vinnytsya during the period of 2020 – 2025.

The diagnosis of early-onset CD was based on the Porto criteria (ESPGHAN) adapted for early childhood [22-24]. Diagnostic evaluation included general clinical examination, laboratory tests, and instrumental investigations.

The general clinical examination included a thorough collection and analysis of anamnestic data, assessment and analysis of the clinical manifestations of the pathology, dynamic follow-up of patients with consideration of changes in gastroenterological and extraintestinal symptoms, evaluation of physical and somatic status, as well as the nature and frequency of bowel movements.

Laboratory investigations included assessment of general and specific inflammatory markers (fecal calprotectin, anti-Saccharomyces cerevisiae antibodies) [25], microbiological stool analysis [26, 27]. An extended immunological evaluation was performed to assess the status of all components of the immune system [28]. In cases of suspected primary immunodeficiency, genetic testing using targeted gene panel sequencing was performed [29].

A mandatory diagnostic measure was the use of endoscopic examination methods (esophagogastroduodenoscopy and ileocolonoscopy) with morphological verification of the diagnosis through histological examination of clinical biopsy specimens [30, 31].

The gold standard for the diagnosis of CD in young children for assessing the condition of the small intestine is magnetic resonance enterography, which provides optimal visualization, enables evaluation of bowel wall thickness and detection of fibrotic changes, while avoiding radiation exposure.

Radiological methods such as irrigography and fistulography were also applied.

In cases of perianal disease, examination under general anesthesia and perineal CT scanning were performed.

The work is a fragment of the scientific-technical work of the Department of Children's Surgery of National Pirogov Memorial Medical University, Vinnytsya «Development of modern and improvement of existing methods of diagnosis, treatment, prevention and rehabilitation of surgical pathology in children» (state registration number 0123U102436).

The research was carried out in accordance with the principles of the Helsinki Declaration of the World Medical Association on the ethical principles of conducting scientific medical research with human participation, approved by the ethics and bioethics committees of the University KROK, Educational and Scientific Institute of Medicine. and National Pirogov Memorial Medical

University, Vinnytsya.

Written informed parental consent for participation in the study was obtained.

REVIEW AND DISCUSSION

The main complaints included chronic diarrhea associated with recurrent respiratory infections, relapsing disease course with a short-term positive effect of conservative therapy, very early disease onset (within the first year of life), and a wide range of extraintestinal and systemic manifestations. Diarrhea in infants with CD persisted for more than 6 weeks, without blood admixture, and was accompanied by abdominal pain.

At admission, the children were in severe condition due to pronounced cramping abdominal pain, intoxication syndrome, and anemia. The abdomen was moderately distended and painful on palpation, with marked intestinal rumbling. Bowel movements occurred up to 10 or more times per day, both during the daytime and at night. Mucus admixtures in the stool were observed, without blood.

Extraintestinal manifestations (EIMs) were observed in all children included in the study and often preceded intestinal symptoms, complicating diagnosis. In one child, erythema nodosum (painful red nodules on

the anterior lower legs) was observed, which showed a close correlation with exacerbations of intestinal disease. In two children, EIMs included eye pain and redness, photophobia, which required urgent consultation with an ophthalmologist; anterior uveitis was diagnosed. Primary sclerosing cholangitis/autoimmune hepatitis occurred in association with ileocecal CD in one 3-year-old child [32]. In three boys (43%), perianal lesions were characterized by deep fissures, fistulas, abscesses, and skin tags. Perianal changes were the only manifestation of CD for a prolonged period, until colitis was detected 1.5–2 years after the onset of perianal lesions.

All patients exhibited growth retardation, which was caused not only by malabsorption but also by the direct effect of proinflammatory cytokines (TNF-α, IL-6) on the bone growth plate and the growth hormone axis, as well as by weight deficit and iron-deficiency anemia.

Laboratory findings showed significantly elevated inflammatory markers (CRP, ESR, thrombocytosis) in all patients. Complete blood count findings included severe anemia in all children, leukocytosis, toxic granulation of leukocytes, and increased ESR. Biochemical blood tests showed hypoproteinemia, elevated C-reactive protein levels, and decreased serum iron. When assessing fecal calprotectin levels, it is important to remember that normal values in infants during the first year of life are higher (<350 μg/g) than in adults (<50 μg/g). In the studied group, fecal calprotectin levels (measured by ELISA) were elevated in all children, ranging from 200 to 250 μg/g.

Serological markers assist in differentiating CD from ulcerative colitis and in predicting disease course; however, their informativeness in children under 6 years of age is limited due to immaturity of the immune response. The main laboratory marker of CD is antibodies to *Saccharomyces cerevisiae* (ASCA), represented by IgG and IgA classes. Positive ASCA results are associated with CD only when correlated with clinical presentation and endoscopic findings. In the studied group, positive ASCA results were obtained in only 4 of 7 children (57.1%). Serological diagnostics is an auxiliary tool; a negative result does not exclude the diagnosis, especially in children under 6 years of age.

Regarding genetic testing, all children with early disease onset underwent molecular genetic testing to detect specific gene mutations. However, the diagnosis was established comprehensively, taking into account clinical symptoms, laboratory findings, and results of instrumental investigations.

Microbiome studies in young children with CD revealed a reduction in bifidobacteria and lactobacilli, which directly correlates with inflammatory damage to the intestinal mucosa and leads to impaired protective mechanisms, increased intestinal permeability, and initiation of systemic inflammation.

Ultrasound examination of the abdominal organs revealed marked intestinal meteorism, moderate thickening, and infiltration of the wall of the descending and transverse colon as well as the cecum.

Esophagogastroduodenoscopy (EGD) and ileocolonoscopy with multiple biopsies are mandatory and constitute the basis for diagnostic verification. During EGD, the gastric mucosa was focally hyperemic, edematous, with isolated hemorrhages. Colonoscopy revealed hyperemic mucosa with an enhanced vascular pattern, fibrinous deposits in the lumen, moderate edema, and mucus clots; in three patients, multiple erosions of the distal colon were observed.

According to the diagnostic evaluation, lesion localization revealed ileocolitis in 4 children (57.1%) and pancolitis with perianal involvement in 3 children (42.9%). In all patients, the diagnosis was morphologically verified.

At present, the diagnosis of early-onset CD remains a challenging issue. The diagnostic findings indicate an aggressive disease phenotype in this patient population, with high inflammatory activity and extensive pathological involvement.

Timely and adequate diagnostic evaluation of CD in children with early manifestation is the basis for initiating treatment, as therapeutic strategy depends on diagnostic findings, taking into account age, phenotype, and the presence of complications. Achieving deep remission (clinical and endoscopic) is possible only with appropriate selection of therapy involving a multidisciplinary team of physicians.

CONCLUSIONS

- Delayed diagnosis of CD in young children is caused by the absence of specific symptoms, diversity of early clinical manifestations, and the presence of extraintestinal and systemic features.
- CD in early childhood represents a significant diagnostic challenge and requires heightened clinical awareness of extraintestinal manifestations (arthritis, erythema nodosum, growth retardation) and perianal lesions.
- Serological testing is an auxiliary diagnostic tool; negative results do not exclude the diagnosis, especially in children under 6 years of age.
- Genetic screening to exclude primary immunodeficiencies is a mandatory component of diagnostics in this age group.

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REVIEW ARTICLE

CONTENTS

Fourth generation human rights in the context of health care

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ABSTRACT

Aim: The aim of the article is to analyze the place and development of the right to health care in the fourth generation concept of human rights, to study the relationship between the formation of new generation rights and the development of science and technology, to investigate the current state of recognition and consolidation of the new concept of rights at the international level and some aspects of application of related international legislation in practice.

Materials and Methods: The authors study the right to health care, its interaction with new technologies and its evolution in the context of scientific and technological progress. General scientific methods such as systematic analysis and dialectical methods were used for the study. The research is based on international legal acts and agreements, scientific articles, and the case law of the ECHR.

Conclusions: In the process of the study, the authors conclude that the main idea of the concept of fourth-generation rights is to find a balance between the introduction of the benefits of technological progress into human life and human dignity and identity. Also, the authors conclude that the classical three generations of rights are insufficient to solve all the problems of modern legal science. Finally, the authors conclude that some important issues of modern law, such as euthanasia and assisted suicide, are not recognized and internationally enshrined in any way.

KEY WORDS: right to health care, fourth generation of human rights, technological development

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INTRODUCTION

Modern technological development is breathtaking in its speed and coordination of changes. New biotechnologies, genetic engineering, and neurotechnologies make possible what was only yesterday the stuff of science fiction writers. But at the same time, the rapid development of technology and science raises many complex issues and challenges in the field of ethics, philosophy, and law. It is believed that today we are already on the verge of the fourth industrial revolution, which is ready to completely change our way of life [1]. One of the most likely directions of these changes may be the widespread introduction of cyber-physical systems, i.e. the integration of electronic computing machines with objects of the surrounding world, including biological ones. This direction of development of modern science alone can raise a number of issues and create a lot of challenges for legal science. However, it is already clear today that modern human rights catalogs are becoming insufficient for all possible situations that the modern world with its rapid development may create. All this is the reason why the concept of fourth-generation rights, the concept of protecting human dignity and integrity in the context of modern technological changes, is rapidly taking shape in legal science. The relevance to research and analyze this topic is dictated by the need to consider such phenomena as editing the human genome, the use of artificial intelligence in diagnosis and treatment, the technology of integrating computer chips into the human nervous system, etc. from the point of view of legal science.

AIM

The aim of the article is to analyze the place and development of the right to health care in the fourth generation concept of human rights, to study the relationship between the formation of new generation rights and the development of science and technology,

to investigate the current state of recognition and consolidation of the new concept of rights at the international level and some aspects of application of related international legislation in practice.

MATERIALS AND METHODS

The article examines the right to health care, its relationship with new technologies and its development in the context of scientific and technological progress. The research is based on such general scientific methods as system analysis and the dialectical method. The comparative legal and technical legal method was used to study international legal norms and the case law of the European Court of Human Rights on the recognition, consolidation and practice of the use of fourth generation rights. The methods allowed us to identify common features, differences and gaps in the legal regulation of these rights. The historical and legal method was used to study the prerequisites for the emergence and historical development of the concept of generations of human rights. The systemic analysis was used for a comprehensive study of the right to health care in the fourth generation human rights system. The logical-formal method was used to define the conceptual apparatus and establish clear definitions of terms and concepts in the paper. The method of legal interpretation was used to interpret the provisions of international legal acts with regard to their application in modern conditions. To achieve the purpose of the study, the authors used the following materials: international legal acts and treaties, scientific articles, and the case law of the ECHR. For example, the article by Shevchuk O. et al. analyzes the legal aspects of the use of smart technologies and virtual reality in various healthcare areas. Schickhardt, C. et al. analyze the ethical and legal aspects of patients' access to their raw genetic data. Tarasevych T. et al. analyze in their article the problems of protecting the rights of the fourth generation through the practice of the ECHR. Baroni MJL. examines in his article the insufficiency of the first three generations of human rights to address the problems posed by the current state of scientific and technological progress and emphasizes the need to recognize the fourth generation. All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

The French-Czech lawyer Karel Vasek was the founder of the concept of human rights generations. The concept characterizes human rights according to their historical development and, in the classical version, contains three generations: the first - political and civil rights inherent in a person from birth (the right to life, free movement, personal integrity, as well as electoral rights, freedom of speech, etc.), the second - economic, social and cultural rights (positive, i.e. those that the state must ensure. The right to work and rest, social security, housing, etc.), and the third is collective or solidarity rights (the right to peace, a clean environment, etc.) [2]. The expansion of the classical concept to include the fourth generation is dictated by scientific and technological progress, which gives rise to qualitatively new human rights issues that are not fully covered by the traditional three [1].

In modern legal science, there is no clear list and content of fourth generation rights. One of the leading and most discussed concepts of modern rights is the concept of somatic rights. In essence, somatic rights are the recognition of a person's inalienable right of ownership of his or her own body and imply the freedom of will to dispose of one's own body, its parts, organs, and tissues, including those already separated from the body [3]. In general, in medical law, it is customary to attribute to the newest generation of law that is in one way or another related to the integration of scientific and technological advances in various sciences, including medical science, into the healthcare sector. There are many aspects of healthcare that are being changed. An example is the current rapid digitalization of the healthcare sector and the gradual introduction of artificial intelligence for diagnosis and treatment. This raises many questions about the protection of personal medical data, privacy of the individual, and the legal regulation and development of standards for algorithms used in diagnosis and treatment. Another example is the rights in the field of biomedicine and genetics. This raises issues of legal regulation of genetic testing, genome editing, cloning, etc. Also at the crossroads of medical rights and the right to life is the problem of legal regulation of such phenomena as euthanasia, resuscitation, and artificial life support. Another debatable area of development is the introduction of virtual reality into medicine. VR can be used in the healthcare sector in many areas, such as education and training of medical personnel, simulation of surgical interventions, psychotherapy and mental rehabilitation, ophthalmology, telemedicine and sports medicine, etc. This is another promising area of medicine using breakthrough technologies, which also requires legal regulation of such aspects as security, confidentiality, protection of medical and private data, etc. [4].

Also, a feature of the concept of fourth-generation rights is that they not only supplement legal science with new definitions and add new ones to the existing list of rights, but also expand long-known rights with new aspects. Thus, the right to life, in the context of the fourth generation, has been supplemented by the concept of "the right to a decent life," which implies the absence of physical and moral suffering and control over one's own life, including the possibility of consciously leaving it. The right to privacy now includes genetic integrity.

Given that the fourth generation rights do not currently have a clear list and scientific discussions about whether a particular right belongs to the new generation are still ongoing, it is important to highlight the general principle on which this generation should be based. Thus, the first two generations were based on the principles of freedom and equality, the third generation - on the principle of solidarity and collectivity. Some authors suggest that the fourth generation be based on the principle of human identity [1]. This means that the development of technologies, genetics, biomedicine, neurotechnology and their implementation in human life is possible only on condition that a person remains a person in the genetic, personal and cultural sense. This principle should prevent uncontrolled interference of science in human life, regardless of the ultimate goal and value of such interference for humanity as a whole.

In the context of medical law, there are several main areas associated with the fourth generation rights. Most of them are currently enshrined in the Oviedo Convention [5]. The first of these is the right to genetic integrity. Its content is the recognition of the human genetic code as unique and inviolable, that is, as the one that cannot be altered or copied. In practice, this means a ban on cloning and a ban on changes to the genome that are transmitted to descendants.

At the international level, this right is enshrined in the Additional Protocol to the Oviedo Convention of January 12, 1998. The Protocol emphasizes that "the intentional creation of genetically identical human beings is incompatible with the concept of human dignity" [6]. Regarding genome modifications, Article 13 of the Oviedo Convention states that they are possible "only for therapeutic, prophylactic and diagnostic purposes and provided that the genome of the descendants is not altered" [5]. All these norms aim to protect the genetic identity and integrity of the individual and prevent uncontrolled interference with the human genome. The second direction is the protection of the confidentiality of biomedical data. Sensitive information about a person's physical condition, characteristics and diseases has always required special protection. At the international level, this is reflected in Article 10 of the Oviedo Convention [5]. The development of medical science requires the storage and processing of an increasing amount of personal biological data. Various medical registries and databases for machine learning AI, etc. are being created, which makes privacy protection particularly important and requires looking at it from a different angle. This area can be called the right to the protection of biomedical data, which can be seen as part of a much broader right to privacy in the digital age. Given the value and sensitivity of biomedical data, their storage, processing, and access can trigger entire scientific discourses. An example is the issue of patients' access to their own genomic data. The specific nature of this data can create a conflict of interest between the patient, their close relatives, researchers and doctors. On the one hand, a patient has the right to know any information related to his or her body, but the complexity of interpreting and understanding genomic data without proper explanation by a specialist can lead to the lack of meaning of such knowledge or to direct harm to the patient due to incomplete or incorrect interpretation of such data. Researchers and research institutions, under the obligation to provide patients with their genome data, may incur additional financial costs and reputational risks, while, again, the data is not useful to the patient. Since the patient's relatives share about half of the patient's genes, the patient's transmission of genetic information about, for example, genetic predisposition may violate their right to informational self-determination [7]. The next area is protection against discrimination on the grounds of genetic heredity, which is enshrined in Article 11 of the Oviedo Convention [5]. An example of such discrimination is the use of genetic tests for health insurance purposes, which is proposed to be prohibited by all Council of Europe states in the recommendation CM/Rec(2016)8 [8] of October 26, 2016. The next right, also defined in Chapter II of the Oviedo Convention [5], is the right to informed consent. It means that no intervention in a person's body can be carried out without the person's informed consent. In the context of a new generation of rights, this right also extends to cases of "digital" interventions, namely telemedical consultations, monitoring of the body's condition through remote sensors or gauges, the use of robotic systems for diagnostics or surgical interventions, etc. Also in the same context, one can highlight the right to refuse

any digital interventions. For example, to refuse the use of algorithms for diagnosis or for developing a treatment plan, or to refuse to enter data into digital registries if the person finds it unacceptable. In this case, the right to traditional methods of treatment or medical records should also be taken into account. For example, a person should be able to receive a paper version of a prescription or his or her appointment sheet even if digital medical records are widespread. This is to ensure that people are not discriminated against if they are unwilling or unable to use digital technologies.

The main document that defines most of the fourth generation rights in the field of medicine at the international level is the Council of Europe Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine, also known as the Oviedo Convention [5]. This is currently the only legally binding document for the states that have ratified it, which establishes legal and ethical norms for the use of scientific and technological achievements in biology and medicine. Having entered into force in 1999, it became the basis of biolaw in European states. The document declares its purpose to protect fundamental rights and freedoms in the context of biology and medicine. The most significant norms established by the Convention, in our opinion, are the norms of Article 2 on establishing the priority of human interests over the interests of society and science. This fundamental statement is very important because it puts an end to the philosophical and scientific discourse on the question of whether the interests of one person can be sacrificed for the sake of scientific and technological progress in the interests of society as a whole. Another equally important achievement is the enshrining of the norm of informed consent in Chapter II of the Convention. No science, no matter how important it is for humanity, should use results obtained in violation of human rights and dignity. Also interesting is the provision of Article 10, which enshrines not only the right to receive comprehensive information about one's health but also the right not to know it, thus recognizing a person's right to informational self-determination. This is very important because it gives people with incurable diseases the right to choose, for example, to live a few years of a decent life without knowing about their illness. Also, in Articles 11 and 12, the Convention prohibits discrimination on genetic grounds and emphasizes that genetic testing can only be used in the interests of health. These norms are intended to prevent the misuse of genetics for the purpose of employee selection or insurance purposes, etc. Article 13 limits genome editing to medical purposes, provided that there is no change to the genetic lineage of descendants. This is intended to protect humanity from possible selection of human beings and genetic enhancement.

In 1998, a protocol on the prohibition of cloning was adopted to the Convention. It states that while cloning can contribute to scientific and technological progress and solve complex questions in medical science, the creation of genetically identical beings is contrary to human dignity [6]. The following three additional protocols to the Convention regulate the issues of transplantation [9], biomedical research [10] and genetic testing [11]. All of them contain rules designed to protect the rights of people in the face of advances in biology and medicine.

The Oviedo Convention was signed by Ukraine on March 22, 2002, but has not yet been ratified. Although signing this Convention indicates an intention to comply with its provisions, the lack of ratification and harmonization of national legislation with the Convention's provisions creates gaps in bio-law legislation.

Also, among the international documents on bio-law there is a UNESCO declaration: Universal Declaration on the Human Genome and Human Rights of 1997 [12]. The 1997 Declaration recognizes the human genetic code as the common heritage of humanity and also contains provisions prohibiting discrimination on the basis of genetic characteristics and prohibiting cloning [12]. However, these provisions are declarative and recommendatory in nature, do not contain obligations, but rather express the agreement of the international community with respect to the phenomena described.

Also, when analyzing the implementation of modern medical law, it is important to consider the related decisions of the European Court of Human Rights. In the practice of the ECHR, there are quite a few decisions related in one way or another to bio- and medical law. Although the ECHR is guided in its practice by the European Convention on Human Rights, which mainly contains the rights of the first two generations, in most cases it is sufficient to consider bio-law cases, although such an interpretation does not always accurately reflect the essence. An interesting example of bio-law cases is the 2002 case of *Mikulić v. Croatia* [13]. In this case, the court was faced with the task of finding a balance between the applicant's right to know her parentage and the right of her alleged father not to undergo a genetic test. The court found a violation

of the right to genetic identity under Article 8 of the European Convention on Human Rights, considering it a violation of the right to respect for private and family life. Another example of the clash between the right to know one's origins and the right of an individual to refuse a genetic test can be found in the case of *Yaggi v. Switzerland* [14]. In this case, the court sided with the plaintiff, considering the refusal to perform a paternity test on a deceased person as a violation of Article 8 of the Convention, and noted that taking DNA tests from a deceased person cannot be considered an interference with his private life.

There are also several cases in the ECHR's practice on the protection of medical and genetic information. For example, the 2008 case of *S. and Marper v. the United Kingdom* [15], in which the applicants complained about the authorities' retention of their fingerprints, cell samples and DNA even after the criminal proceedings against them had been closed. The court considered the fact of storing a person's biological materials regardless of the existence of a criminal case and without the possibility of the acquitted person to delete data about himself or herself and destroy the materials as an interference with privacy in violation of Article 8 of the Convention. In the 2008 case of *K. and T. v. Finland*, the ECHR found that the leaking of information about the applicant's HIV status to her colleagues was a violation of Article 8 of the Convention [16].

The ECHR's decisions on informed consent and the patient's right to control his or her own life are also noteworthy. In the 2004 case of *Glass v. the United Kingdom*, the Court considered a complaint by a mother whose sick child, during a severe seizure, was given a drug that could have hastened the death, assuming that the child would not recover anyway. The mother did not agree with the doctors' conclusions and did not give her consent to these manipulations. The child later recovered and was discharged home. The ECHR considered the doctors' actions as a violation of Article 8 of the Convention [17]. In the case of *Pretty v. the United Kingdom*, a seriously ill woman asserted her right to assisted suicide. Since she was unable to commit suicide due to complete paralysis caused by her serious illness, her lawyer appealed to the state authorities for guarantees that her husband would not be held criminally liable if he assisted her. The Court decided that although the right to voluntary cessation of life may fall within the concept of "respect for private life", the refusal of state authorities not to prosecute cannot be regarded as a violation of Article 8 of the Convention, since the assessment of the risks of abuse in the event of permission to commit suicide falls within the competence of the state [18].

Cases concerning reproductive technologies can also be found in the case law of the European Court of Human Rights. For example, in the case of *Evans v. the United Kingdom* in 2007, the Court held that the reluctance of the applicant's partner, who withdrew his consent to the implantation of an embryo artificially fertilized with his biological material, was fully compatible with his right to private life [19]. In the 2012 case of *Costa and Pavan v. Italy*, the Court considered Italy's ban on pre-implantation embryo screening as denying a family the opportunity to have a healthy child, i.e. as a violation of the right to private life [20].

CONCLUSIONS

Given the above, a number of conclusions can be drawn. Firstly, the concept of fourth-generation rights in the field of medicine emerged as a response to new challenges created by the implementation of the results of scientific and technological progress in the field of human health. Their main meaning is to find a balance between the development of science, technology and society and human dignity and identity. All international documents on modern human rights are permeated with the idea of the inadmissibility of a situation in which, after all changes to the human body or genetic code, even for the purpose of treatment and prevention, a person ceases to be a person in the genetic and personal sense. Uncontrolled implementation of scientific achievements in the field of healthcare can lead to abuses that contradict human dignity, such as gender selection or external data of the future child, attempts to improve human qualities, etc. On the other hand, editing technologies open up great opportunities in the treatment of previously incurable diseases. Neurotechnology through the implantation of a neuroimplant may also be the only method of treating some diseases of the nervous system, but can't a neurochip be used to control a person? Euthanasia is a salvation for terminally ill people who are doomed to die in pain and humiliation, but can't it be used for legalized murder? Answers to such questions should be embodied in legislation at the international and national levels. It is the search for a balance between the interests of the individual and society as a whole that is the main idea of fourth generation rights.

Secondly, the traditional human rights of the first three generations are no longer sufficient to protect against all possible challenges of today. That is why the rights of the fourth generation require the earliest

possible detailed analysis, cataloguing, and constant adaptation of international and national legislation to them. Given the rapid development of science and technology, this will be an increasingly difficult task every year.

Third, some important rights, such as the right to euthanasia and assisted suicide, are not in any way enshrined at the international level. That is, no international document recognizes it as a separate right and does not oblige states to recognize it. Euthanasia and assisted suicide are only allowed in some countries on the basis of national legislation. The ECHR considers these concepts as part of the right to life and the right to respect for private life. Given the importance of this right for society and its prevalence in the legislation of European countries, it would be advisable to recognize and consolidate it at the international level.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Microbiology of dental decay and periodontal disease: A review

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ABSTRACT

Aim: This review attempts to examine the microbiology, pathogenesis and current therapeutic approaches of dental caries and periodontal diseases with a special focus on the role of polymicrobial biofilms, the host-microbe interaction and the major pathogenic species involved in disease progression.

Materials and Methods: A thorough literature review was performed using major scientific databases such as PubMed, Scopus, Web of Science and Google Scholar. Studies that were published between 2000 and 2025 were included. Relevant experimental, clinical and review articles that focused on the etiology, microbial composition, virulence mechanisms, host immune responses and therapeutic approaches of dental caries and periodontal disease were analyzed.

Conclusions: The oral cavity harbors over 700-800 bacterial species, of which the primary cariogenic pathogen is *Streptococcus* mutans and *Porphyromonas* gingivalis has been implicated as a major cause of periodontal disease. Dental caries progression is mostly attributed to acid production and demineralization of enamel, whereas periodontal disease is a result of dysbiotic shift in the subgingival microbiome with destructive host inflammatory responses. The "red complex" (*P. gingivalis, Treponema denticola and Tannerella forsythia*) has a high degree of synergistic virulence in advanced periodontitis. Biofilm formation, production of extracellular polysaccharide (EPS) matrix, quorum sensing and immune components (neutrophils, macrophages and matrix metalloproteinases or MMPs) are all factors that contribute to disease formation. Prevention strategies include oral hygiene measures, fluoride exposure, dietary modification, and antimicrobial agents, whereas treatment measures include mechanical debridement, systemic antibiotics, antimicrobial peptides, probiotics, and photodynamic therapy. Dental caries and periodontal diseases are the result of complex interactions between polymicrobial biofilms and immune responses by the host. A better understanding of the microbial ecology, virulence pathways and host-pathogen interactions is crucial in the process of improving prevention and treatment. Advances in targeted antimicrobial therapies and innovative therapeutic approaches hold promise for enhancing global oral health outcomes.

KEY WORDS: dental carriers, periodontal disease, biofilm, *Streptococcus mutant*, quorum sensing

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INTRODUCTION

Periodontal diseases and dental caries are the most common chronic infectious complications of the mouth cavity in the world, which place significant burdens on the health system of an individual and the population at large. Both pathologies are due to complicated biofilm-mediated mechanisms whereby the resident microbiome of the oral cavity alters its state of being symbiotic and homeostatic to a state of dysbiosis and pathogenicity. The microbial ecology, molecular mechanisms and host-microbe interactions that contribute to these diseases are important to understand so that evidence-based preventive and therapeutic interventions can be developed [2].

The oral microbiota can be defined as a dynamic and diverse ecosystem of microbes consisting of about 500 to 700 different bacterial species, which further contributes to fungal and viral species to oral health or disease states. Unlike the historical conception of oral disease as resulting from single pathogenic agents, modern microbiological knowledge of oral diseases is based on the now commonly accepted view that dental caries and periodontitis are polymicrobial diseases that involve complex synergistic interactions involving multiple microorganisms, with individual host factors and environmental factors determining the clinical manifestation and progression of these diseases [1].

Dental caries has come to be recognised as a biofilm-mediated, sugar driven, multifactorial disease involving the progressive demineralisation of dentine and enamel caused by persistent acid production from acidogenic and aciduric microbial communities. Rather than being caused by a single pathogen, caries results from ecological changes that take place in supragingival dental plaque biofilm communities in which high frequency consumption of fermentable carbohydrates (especially sucrose, refined sugars) selects for acid producers such as *Streptococcus mutans*, *lactobacilli,*

Actinomyces, Bifidobacterium, and Veillonella, while inhibiting acid-sensitive commensal organisms. These cariogenic communities have three cardinal virulence traits: acidogenicity (rapid fermentation of dietary carbohydrates into organic acids), aciduricity (survival and metabolic activity in low pH) and robust extracellular polysaccharide (EPS) synthesis that increases biofilm adhesion, structural integrity and diffusion resistance [3].

In contrast, periodontal diseases (including gingivitis and periodontitis) have been linked to dysbiotic subgingival biofilms enriched in Gram-negative anaerobes and proteolytic species that are responsible for chronic inflammation, irreversible destruction of the connective tissue and resorption of alveolar bone. Pathogenesis Periodontitis is not only caused by the growth of the total bacterial biomass, but also by changes in microbial composition and activities, which are frequently mediated by low-abundance keystone pathogens like Porphyromonas gingivalis.

The pathogenesis of periodontitis is driven not merely by an increase in total bacterial biomass but by qualitative shifts in microbial composition and function, often orchestrated by low-abundance "keystone pathogens" such as *Porphyromonas gingivalis*. These organisms manipulate host immune signaling, specifically the complement signaling and TLR signaling, to induce an inflammatory environment that favors the outgrowth of proteolytic and asaccharolytic bacteria as well as nutrient-rich conditions through gingival crevicular fluid and perpetuates tissue breakdown through bacterial proteases and gingipains as well as host matrix metalloproteases.

The progression from health to disease, both in caries and periodontitis can be explained by the ecological plaque hypothesis, which holds that environmental stressors such as frequent sugar consumption, low salivary flow, poor oral hygiene, smoking, diabetes, and immunocompromise affect the state of microbial homeostasis and select for pathogenic communities. Advances in high-throughput sequencing, metagenomics, metatranscriptomics, and metabolomics have shown that both diseases are polymicrobial and community-level diseases with unique dysbiotic signatures in terms of altered species diversity, functional gene expression, and metabolic products. Notably, oral dysbiosis has a more extensive pathophysiology systemic effect because pathogen-associated oral biofilms are reservoirs of respiratory pathogens, inflammatory cytokines and proteolytic enzymes that cause cardiovascular disease, diabetes, rheumatoid arthritis, adverse pregnancy outcomes, and respiratory infections [4].

The present review gives a concise overview of the available microbiological knowledge of dental caries and periodontal disease, its structure, pathogenicity, biofilm structure and dynamics, bacterial communication systems, host immune response, and the recent prevention and treatment options. Microbiological, immunological, and clinical perspectives integration offers a basis for interpreting available literature and designating future research objectives and treatment possibilities in oral disease management.

AIM

The purpose of the review is to assess the microbiology, pathogenesis, and current treatment regimens of dental caries and periodontal disease, along with the role of multispecies biofilms, host-microbe interactions, and pathogenic microorganisms that play a role in the progression of the disease. The study is also aimed at giving in depth insight into the correlation between oral microbes and host immunity to guide the progress in developing improved preventive and therapeutic interventions.

MATERIALS AND METHODS

Major databases were used to conduct a systematic literature review, i.e., PubMed, Scopus, Web of Science, and Google Scholar.

Studies on the period 2000-2025 were included and were experimental, clinical and review articles addressing:

- Etiological agents of dental caries and periodontal diseases,
- Composition and dynamics of oral microbiota,
- Mechanisms of biofilm formation and interspecies interactions,
- Host immune responses,
- Preventive and therapeutic strategies.

Relevant studies were critically reviewed in order to present a comprehensive overview of the most recent scientific findings on oral microbiology and its association with dental caries and periodontal diseases.

REVIEW AND DISCUSSION

OVERVIEW OF ORAL MICROBIOTA

COMPOSITION AND DIVERSITY

The human oral cavity is one of the most complex and dynamic microbial ecosystems that is associated with human health. The oral microbiota includes bacteria, archaea, fungi, viruses and bacteriophages, and bacteria are a dominant component of the oral microbiota.

The monumental diversity of the oral microorganisms has been demonstrated by cultivation-independent molecular methods, especially 16S ribosomal RNA gene sequencing and metagenomics. The oral microbiota contains over 700-800 species of seven major phyla, such as *Actinomycetota (formerly Actinobacteria), Bacteroidota (Bacteroidetes), Bacillota (Firmicutes), Fusobacteriota, Pseudomonadota (Proteobacteria), Saccharibacteria* and *Spirochaetota*. Recent metagenomic catalogs have revealed more than 3,400 species-level clusters containing about 60 percent of uncharacterized taxa, suggesting that most of the oral microbial diversity is uncultivated and poorly characterized [5].

In addition to bacteria, the oral microbiome harbors various non-bacterial microorganisms, which play an ecological role and pathogenesis of diseases. Fungi, predominantly *Candida* species, are detected in ~70% of healthy individuals and can form biofilms, with over 150 species documented in the oral mycobiome. The archaea, mainly methanogens e.g. *Methanobrevibacter* oralis, are found in low levels in health, but in periodontal disease and in peri-implantitis, they may work synergistically with the pathogens. The most common viral element is bacteriophages, more than 60,000 phage groups by the species are known; most of these are temperate phages that actively control the growth of bacteria and mediate horizontal gene transfer and genetic variation. The functions of these non-bacterial elements in oral disease and health are not fully studied yet [6].

The oral microbiota is very site-specific, and dental plaque and tongue dorsum, buccal mucosa, saliva and gingival sulcus communities exhibit different oxygen tension, nutrient availability, pH, and salivary flow. The most diversity is found in supragingival and subgingival plaques, with subgingival plaque having less oxygen and more anaerobiosis. Fluorescence in situ hybridization (FISH) biogeographic studies have shown that oral biofilms are organized at micron scales and certain genera of bacteria have a distinct location and multispecies consortia [7].

Primary colonizers of the oral cavity are bacteria initially colonizers of the oral cavity, which are mostly Gram-positive bacteria, mainly streptococci: *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus mitis* (the mitis group), and the species of *Actinomyces*, *Haemophilus*, and *Neisseria*. These organisms can stick to tooth surfaces that are covered by salivary pellicles and form early biofilms.(29). Primary colonizers also generate extracellular enzymes and metabolic products that alter the local microenvironment, decreasing oxygen tension and pH, and allowing secondary colonization by the fastidious anaerobes such as the members of the red and orange bacterial complexes within a few hours of biofilm formation [8].

There is significant compositional complexity of the subgingival microbiota, and anaerobic species are predominant because of the oxygen-deprived nature of the periodontal pocket. The important subgingival microbes are the members of the red complex *(Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia)*, the orange complex flora (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella nigrescens*, *Peptostreptococcus micros*), and the yellow complex organisms (many species of *Streptococcus* such as *S. mitis*, *S. oralis*, *S. sanguinis*, *S. gordonii*, *S. intermedius*) [4, 9].

The oral biofilm is spatially organized by having specific bacterial aggregates and layered structures spread throughout the matrix to provide micro environments with different levels of PH and oxygen tension, and varying nutrient levels.

The stratification of subgingival plaque has been shown to be divided into various morphologic layers, the most important layer of which is the basal layer that is close to the tooth surface, the intermediate layer with a high number of filamentous bacteria, Corynebacterium and Actinomyces, and the outermost layer composed of a high percentage of motile spirochetes and other late colonizers. This spatial arrangement records metabolic collaboration and substrate trade among the species and is essential in the functions of a biofilm as well as pathogenicity [10]

ORAL MICROBIOTA IN HEALTH VERSUS DISEASE

In periodontally healthy conditions, the oral microbiota is symbiotic in contact with host tissues, in complex metabolic interrelationship and immune tolerance mechanisms. Healthy oral biofilms have a rich microbial diversity, resident commensals excluding colonization by pathogenic species by competitive exclusion, antimicrobial compound production, and creation of local environmental conditions adverse to pathogenic species [2].

The pathological change in the composition of microbial communities, dysbiosis, is an indicator of a shift between an oral health state and a disease state. The microbial diversity in dysbiotic biofilms related to caries is reduced, whereas acid-tolerant and acidogenic bacteria such as *Streptococcus mutans*, *Lactobacillus species*, and *acid-tolerant streptococci* increase in number. These organisms cause acidic micro environments favorable to their existence but unfavorable to acid sensitive species, triggering a self-reinforcing loop of dysbiosis [1].

Shifts in microbial composition towards pathogenic consortia that are enriched in proteolytic species and

can invade tissues and synthesize virulence factors are present in periodontal disease as dysbiosis. It is not merely an increase in the abundance of pathogens that is involved in health-disease transitions, but rather a fundamental restructuring of microbial community structure, changes in the pattern of gene expression and a shift in metabolic interactions among biofilm constituents [2].

DENTAL CARIES: MICROBIOLOGY AND PATHOGENESIS

ETIOLOGY AND DISEASE MECHANISM

Dental caries is a biofilm-mediated pathology that develops as a consequence of the interaction between cariogenic microbes, dietary carbohydrates, especially sucrose and host factors. There is a series of steps in the disease process that involve the colonization of the tooth surfaces by bacteria, formation of biofilms, fermentation of carbohydrates to produce organic acids and demineralization of the tooth structures. The pathogenesis of caries is the classical three-factor triad, including susceptible host (saliva composition or flow), cariogenic bacteria with an ability to convert carbon dioxide into acids and withstand acid levels), and the frequent intake of carbohydrates to feed bacteria [1, 11].

Special attention should be paid to the role of the dietary sucrose. The ability of sucrose to be the preferred substrate for extracellular polysaccharide (EPS) synthesis is the reason behind its special status as compared with other fermentable carbohydrates. Although all fermentable carbohydrates produce acids due to the activity of the bacterial metabolism, the characteristic structural features of sucrose allow enzymes of glucosyltransferase (Gtfs) of *Streptococcus mutans* to break down sucrose to glucose and fructose units and produce glucose polymers (glucans) in a particular 1,3 and 1,6 glycosidic structure. S. *mutans* express three different Gtfs: GtfB expresses mostly water-insoluble, 1,3-linked glucans, which enable strong adhesion of the bacteria to the surface and give the biofilm structure a rigidity; GtfC expresses a mix of soluble and insoluble glucans; GtfD expresses primarily soluble 1,6-linked glucans, which are used as extracellular energy stores [12].

PRINCIPAL PATHOGENIC ORGANISMS

Streptococcus mutans holds a superior role as the main etiological cause of dental caries. This is a Gram-positive, facultative anaerobic coccus that has several cariogenic pathogenicity features. This is because *Streptococcus* mutans synthesizes glucosyltransferase (Gtf) enzymes that mediate the production of insoluble and soluble glucans on sucrose to produce an extraordinarily adhesive biofilm matrix. The organism is truly acid-tolerant with a viability and even growth down to pHs of 3.5, which is nearly unique in oral bacteria[1, 13].

Streptococcus mutans is present as serotype c, e, f, and k of which serotype c is the most common oral serotype with about 70-80% of oral S. mutans isolates having this serotype whereas serotype e has about 20, serotype f and k each form represents less than 5% cases [1]. These serotypes differ in their virulence properties, geographic distribution and other oral isolates in their association with extraoral pathologies; some serotype k and some serotype e strains are found more abundantly in cardiovascular specimens and cases of infective endocarditis than oral isolates, indicating a potentially higher invasive capacity. A small proportion of strains are unable to be typed using conventional PCR methods, which reflects a lack of RGP diversity beyond the standard four serotypes; however, some clinical isolates are dual serotype (commonly c and k) and thus display diverse RGP populations [14].

Other secondary pathogenic agents that help in the pathogenesis of caries are *Streptococcus sobrinus*, which is also a mutans streptococci member and a number of species of *Lactobacillus*. The role of the latter organisms on caries pathogenesis is yet to be fully defined [15].

VIRULENCE FACTORS AND PATHOGENIC MECHANISMS

Streptococcus mutans has numerous virulence factors coordinating its cariogenic capability. The glucosyltransferase enzyme family is an important virulence factor. The glucan synthesis involved three main Gtf enzymes (GtfB, GtfC, and GtfD), which have different linkage specificities: GtfB synthesizes insoluble α1,3-linked glucans, which shape the biofilm matrix, GtfC synthesizes soluble and insoluble α1,6-linked glucans [12]. The glucans containing heterogeneous biofilm microarchitecture enabling colonization by microbes and structural stability is determined by the compartmentalized distribution of glucans with dissimilar linkage features [16].

The synthesis of fructans by the use of fructosyltransferase (Ftf) enzymes catalyzes the production of further matrix polysaccharides and is also an ancillary virulence mechanism that uses fructose as its substrate. GbpA, GbpB and GbpC are glucan-binding proteins (Gbp) that facilitate bacterial adherence to glucan molecules on the surface of the bacterium, connecting microbial cells with the scaffold of the matrix. The regulation of these

virulence determinants via a gene is through a series of regulatory systems, such as the AtpF (F1F0-ATPase) and LuxS (Autoinducer-2 production) and ComABCDE systems (competence and biofilm regulation), which coordinate the expression of virulence factors in response to environmental signals [1].

Carbohydrate fermentation resulting in acid production is one of the key pathogenic processes. The dentin is directly eroded by lactic acid and other organic acids produced by Streptococcus mutans by glycolytic pathways at acidic pH. The organism stores intracellular polysaccharides, which are formed using various carbohydrates, this allows it to produce acid even when carbohydrates are starved. This intracellular store of polysaccharides maintains the ability of the organism to produce acid over a long period, continuing to maintain the acidic microenvironment despite the scanty external supply of carbohydrates [1, 13].

PERIODONTAL DISEASE: MICROBIOLOGY AND PATHOGENESIS

DISEASE CLASSIFICATION AND PROGRESSION

Periodontal diseases represent a continuum of inflammatory diseases of tissues of the tooth support, which include gingivitis (inflammation of gingiva tissue without loss of underlying alveolar bone) and periodontitis (loss of alveolar bone and periodontal attachment). Periodontitis is divided into aggressive periodontitis (faster developmental stages, the age of the patient at the time of the onset of the disease) and chronic periodontitis (slower development, mostly in middle-aged and elderly people). Periodontal disease pathogenesis entails a polymicrobial threat to periodontal tissue and an untamed host inflammatory reaction that leads to the deterioration of periodontal tissues [2, 17].

THE RED COMPLEX AND ORANGE COMPLEX BACTERIAL CONSORTIA

Modern periodontal microbiology has conceptualized the pathogenic bacteria as being formed into color-coded groups of bacteria on the basis of association with each other, and temporal emergence through biofilm formation was introduced by Socransky et al in 1998. The orange complex has such secondary colonizers as *Fusobacterium nucleatum, Prevotella intermedia, Prevotella nigrescens, Peptostreptococcus micros, Eubacterium nodatum, Campylobacter rectus, and Streptococcus constellatus.* They mediate the transition between early colonizing streptococci and actinomyces and late colonizing red complex pathogens, and are scaffolding bacteria that form microenvironmental conditions (deoxygenated, increased nutrients of gingivally secreted crevicular fluid) that are conducive to the highly fastidious anaerobes [8]. There are three pathogenic species forming the red complex: *Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia (formerly Bacteroides forsythus*). They are the most significant pathogens of adult periodontal disease and have a strong relationship with the clinical development of advanced periodontitis [18]. The red complex bacteria do not occur in isolation; instead, they are usually present in periodontal pockets in synergistic polymicrobial consortia. Complex anaerobic fermentation of amino acids, extracellular proteolytic activity, the synthesis of toxic metabolites such as lipopolysaccharides and outer membrane vesicles, and increased interspecies virulence are the homologous pathogenic features [19].

PORPHYROMONAS GINGIVALIS: THE KEYSTONE PATHOGEN

Porphyromonas gingivalis has come to be a model of a key pathogen in periodontitis- an organism that is found in comparatively low abundance but has a disproportionate effect on the biofilm microbial composition and virulence by a variety of immune subversion mechanisms. This is an obligate asaccharolytic, anaerobic, Gram-negative rod that expresses a wide range of virulence factors that allow it to penetrate tissues, avoid the immune response, and promote pathobiont status [2].

Gingipains (Rgp and Kgp arginine-specific and lysine-specific cysteine proteases) are some of the virulence factors in *porphyromonas* gingivalis, which are some of the important pathogenic determinants. Gingipains destroy host proteins such as immunoglobulins, complement, cytokines, and chemokines that inhibit host defence systems and produce peptides and amino acids to aid bacterial metabolism. This organism synthesizes lipopolysaccharides that have distinctive lipid A frameworks that cause attenuated Toll-like receptor 4 signaling, which controls host inflammatory reactions [20]. Major fimbriae (FimA) mediate epithelial cell invasion and co-aggregation with other bacterial species, whereas minor fimbriae (mfa) mediate biofilm formation [19].

Particularly notable is *P. gingivalis'* inflammophilic nature—the organism thrives within inflammatory environments, utilizing hemorrhage products and inflammatory exudates as nutrient sources. The production of complement and cytokines is mediated by the bacterial lipopolysaccharide and peptidoglycan that produce nutritionally advantaged inflammatory microenvironments. Additionally, *P. gingivalis* actively evades antimicrobial

responses such as neutrophil oxidative burst and phagocytosis by a variety of mechanisms, such as degradation of the TLR2 adaptor protein MyD88 by cysteine proteases, which facilitates disease-sustaining dysbiosis [2].

TREPONEMA DENTICOLA AND TANNERELLA FORSYTHIA

Treponema denticola is a motile spirochete which also leads to the pathogenicity of the red complex in several ways. The organism secretes hydrolytic enzymes such as collagenases, hyaluronidases and proteins that directly break down host extra cellular matrix proteins. It is also important to note that *T. denticola* shows nutritional interdependence with *P. gingivalis*: the latter organism synthesizes isobutyric acid, which favors the growth of *T. denticola*, and *T. denticola* produces succinic acid, which favors the growth of *P. gingivalis*. This example of metabolic cooperation is an example of polymicrobial synergism in periodontal biofilms [2].

Tannerella forsythia is an organism that has been recovered as a late colonizer in progressive periodontal disease and also produces dentilisin (a serine protease), collagenase and other proteolytic enzymes involved in degrading periodontal tissue. It is covered with special O-glycans, which mediate the immune evasion and survival of the organism in periodontal pockets. Interestingly, *T. forsythia* is often colonized prior to *P. gingivalis* colonization in subgingival biofilms, indicating primary ecological colonization functions [18].

BIOFILM FORMATION AND MATRIX COMPOSITION

STAGES OF BIOFILM DEVELOPMENT

The formation of dental plaque biofilm occurs in a series of sequential phases with each stage defined by a particular microbial community, composition of the matrix and its functional features. Knowledge of these developmental stages can give information on intervention points in biofilm control and disease prevention [21].

Stage 1: Pellicle formation and initial microbial attachment
When teeth are cleaned or ejected to the oral cavity the acquired salivary pellicle is quickly colonized by the salivary pellicle, a proteinaceous film of more than 180 proteins, glycoproteins, phosphoproteins and lipids. Pellicle formation occurs several seconds after exposure of the tooth surface to saliva, with precursor proteins being proline-rich proteins, statherin, and histatins binding directly to the hydroxyapatite crystals via calcium-mediated interactions. The formation of pellicle begins with a pellicle thickness of roughly 10-20 nm within minutes, the growth of the pellicle through protein-protein interactions occurs after 30-45 minutes, and the maturation of the pellicle occurs after 90-120 minutes as the adsorption of high-molecular-weight mucins onto the pellicle structure occurs [22]. This is due to the fact that pellicle composition has a very strong effect on the later colonization by microbes. Salivary pellicle gives certain receptors mediating bacterial adherence: glycosylated proteins and histatins mediate streptococcal adhesion, proline-rich proteins mediate Actinomyces species adhesion, whereas statherin mediates Fusobacterium nucleatum adhesion. Enzymes that are functionally active, such as peroxidases, lysozyme, and amylase are also found in the pellicle and regulate adhering bacterial physiology [22].

Stage 2: Primary colonization and microcolony formation
Gram-positive facultative bacteria, especially streptococci and actinomyces are the ones that preferentially colonize pellicle-coated surfaces in 2-4 hours of pellicle formation. These major colonizers divide further to create microcolonies of the growing biofilm. Primary colonizers show glycosidase activities, which allows them to get access to the salivary glycoproteins as a nutritional source. Preventing the elimination of other anaerobic species through the initial metabolic operations of oxygen-depleted microenvironment is enabled by the process of oxygen consumption. Primary colonizers produce novel bacterial cell surface receptors that permit secondary bacterial adhesion by coaggregation, where bacteria, by complementary adhesin-receptor interactions [21].

Stage 3: Secondary colonization and biofilm maturation
Primary colonizers are bound by secondary colonizers which include Fusobacterium nucleatum and other anaerobic species via coaggregation. Fusobacterium nucleatum is a so-called coaggregation hub that interacts with nearly all oral bacterial species, thus facilitating multispecies biofilm formation. Subsequent growth of biofilm provides heterogeneous micro environments that have oxygen tension, pH, nutrient, and metabolic concentration gradients. The maturation of biofilms is characterized by the formation of 3-dimensional design with mushroom or clumped microcolonies embedded in extra-cellular matrix [22].

EXTRACELLULAR POLYMERIC SUBSTANCE MATRIX COMPOSITION

Extracellular polymeric substances (EPS) that include polysaccharides, proteins, extracellular DNA (eDNA), and lipids predominate, but cell wall polymers, including lipoteichoic acids and peptidoglycan fragments are present

in lesser quantities in the biofilm extracellular matrix. EPS is particularly highly concentrated with insoluble and soluble glucans and fructans produced by Streptococcus mutans glucosyltransferases (GtfB, GtfC, GtfD) and fructosyltransferase (Ftf) in cariogenic supra-gingival plaque, creating a diffusion restrictive, mechanically stiff matrix to facilitate local bacterial concentration and acid retention. By comparison, the periodontitis-associated biofilms subgingival matrix has a more complex biofilm composition (bacterial and host-derived components: polysaccharides, proteins, eDNA, lipids, serum proteins, and inflammatory exudate), although its actual chemical composition is not so well defined [23].

POLYSACCHARIDE COMPONENTS

Exopolysaccharides are large matrix constituents that are found as glucans and fructans in cariogenic biofilms. The glucosyltransferase enzymes of *Streptococcus mutans* produce glucans of various structures: α1,3-linked insoluble glucans are the major scaffold of the matrix, and α1, 6-linked soluble glucans are the secondary binding sites. The glucan matrix has a compartmentalized structure which has spatial heterogeneity that forms different microenvironments. Fructose is further enriched with fructan constituents produced by fructosyltransferase [11, 12].

Exopolysaccharide compositions also vary significantly in subgingival biofilms related to periodontitis compared to cariogenic biofilm maturation, but the present knowledge about periodontal biofilm matrix polysaccharides is incomplete. But, there are bacterial species such as *Prevotella* and *Fusobacterium* species that produce exopolysaccharides, which lead to the formation of the matrix.

PROTEIN COMPONENTS

Proteins are 10-20% of the biofilm mass that is of bacterial and host origins. The bacterial surface protein such as glucan-binding proteins, adhesins (especially antigen I/II and other cell-surface proteins) and enzymes are incorporated into the matrix. Amyloid structures are produced by a large number of surface proteins, which may have a structural role in assembling the matrix. Host-derived proteins such as immunoglobulins, complement components and tissue-derived proteins are deposited in biofilm matrices either by entrapment or active integration [23].

EXTRACELLULAR DNA

Extracellular DNA (eDNA) makes up 1- 20 percent of the biofilm dry weight, depending on biofilm type and conditions, and has important structural functions, which involve biofilm cohesion and mechanical stability due to electrostatic interactions with positively charged matrix constituents, acting as a horizontal gene transfer vehicle and antimicrobial peptide sequestrant, increasing biofilm tolerance. The sources of eDNA can be bacterial autolysis, active secretion, and neutrophil extracellular traps (NETs) that are released to combat infection, and the presence of NETs in subgingival periodontal biofilms of periodontitis patients. The structural significance of eDNA and its therapeutic potential may be supported by the ability of enzyme degradation of oral biofilms and its dispersal [24].

LIPID AND LIPOPOLYSACCHARIDE COMPONENTS

Components of lipid and lipopolysaccharides (LPS) form a significant yet quantitatively minor part of the oral biofilm matrix compared to the polysaccharides and proteins, and the composition of the LPS is different between supragingival and subgingival biofilms. Gram negative species (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella* spp.) release LPS in gram negative biofilms in their outer membranes, which, together with outer membrane vesicles (OMVs) lose into the outer membrane, supply important lipid and glycolipid components, which are incorporated into the extracellular matrix. These anionically charged molecules combine with matrix polysaccharides, proteins and divalent cations to facilitate matrix cohesion and also serve as strong pro-inflammatory pathogen-associated molecular patterns (PAMPs) to stimulate host immune response through Toll like receptors in periodontal tissues. Lipids (bacterial membrane lipids, LPS of minor Gram-negative constituents and host derived lipids) often constitute around 10-15% of the organic matrix in cariogenic supragingival biofilms, where they affect hydrophobicity and diffusion and mechanical behavior, but exopolysaccharides are still the major structural component [23].

MATRIX FUNCTIONS IN BIOFILM PATHOGENESIS

The biofilm matrix is essential in biofilm pathogenicity which has a variety of mechanisms. The matrix offers primary adhesion binding loci in the form of glucan and other polysaccharide receptors, which allow primary bacterial adhesion and further biofilm formation. The matrix serves as a 3-dimensional framework that assembles the biofilm structure and, at the same time, facilitates biofilm integrity, keeping the multispecies

communities in tight contact with each other such that they can experience interdependence of their metabolic activity and polymicrobial synergy [25].

Biofilm matrix has a dynamic role in the antimicrobial tolerance and enamel demineralization instead of a passive scaffold. It forms diffusion limiting micro-environments which inhibit penetration and effective concentration of agents like chlorhexidine, fluoride and antibiotics, thus biofilm embedded bacteria develop significantly increased resistance in comparison to planktonic cells. The polysaccharide, eDNA, protein, lipid, and lipid-based network of negatively charged EPS binds and retards the incoming cationic antimicrobials, which enables even exposed multispecies biofilms sustained over high concentrations of chlorhexidine to recover. This type of 3D architecture preserves acidic focal points at the biofilm-enamel interface by preventing the efflux of acid as well as the influx of hydroxyl ions in the cariogenic biofilms, which is crucial in maintaining the pH at levels not permissible to remineralization and supporting enamel demineralization [26].

BACTERIAL COMMUNICATION AND COORDINATION

QUORUM SENSING MECHANISMS

Bacterial quorum sensing is a cell-density dependent signaling pathway that allows bacteria to carry out collective behavior with regard to population density. Quorum sensing is the process of producing diffusible signaling molecules (autoinducers) that are released into the culture supernatants; when the required concentrations are reached, the molcules bind the receptor proteins, causing widespread changes in gene expression and physiological behavior [27].

AUTOINDUCER-1 (AI-1) SYSTEMS

Oligopeptide-based AI-1 systems are used by gram-positive bacteria such as Streptococcus mutans to communicate with each other within the species. Autoinducer peptides are generated as precursors which are protein processed through proteins and recognized by the two-component regulatory systems, such as the serine/threonine kinase and response regulator proteins. In S. mutans, AI-1 system controls the biofilm formation, bacteriocin production, competence development and expression of virulence factors [27].

Porphyromonas gingivalis has a system of AI-1 that controls the expression of virulence factors and the formation of biofilms. Recent findings indicate that *P. gingivalis* AI-1 signals mediate interactions between *P. gingivalis* and other species that include *Streptococcus mutans* in mixed-species biofilms, and thus functions of AI-1 are extended beyond intraspecies communication, suggesting interspecies signaling.

AUTOINDUCER-2 (AI-2) SYSTEMS

The signal molecule that is involved in interspecies communication between Gram-positive and Gram-negative bacteria is autoinducer-2 (AI-2), a furanone-based signal molecule. The production of AI-2 is catalyzed by the LuxS enzyme (autoinducer-2 synthase), and the LuxS activity is controlled by the metabolism of methionine. The cross-kingdom communication coordinating the multispecies biofilm behavior is possible due to the ubiquitous nature of AI-2 signaling in the oral bacterial species [27].

P. gingivalis AI-2 signaling promotes cooperative interactions with other periodontal pathogens in oral biofilms, through an alteration of biofilm architecture and virulence. The nutritional dependencies that define synergistic pathogenicity between *P. gingivalis* and *T. denticola* are regulated by AI-2 signaling. Likewise, *S. mutans* AI-2 signaling is an interaction in multispecies biofilms in caries.

BIOFILM REGULATION GENES AND ENVIRONMENTAL SENSING

The ComCDE quorum-sensing pathway in *Streptococcus mutans* links cell density with virulence by the competence -stimulating peptide (CSP) to signal the ComD/ComE two-component system, which subsequently enhances bacteriocin production, genetic competence, stress responses, and normal 3-D biofilm structure. Interventions with comC, comD, or comE lead to the appearance of uncharacteristic and low-biomass biofilms, which underscores ComCDE as an important regulator of *S. mutans* biofilm formation and pathogenicity [28].

LuxS/AI 2 is a highly conserved quorum -sensing system that regulates biofilm growth, carbohydrate catabolism, stress resistance, and the expression of virulence factors in *S. mutans* and other oral bacteria. LuxS mutation disrupts AI -2 production, results in compromised biofilms dependent on sucrose and Abnormal glucosyltransferase expression, and attenuates mixed-species biofilms with *Streptococcus gordonii* and *Porphyromonas gingivalis*, making LuxS/AI -2 a key virulence factor and a possible anti-biofilm agent [29].

QUORUM SENSING AND HOST INTERACTIONS

Quorum sensing (QS) signals are now accepted to be two active mediators which regulate microbial behavior, as well as host responses. QS molecules generated by oral and

other bacteria are capable of being sensed by the epithelial cells, neutrophils and macrophages among other immune cells and modulate cytokine production, barrier properties and cell survival in a manner that may either amplify or suppress inflammation depending on the context [27].

N acyl homoserine lactones (AHLs), traditionally connected to Gram-negative bacteria, and AI 2 type signals can activate pattern recognition and danger sensing pathways in host cells, such as Toll like receptors (TLRs), NOD like receptors and inflammasome components, which result in the activation of NF κB, MAPK signaling and IL1β/IL18 processing. There is experimental evidence that AHL exposure can regulate the expression of pro inflammatory mediators (e.g. IL6, IL8, TNFα), affect epithelial barrier integrity and alter phagocyte activity, suggesting that QS signals are inter kingdom communication molecules that control the outcome of oral and systemic hostpathogen interactions instead of coordinating the behavior of bacterial groups [27].

HOST IMMUNE RESPONSE AND INFLAMMATION

COMPONENTS OF ORAL INNATE IMMUNITY

Elements of oral innate immunity constitute a layered primary line of defense that is constantly surveilling and acting against biofilm communities of the mouth. Innate and adaptive pathways are used in conjunction to identify pathogenic organisms and to generate inflammatory responses when required, and to limit the growth of microorganisms, although innate mechanisms offer the quick, nonspecific reaction that can take minutes to hours [5]. Physical barriers entail intact oral epithelium having tight junctions, the keratinized gingival epithelium and uninterrupted salivary flow, which mechanically removes microbes and dilutes soluble virulence factors. Saliva also has mucins and agglutinins which trap and cluster bacteria making their elimination easier by swallowing instead of mucosa or teeth being stuck [30].

Salivary and GCF humoral intrinsic factors are lysozyme, lactoferrin, peroxidase systems, complement components, antimicrobial peptides (defensins, cathelicidins), and all of which cause direct damage to bacterial membranes, chelate essential iron, or opsonized microbes to be phagocytosed. Phagocytic clearance, release of reactive oxygen species and proteases and antigen presenting to T and B cells are provided by cellular components, in particular neutrophils, macrophages, and dendritic cells recruited via the junctional epithelium, facilitating an interface between innate and adaptive responses to pathogenic biofilms [2].

SALIVA AND MUCOSAL BARRIER FUNCTION

Saliva also performs several antimicrobial roles such as mechanical cleansing, acid buffering of food and bacterial organic acids, remineralization enhancement by calcium and phosphate transport and antimicrobial proteins and peptides. Salivary immunoglobulins, such as secretory IgA avails immune exclusion against pathogenic biofilm adhesion. Salivary lysozyme has bacteriolytic activity on many organisms. Lactoferrin has iron-withholding antimicrobial activities. Salivary histatin antimicrobial peptides have antifungal and antibacterial effects [27].

The junctional epithelium forms a critical physical and immunological barrier at the gingival sulcus, where tight junction proteins and specialized cell–cell contacts help maintain epithelial integrity and restrict bacterial invasion into underlying connective tissues. Pattern recognition receptors are expressed by oral epithelial cells, including Toll -like receptors (TLRs) and NOD -like receptors (NLRs) that perceive pathogen-associated molecular patterns and trigger downstream signaling cascades (e.g., NF- -B, MAPK), which result in the production of antimicrobial peptides and release of pro-inflammatory mediators which coordinate innate and adaptive immune responses to dental biofilms [2].

NEUTROPHILS AND INNATE CELLULAR RESPONSE

Neutrophils (polymorphonuclear leukocytes) constitute the major cellular constituent of innate inflammation of the oral tissues. They are found in gingival crevicular fluid and periodontal pockets, activated by biofilm-generated chemotactic factors and complement activation. Neutrophils infiltrate periodontal tissues in a low concentration on a regular basis, which is a homeostatic state that occurs under steady-state conditions and that actively involves antimicrobial activity and tissue support [31].

Neutrophil antimicrobial mechanisms include: (1) phagocytosis - internalization and intracellular degradation of pathogens through fusion of phagolysosomes containing hydrolytic enzymes and antimicrobial proteins; (2) degranulation - extracellular release of granule contents including elastase, lactoferrin, lysozyme, and antimicrobial peptides; (3) reactive oxygen species (ROS) production—generation of superoxide, hydrogen peroxide, and other reactive species directly toxic to microbes; and (4) neutrophil extracellular trap (NET) formation - release of decondensed chromatin decorated with histones and granule contents entrapping microorganisms [31].

Neutrophil extracellular traps (NETs) have become a significant immune response that plays two roles. The antimicrobial proteins and peptides in NETs increase

local antimicrobial response against periodontal pathogens. Nevertheless, excessive NET leading to the buildup of the host tissue is associated with the release of damaging enzymes such as neutrophil elastase, collagenase, as well as other enzymes that degrade the matrix. There is a role of NET-derived enzymes in the alveolar bone loss in progressive periodontitis.

INFLAMMATORY CYTOKINES AND CHEMOKINES

Bacterial proteins, lipopolysaccharides, and peptidoglycans produced in biofilms activate epithelial cells and macrophages to release pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1- beta (IL-1β), and interleukin-6 (IL-6). These cytokines amplify the effects of inflammatory responses in several ways, such as augmenting neutrophil recruitment, augmenting the vascular permeability as well as augmenting the expression of adhesion molecules [2].

Chemokines, including IL-8, MCP-1 and RANTES coordinate selective leukocyte transport to the locales of biofilm triggered inflammation, with an enhancement in MCP 1 and RANTES concentrations in gingival crevicular fluid and tissues positively associated with probing depth and clinical attachment loss in periodontitis. It is also worth noting that *Treponema denticola* expresses a surface protease dentilisin and a complex of a chymotrypsin like protease, which can degrade host inflammatory mediators, such as IL1β, IL6, TNFα, and a number of chemokines, to disrupt normal neutrophil and monocyte recruitment and lead to a dysregulated inflammatory environment and dysbiosis in periodontal epithelial pockets [32].

MATRIX METALLOPROTEINASES AND TISSUE DESTRUCTION

MMPs are major mediators of periodontal tissue destruction and include a family of zinc and calcium-dependent endopeptidases that cleave extra-cellular matrix components (fibrillar collagens (type I, II, III), elastin, gelatin, laminin, fibronectin, and proteoglycans). The neutrophil collagenase (MMP-8) and interstitial collagenase (MMP-1) activated by periodontitis trigger the breakdown of periodontal ligament type I collagen at select Gly-Ile/Leu bonds to form collagen telopeptides cleaved by gelatinases MMP-2 and MMP-9 to peptides. MMP-13 (collagenase-3) is also increased in pathologic gingivae and leads to bone erosion [33].

Periodontitis MMP activation is a process with bacterial and host pathways. *Porphyromonas gingivalis* gingipains (RgpA/B, Kgp) are a direct proteolytic activator of latent pro-MMP-9/2 and pro-MMP inhibitors, and induce more extensive matrix destruction. Host pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) induce MMP-1, MMP-3, MMP-8, MMP-9, and MMP-13 expression in fibroblasts, macrophages, and gingival epithelial cells via NF-κB and AP-1 signaling. Bacterial LPS and other PAMPs further upregulate MMP production through TLR signaling

In physiological conditions, MMP activity is under the strict control of tissue metalloproteinase inhibitors (TIMPs 1 4), which are able to form 1:1 metal protease active complexes with active MMPs to keep extracellular matrix homeostatic. In periodontitis, net proteolytic unbalance supporting tissue destruction, an increase in the levels of MMP-8 and MMP-9 in gingiviable crevicular fluid and diseased tissues are always accompanied by MMP overexpression and relative deficiency of TIMP (especially TIMP-1 and TIMP-2) [2].

ADAPTIVE IMMUNE RESPONSE

Immunogenicity of oral pathogens is best characterized as adaptive immunity that is partially protective. Periodontal pathogen specific antibodies Periodontal pathogen specific antibodies (primarily IgA in saliva and IgG in serum) can be detected in caries and periodontitis respectively, respectively, in individuals with antigen mediated humoral responses. Increased salivary anti s. mutans IgA may be related to reduced caries experience in certain cohorts, but in most periodontal and caries research, high levels of pathogen specific antibody are related to disease presence or disease severity rather than protection, which may indicate that antibody production is often a measure of persistent antigenic stimulation and ineffective immune control as opposed to the presence of optimal sterilizing immunity [34].

Tcell responses in periodontal disease involve multiple $CD4^-$ Thelper subsets with distinct effector profiles. Th1 cells produce IFNγ and TNFα and support cellmediated immunity; Th2 cells secrete IL4 and IL10 and favor humoral responses; Th17 cells produce IL17 and IL22, driving neutrophil recruitment, osteoclastogenesis, and proinflammatory cytokine cascades; and regulatory T cells (Tregs) expressing FOXP3 secrete IL10 and TGFβ to restrain excessive inflammation. In periodontitis, an imbalance characterized by increased Th1/Th17 activity and reduced Th2/Treg function is consistently associated with heightened inflammatory mediator levels and alveolar bone loss, and Th17/Treg ratios in blood and periodontal tissues correlate with disease severity and response to therapy. More recently, γδ T cells have emerged as important regulators of gingival immunity: oral barrier γδ T cells can either limit or exac-

erbate periodontal pathology depending on context, contributing to tissue repair via amphiregulin production but also promoting bonedestructive responses in some infection models, highlighting their dual role in chronic periodontal inflammation [2].

PREVENTION STRATEGIES

ORAL HYGIENE AND MECHANICAL PLAQUE REMOVAL

Toothbrushing and interdental cleaning are the bases of caries and periodontal disease prevention as they remove plaque, which is entirely mechanical. Removal of pathogenic biofilms before they become pathogenic consortia of pathogenic bacteria by means of daily plaque removal through brushing of the teeth. Proper fluoridated toothpasting with fluoride concentrations of 1000 ppm fluoride or higher reduced caries incidence significantly, and there is evidence that it is effective, especially in fluoride levels of 1000-1500 ppm fluoride (to prevent caries) and 5000 ppm fluoride (to protect high-risk individuals)[35].

Interdental cleaning (floss, interdental brushes, wood sticks, oral irrigators) is required to access interproximal and subgingival spaces that can not be reached by toothbrush bristles and it can prevent proximal caries and interproximal periodontal disease. Systematic and scoping reviews show that interdental devices have weak to moderate adjunctive effects in the control of plaque and gingivitis, with interdental brushes demonstrating more consistent results than floss due to the restricted nature of the evidence and the significant effect of compliance and technique on results.

FLUORIDE APPLICATION AND MECHANISMS

Fluoride exhibits multiple anti-caries mechanisms: (1) inhibition of demineralization through fluorapatite formation, which is more acid-resistant than hydroxyapatite; (2) enhancement of remineralization through deposition of fluoride-containing minerals; (3) inhibition of bacterial glycolytic enzymes, including enolase and lactate dehydrogenase, thereby reducing acid production and bacterial growth [35].

Some of the modalities of delivering fluoride are through water fluoridation, fluoride toothpaste, mouthrinses, professionally applied gels and varnishes, and fluoride supplements in the diet. The efficacy of 0.7-1.0 ppm in water fluoridation is that it virtually eliminates caries by a margin of 25-30 percent among the population with sufficient water fluoridation. There is moderate-level evidence in the use of professionally administered fluoride gels (1% sodium fluoride) and varnishes (22,600 ppm) as caries prevention methods. Recent systematic reviews show that professionally applied fluoride varnishes have clinical benefit in caries prevention, as resin-based fissure sealants have the same or even higher effectiveness in caries prevention as fluoride varnish applications.

PIT AND FISSURE SEALANTS

Dental sealants (resin-based and glass ionomer) are physical barriers to cover occlusal pits and fissures of posterior teeth, inhibiting the accumulation of plaque and inhibiting the access of nutrients to fissure microorganisms. The majority of caries in children and adolescents happens on the occlusal surfaces, on which the fissure anatomy is slender and complex, making the brush bristles of the toothbrush inaccessible; thus, sealants are effective among primary and permanent molars.

There is high-grade evidence indicating that pit-and-fissure sealants can significantly prevent the incidence and progression of caries in the pit-and-fissure area compared with no sealant or fluoride varnish alone, with the guideline panels indicating that the risk is reduced by about 70-80 percent over 2-3 years in children and adolescents. Modern reviews find that resin-based sealants offer better retention and caries prevention effect where adequate isolation and moisture control is possible, but glass ionomer sealants, though with low retention, are more beneficial in partially erupted molars or where moisture control cannot be adequately controlled, and may be equally effective in the long term prevention of caries in those situations.

DIETARY MODIFICATION AND CARBOHYDRATE RESTRICTION

Dietary change and carbohydrate restriction contain the risk of caries by reducing the number and severity of biofilm acid challenges. Frequent consumption of free sugars, especially sucrose in snacks and beverages between meals, is strongly associated with higher caries activity in children, adolescents, and adults, whereas limiting free sugars to less than 10% (ideally <5%) of total energy intake and restricting sugar exposures to ≤3–4 times per day substantially reduces caries risk. Frequent consumption of sugary snacks by high frequency raises the odds of caries several folds as compared to low frequency, and epidemiologic evidence indicates consistently that the frequency and quantity of sugar consumption, especially outside of mealtime, is a crucial factor in caries experience [35].

On the biofilm level, sucrose is the only cariogenic fermentable material since it can be used as a fer-

mentable material to produce acids, and it can be used as an extracellular polysaccharide material to produce EPS-rich, acidogenic, and aciduric biofilms. Decreasing the amount of carbohydrate intake, reducing the amount of sucrose and refined carbohydrates reduces substrate availability to *Streptococcus mutans* and other cariogenic organisms, decreases EPS production and biofilm acidogenicity, and restricts ecological selection of acid-tolerant species. Substituting sugar-free alternatives - particularly xylitol- or sorbitol-based chewing gums and confections - further reduces cariogenic potential, as polyols are poorly fermented by plaque bacteria and can stimulate salivary flow; multiple clinical trials and meta-analyses show that regular xylitol gum use decreases caries incidence and *S. mutans* levels compared with sugared controls, especially when used several times daily after meals [35].

XYLITOL AND SUGAR ALCOHOL CARIES PREVENTION

Xylitol, a naturally occurring five-carbon sugar alcohol, exhibits caries-preventive properties through multiple mechanisms: (1) nonfermentability by oral bacteria - xylitol is scarcely metabolized by cariogenic microorganisms, preventing fermentation and acid production that drive enamel demineralization; (2) antimicrobial effects through metabolic disruption - when *Streptococcus mutans* takes up xylitol, it is phosphorylated but not further metabolized, creating a futile cycle that depletes cellular energy, inhibits bacterial growth, and reduces acid and virulence factor production; (3) reduced extracellular polysaccharide synthesis by *S. mutans*, thereby decreasing plaque adhesiveness and biofilm accumulation; (4) saliva stimulation through mastication, enhancing saliva-mediated remineralization, pH buffering, and antimicrobial clearance; and (5) microbial selection toward less cariogenic xylitol-resistant *S. mutans* strains with attenuated virulence, although the long-term clinical significance of this shift is still being clarified [36].

Meta-analysis demonstrates xylitol reduces DMF/dmf (decayed, missing, filled surfaces/teeth) scores with a standard mean reduction of -1.09 compared to all controls, and -1.87 reduction compared to fluoride varnish controls [36]. Newer systematic reviews of xylitol versus other polyols like sorbitol and erythritol reveal that xylitol lowers caries increment and *S. mutans* counts, and erythritol may have potentially better efficacy in some long-term studies, but the clinical implications of the differences have not yet been studied. In particular, erythritol showed inferior and slower caries formation relative to xylitol and sorbitol during a 3 year follow-up type study in child groups, and had reduced dental treatments.

Xylitol can be found in various products, which are chewing gum, lozenges, mints, candies, syrups, toothpastes, etc. American Academy of Pediatric Dentistry recommends xylitol 3-8g/day in the form of syrup or wiping to children at ages below 3 years; and age-specific products like gum or candies to children aged 4 and above years. Among those who are at risk of cavities (adolescents and adults), and those with high caries rates, clinical evidence indicates that a 5-10g/day, 3-5 times regimen is the most effective regimen in preventing caries incidence.

ANTIMICROBIAL AGENTS AND CHLORHEXIDINE

Chlorhexidine (CHX) is a cationic biguanide that has been shown to have a broad-spectrum antimicrobial activity and a high activity against both aerobes and anaerobes. The CHX antimicrobial action is based on attraction of the positive charge to negatively charged bacterial cell surfaces, which results in the strong adsorption on the bacterial cell membranes, cell wall disruption, cytoplasmic leakage, and bacterial death at increased concentration. CHX demonstrates substantivity, i.e. antimicrobial activity remaining after as long as 12 hours after application, by retention on oral surfaces such as tooth enamel, mucosa and salivary glycoproteins.

Chlorhexidine mouthwash at 0.12% concentration demonstrates superior efficacy for plaque control; rinsing for 60 seconds twice daily with 10 ml of chlorhexidine inhibits plaque growth by approximately 60% and reduces gingivitis by 50-80%. Clinical use of chlorhexidine in chronic prevention is however, limited because of the side effects such as tooth discoloration, taste disorder and mucosal erosions. The existing knowledge underlies short-term use (weeks to several months) of chlorhexidine in specific indications, not in chronic prevention.

It is worth noting that exposure to chlorhexidine causes microbiota changes that can lead to disease-related dysbiosis in certain instances. Exposure of biofilm to chlorhexidine leads to initial microbial inactivation and a rapid increase in biomass with altered microbial composition, in certain cases with an increase in the abundance of pathobiont strains and a shift in metabolic activity towards disease-associated lactate production.

PROBIOTICS AND PREBIOTICS

Probiotics are live microorganisms that, when ingested in sufficient quantities, provide a health benefit to the

host and have been explored as adjunctive measures, not as treatment in themselves, in the prevention of caries and periodontitis. Competitive exclusion, co-aggregation as well as secretion of antimicrobial compounds including organic acids, hydrogen peroxide and bacteriocin-like peptides along with the regulation of local immune responses can inhibit the growth and virulence of cariogenic and periodontopathogenic species by oral probiotic strains like *Lactobacillus reuteri*, *Streptococcus salivarius* (K12, M18) and various Bifidobacterium species [37].

In oral health, probiotic mechanisms are better framed as:

1. Competing with pathogens for adhesion sites and nutrients on oral surfaces and within biofilms, and, as a result, colonization by cariogenic and periodontal pathogens [37]
2. Instead of preventing the formation of calculus, producing antimicrobial metabolites (e.g. bacteriocins, hydrogen peroxide, organic acids) that are able to inhibit biofilm formation and modify biofilm composition
3. Modulating inflammatory responses by down-regulating pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and MMP-8 and enhancing anti-inflammatory mediators like IL-10 and TIMP-1
4. Enhancing tissue remodeling and periodontal stability by decreasing the burden of inflammation and changing the microbiota composition to a less pathogenic state indirectly.

Recent clinical trials prove that periodontal disease clinical markers, such as bleeding on probing and probing pocket depth, are decreased by probiotics in chewing gum or in lozenges. A meta-analysis of the effectiveness of probiotics in the management of periodontitis suggests that probiotics decrease periodontal inflammatory (IL-1β, MMP-8) and improve the decrease in probing pocket depth and gain in clinical attachment level.

Prebiotics are non-digestible substances that are selectively used by host microorganisms with a health effect; in the mouth, inulin -type fructans and fructo -oligosaccharides can stimulate growth of desirable lactobacilli and bifidobacteria and could aid in repositioning the oral microbiome to be less dysbiotic. Combined prebiotics add to the particular probiotics have been identified as synbiotics that seem to have improved efficacy due to synergistic effects, and the recent reviews and preliminary clinical trials indicate that synbiotic or postbiotic preparations can be more beneficial to caries outcomes in children and periodontal treatment response in adults compared to the individual components.

TREATMENT APPROACHES

MECHANICAL DEBRIDEMENT: SCALING AND ROOT PLANING

Scaling and root planing (SRP) is the gold standard non-surgical mechanical therapy of periodontal disease, and the central part of primary therapy in most patients with chronic periodontitis. SRP in periodontal therapy mechanically debridges tooth surfaces with supra- and subgingivally located biofilm and calculus, destabilizes organized biofilm communities, and smoothes root surfaces and, thus, reduces pathogenic biomass, enhancing periodontal healing. Even though mechanical debridement and plaque removal also feature in the caries control, SRP *per se* is instead chiefly established in terms of periodontal, not coronal, caries management.

SRP induces treatment responses in a number of ways, with direct destruction of bacteria by destabilizing biofilm structure, exposing anaerobic microorganisms to oxygen, which creates an unfavourable environment to obligate anaerobes, and by eliminating bacterial lipopolysaccharides and other pathogenic surface composites of root cementum. Such changes alter the subgingival environment, decrease inflammation, and on average have an average probing depth decrease of about 1-2 mm and clinical attachment improvements of about 0.5-1 mm based on initial pocket depth.

Nonetheless, there are significant drawbacks to SRP. The difficulty of removing 100 percent of calculus and biofilm is increasingly challenging in deep pockets, furcation sites and complex root morphologies, and research reveals that, even after closed SRP, 30-60 percent of the root surfaces might still have some residual subgingival calculus, particularly when the pocket depth is more than 6 mm. Clinically, an impressive percentage of locations or patients (usually estimated at about 20-40% of moderate-severe cases of chronic periodontitis) have residual deep pockets and evidence of ongoing inflammation following SRP alone and require adjunctive measures (usually considered surgical access, local or systemic antimicrobials, or photodynamic therapy).

SYSTEMIC ANTIBIOTICS

A combination of adjunctive systemic antibiotics with SRP augments the therapeutic effect, especially in aggressive periodontitis and in chronic periodontitis patients with poor response to monotherapy when using SRP. Various combinations of antibiotics are effective, the most well-researched and clinically validated combinations being metronidazole (used or used with amoxicillin or azithromycin). Recent systematic reviews

affirm that amoxicillin, metronidazole, and azithromycin offer the most uniform extra benefits in probing depth reduction and clinical level of attachment, particularly in deep pockets and in the progressively advancing disease.

The antibiotic efficacy mechanisms include: decreasing obligate anaerobic periodontal pathogens by direct antimicrobial attack, altering polymicrobial synergism by selective destruction of critical species, and attenuation of bacterial virulence factor expression. Systemic antibiotics combined with SRP show a mean additional clinical attachment gain of between 0.2-0.4 mm and a probing depth reduction of between 0.3-0.6 mm on average compared to SRP monotherapy, with the greatest effect reported with amoxicillin-metronidazole combinations. The positive effects are seen in the aggressive periodontitis patients, where the mean gain of attachment is significantly higher than in chronic periodontitis, especially in stage III/grade Cs.

Nevertheless, developing antimicrobial resistance is a severe disadvantage of long-term or repeated systemic antibiotics, and emerging resistant pathogens diminish clinical efficacy with time and represent a more general public-health threat. Recent umbrella reviews have determined that, whilst statistically significant improvement is obtained with adjunctive systemic antibiotics, the effect size on a population level is small, and it is not justified to use this in all periodontitis cases. Recent evidence-based recommendations and guidelines encourage a restrictive, case-by-case approach and administration of systemic antibiotics only on specific high-risk patients or non-responsive patients, with short and well-defined courses of antimicrobials in coordination with completion of SRP and focus on concomitant mechanical debridement and antimicrobial mouthrinses to reduce the duration of treatment and risk of resistance.

ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs) are innovative therapeutic tools that have a strong anti-oral biofilm activity and a low tendency to develop resistance to them due to their fast and multitarget effects on bacterial membranes and intracellular elements. Peptide 1018 (IDR-1018) is a synthetic cationic amphiphilic peptide, the analogue of bovine host-defense peptide bactenecin, with a broad-spectrum antibiofilm activity against oral bacteria such as Streptococcus mutans, Enterococcus faecalis and mixed-species oral biofilms at significantly lower concentration levels than its MIC values against planktonic cells [38].

Mechanisms of peptide 1018 triggering the ability to induce biofilm cell death by targeting and inducing the degradation of the nucleotide second messenger (p) ppGpp, a central regulator of the stringent response and the survival of biofilms, and cause cell lysis and disruption of the biofilm structure as observed under electron microscopy. The peptide disperses and kills preformed biofilms as well as prevents biofilm formation, and there are large changes in viable biofilm bacteria at sub-MIC concentrations.

Notably, peptide 1018 does not lose its antibiofilm capability when combined with saliva, which is also clinically significant because, in vivo, most AMPs will be inactivated by salivary and bacterial proteases in the oral cavity. The combination treatment of peptide 1018 at low-concentration chlorhexidine (2%) significantly increases the efficacy of antibiofilm treatment, killing biofilm by greater than 50 and dispersing biofilm by more than 35 of 3-minute exposure in multispecies oral biofilm models.

Human β-defensin-3 (HBD-3) demonstrates enhanced bactericidal activity against mixed-species oral and endodontic biofilms compared to conventional agents such as calcium hydroxide and chlorhexidine, particularly against *E. faecalis* within dentinal tubules. HBD-3 synthetic HBD 3 derivatives (e.g. HBD3 -C15) have been demonstrated to be more penetrative and kill biofilm bacteria in dentin models and can act synergistically with traditional irrigants to promote HBD3 -based formulations to be used in endodontic and periodontal practice.

PHOTODYNAMIC THERAPY

The antimicrobial photodynamic therapy (aPDT) is an academic technique of treatment using light irradiation in combination with a photosensitizer molecule to produce reactive oxygen species (ROS) that are harmful to microorganisms, such as singlet oxygen. aPDT is a non-invasive method that requires interaction of a photosensitizer, molecular oxygen, and visible light to create reactive oxygen species (ROS). The treatment is usually administered as a topical photosensitizer (usually indocyanine green -ICG) application and later irradiation of the skin with lasers with certain wavelengths corresponding to the spectral absorptions of photosensitizers. Indocyanine green combined with a diode laser of 810 nm produces singlet oxygen and the effect of other reactive oxygen species with bactericidal activity against periodontal pathogens. The process entails the electron transfer under the influence of a photon via Type II photosensitization where ICG causes intense cellular damage when exposed to a near-infrared-light. The produced singlet oxygen quickly interacts with the bacterial biomolecules, which results in cross-

linking lipids of the membrane, damage to proteins and ion channels, and the removal of metabolic enzymes.

A clinical trial has shown that aPDT when used as an adjunct to scaling and root planing (SRP) and laser-assisted is very effective in reducing probing pocket depth (PD) along with increasing clinical attachment level (CAL) over SRP used alone. Repeated applications of aPDT significantly improved outcomes, reducing residual pockets greater than 5 mm by more than 40%, with effects more pronounced in deep sites (PPD ≥ 6 mm) [39].

aPDT demonstrates efficacy against periodontal pathogens, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Tannerella forsythia*, with bacterial load reductions of 95-99%. Beyond bacterial killing, aPDT promotes healing through immunomodulatory effects by suppressing inflammatory mediators such as TNF-α and IL-1β, resulting in decreased inflammation and enhanced tissue regeneration.

The benefits associated with the use of eye products are non-development of antibiotic resistance, non-invasive use, and possible repetitive use without the occurrence of resistance. The multi-target mode of action also results in less resistance, unlike antibiotics, since the ROS floods antibacterial antioxidant responses at the same time. It has been proposed through comparative studies that aPDT is an effective alternative to systemic antibiotics in the treatment of periodontitis. The existing evidence confirms the efficacy of aPDT as an adjunctive therapy, but concerns the cost-efficiency and the best protocols. Studies on photosensitizers differ significantly in the choice of treatment regimens, laser settings, and frequency of use. When using multiple applications (2-4 sessions), the results are superior to single applications. It is justified that future standardized clinical studies involving larger cohorts with a longer follow-up period should be conducted to develop some standard guidelines.

PROBIOTICS AS ADJUNCTIVE THERAPY

New data are in favor of probiotics as a complement to traditional periodontal therapy. The mechanistic explanation includes the replacement of pathogenic species by the competitive exclusion, disruption of biofilms by antimicrobial compounds production, and the immune modulation in favor of healing. Clinical trials prove the use of *Lactobacillus reuteri* strains integrated into chewing gum when using it together with the traditional SRP lead to better clinical parameter changes such as the decrease of bleeding on probing, the decrease of probing pocket depth, and the improvement of the clinical level of attachment in comparison with SRP and placebo [40].

CONCLUSIONS

Clinical trials prove that the use of *Lactobacillus reuteri* strains integrated into chewing gum, when used together with the traditional SRP lead to better clinical parameter changes, such as the decrease of bleeding on probing, the decrease of probing pocket depth, and the improvement of the clinical level of attachment in comparison with SRP and placebo.

There are more than 500 different types of bacteria in the oral microbiota that remain in symbiosis in a state of health. The selective growth of disease-causing consortia such as Streptococcus mutans and related acid-tolerant pathogens in caries, and the red complex (*Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*) in periodontal disease are dysbiotic changes to disease. These pathogens have complex virulence factors such as biofilm formation, production of extracellular polysaccharides, synthesis of proteolytic enzymes as well as immune evasion mechanisms that enable pathogenesis even in the presence of strong host immunity.

The formation of biofilms is the key pathogenic process in both pathologies, and the extracellular polymeric substance matrix offers key scaffolds, diffusion-restricting microenvironment, and antimicrobial resistance. Bacterial communication Quorum sensing, regulates the expression of virulence factors and biofilm formation in multispecies communities and creates regulatory mechanisms to switch between commensal existence and pathogenic phenotype.

The host immune responses to biofilm challenge include innate responses such as neutrophil recruitment and production of matrix metalloproteinase and adaptive responses (antibody and T cell). Although the roles of these immune processes are protective, overreactivity or malregulation of these mechanisms, especially neutrophil and MMP activation, is involved in the processes of destructive periodontal tissue destruction that accelerates the onset of the disease.

Evidence-based prevention strategies are mechanical plaque removal, fluoride application, dietary modification, and emerging ones that involve the use of antimicrobial agents that have lower resistance potential. The paradigms of treatment are still developing past the traditional use of mechanical debridement and antibiotics to modality specific approaches such as antimicrobial peptides, photodynamic therapy and antibiotic-based approaches based on dysbiotic restoration of oral microbiota.

Future research should prioritize: (1) identification of dysbiosis markers enabling early disease intervention; (2) development of interventions specifically targeting keystone pathogens and dysbiosis reversal rather than

non-specific biofilm removal; (3) integration of omics technologies providing systems-level understanding of host-microbe interactions; (4) development of drug delivery approaches improving therapeutic agent penetration into biofilm microenvironments; and (5) personalized treatment approaches incorporating individual host susceptibility factors and microbiota profiles guiding intervention selection.

The microbiology of dental caries and periodontal disease has also been undergoing advanced development with complex molecular applications and systems thinking. The increased knowledge provides the possibility of rational, mechanism-based intervention development that enhances the effectiveness of disease prevention and treatment and minimizes the antimicrobial resistance and other adverse effects of traditional methods.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

CONTENTS

Before and after mastopexy: Expectations and reality (meta-analysis)

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ABSTRACT

Aim: To systematically analyze and compare the expectations and actual postoperative outcomes of patients undergoing mastopexy procedures. By conducting a comprehensive meta-analysis of current clinical literature, the study seeks to: evaluate patient-reported satisfaction rates following mastopexy; identify the most common discrepancies between preoperative expectations and achieved aesthetic results; analyze the frequency and nature of postoperative complications; provide evidence-based recommendations for improving preoperative counseling and aligning patient expectations with realistic surgical outcomes.

Materials and Methods: A systematic review and meta-analysis were conducted using PRISMA guidelines. Databases searched included PubMed, Scopus, and Web of Science from 2010 to 2024. Inclusion criteria were studies that reported both subjective (patient satisfaction) and objective (clinical outcome) data pre- and post-mastopexy.

Conclusions: Mastopexy demonstrates good safety and satisfaction profiles, but better preoperative counseling is essential to align expectations with surgical outcomes.

KEY WORDS: mastopexy, breast lift, aesthetic surgery, meta-analysis

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INTRODUCTION

Mastopexy, or breast lift surgery, is a cosmetic procedure designed to correct breast ptosis by reshaping and elevating the breast mound. The popularity of this procedure has increased significantly over the past two decades, particularly among women aged 30 to 55, due to greater awareness, improved surgical techniques, and rising aesthetic expectations fueled by social and digital media platforms [1, 2-6].

Breast ptosis can result from aging, postpartum changes, weight fluctuations, or genetic predisposition. It is classified according to the position of the nipple-areolar complex relative to the inframammary fold, commonly using Regnault's classification (grades I–III). Mastopexy techniques vary depending on the degree of ptosis and surgeon preference and may include periareolar, vertical, or Wise-pattern incisions, often combined with augmentation using breast implants to improve volume and upper pole fullness [3,7].

Despite the overall high success rate of mastopexy in restoring breast aesthetics and improving body image, a persistent gap remains between patient expectations and postoperative outcomes. Many women envision a dramatic and lasting transformation with minimal scarring, ideal symmetry, and youthful projection. However, the biological limits of skin elasticity, healing variability, and procedural limitations may lead to postoperative dissatisfaction if expectations are not realistically managed [4, 8].

Previous studies have focused predominantly on surgical techniques, complication rates, and aesthetic scoring, with relatively fewer works assessing the psychosocial dimension of cosmetic satisfaction or the discordance between anticipated and achieved results. The subjective nature of beauty, compounded by individual psychological and emotional drivers, complicates standardized outcome assessments [5, 9].

This meta-analysis aims to synthesize current literature on the perceived and actual outcomes of mastopexy. Specifically, it evaluates patient satisfaction levels, the alignment between preoperative expectations and clinical realities, common complications, and the overall aesthetic success from both subjective and objective perspectives. Through this analysis, we aim to provide clinicians with evidence-based recommendations for optimizing patient education and improving procedural planning and outcomes.

AIM

The aim of this study is to systematically analyze and compare the expectations and actual postoperative outcomes of patients undergoing mastopexy procedures. By conducting a comprehensive meta-analysis of current clinical literature, the study seeks to: evaluate patient-reported satisfaction rates following mastopexy; identify the most common discrepancies between preoperative expectations and achieved aesthetic results; analyze the frequency and nature of postoperative complications; provide evidence-based recommendations for improving preoperative counseling and aligning patient expectations with realistic surgical outcomes.

MATERIALS AND METHODS

This research is a systematic review and meta-analysis conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The study protocol was pre-registered in the PROSPERO database.

A comprehensive literature search was performed using the electronic databases PubMed, Scopus, Web of Science, and EMBASE to identify relevant articles published between January 2010 and March 2024. The following Medical Subject Headings (MeSH) and free-text terms were used in combination: "mastopexy"; "breast lift"; "aesthetic surgery"; "cosmetic outcome"; "patient satisfaction"; "expectations vs reality"; "postoperative evaluation".

Boolean operators (AND, OR) were used to refine the searches. References of selected articles and relevant reviews were also manually screened to identify any additional eligible studies.

Studies were included if they met the following inclusion criteria:

- Original clinical research (prospective, retrospective, or cross-sectional);
- Sample size ≥ 30 patients;
- Reports on both preoperative patient expectations and postoperative outcomes;
- Quantitative assessment of patient satisfaction or aesthetic results;
- Published in English;
- Full-text available.

Exclusion criteria were: case reports, reviews, editorials, conference abstracts;

studies not reporting on patient-reported outcomes; non-English publications.

Two independent reviewers (Reviewer A and Reviewer B) screened all titles and abstracts for eligibility. Full-text articles were then assessed for inclusion. Discrepancies were resolved through discussion or third-party adjudication. A standardized data extraction form was used to collect:

- Author(s), year of publication.
- Study design and setting.
- Sample size and demographics.
- Type of mastopexy technique.
- Use of augmentation (if applicable).
- Reported patient expectations (preoperative).
- Postoperative outcomes (subjective and objective).
- Fo1llow-up duration.
- Satisfaction scores.
- Com1plication rates.

The Newcastle-Ottawa Scale (NOS) was used to assess the quality of observational studies. Each study was rated on three domains: selection, comparability, and outcome. Studies scoring ≥7 were considered high-quality.

Meta-analytic pooling of outcome measures (satisfaction rate, complication rate, expectation vs. reality alignment) was conducted using Review Manager (RevMan 5.4). The random-effects model was used due to expected heterogeneity among studies. The I^2 statistic was calculated to assess heterogeneity (with thresholds: 25% = low, 50% = moderate, 75% = high). Publication bias was evaluated using funnel plots and Egger's regression test.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

Out of 1,128 identified records, 856 remained after removing duplicates. After abstract screening, 72 full-text articles were reviewed. Ultimately, 14 studies fulfilled all inclusion criteria and were included in the final meta-analysis. The detailed selection process is depicted in the PRISMA flowchart (Fig. 1).

The 14 studies spanned from 2010 to 2023 and involved a total of 1,213 female patients aged between 21 and 58 years (mean age: 38.4 ± 6.2 years). Mean follow-up time was 14.8 months (range: 6 to 36 months). Different techniques were used: Wise-pattern mastopexy (6 studies), vertical scar (4 studies), periareolar (2 studies), and augmentation-mastopexy (7 studies). Geographical distribution included 5 studies from Europe, 4 from North America, 3 from South America, and 2 from Asia (Table 1).

The pooled patient satisfaction rate was 84.7% (95% CI: 79.2–89.3%). Patient satisfaction was measured via self-report questionnaires, Visual Analogue Scales (VAS), and validated tools such as the BREAST-Q in 5 studies.

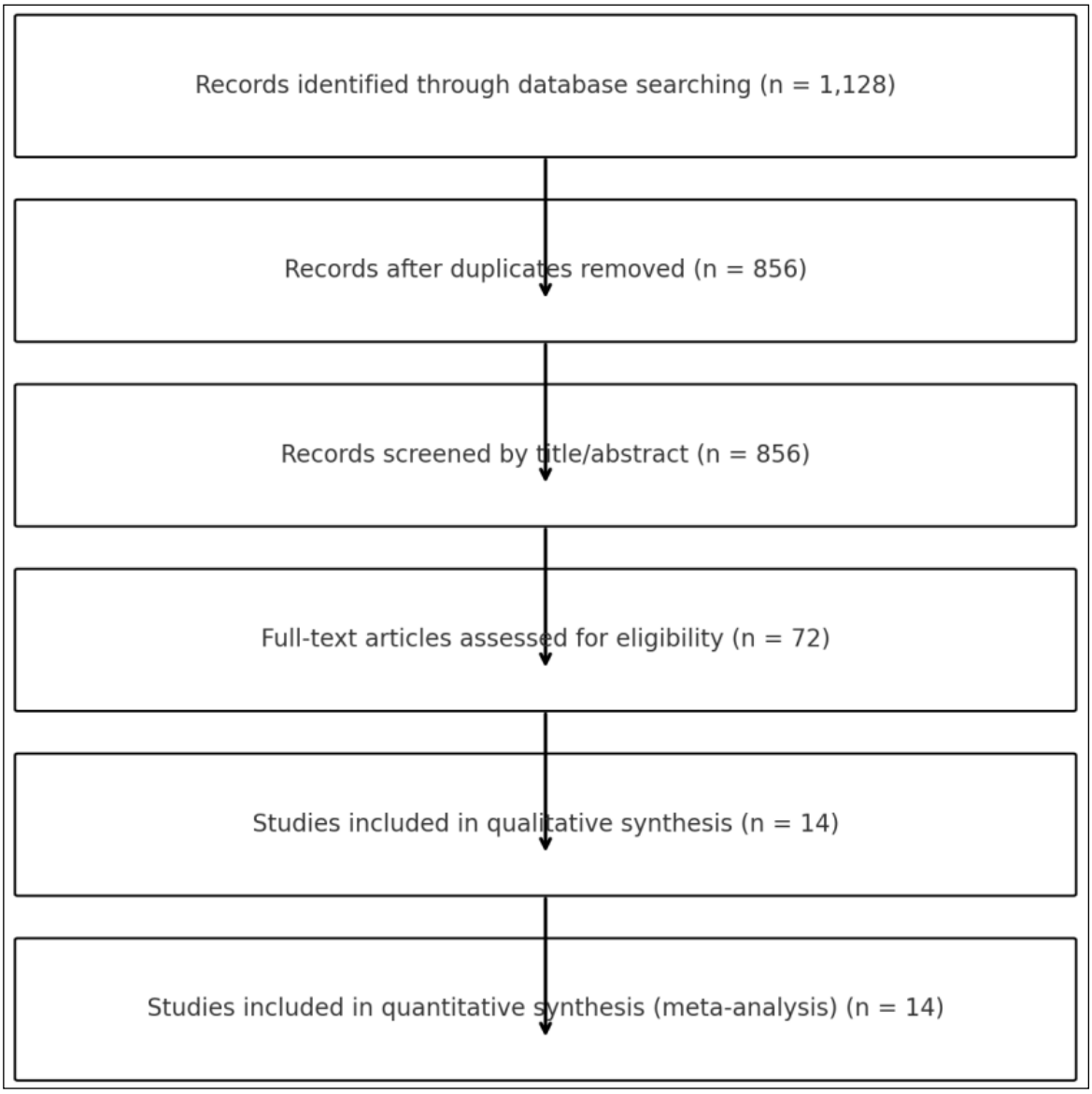

Fig. 1. PRISMA flowchart for selection process
Source: compiled by the authors of this study

Key satisfaction drivers included:
- Improvement in breast shape and contour (89%).
- Restoration of nipple height (85.5%).
- Psychosocial benefit and improved body image (78%).

Subgroup analysis revealed that: patients who received augmentation-mastopexy had significantly higher satisfaction (88.4%) than those who received mastopexy alone (80.5%, $p < 0.05$). Patients aged 35–45 had the highest satisfaction (87.2%), likely due to more realistic expectations and better tissue quality.

Mismatch between expectation and reality was especially noted in the following areas (Table 2).

Despite high overall satisfaction, patients often overestimated the invisibility of scars and the duration of lifted results. Importantly, preoperative counseling involving photographic simulations reduced this mismatch by 30% ($p < 0.01$ in 3 studies).

The overall complication rate across the pooled sample was 9.6% (95% CI: 7.2–12.4%) (Table 3). Complications were predominantly minor and self-limiting.

There were no major infections, necrosis, or implant-related capsular contracture reported. All cases of wound healing issues resolved conservatively.

A random-effects model meta-analysis revealed: effect size (pooled satisfaction): 0.82 (95% CI: 0.76–0.88), indicating high satisfaction. Heterogeneity: moderate ($I^2 = 58\%$). Egger's regression intercept: $p = 0.21$, suggesting no significant publication bias. A forest plot is presented in Fig. 2.

Wise-pattern technique had the highest correction of ptosis but also the highest scarring rate. Vertical technique showed a favorable balance between lift and scar visibility. Augmentation group showed greater upper pole projection and longevity (≥18 months), contributing to enhanced satisfaction (Fig. 3).

Table 1. Study Characteristics

Category	Details
Study period	2010–2023
Total number of studies	14
Total patients included	1,213
Age range (years)	21–58
Mean age ± SD	38.4 ± 6.2
Follow-up duration (months)	14.8 (range 6–36)
Wise-pattern mastopexy	6 studies
Vertical scar mastopexy	4 studies
Periareolar mastopexy	2 studies
Augmentation-mastopexy	7 studies
Studies from Europe	5 studies
Studies from North America	4 studies
Studies from South America	3 studies
Studies from Asia	2 studies

Source: compiled by the authors of this study

Table 2. Mismatch between expectation and reality

Outcome Domain	Expected (%)	Observed (%)	Δ Difference	Significance (p)
Scar visibility	89.2	63.5	–25.7%	<0.001
Upper pole fullness	94.1	76.6	–17.5%	<0.01
Symmetry	90.3	82.1	–8.2%	0.05
Longevity of correction	91.5	69.2	–22.3%	<0.01
Overall body confidence	85.0	79.6	–5.4%	ns

Source: compiled by the authors of this study

Table 3. Complication rate

Complication Type	Frequency (%)
Hypertrophic or wide scarring	4.1%
Wound dehiscence (minor)	2.8%
Nipple-areolar complex hypoesthesia	1.6%
Minor asymmetry requiring revision	1.1%
Seroma or hematoma	0.7%

Source: compiled by the authors of this study

Five studies reported validated psychological outcomes using BREAST-Q or Body Image Scale (BIS) (Fig. 4):
- 78% reported increased body confidence.
- 66% noted improvements in sexual wellbeing.
- 58% reported enhanced self-esteem in social settings.

Patients with preoperative anxiety or unrealistic expectations showed lower satisfaction and higher request for revision.

The findings from this meta-analysis demonstrate that mastopexy is associated with a high level of overall patient satisfaction, with a pooled rate of 84.7%. The majority of patients reported significant improvements in breast shape, nipple positioning, and body image perception. However, notable discrepancies between expectations and outcomes were observed—particularly in the domains of scar visibility, upper pole fullness, and the longevity of aesthetic results.

Subgroup analyses revealed that patients undergoing augmentation-mastopexy experienced higher satisfaction, especially in relation to breast projection and upper pole volume. Technique-specific outcomes also varied, with Wise-pattern mastopexy offering more significant lift at the expense of increased scarring, whereas vertical and periareolar approaches offered less conspicuous scars but reduced reshaping potential.

Importantly, the psychological impact of mastopexy was considerable. Among the five studies that reported validated psychosocial outcomes using BREAST-Q or

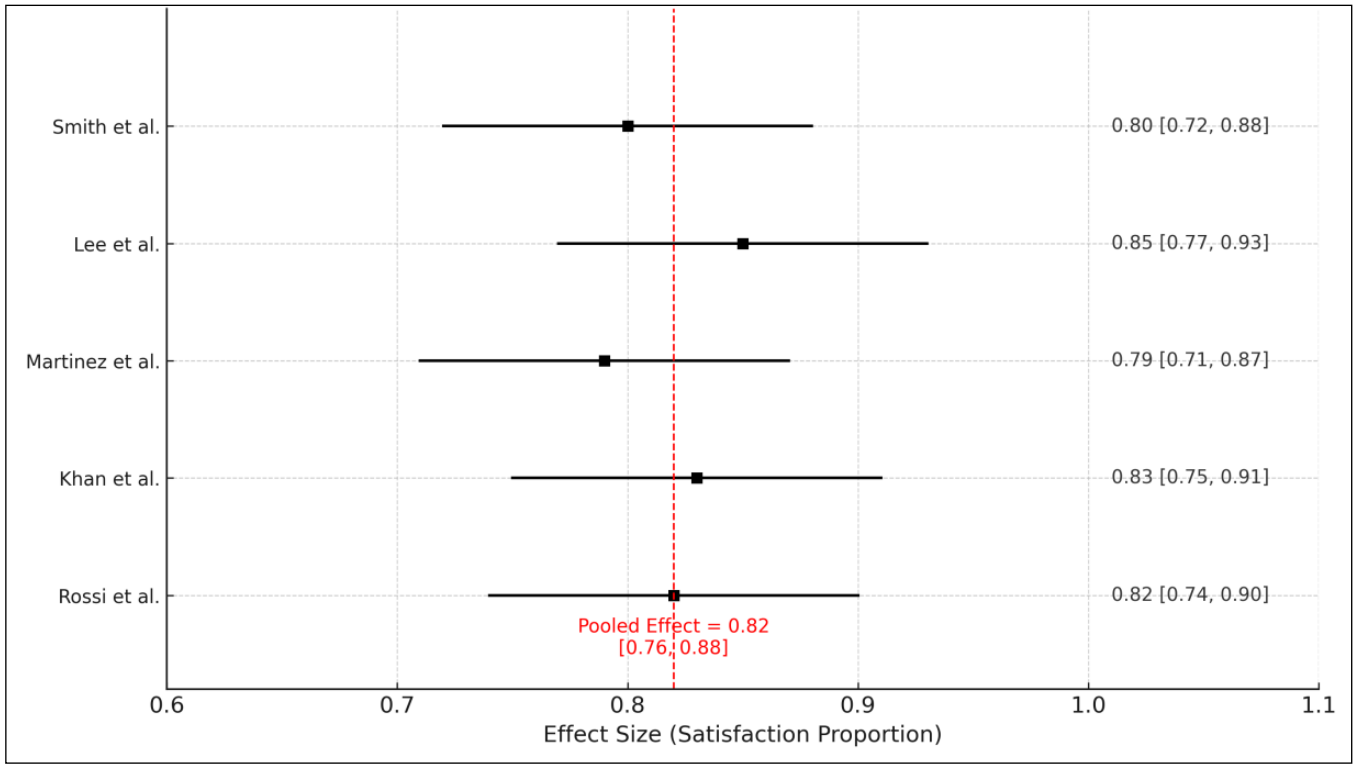

Fig. 2. Forest plot of patient satisfaction after mastopexy
This forest plot displays the individual study satisfaction rates and the pooled estimate (0.82, 95% CI: 0.76–0.88), based on a random-effects model. Moderate heterogeneity was observed ($I^2 = 58\%$), and Egger's test suggested no significant publication bias ($p = 0.21$)
Picture taken by the authors

the Body Image Scale (BIS), most patients indicated enhanced body confidence (78%), improved sexual wellbeing (66%), and better self-esteem in social situations (58%). Nevertheless, patients with preoperative anxiety or unrealistic aesthetic expectations were significantly more likely to experience dissatisfaction or seek revision surgery.

These results underscore the critical role of preoperative counseling in aligning patient expectations with achievable outcomes, thus maximizing both physical and psychological benefits of mastopexy.

This meta-analysis provides a comprehensive synthesis of current evidence regarding aesthetic and psychological outcomes following mastopexy, with particular attention to the divergence between patient expectations and postoperative reality. The findings confirm that mastopexy is generally a safe and effective procedure with high rates of patient satisfaction. However, they also reveal critical areas where expectations may not align with achievable results, emphasizing the importance of individualized patient education and surgical planning.

The high expectation for idealized breast aesthetics—often influenced by social media, advertising, and digitally altered images—can lead to unrealistic patient goals. Our findings show a marked discrepancy between expected and observed outcomes in domains such as scar visibility (–25.7%), upper pole fullness (–17.5%), and longevity of lift effect (–22.3%). These mismatches may contribute to psychological distress or requests for revision surgery, especially among patients lacking comprehensive preoperative counseling.

Augmentation-mastopexy patients reported higher satisfaction rates, particularly in regard to breast projection and contour, supporting previous literature suggesting that combining volume enhancement with lifting offers more durable and aesthetically pleasing results [1,2].

The choice of technique plays a critical role in determining both aesthetic and functional outcomes. Wise-pattern mastopexy provides superior lift and reshaping but has a higher risk of noticeable scarring. Vertical and periareolar techniques offer more concealed incisions but may be limited in their ability to correct severe ptosis or improve upper pole fullness. These findings reinforce the need for surgeon-patient dialogue tailored to individual anatomy, ptosis grade, and cosmetic goals [3].

An equally important yet often overlooked dimension of mastopexy outcomes is its psychological and social impact. Data from five included studies utilizing BREAST-Q and Body Image Scale (BIS) instruments

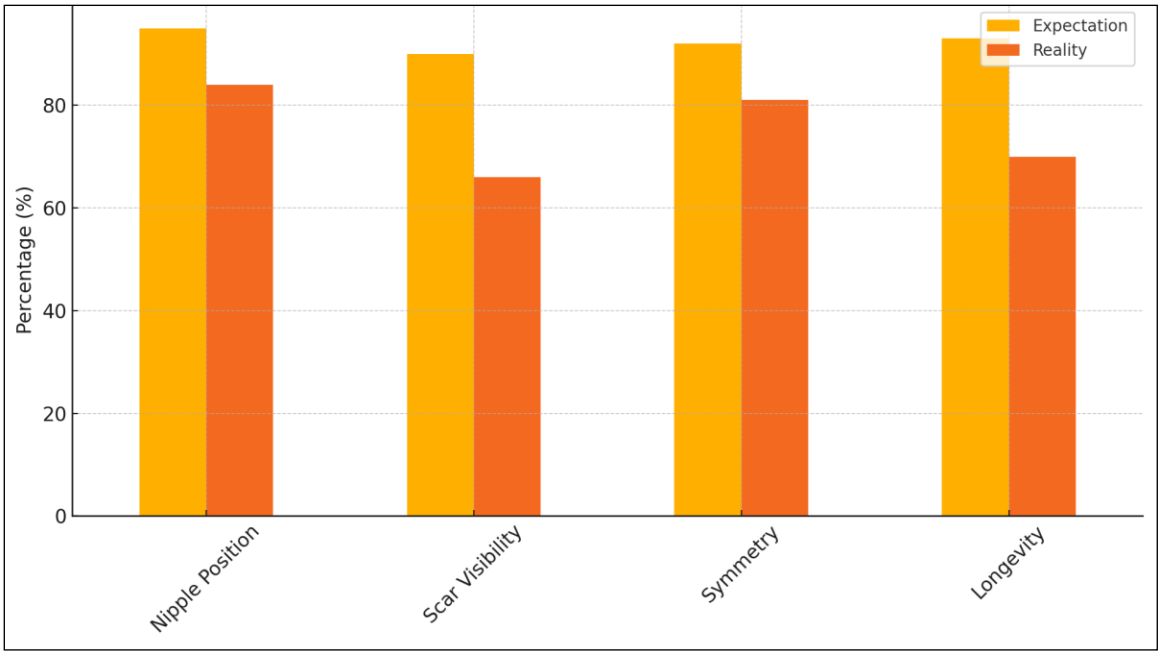

Fig. 3. Comparison between preoperative expectations and postoperative realities in common mastopexy outcomes
Picture taken by the authors

highlight significant positive effects on self-image, sexual wellbeing, and confidence in social interactions. This supports the notion that cosmetic surgery extends beyond physical correction, contributing to improved quality of life. However, our review also indicates that patients with preoperative anxiety, body dysmorphic traits, or external pressures (e.g., partner influence) had a higher incidence of dissatisfaction or revision, suggesting the importance of preoperative psychological screening and expectation management [4].

These findings have direct implications for clinical practice: realistic expectation management is paramount. Use of preoperative imaging, outcome galleries, and standardized scales (e.g., BREAST-Q) can help ground expectations in reality. Shared decision-making should consider both aesthetic goals and lifestyle factors (e.g., future pregnancy or weight fluctuations) that may affect long-term outcomes. Postoperative follow-up should include not only wound assessment and aesthetic evaluation but also discussions about body image and emotional well-being.

Incorporating these strategies may reduce postoperative regret and increase the likelihood of positive long-term outcomes.

This meta-analysis has several limitations. First, there is heterogeneity among the included studies, particularly in terms of surgical technique, follow-up duration, and outcome measurement tools. While the random-effects model accounted for statistical variability, clinical variation may still influence results. Second, subjective outcomes like satisfaction and confidence are inherently difficult to quantify and may be influenced by cultural or individual psychological factors. Lastly, only English-language studies were included, potentially introducing language bias.

Future studies should aim for standardized outcome reporting, including the use of validated tools like BREAST-Q at multiple postoperative intervals. Long-term follow-up (>3 years) is particularly needed to assess the durability of aesthetic improvements and patient satisfaction. Additionally, more research is warranted on the psychological predictors of dissatisfaction and the development of preoperative screening protocols to identify patients at risk for negative postoperative experiences.

CONCLUSIONS

This meta-analysis confirms that mastopexy is a generally safe and effective surgical procedure with a high rate of patient satisfaction, particularly when individualized techniques are selected and patient expectations are appropriately managed. Despite the overall positive outcomes, our findings highlight several persistent gaps between preoperative expectations and postoperative reality—especially in areas such as scar visibility, longevity of the lifted appearance, and upper pole fullness.

Key determinants of patient satisfaction include the choice of surgical technique, use of augmentation when indicated, and adequate preoperative counseling. The addition of implants tends to enhance upper pole fullness and improve the perceived longevity of results, while

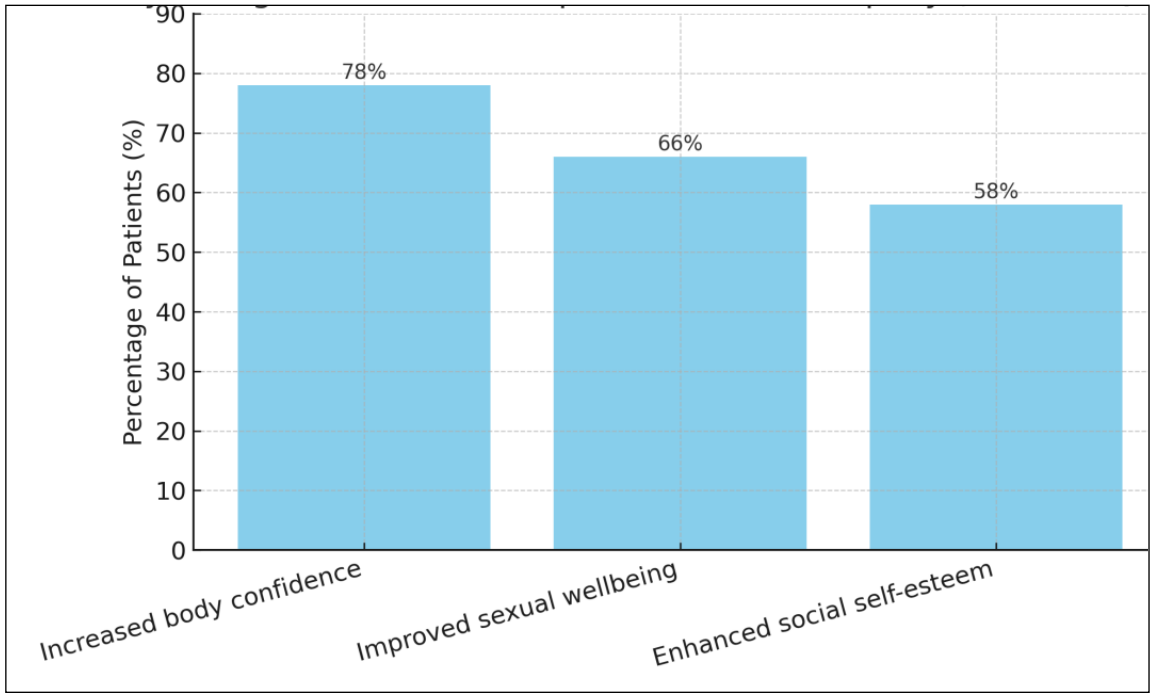

Fig. 4. Psychological outcomes using BREAST-Q or Body Image Scale (BIS)
Picture taken by the authors

technique-specific trade-offs (e.g., visible scarring with Wise-pattern incisions) must be transparently discussed.

Furthermore, the psychosocial impact of mastopexy should not be underestimated. Patients report significant improvements in self-image, body confidence, and sexual wellbeing post-surgery, suggesting that the benefits extend beyond aesthetic restoration. However, patients with unrealistic expectations or underlying psychological concerns are more prone to dissatisfaction and revision surgery, underscoring the need for holistic patient assessment.

In light of these findings, the following clinical recommendations are proposed: incorporate standardized preoperative tools (e.g., BREAST-Q, 3D simulation) to align expectations with likely outcomes. Emphasize shared decision-making tailored to individual anatomy and aesthetic goals. Include psychological evaluation as part of the consultation process for patients with high-risk profiles.

Ultimately, mastopexy can significantly enhance both physical appearance and quality of life when performed with technical precision and supported by thoughtful, expectation-based patient care. Ongoing research and standardized outcome reporting are needed to further refine best practices and ensure long-term patient satisfaction.

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REVIEW ARTICLE

CONTENTS

Radiological follow-up after endovascular aortic repair – review of surveillance modalities

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ABSTRACT

Aim: This review aims to present diagnostic modalities suitable for monitoring patients after endovascular aortic repair.

Materials and Methods: A literature search was conducted across electronic databases from January 2024 to May 2025 using a combination of keywords and Boolean operators.

Conclusions: While computed tomography angiography remains the reference standard for monitoring after endovascular aortic repair, with 92% sensitivity for endoleak detection, magnetic resonance imaging and ultrasonography offer viable alternatives that eliminate radiation exposure and may reduce contrast agent requirements. Magnetic resonance imaging detects twice as many endoleaks as computed tomography angiography, whereas contrast-enhanced duplex ultrasonography achieves 98% sensitivity and 88% specificity.

Treatment of abdominal aorta aneurysm with endovascular aortic repair offers a significant opportunity for a carefully selected patient population. The selection of surveillance modalities should be tailored to individual patient characteristics.

KEY WORDS: computed tomography angiography, magnetic resonance imaging, ultrasonography, contrast media, endovascular aortic repair

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INTRODUCTION

Abdominal aortic aneurysm (AAA) is a chronic, potentially lethal disease characterized by a permanent and irreversible dilatation of the aorta, which may progress to a life-threatening aortic rupture with mortality rates exceeding 80%. Prevalence rates range from 1% to 8.9%, with AAA being more frequent in men [1]. Despite the steadily increasing prevalence of AAA over the past decades, recent data underline a decline in AAA incidence and mortality, particularly in high-income countries. This trend may be attributed to a decrease in tobacco — one of the most significant risk factors - and the significant impact of antihypertensive therapy in preventing AAA [2]. Conversely, in middle- and low-income countries with inefficient healthcare systems, the prevalence of AAA continues to rise [3]. Although AAA formation is complex and results from multiple risk factors, the most significant predictive risk factors remain advanced age, a history of tobacco smoking, male sex, family history of AAA, cardiovascular diseases, and hypertension [1].

Open surgical repair (OSR) and endovascular aortic repair (EVAR) with a stent graft placement are two available treatment approaches [4]. The primary goal of OSR is to replace the aneurysmal segment of the aorta with a synthetic graft, with anastomoses performed as close as possible to the renal arteries in the infrarenal aortic segment [4]. In selected cases, the reimplantation of the inferior mesenteric artery may be performed [5]. The OSR is considered a high-risk intervention, with a cardiovascular mortality rate exceeding 5% in the 30-day postoperative period [6]. Moreover, OSR may be burdened with significantly higher systemic complications compared to EVAR, including cardiac, pulmonary, and renal complications [7].

On the other hand, the primary objective of EVAR is to exclude the aneurysmal sac from the circulation by placing an endograft. The endograft is inserted over the aneurysmal sac via the common femoral artery under angiographic guidance. After proper positioning of the main endograft seal, distal endograft extensions are placed below the main body and extend

to the common iliac arteries. Finally, angiography confirms proper graft positioning and excludes early endovascular leaks [8]. Compared with OSR, EVAR is considered an intermediate-risk intervention, with a cardiovascular mortality risk of 1-5% [9], and it has a better safety profile, associated with a significant reduction in the postoperative complication rate and a shorter hospitalization duration [10]. The most common complications in the 30-day postoperative period after EVAR are graft endoleak, endograft migration, or graft limb thrombosis. [11]. Nevertheless, according to current guidelines, EVAR is considered the preferred treatment modality for AAA in selected patients [12]. However, in some cases, OSR may be beneficial, particularly in patients with aortic angulation and tortuosity, unfavorable aortic branch anatomy, or iliac artery obstruction [13].

Patients after aortic repair should be monitored closely to detect postoperative complications and initiate appropriate treatment [14]. The purpose of this narrative review is to present diagnostic modalities for monitoring patients after EVAR.

AIM

This review aims to present diagnostic modalities suitable for monitoring patients after endovascular aortic repair.

MATERIALS AND METHODS

To prepare this narrative review, a comprehensive literature search was conducted across multiple electronic databases, including PubMed, Scopus, and Google Scholar, covering the period from January 2024 to May 2025. The search strategy employed a combination of keywords, including "endovascular aortic repair," "computed tomography angiography," "magnetic resonance imaging," "ultrasonography," and "abdominal aortic aneurysm". Boolean operators (AND, OR) and Medical Subject Headings (MeSH) terms were utilized to optimize search sensitivity and specificity for each database.

Inclusion criteria encompassed peer-reviewed publications, including original research articles, systematic reviews, narrative reviews, letters to the editor, and meta-analyses with full-text availability in English. Exclusion criteria included conference abstracts, case reports, and non-peer-reviewed publications.

The authors independently conducted title and abstract screening in accordance with predetermined eligibility criteria. Subsequently, full-text articles of potentially relevant studies were comprehensively evaluated for final inclusion. Inter-reviewer discrepancies were resolved through consensus discussion. Additional relevant publications identified through citation tracking and reference list screening were incorporated when they provided essential information pertinent to specific aspects of the review.

REVIEW AND DISUSSION

POST-EVAR DIAGNOSTIC METHODS

During the first 5 years after EVAR, the risk of adverse events is estimated at approximately 20% [15]. These events may include endoleaks, stent graft migration, or endograft infection [13]. Current guidelines recommend assessing the stent graft at 30 days and 12 months post-procedural [13,15–17]. If any EVAR-related pathology is detected, additional imaging should be performed within six months of the initial examination [16]. Lifelong surveillance is recommended annually [13,15,16,18,19]. Typical assessment involves computed tomography angiography (CTA), but it can be continued with ultrasound (US) when no abnormality is noted in the first year of follow-up [16]. However, this management is considered financially ineffective and exposes patients to radiation and nephrotoxic contrast agents [15,18]. Moreover, approximately one-third of patients discontinue follow-up, which significantly increases the risk of mortality within three years post-EVAR [15]. Follow-up imaging modalities include conventional radiography and angiography, CTA as the gold standard, magnetic resonance imaging (MRI), and US [13,19,20]. Nevertheless, the best diagnostic outcomes are observed when different methods are combined [16].

CONVENTIONAL RADIOGRAPHY AND ANGIOGRAPHY

Currently, conventional radiography is rarely in use. When performed, it is typically acquired in an anteroposterior (AP) projection to assess stent graft position and integrity, and to detect potential fractures and tears [13, 21, 22]endovascular aneurysm repair or endovascular aortic repair (EVAR. Its main advantages are low cost and minimal radiation exposure [13].

Digital subtraction angiography is utilized preoperatively or during secondary interventions to visualize vessels feeding the aneurysm sac in type II endoleaks [13]. However, conventional angiography has limited sensitivity (63%) and specificity (77%) [23] and is associated with risks such as hematoma, pseudoaneurysm formation, retroperitoneal hemorrhage, and even vessel rupture [13].

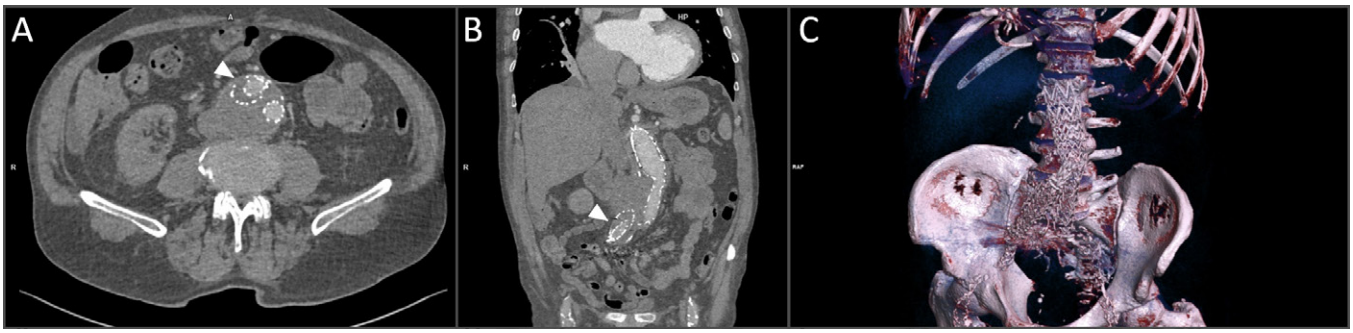

Fig. 1. Patient after endovascular aortic repair, and adding an additional stent to the right common iliac artery due to endoleak. On axial **(A)** and coronal **(B)** images, a stent graft body is visible with an additional stent inside the right stent graft extremity (arrowheads). The entire stent graft body is visible in the 3D reconstruction **(C)**
Source: Own materials

CTA

CTA assessment of stent grafts after EVAR is recognized as the gold standard [11,13, 19, 20, 24–26]. It enables precise visualization of the aneurysm sac, its diameter, and the anchoring of the vascular prosthesis (Fig 1) [11, 24]. Standard CT protocols comprise three phases: non-contrast, arterial (post-contrast), and delayed-phase imaging (120–300 ms post-contrast). The non-contrast phase enables the assessment of dense structures such as calcifications, while the contrast-enhanced phases allow assessment of graft integrity, detection of endoleaks, infection, or vessel occlusion. CTA has a sensitivity of 92% for endoleak detection [13].

Bley et al. [19] investigated a protocol using nonenhanced CT for follow-up after infrarenal EVAR, as the radiation dose is smaller and the contrast material may be omitted. Initial scans (0-3 months after EVAR) were performed with contrast medium to detect leakage. If no endoleak was present, the patient was asymptomatic, and the aneurysm sac expanded by less than 2%, subsequent follow-up was conducted with nonenhanced CT. If these criteria were not met, contrast-enhanced CT was introduced. This approach reduced the radiation dose by 57%–72% compared with conventional contrast CT angiography and by 69%–82% compared with protocols that included a delayed-phase post-contrast acquisition. This protocol allowed for limiting the contrast material usage, which can be beneficial for nephrologically burdened patients. Furthermore, the study demonstrated that high-pressure endoleaks (types I and III) increase aneurysm sac volume by approximately 10%, whereas low-pressure type II endoleaks are associated with a 5.4% increase. Volumetric analysis appears particularly useful for detecting type V endoleaks, in which the only indicator may be a slow, subtle sac enlargement despite the absence of contrast extravasation. Additionally, small type II endoleaks may be observed even when sac volume decreases, and the surgical approach can be waived, as the risk of rupture is primarily associated with increased sac volume [19].

Double-energy CT (DECT) employs two different kilovoltages to acquire images of the same volume. The energy difference enables differentiation of materials based on atomic number, k-edge properties, and attenuation profiles. Various acquisition techniques exist, such as rapid-kilovoltage-switching DECT, double-source DECT, split-filter DECT, and multilayer detectors. DECT is used to generate virtual noncontrast images and iodine map reconstructions based on the iodine spectrum and its distribution in tissues. Although virtual noncontrast images are comparable to true noncontrast images, their appearance may be influenced by residual iodine. Iodine maps have been shown to be useful for detecting endoleaks. In addition, DECT can generate "noncalcium" images to assess vessels narrowed by calcified plaques [18]. Furthermore, the radiation dose with DECT is comparable to or lower than that of conventional CT, as the noncontrast phase is virtually reconstructed [13, 18, 20, 27]. This dose reduction is possible by limiting the examination to the phase following contrast agent administration and then virtually reconstructing noncontrast images. Some studies suggest that the arterial phase can be omitted, as delayed-phase images maintain high sensitivity for detecting endoleaks [18, 27]. However, the arterial phase remains essential for accurate identification of type I and III endoleaks and for a thorough assessment of the abdominal vessels and other potential pathologies [18]. Stolzmann et al. [27] reported high interobserver agreement for DECT protocols, although readers exhibited lower confidence when relying on virtual noncontrast images or omitting the arterial phase. Furthermore, DECT protocols can reduce radiation dose by 41–61% when the arterial phase is omitted, compared with single-energy CT. This is explained by the fact that

each scan in single-energy CT delivers less radiation than in DECT; more scans are required to produce the final images, which is responsible for the higher radiation dose in single-energy CT. Early imaging with DECT may be confounded by residual contrast that mimics endoleaks [27]. Javor et al. [28] described a split-bolus DECT technique, wherein two contrast boluses are administered sequentially to capture both arterial and delayed phases in a single acquisition, reducing the radiation dose by up to 42% [28].

Dynamic CT, which uses longitudinal table movement to capture the dynamic enhancement pattern, is a potential post-EVAR assessment technique. It is useful when there is no regression in sac diameter or when leakage is unclear. The method detects and differentiates endoleaks by time-to-peak attenuation [26]. Despite a higher radiation dose than other protocols [29], dynamic CT may improve the classification of endoleaks. Further prospective studies are needed to fully validate this technique [26].

IODINE-BASED CONTRAST AGENTS

Iodine-based contrast agents for CT may induce various complications, including skin reactions (rash, itching, blistering) and respiratory or gastrointestinal disturbances [30]. A significant concern is contrast-induced acute kidney injury, particularly in patients with pre-existing renal impairment, with an incidence of up to 30% in high-risk groups [11,31–33]. However, in patients with normal renal function, the probability of contrast-induced acute kidney injury is low, typically <5% [33]. Nevertheless, it is important to adequately hydrate the patient before iodine contrast use or before the use of diluted contrast agents [20]. Low-osmolar agents can reduce the incidence of contrast-induced acute kidney injury to around 2%, regardless of comorbidities. Additionally, statin use may decrease the risk of nephrotoxicity by up to 80% [34]. Notably, intra-arterial administration is associated with higher nephrotoxicity due to increased renal contrast concentration [33].

MRI

MRI is a complementary imaging modality to CT, particularly when CT is contraindicated [13,25]. It has demonstrated higher sensitivity in detecting endoleaks—especially those of unknown origin—owing to superior soft-tissue contrast [16,25,35]. Standard MRI protocols typically include T1-weighted images acquired before and after gadolinium contrast administration [13]. In a meta-analysis, Habets et al. [25] found that MRI detected nearly twice as many endoleaks as contrast-enhanced CT, with only two type I endoleaks being missed. Many of the additional endoleaks identified are type II endoleaks, whose clinical importance remains uncertain. Only those with AAA growth require surgical treatment. MRI's ability to identify endoleaks missed by CT is a significant advantage and is recommended as an adjunct in cases of aneurysm sac expansion with no evident type II endoleak on CT [25]. However, artifacts from stent grafts composed of stainless steel or nickel alloys may compromise image quality; nitinol grafts are preferred [17,20].

The 4D-FLOW technique provides additional post-EVAR information by distinguishing endoleak types (except type V), quantifying leak volume, and assessing flow dynamics [20,36]. It can differentiate between type IIA endoleaks (bidirectional flow) and type IIB endoleaks (unidirectional flow) [36]. Katahashi et al. [36] demonstrated that higher peak flow velocity and greater amplitude of blood flow dynamics in tributary arteries are significantly associated with aneurysm sac expansion (OR 42.787, 95% CI 1.256–1463.57, p = 0.037). The researchers established that this technique has 85.7% sensitivity and 76.2% specificity for predicting sac growth when a cutoff value of 3750 mm3/min is used. In addition, detection of tributary vessels with 4D-FLOW MRI achieved 100% sensitivity and specificity [36]. This modality enables detailed hemodynamic assessment that is challenging with contrast-enhanced CT and may help select patients with type II endoleaks who require more aggressive management [22, 36].

In patients with contraindications to contrast agents (e.g., pregnancy, renal impairment), the time-of-flight (TOF) sequence provides a useful, albeit less sensitive (approximately 54%), alternative for image acquisition [13, 37]. When combined with contrast, TOF can achieve up to 97% concordance with angiographic images [13]. Additionally, TOF allows physicians to assess the direction of blood flow within vessels, and some studies suggest that this method outperforms CT in evaluating nitinol stent grafts [13, 20].

Despite its advantages — lack of ionizing radiation and reduced contrast requirements — MRI is limited by accessibility, high cost, and longer acquisition times [13, 17]. Furthermore, despite improved detection sensitivity, the clinical relevance of additional type II endoleaks remains uncertain; only those associated with AAA enlargement warrant surgical intervention.

GADOLINIUM-BASED CONTRAST AGENTS

Adverse reactions after gadolinium contrast agents are less common than after iodine-based agents [30]. However, acute side effects, such as paresthesia, nausea,

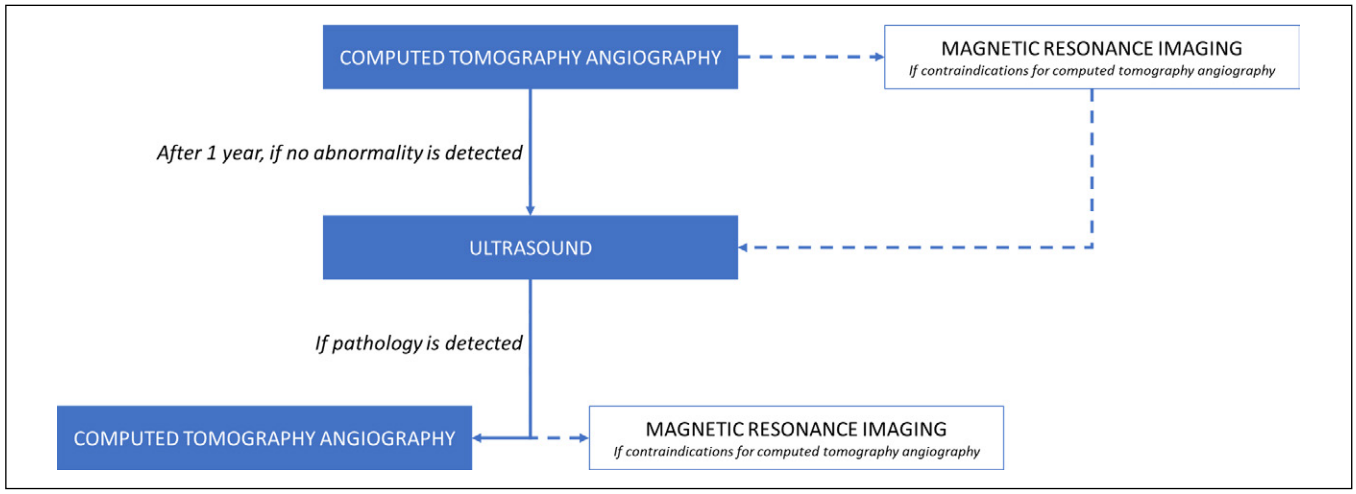

Fig. 2. Proposed diagnostic surveillance pathway following endovascular aortic repair
Picture taken by the authors

vomiting, headaches, or dizziness, may still occur [38]. There is also concern regarding gadolinium deposition in the central nervous system, even in patients with normal renal function, though the clinical implications remain uncertain [20,39]. Additionally, there is a risk of nephrogenic systemic fibrosis, primarily in patients with pre-existing renal disease [20, 32, 38, 39]. Notably, the incidence of NSF has declined in recent years, and gadolinium deposition disease is recognized as a possible, albeit rare, complication following intravenous administration [38].

US IMAGING

US is a cost-effective and widely available method for post-EVAR follow-up, particularly in patients for whom CT or MRI are contraindicated. Its primary advantages are the absence of ionizing radiation and contrast agents [13, 24]. B-mode US evaluates the abdominal aorta, iliac and femoral arteries, the aneurysm sac, and the stent graft, while Doppler US assesses blood flow direction and detects endoleaks [13]. However, its diagnostic utility is controversial. Some studies report Doppler US sensitivities of 70–82% and specificities of 93–94% [14, 27], whereas others indicate that duplex US may be inferior to CT [35]. Some authors note that, despite lower sensitivity compared to contrast-enhanced CT, Doppler US may exhibit higher specificity [16]. Differences in reported sensitivities and specificities may be attributable to the experience of both the medical center and the physicians, given that US requires expertise. On the other hand, contrast-enhanced US can match [16] or even exceed CT in endoleak detection [35]. Contrast-enhanced duplex US can reach a sensitivity of 98% and a specificity of 88% [26]. A pilot study by Esposito et al. [40] suggests that a combination of duplex US, contrast-enhanced US, and plain CT may be sufficient for post-EVAR surveillance. Nevertheless, operator dependence and variability in image quality — owing to patient body habitus and cooperation — remain significant limitations [13, 18, 21].

PROPOSED PRACTICAL IMAGING PROTOCOL FOLLOWING EVAR

Postoperative surveillance after EVAR is crucial for post-procedural outcomes. The initial examination should be performed with CTA, which serves as the reference modality [13]. It should be the first-choice imaging modality due to its availability and reproducibility, with excellent detection of endoleaks. MRI, especially with advanced sequences such as 4D-FLOW and TOF, should be used in patients with contraindications to CTA [13, 25]. This modality offers excellent soft-tissue resolution, enabling detection and accurate classification of endoleaks. However, limited access and high cost may restrict MRI use to selected patients. For long-term follow-up, if prior examinations show no abnormalities, US can be recommended after one year [16]. It remains a viable option because of its availability and lack of radiation [13, 24]. It is noninferior to CTA and MRI, and when pathology is detected, additional examination can be performed with other modalities. Proposed diagnostic options over time are presented in Figure 2.

CONCLUSIONS

While CTA remains the reference standard for post-EVAR surveillance, MRI and US are viable alternatives. These techniques demonstrate comparable diagnostic accuracy while eliminating ionizing radiation exposure and potentially reducing contrast medium requirements.

This approach may confer particular benefit for patients with chronic kidney disease or those at risk for contrast-induced nephropathy. Clinical decision-making should incorporate a comprehensive assessment of imaging modality selection, with careful consideration of cumulative radiation exposure and contrast agent administration in the context of individual patient risk factors and long-term surveillance requirements.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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REVIEW ARTICLE

CONTENTS

Cervical pathology: Diagnosis, treatment and management of patients with *Human Papillomavirus*

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ABSTRACT

Aim: The objective of this literature review was to identify current aspects of the diagnosis, treatment, and management of patients with human papillomavirus.

Materials and Methods: 15 articles published from 2018 to 2024 were analyzed using the keywords: cervix, *Papillomavirus*, precancerous cervical pathology, cervical cancer, cervical screening, cytological method, colposcopy, cervical biopsy, for which a review of the available literature was conducted. Pubmed, Google Scholar, Web of Science, and Scopus databases were used to search for materials on current aspects of the diagnosis, treatment and management of patients with *Human Papillomavirus*. Inclusion criteria were cervical pathology caused by human papillomavirus.

Conclusions: The goal of the screening program is to detect and treat high-grade precancer and prevent the development of cancer. Detection of LSIL (CIN1) is carried out by HPV testing and cervical screening, and the diagnosis is verified by histology. Most guidelines talk about surveillance from 18 years of age for 2 years for CIN 1. Diagnosis of persistent infection with HPV 16, 18, 31, 33 and others, and not the presence of CIN1, determines the risk of developing CIN 3.

KEY WORDS: papillomavirus, cervical cancer

Wiad Lek. 2026;79(4):863-876. doi: 10.36740/WLek/216928 DOI

INTRODUCTION

Every year, cervical cancer is diagnosed in more than 600,000 women worldwide. In Ukraine, the disease is diagnosed annually in 7,500 women, of whom 2,500 die (including 500 of working age).

Since February 24, 2022, a war has been ongoing in Ukraine, which has a profound impact on the health of the population. The adverse consequences of armed conflicts include both combat injuries and stress, as well as chronic somatic diseases, including cancer. Diseases that emerged during armed conflicts are very persistent [1]. They continue even after the cessation of hostilities. According to statistics from some countries that have experienced armed conflicts, the incidence of cancer among military personnel and civilians increases by more than 100% over several years [2].

Along with carcinogens, important factors influencing the development of cancer during and after war are mass population movements, which increase the risk of transmission of oncogenic bacteria and viruses.

It is known that a surge in cervical cancer incidence of more than 260% was recorded after the end of the Vietnam War. Unfortunately, this trend can also be observed in Ukraine.

Considering the experience of countries that participated in previous military conflicts, we can conclude that the most important measures for reducing cancer morbidity and mortality may be state programs for prevention, screening, and early diagnosis of cancer.

AIM

The objective of this literature review was to identify current aspects of the diagnosis, treatment, and management of patients with *Human Papillomavirus*.

MATERIALS AND METHODS

15 articles published from 2018 to 2024 were analyzed using the keywords: cervix, papillomavirus, precancerous cervical pathology, cervical cancer, cervical screening, cytological method, colposcopy, cervical biopsy, for which a review of the available literature was conducted. Pubmed, Google Scholar, Web of Science,

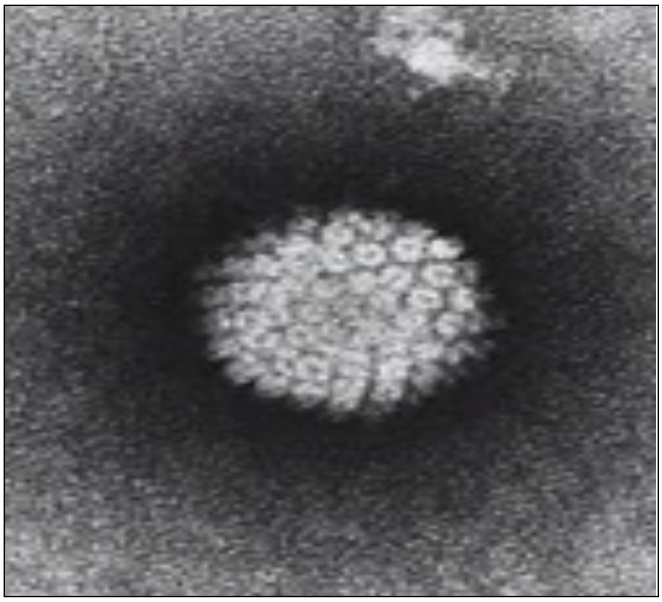

Fig. 1. *Human Papillomavirus*
Source: compiled by the authors based on [5]

and Scopus databases were used to search for materials on current aspects of the diagnosis, treatment and management of patients with human papillomavirus. Inclusion criteria were cervical pathology caused by human papillomavirus. EHTICS

All sources used in this literature review are publicly available

REVIEW AND DISCUSSION

Cervical pathology has an etiological factor that causes this pathology – it is the human papillomavirus. Cervical cancer is a completely infectious disease. Over the past 20 years, approaches to the diagnosis, treatment, and management of patients with *Human Papillomavirus* have completely changed. *Human Papillomavirus* is the most common sexually transmitted pathogen, one of the few that leads to cancer and oncological changes in the human body. Everything we know about the virus comes from its physiological properties.

In 1983, Harald zur Hausen and his colleagues isolated *Human Papillomavirus* type 16 (HPV-16), and in 1984, type 18 (HPV-18). He also examined a culture of Hela cells, which turned out to be infected with HPV-18. In 2008, Harald zur Hausen received the Nobel Prize in Medicine and Physiology for his discovery. Thus, an equal sign was drawn between the papilloma virus and cervical cancer. The whole world received the vaccine, and began vaccinating girls, older women who had already been exposed to the *Human Papillomavirus* and who already had precancerous conditions [3]. Today, there is experience from those countries that have begun to implement this. Currently, Australia, New Zealand, and some parts of America have already overcome the problem of cervical cancer (Fig. 1).

In 2023, a congress was held on the *Human Papillomavirus*, where work was published, studying cervical cancer microsamplings from women from different regions - Greece, Australia, New Zealand, America, Europe. Regardless of the region, the disease was caused by *Human Papillomavirus* type 16 (HPV-16).

In Ukraine, women do not accept the topic of cervical cancer vaccination for themselves and their children, but the war changed everything and many women ended up in other countries where they will not be asked whether they want to be vaccinated or not, children

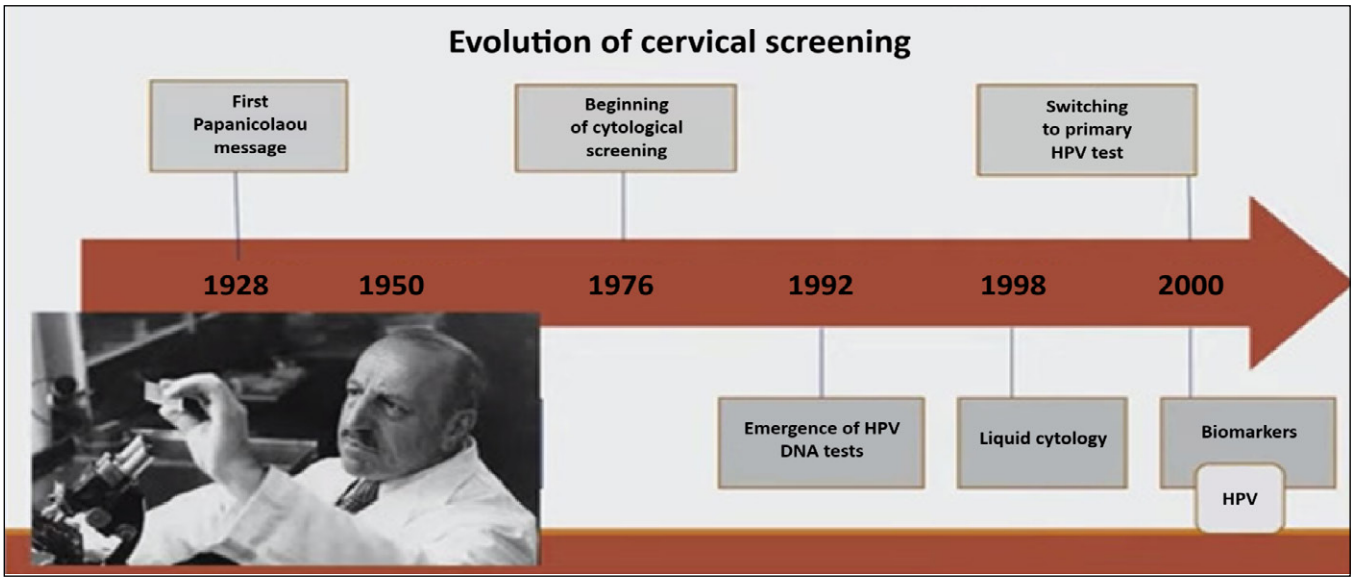

Fig. 2. The evolution of cervical screening
Source: compiled by the authors based on [1]

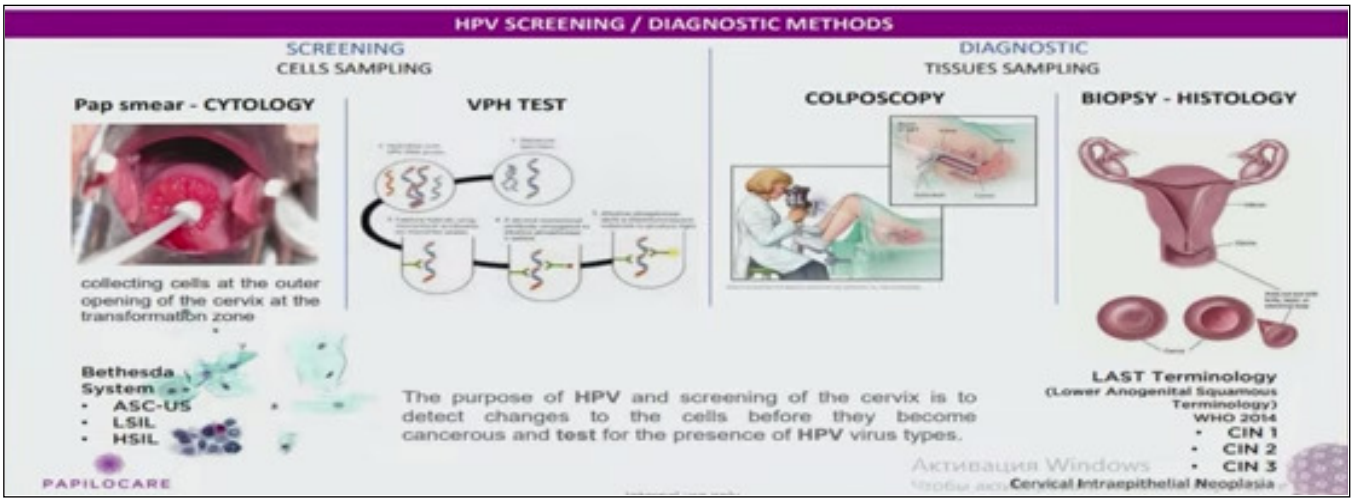

Fig. 3. Patient route depending on screening results
Source: compiled by the authors based on [2]

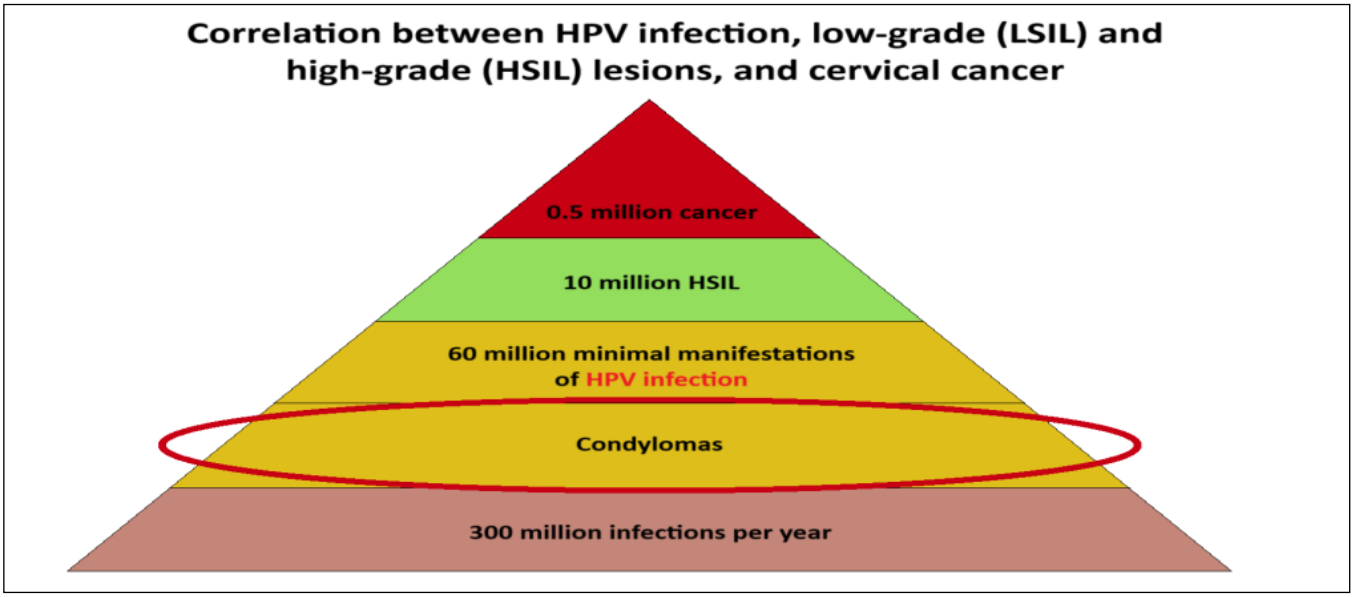

Fig. 4. Correlation between HPV infection, low-grade (LSIL) and high-grade (HSIL) lesions, and cervical cancer
Source: compiled by the authors based on [4]

are vaccinated and everyone understood what a good thing it is [4]. And in Ukraine, the approach to this issue is also changing, because every day we receive more and more evidence that the vaccine is not only prevention of infection, it is prevention of cervical cancer.

The vaccine has its present: the problem in the world is the shortage of vaccines. Many countries have lined up to get these doses of vaccines for their children, women, and men to protect their people from this disease. For example, Japan, which for a long time did not allow the HPV vaccine, but today they are standing in line.

As of 2022, 125 countries have included vaccination in their national vaccination program. The vaccine also has its future - it is the elimination of HPV in the population, and for this it is necessary to cover up to 90% of girls with vaccination, and this was introduced by the WHO. Currently, vaccination and screening are being carried out in age groups that are already carriers of the *Human Papillomavirus* (HPV-faster program). Girls who are past the age of vaccination and who have already been exposed to the *Human Papillomavirus*, currently under the age of 30, without gender equality, and boys should also be vaccinated. This is a late vaccination, but it is also protection.

Vaccination protects against disease, and screening protects those who were not helped by vaccination. That's why a screening program is important today. The first report of screening was in 1928, when the Papanicolaou test was introduced, and every year and every decade it has changed and evolved. Currently, there are tests for *Human Papillomavirus*, a more sensitive study

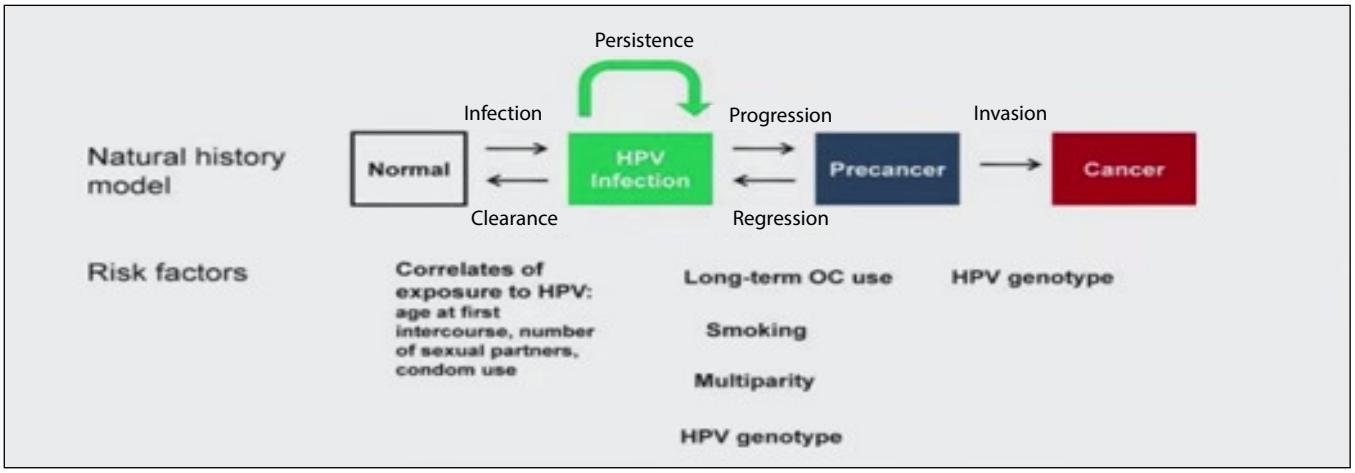

Fig. 5. The natural history of CIN and cervical cancer
Source: compiled by the authors based on [3]

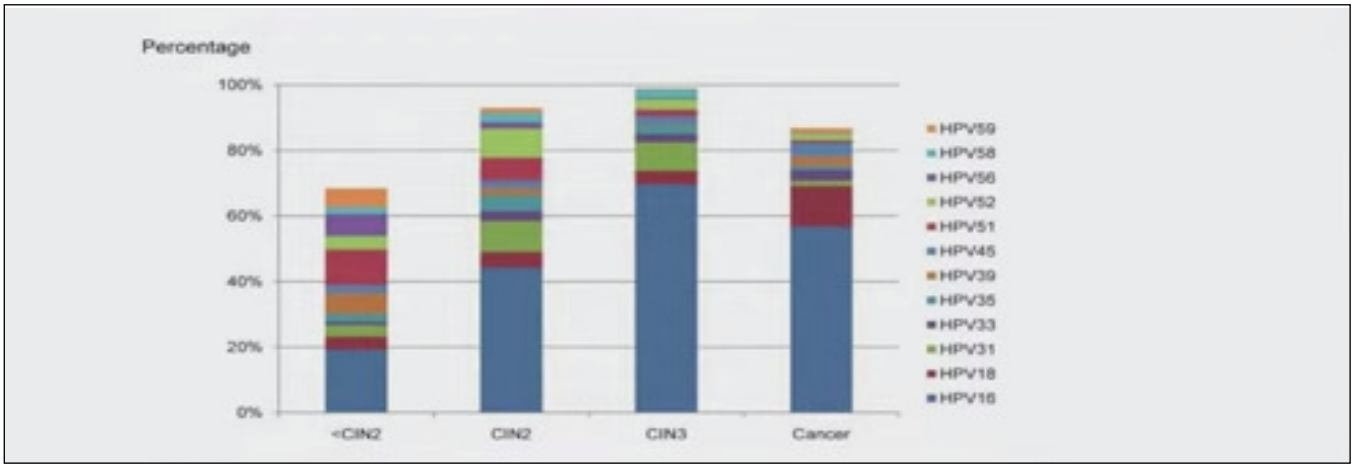

Fig. 6. HPV-16 is the most important viral factor in the development of cervical cancer
Source: compiled by the authors based on [7]

is being conducted, a risk group is identified that needs to be monitored, liquid cytology, and biomarkers have appeared (Fig. 2).

However, it is important to remember that screening is a test that does not establish or exclude a diagnosis, but only identifies women in the population with a high probability of having the disease. Today, a Co-testing program has been developed, where cells are examined and tested for the presence of oncogenic strains of the *Human Papillomavirus*.

PURPOSE of cytological and HPV screening

- Detection and treatment of high-grade HSIL precancer and prevention of the development of squamous cell carcinoma of the cervix;
- Avoiding overtreatment of HSIL and its consequences (cervical stenosis and pregnancy complications).

The patient's route, depending on the screening results, is known (Fig. 3)

- Cytology
- ASC-US
- LSIL
- HSIL
- HPV test – virus detection
- Colposcopy
- Biopsy-histology

Every doctor who treats patients with *Human Papillomavirus* needs to know the following:

- Clearance
- Latency
- Persistence

Millions of people around the world are infected with the *Human Papillomavirus*. This can happen on the first or the hundredth sexual intercourse, but it still happens at some point [5]. The virus can leave the body of a woman or man, but it can also spread further (Fig. 4).

It is necessary to know the natural occurrence of dysplasia and cervical cancer (Fig. 5).

First, it matters which virus is causing the infection, because this is the most important factor.

Secondly, you need to know the woman's lifestyle. Studies show that if an infected couple uses condoms, the viral load

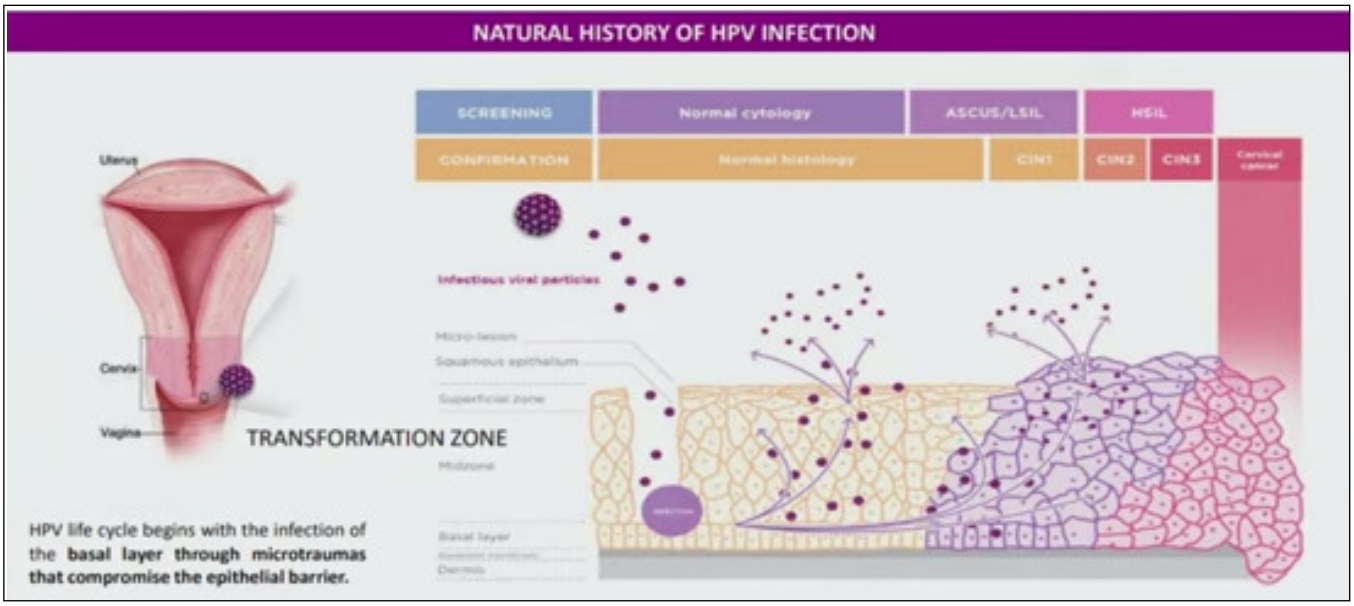

Fig. 7. The role of the transformation zone in the development of the cervical spine
Source: compiled by the authors based on [6]

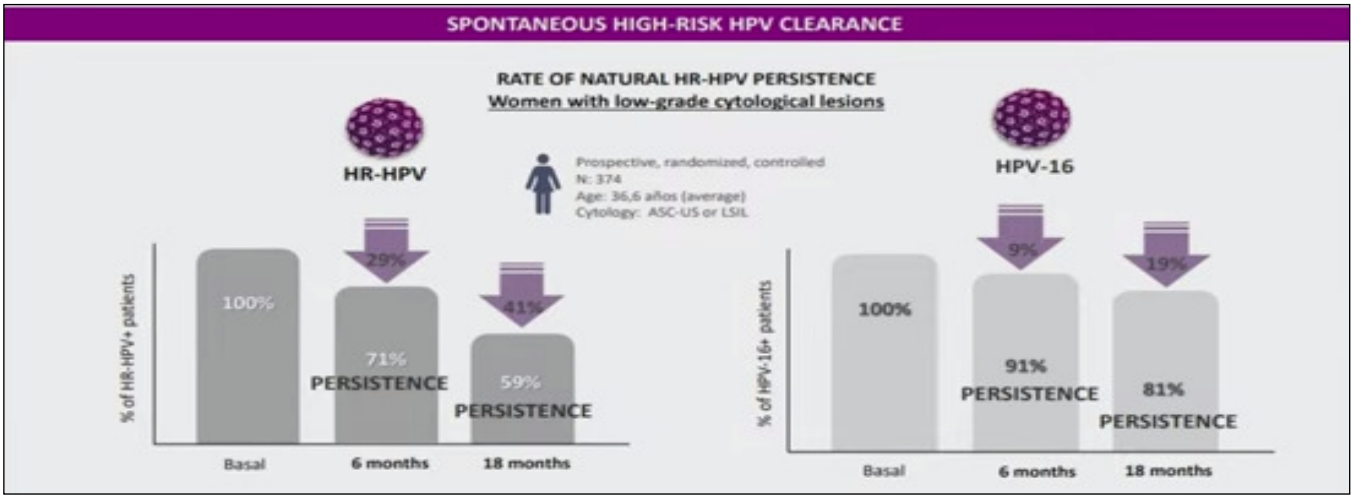

Fig. 8. HPV persistence
Source: compiled by the authors based on [9]

drops after a month. If a woman uses contraceptives, the amount of estrogen in the body decreases and due to this, lactobacteria also decrease, because it is estrogens that secrete them [6]. Women who take contraceptives reduce the protection in their body that occurs due to lactobacilli. Therefore, it is this mechanism that will affect women who use contraceptives for a long time. This is also something to keep in mind and to take courses of lactobacilli, etc.

Smoking is an important factor because smoking suppresses the very necessary P53 gene, which is responsible for apoptosis - one of the most important factors in the pathogenesis of the integration of *Human Papillomavirus* infection into the genome. Therefore, smoking is certainly a very important factor.

Thirdly, it is a type of *Human Papillomavirus*. Fig. 6 shows precancerous conditions and cervical cancer, where *Human Papillomavirus* type 16 is marked in blue. If we see that a woman has type 16 *Human Papillomavirus*, then special attention should be focused on her. *Human Papillomavirus* type 16 is the most virulent, fastest, and most common virus that causes the development of cervical cancer [7].

Fourth, the moment that will be of great importance in the development of precancerous conditions of the cervix is the transformation zone. The transformation zone can be several millimeters and can reach the vaginal vaults, and it is important to remember that the congenital transformation zone is centimeters on the mucous membrane. Sometimes doctors do not notice that there are such changes and believe that this is a type 3 transformation zone, which is actually not true. Infection can occur in any part of the zone, because

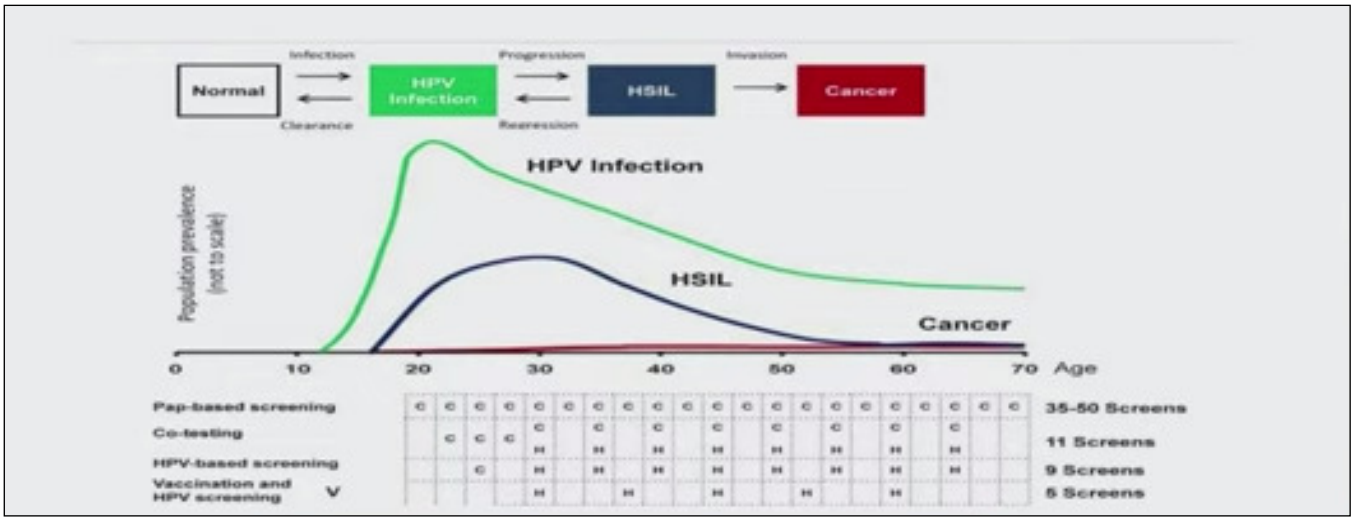

Fig. 9. Clinical perspective and history of CIN and cervical cancer
Source: compiled by the authors based on [8]

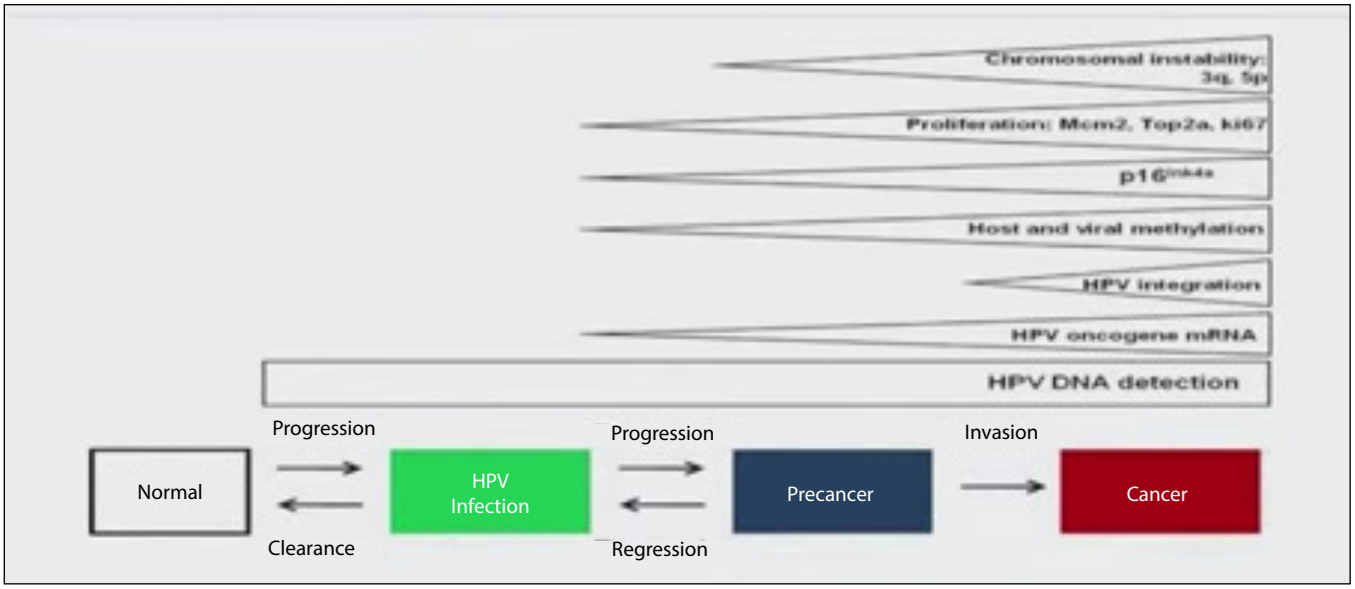

Fig. 10. Biomarkers for cervical cancer incidence categories
Source: compiled by the authors based on [11]

this metaplastic epithelium is an epithelium that has minimal immunological protection and foci of *Human Papillomavirus* interference can form in any area (Fig. 7).

Persistence is one of the main stages of finding the *Human Papillomavirus* for a fairly long time and in most cases, if there are more than one of those viruses, HPV-16, HPV-18, HPV-35, etc [8,9]. Therefore, we do HPV today, then observe in a year, repeat in a few years and thereby determine whether the virus persists or not (Fig. 8).

A woman becomes infected and by the age of 25-30 her future fate is determined, either the virus will leave the body, or there will be an acute intervention of the *Human Papillomavirus* and we have low-grade intraepithelial lesions (LSIL), which corresponds to CIN1, and all this will be eliminated and will be the norm, or there will be a severe precancerous condition, or there will be invasive cervical cancer [10,11]. This age is of great importance 25-30-35 years old. It is during this period that it is necessary to actively conduct screening. If it is a traditional PAP test, then it is annually, if it is Co-testing (liquid cytology + PCR of papillomavirus 28 types), then it can be in a year (Fig. 9).

After 2020, we will have data on biomarkers that help diagnose where there are weak lesions and where there are true precancerous conditions, and all of this will be reflected in the histological report. Cervical intraepithelial neoplasia CIN 1 (LSIL) is a premalignant squamous lesion of the cervix that is diagnosed by biopsy and histological examination. Cervical intraepithelial neoplasia CIN 1 (LSIL) is a premalignant squamous lesion of the cervix that is diagnosed by biopsy and histological ex-

Table 1. Results of revised biopsies with a primary diagnosis of CIN

Diagnosis of CIN1 confirmed	Reduced to normal	Increased to CIN 2.3
43%	41%	16%

Source: compiled by the authors of this study

Table 2. Terminology of cervical disease categories

Natural history model	Histology			Cytology	
	Dysplasia nomenclature	CIN nomenclature	LAST nomenclature	Papanicolaou classification	The Bethesda system
Infection	Negative	Negative		I	NILM
	Squamous atypia	Squamous atypia		II	ACS-US
Precancer	Mild dysplasia	CIN 1	LSIL		LSIL
	Moderate dysplasia	CIN2		III	
	Severe dysplasia Carcinoma in situ	CIN 3	HSIL	IV	HSIL
Cancer	Carcinoma	Carcinoma		V	Carcinoma

Source: compiled by the authors of this study

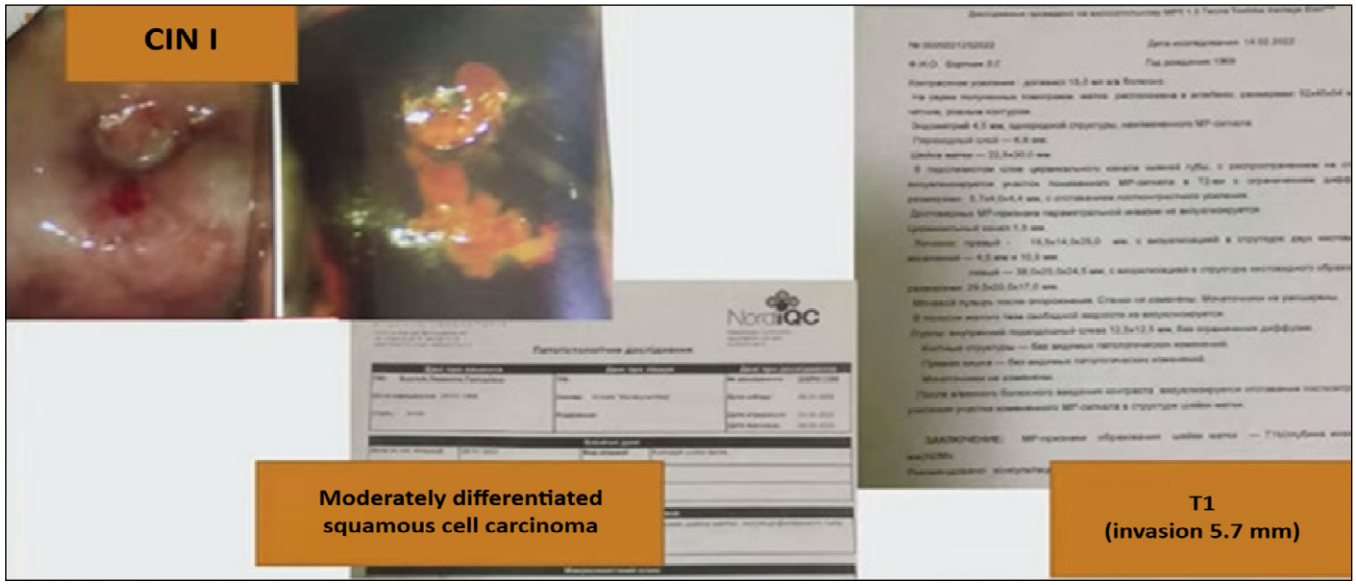

Fig. 11. CIN 1 – cytologically, colposcopically – metaplastic epithelium, histologically – moderately differentiated squamous cell carcinoma
Picture taken by the authors

amination. The diagnosis cannot be made by cytology and in any case must be confirmed by biopsy.

In the late 1960s, Richard proposed the concept of intraepithelial neoplasia.

CIN 3 included severe dysplasia and carcinoma in situ.

CIN 2 was replaced by moderate dysplasia.

CIN 1 combined both cytological signs of HPV infection (koilocytic atypia) and mild dysplasia. CIN 1 is an acute infection with *Human Papillomavirus*, which can be influenced and which can progress to the normal stage.

The severity of the diagnosis is determined by the degree of replacement of normal stratified epithelium with mitotically active basal-like epithelium. (<1/3=CIN 1, <2/3=CIN 2, >2/3=CIN 3). CIN is seen as a stepwise progression with a high probability of transitioning from more minor to more aggressive precursors of cancer.

CHANGES IN VIEWS ON THE DIAGNOSIS, TREATMENT AND MANAGEMENT OF PATIENTS WITH *HUMAN PAPILLOMA VIRUS* (HPV)

After the 20s of the 21st century, we already have data on biomarkers that help establish a diagnosis, help identify where there are weak lesions, and where there are already true precancerous conditions. All this will be reproduced in the histological report (Fig. 10).

In 2012, the Lower Anogenital Cancer Classification (LAST) conference adopted the LAST nomenclature

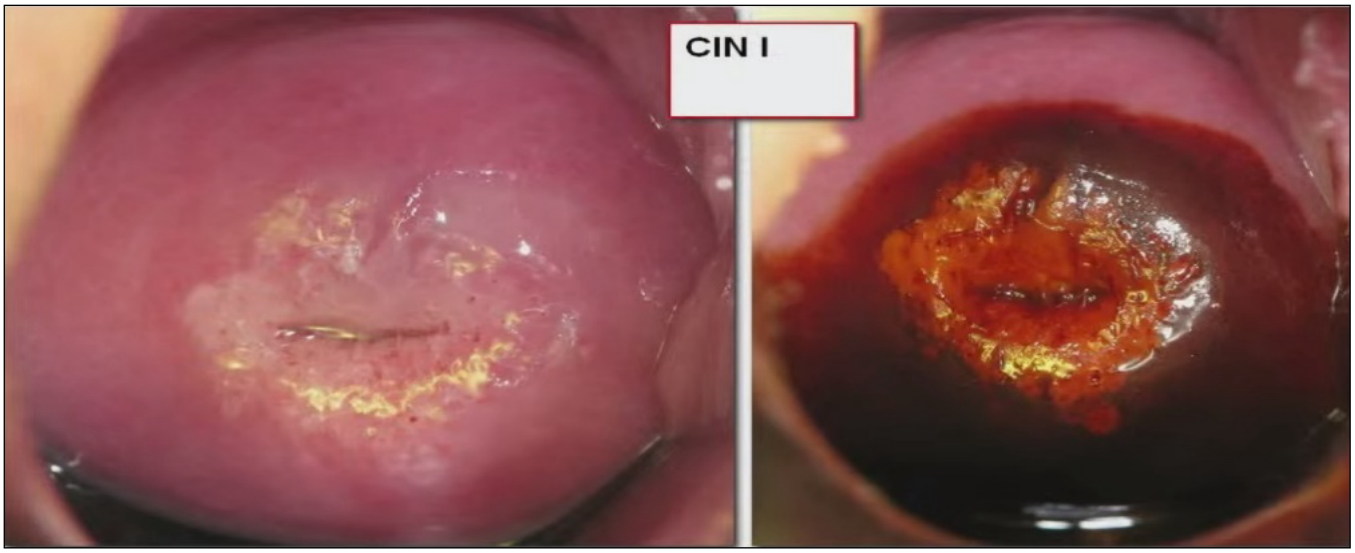

Fig. 12. CIN 1 Metaplastic epithelium, transformation zone
Picture taken by the authors

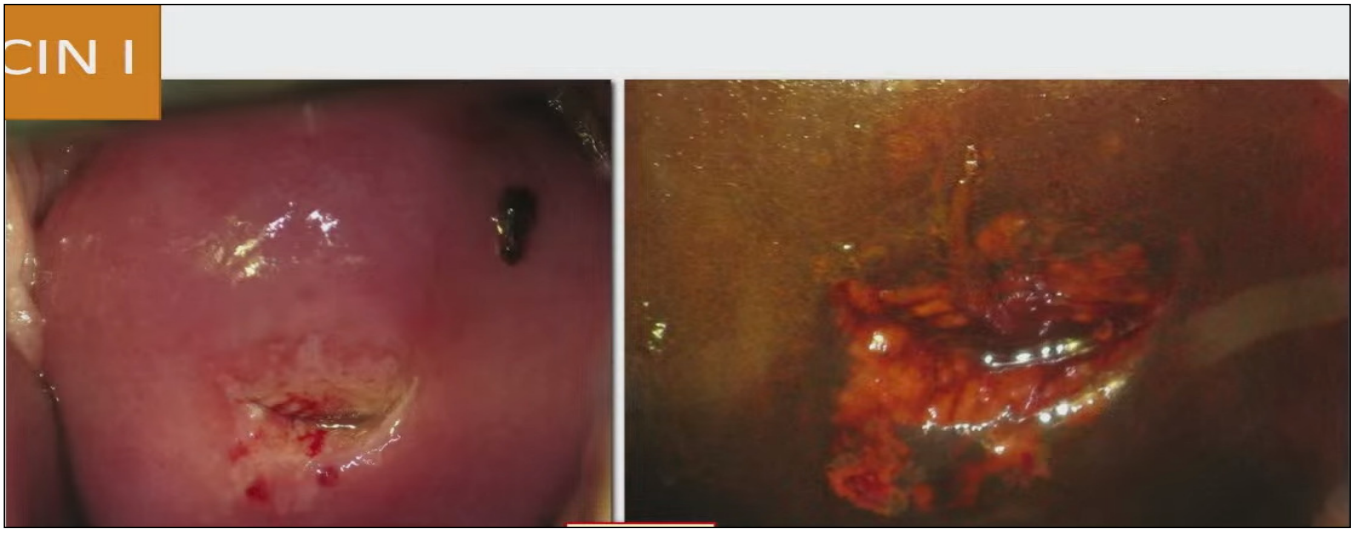

Fig. 13. CIN 1 Metaplastic epithelium
Picture taken by the authors

based on p16 staining for grading CIN 2; p16-positive combines with CIN 3 to form high-grade squamous intraepithelial lesion (HSIL), which is an immediate precursor to cervical cancer. CIN 2, negative for p16, combines with CIN1 to form low-grade squamous intraepithelial lesion (LSIL).

HPV studies have demonstrated the high prevalence and transient nature of most cervical HPV infections and it has become clear that there is no mandatory progression to CIN. Not all CIN will lead to cancer [12, 13].

CIN 1 has been found to be a poorly reproducible and insensitive histological diagnosis of an acute and generally transient infection.

When a number of histologists examined microscopy specimens from women diagnosed with CIN 1, some of them confirmed the diagnosis, some reduced it to normal, and some increased it to CIN 2 and CIN 3. Biopsies with a primary diagnosis of CIN 1 were reviewed by a panel of experts.

Stoler M.N. Schiffman M. From the ASCUS / LSIL. Triade Stady.

Conclusions should be made based on the HPV test, cytological test, and sometimes using markers to establish the diagnosis, which is very important. Table 2 presents the compatibility of cytological and histological diagnoses.

Cytology does not diagnose dysplasia, it identifies those patients who need to be worked with, who need to be isolated, examined, and biopsied.

Fig. 11 shows CIN 1 - cytologically, colposcopically - a region of metaplastic epithelium, and histologically - moderately differentiated squamous cell carcinoma.

CIN 1 is presented in Fig. 12, Fig. 13, where metaplastic epithelium is visible, the transformation zone of type 1, can be regarded as acetowhite epithelium, but without

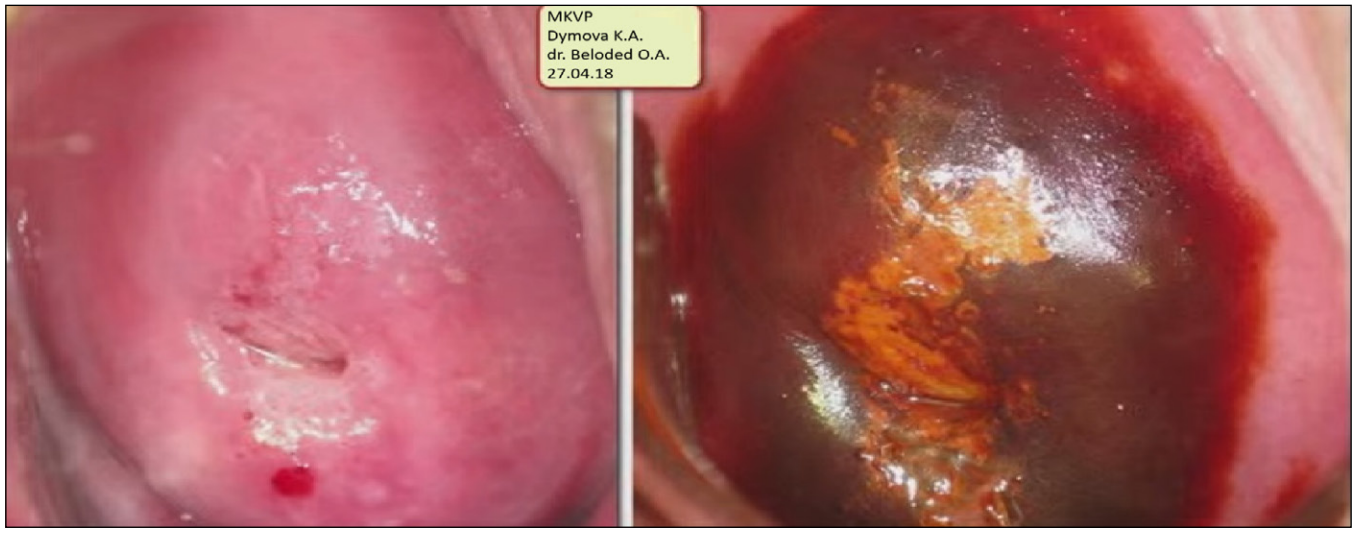

Fig. 14. Acute inflammation, reactive changes with atypia

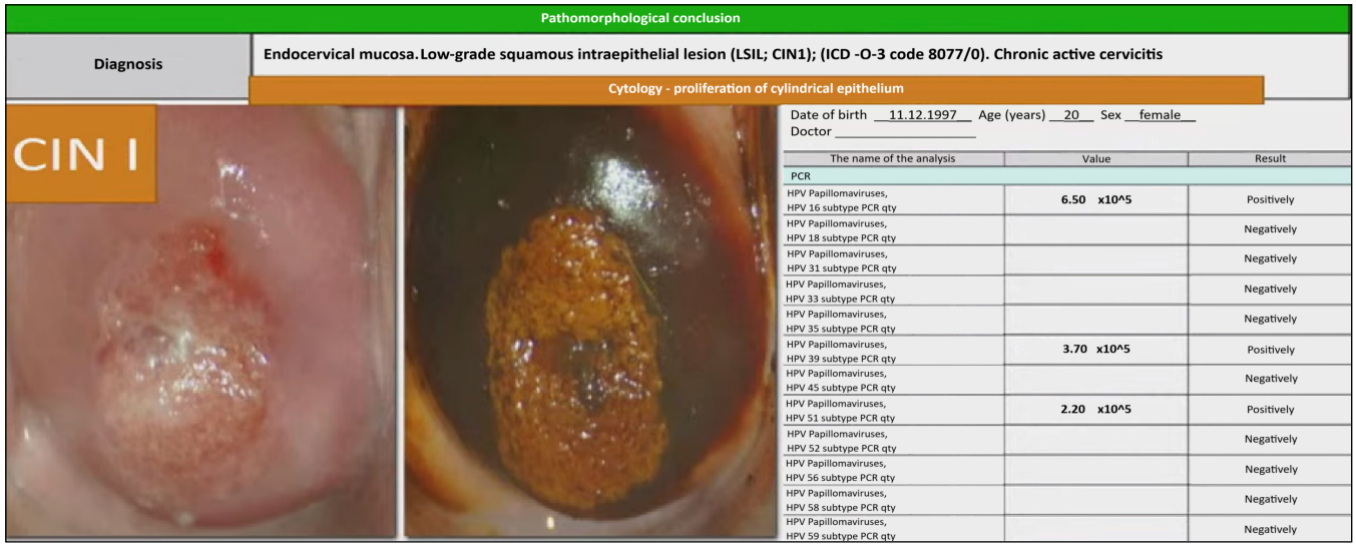

Fig. 15. Ectopia of the cervix of a woman whose biopsy revealed CIN 1
Picture taken by the authors

a biopsy we cannot say for sure what this will be in true manifestation.

Fig. 14 shows acute inflammation, no changes were detected cytologically, bacterial vaginosis, reactive changes with atypia of epithelial cells are present, because the epithelium suffers from an inflammatory reaction.

Fig. 15 shows a long-term ectopia in a woman with altered hormonal background, PCOS, unstable hormones, with a high viral load, in whom CIN 1 was detected during biopsy.

MODERN LSIL TREATMENT ALGORITHMS

Currently, the following issues are problematic:

- There is no true verification of the diagnosis. Treatment is planned and carried out based on screening.
- "Quick" treatment is carried out, sometimes almost without examining HPV status.
- Treatment is dominated by surgical and ablative methods, the infectious state is not taken into account.
- The median observation time for HPV clearance is not tracked.

CIN 1 – persistent HPV has:

- Viral cytopathic effect
- Damage to 1/3 of the epithelium
- Detection as a result of (HPV and cytological) screening programs
- Diagnosis = biopsy

CIN 1 regresses in 60-80% of cases within 2-5 years, very often regresses due to pregnancy, so there is no need to endlessly cut off pieces on the cervix in women planning pregnancy. There are studies that show that CIN 1 becomes normal due to pregnancy.

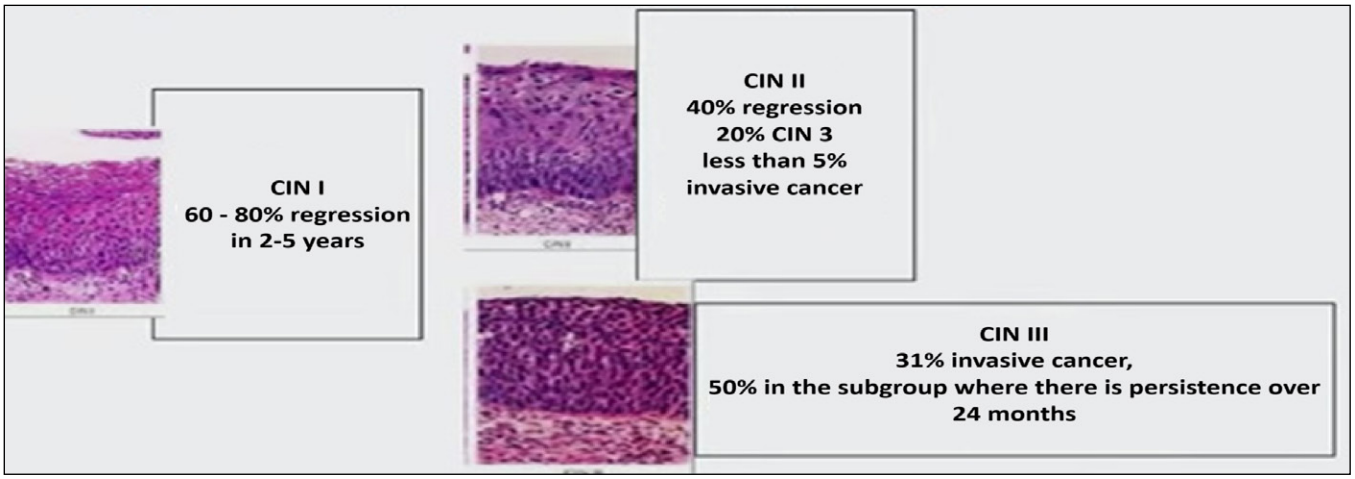

Fig. 16. CIN risk assessment
Source: compiled by the authors based on [12]

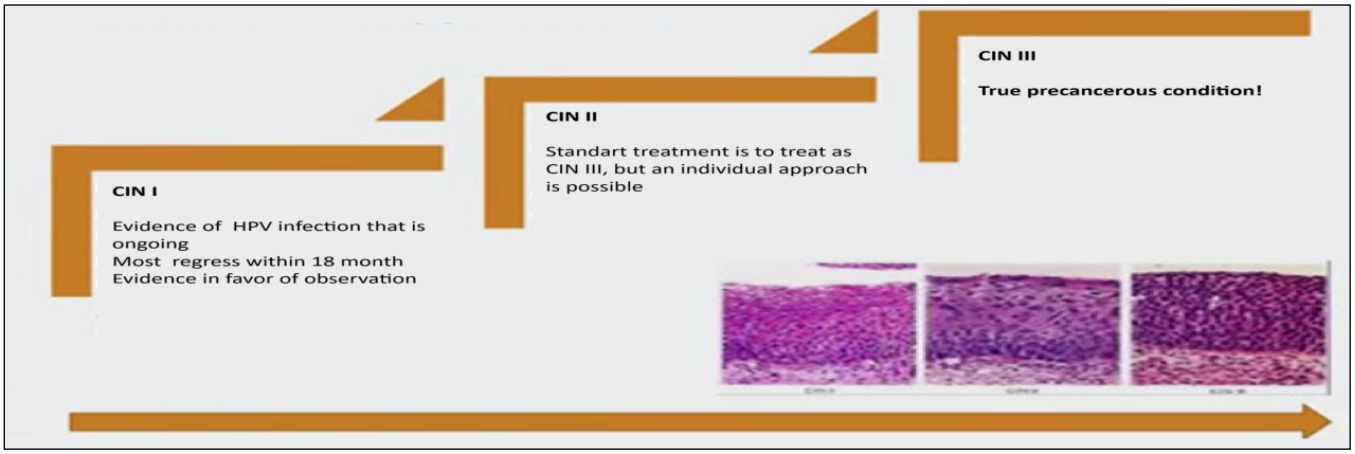

Fig. 17. CIN Management
Source: compiled by the authors based on [13]

CIN 2 40% regress, but 20% definitely transform into invasive cancer, but without having HPV-16 in this diagnosis, regression can be observed.

CIN 3 has a very small percentage of spontaneous recovery – less than 5%, and this occurs when the woman's viral aggression disappears.

CIN 3 progresses to invasive cancer in 31%, 50% in the subgroup where there is persistence for 24 months (Fig. 16).

CIN management is presented in Fig. 17.

DIFFICULTIES IN DIAGNOSIS AND TREATMENT

The following problematic issues very often arise (Fig. 18):

- this is a positive HPV test and normal cytology or colposcopic changes are detected, a biopsy is taken, a diagnosis is made, etc.
- is a positive HPV test and cytological changes to LSIL →refer for colposcopy →biopsy →observe →treat
- this is a positive HPV test, LSIL, CIN 1 diagnosis, quantitative viral load should be taken into account
- whether the place that causes changes in histology was correctly found colposcopically
- age: women who are 30-35 years old and older and may have already been infected with the *Human Papillomavirus* during their sexual life

CIN 1+high-risk HPV

– Most guidelines suggest treatment or continued observation for CIN 1 (LSIL) if the changes persist for at least 2 years
– Regression is defined as a CIN 1 lesion that returns to normal cytology (70-80%)
– Persistence is defined as CIN 1 lesions demonstrated cytologically after 2 years (5-20%)
– Progression is defined as histologically confirmed CIN2+ (various studies 1.5– 4% to 10% in one study)
– Менш, ніж 1 % призведе до інвазивного раку
– Less than 1% will lead to invasive cancer
– Both progression and persistence are correlated with HPV infection

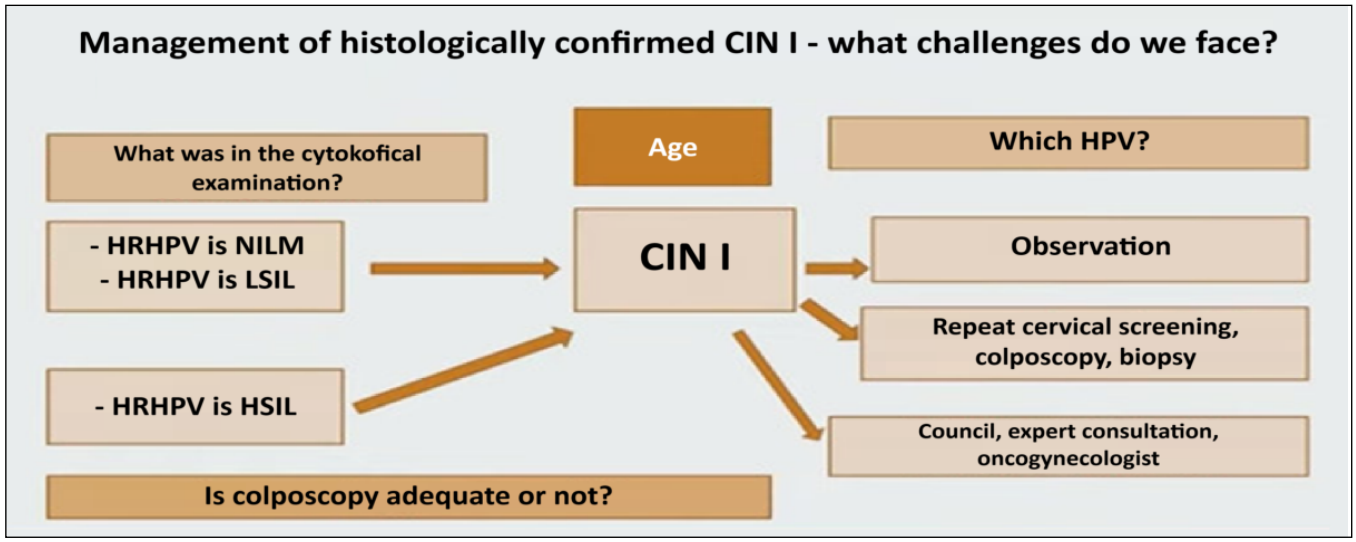

Fig. 18. Management of histologically confirmed CIN 1
Source: compiled by the authors based on [14]

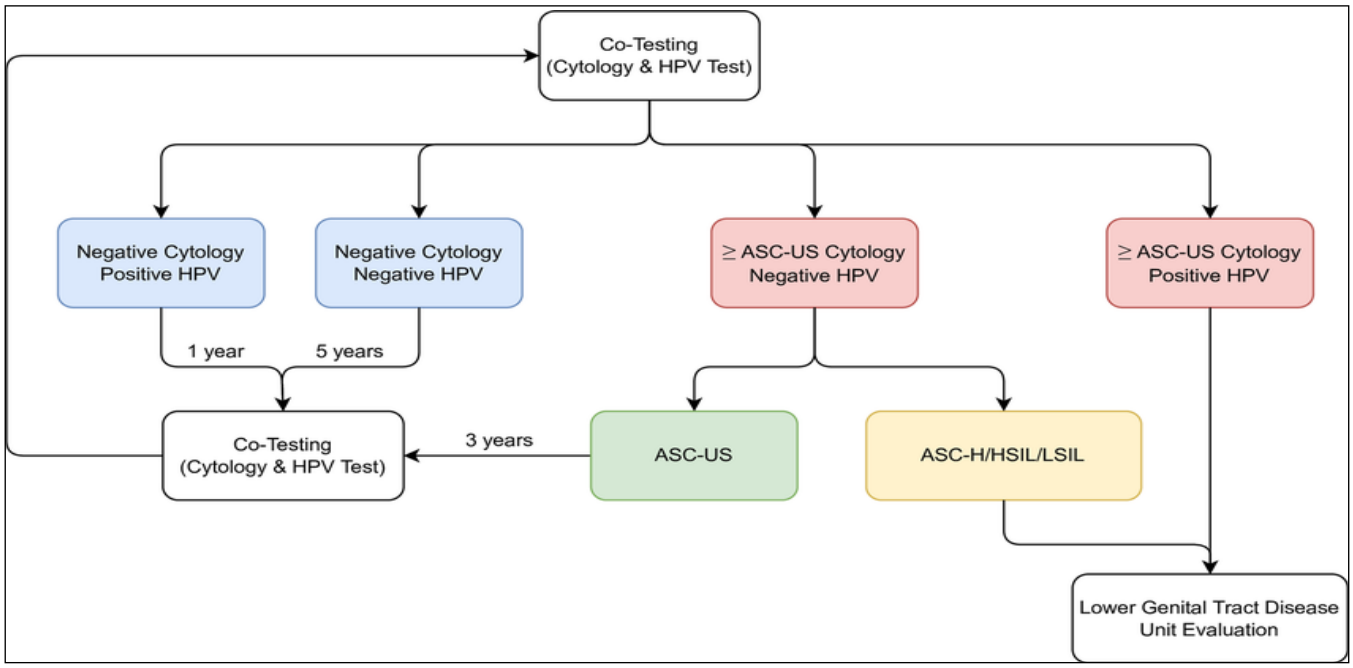

Fig. 19. Cervical cancer screening algorithm via co-testing
Source: compiled by the authors based on [14]

Progression
– CIN 3 may occur *de novo*, rather than being the result of progression of CIN 1 (infection with a different viral genotype PARK, Quint, Litjens studies)
– It is not the presence of CIN 1 that determines the risk of developing CIN 3, but the diagnosis of persistent HPV infection 16,18,31,33, etc. (Patricia, Viviant, and others).

The goal of treatment
– Recognition of occult CIN 3
– Treatment of low-risk abnormal cells
– Return to normal cytology
– Patient support

HPV
– Clearance
– Latency
– Persistence

The algorithm for screening for cervical cancer is presented in Fig. 19.

The main question of the congress on *Human Papillomavirus*, held in Washington in 2023, is whether HPV can leave the body forever? The world must answer this question. But today there is a tool that, within 2 years, has changed the perception of cervical cancer treatment. Today, they don't treat something, they don't cut something out, they treat the virus, and this process is called the elimination of the

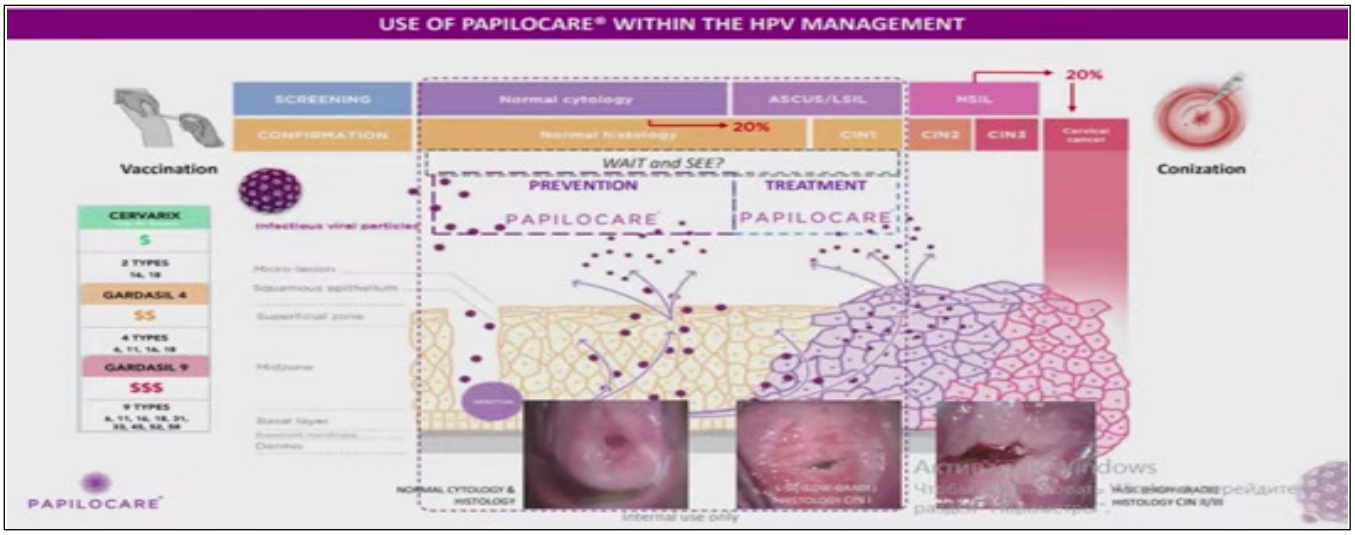

Fig. 20. The path of Papilocare gel in the human body
Source: compiled by the authors based on [15]

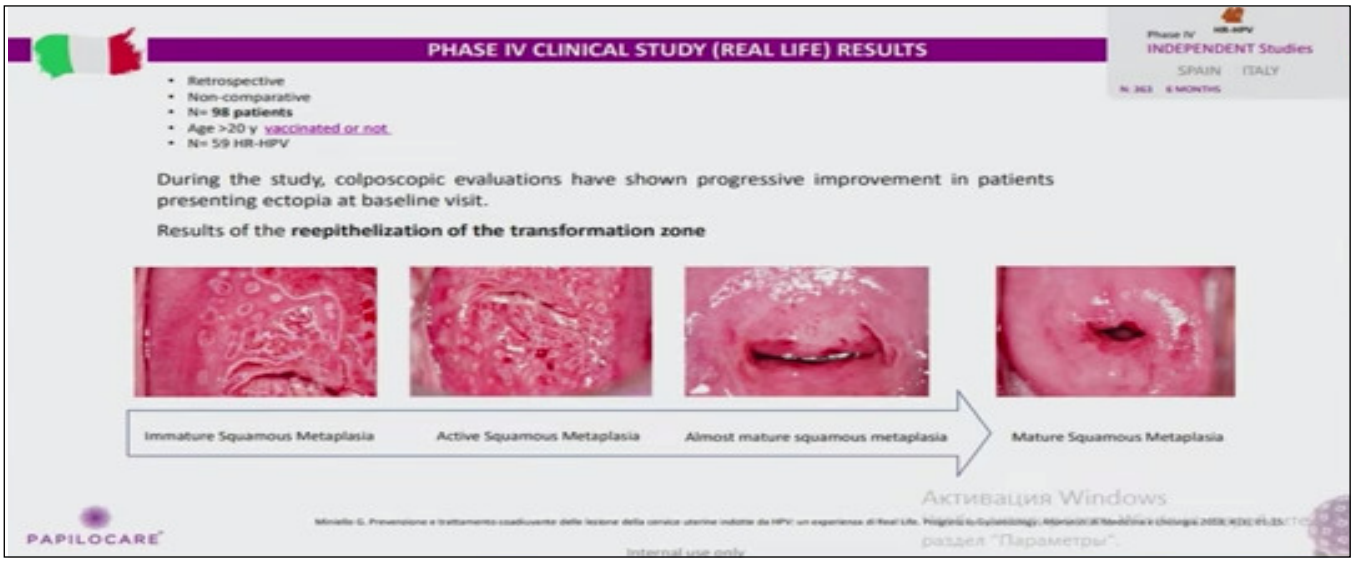

Fig. 21. Incomplete epithelialization with Papilocare gel
Source: compiled by the authors based on [12]

Human Papillomavirus pathogen. This is something that the body couldn't handle, something that lactobacteria should have handled. Interferon α should strengthen the immune system, it should clear on its own. But this did not happen and the process continued. The virus began to infect the epithelium and divide cells similar to itself, so there is a means of eliminating the human papillomavirus – papilocare.

– A medical product that combines ingredients of natural origin
– Vaginal gel that forms a protective and healing barrier in the "transformation zone"of the cervix.

Papilocare gel follows the same path as the human papillomavirus, cleansing layer by layer of stratified squamous epithelium. The entire epithelium has 5 layers. 1 superficial – exfoliates and definitely the human papillomavirus affects all layers from below, all layers are cleaned, 5 months of cleaning are required for 5 layers. After two years of administration, excellent results in eliminating the pathogen have been observed (Fig. 20).

Indications for use of Papilocare gel:

– Monitoring and assisting in the reepithelialization of the cervical transformation zone to prevent the risk of HPV-induced lesions (LSIL)
– Use as an adjunctive treatment for intraepithelial lesions caused by HPV
– Restoration and assistance in re-epithelialization of lesions of the cervical mucosa
– Treatment of dryness of the cervical and vaginal mucosa
– Restoring the balance of vaginal microbiota
– Improving the overall condition of the vagina
– Creating conditions for rapid healing of scratches caused by inflammation or itching

– Formation of a protective film that quickly reduces irritation, creating the right conditions to promote the natural healing process.

The action of Papilocare gel is explained by the fact that it is a very small molecule in structure, like the human papillomavirus, therefore, starting layer by layer, the cleansing process takes place. The main component of Papilocare gel is Coriolus versicolor, an antitumor fungus that has the property of restoring apoptosis.

Coriolus versicolor

– Induction of anti-inflammatory cytokines, interferon-γ, effects on NK cells
– Expression of tumor necrosis factor – stimulation of apoptosis

To date, 50% to 70% of patients have HPV clearance after 6 months in 4 studies. An example of incomplete epithelialization using Papilocare gel is shown in Fig. 21.

Who can use Papilocare gel:

– It is recommended for women over 18 years of age infected with human papillomavirus, regardless of whether they have lesions caused by the virus (LSIL) with appropriate colposcopy results.
– Women whose histological results are: CIN 1 or who do not require surgical treatment of CIN2.
– Duration of treatment is 5-6 months, administered every day for 21 days, and then every other day for 2,3,4 months for 21 days.
– Control tests after 6 months.

Treatment options depend on:

– Vasibility of the junction of the squamous and columnar epithelium (how adequate was the colposcopy)
– Age (greater caution in reproductive age)
– Ablation (cryo and thermal ablation)
– Excision
– Hysterectomy only in the presence of concomitant gynecological diseases

PREVENTION

Vaccination is not currently considered a purely preventive measure. Currently, vaccination is one of the parallel ways to eliminate the *Human Papillomavirus*, to protect women from cervical cancer and subsequently their healthy cells, as a means of preventing the recurrence of severe precancerous conditions.

Today, vaccination is mandatory after each treatment for severe cervical dysplasia, especially if it concerns the viruses that caused the dysplasia. Research into vaccinating infected women is ongoing.

In the UK and Europe, women receive preventive vaccines at the time of conization or other treatment and are re-examined 6 months later [14]. In 2021, at the XVII Congress, which was held in India, there was a report by Dr. Eva Lois from the Department of Oncology and Gynecology from New Zealand, who reported that the population practically does not have HPV-16, they may have other precancerous conditions, condylomas, but they are not caused by HPV-16, HPV-18, because due to the vaccination introduced in the country, HPV-16 has almost completely disappeared from the human population and due to this, the incidence of cancer has decreased [15]. New Zealand is a country that currently has a cervical cancer incidence rate of 5 cases per 100,000, which is almost the rate that the WHO calls for - 4 cases per 100,000.

CONCLUSIONS

1. Detection of LSIL (CIN1) is carried out by HPV testing and cervical screening, and the diagnosis is verified by histology.
2. The goal of the screening program is to detect and treat high-grade precancer and prevent the development of cancer.
3. Avoiding overtreatment and its consequences (cervical stenosis and pregnancy complications).
4. It is not the presence of CIN 1 that determines the risk of developing CIN3, but the diagnosis of persistent HPV infection 16, 18, 31,33, and others.
5. Most guidelines talk about surveillance from 18 years of age for 2 years for CIN 1.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Complications of immediate-loading dental implants: Current insights into etiology and pathogenesis

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ABSTRACT

Aim: To analyze the scientific literature on the etiology and pathogenic mechanisms underlying complications arising from dental implantation under immediate loading protocols and to outline promising approaches to their prevention and management.

Materials and Methods: A review of the scientific literature addressing the complex mechanisms underlying potential complications of dental implantation under immediate loading protocols was conducted.

Conclusions: The literature suggests that the primary causes of early dental implant failure include compromised mechanical stability during the critical phase of bone remodeling, inadequate seal control, and violation of the biological width. Soft tissue thickness deficiency (thin biotype) or the absence of keratinized mucosa triggers pathological bone destruction, which, in turn, facilitates infection and inflammation, further exacerbating destructive changes. These mechanisms are interrelated and mutually reinforce one another.

The findings underscore the need to develop protocols for guided implant therapy in high-risk patients, to implement clear algorithms for objective monitoring of implant stability at all stages of treatment, and to identify the optimal sealing material. These measures have the potential to improve approaches to the fixation of prosthetic restorations.

KEY WORDS: dental implantation, biomechanical, biological, infectious inflammatory processes, immediate loading

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INTRODUCTION

In recent decades, alongside the two-stage protocol, immediate loading with simultaneous healing abutment placement has been increasingly adopted in dental implantology, driven by the need to minimize rehabilitation time and to optimize aesthetic outcomes [1, 2]. At the same time, inadequate dental care results in dysfunction of the stomatognathic system, disorders of the digestive and respiratory systems, and the development of aesthetic defects, ultimately leading to social and psychological maladaptation in patients. Consequently, the steadily increasing number of forensic medical examinations related to dental service quality, together with the associated litigation risk, contributes to stress and burnout among dentists [3]. Despite the high success rates of dental implant osseointegration, early and delayed complications remain a significant concern [4]. Unlike the delayed protocol, in which the implant is isolated from external forces during the osseointegration phase, immediate loading transforms the surgical site into a dynamic system, where even minimal disturbance to equilibrium can lead to adverse outcomes [5].

AIM

To analyze the scientific literature on the etiology and pathogenesis of complications arising from dental implantation under immediate loading protocols and to examine key aspects of their prevention and management.

MATERIALS AND METHODS

A review of the scientific literature on the etiology and pathogenesis of complications arising from dental implantation under immediate loading protocols was conducted. Selected studies from the 1980s and 1990s

were included to provide historical context regarding past successes and failures, while the literature published between 2000 and 2024 was analyzed for insights into the complex mechanisms underlying potential complications.

REVIEW

According to the literature, these causes can be classified as *biomechanical* (loss of primary stability), *biological* (impaired osseointegration and altered biological width), and *infectious-inflammatory* (peri-implantitis) [6].

Biomechanical factors associated with implant failure under immediate loading. Primary (mechanical) stability is achieved solely through the macromechanical retention of the implant threads within the trabecular bone and cortical plate and is maximal at the time of placement. Secondary (biological) stability arises from new bone formation on the titanium surface. Between these phases, a transitional period known as the "stability dip" occurs, typically between the third and fifth postoperative weeks [7].

Compression of bone tissue required to achieve high insertion torque (35–45 Ncm) leads to local ischemia and osteocyte micronecrosis at the bone–implant interface [8]. In response, a remodeling process is initiated, during which osteoclasts resorb the traumatized bone tissue supporting the dental implant. During the interval between extensive resorption of intact bone tissue and the presence of newly formed, insufficiently mineralized bone, the overall stability of the system reaches its minimum. At this stage, masticatory forces during immediate loading are transmitted to the weakened remodeling zone. Between the third and fourth postoperative weeks, the risk of implant loss is highest, as mechanical support has been lost, and biological stability has not yet developed [9]. Excessive compression (over 60–70 Ncm) can lead to pathological bone resorption by compressing the Haversian canal vessels [2]. When the pressure on the implant bed walls exceeds capillary blood pressure (approximately 30–40 mmHg), ischemia occurs, and if it persists, osteocytes die from hypoxia [10]. This is particularly critical in cortical bone (D1 type) due to its low vascularity; therefore, adherence to a thread-tapping protocol or the use of implants with active, knife-edge threads, rather than compressive threads, in dense bone is an essential preventive measure [11]. Indeed, surgical complications under immediate loading correlate with bone type. In D3 and D4 bone (predominantly in the posterior maxilla), achieving the required insertion torque is challenging due to low trabecular density, which provides inadequate rigidity for fixation. Consequently, the "stability dip" is more pronounced and prolonged in these patients. The incidence of early implant failure under immediate loading in D4 bone is three to four times higher than in D1/D2 bone [2].

In immediate implantation into an extraction socket, the most common error is positioning the implant in the center of the socket rather than toward the palatal side. This often results in negative outcomes, as the buccal bone wall may be less than 2.0 mm thick or entirely absent. The buccal bone plate is a tooth-dependent structure that undergoes resorption after tooth extraction. Consequently, if an implant is positioned too far buccally, it will inevitably become exposed during the remodeling process. To achieve a stable and esthetic outcome, buccal bone thickness should be at least 2.0 mm [12].

During this period, micromotion amplitude is critical. Micromotions within the 30–50 μm range are within physiological limits and may even stimulate osteogenesis via the piezoelectric effect. However, maintaining this range in clinical practice is extremely challenging, particularly when removable prosthetic restorations or single crowns are used [13]. A micromotion threshold of 100–150 μm is generally accepted as the upper limit for successful dental implant osseointegration [14]. If micromotion within the socket exceeds 150 μm under occlusal loading, neoangiogenesis is disrupted, and fibrin fibers, which serve as a matrix for osteogenic cell migration, are ruptured. Under these conditions, multipotent mesenchymal cells differentiate into fibroblasts rather than osteoblasts, forming a connective tissue capsule. Clinically, this presents as implant mobility without signs of purulent inflammation (aseptic fibrous integration) 6–8 weeks postoperatively [15].

Regardless of the protocol, dental implant placement elicits a foreign body reaction: osseointegration represents a protective host response in which the body attempts to isolate the titanium implant by forming a bony barrier ("shielding off") around it [16]. In successful cases, an immunological equilibrium is achieved; however, under immediate loading, this equilibrium is only relative. Overall, mechanical stress and micromotion activate the pro-inflammatory M1 macrophage phenotype. Rather than transitioning to the reparative M2 phenotype (which promotes osteoblast activity), these cells continue to secrete pro-inflammatory cytokines, thereby sustaining chronic inflammation. Consequently, peri-implantitis is not regarded as a distinct disease but rather as a disruption of the foreign body equilibrium that progresses to implant rejection [17].

Osteotomy site preparation generates frictional heat. Heating the bone to 47 °C for 1 minute induces irreversible protein denaturation (particularly alkaline phosphatase), vascular coagulation, and osteocyte necrosis [18]. The risk of thermal necrosis increases when using guided surgical templates, as temperatures in the apical region of the implant osteotomy can reach 50–55 °C, thereby inducing protein denaturation [19]. Thermal shock inhibits the expression of heat shock protein 70 (HSP70), leading to bone necrosis around the implant. By the fourth to fifth postoperative week, as the body begins to sequester necrotic tissue, the implant loses stability and fails. Under immediate loading, this process is accelerated by micromotion [8].

The direction of loading is critical. Axial (vertical) forces are well tolerated by the bone and dental implant, promoting bone compaction. In contrast, horizontal (lateral) forces arising from premature contacts or steep cusp inclines of temporary crowns generate destructive bending moments in the crestal region of the implant. Under immediate loading, the cortical plate at the implant neck experiences the highest stress. If this area is thin (<1 mm) or damaged during tooth extraction, crater-like bone resorption may occur. Marginal bone loss of 1–2 mm compromises esthetics and reduces bone–implant contact, thereby reducing the system's overall load-bearing capacity [13].

Impaired osseointegration and biological width violation. A fundamental challenge of any two-piece implant system is the inability to achieve a hermetic seal between the implant and abutment, as microgaps inevitably form at the interface. The size of these microgaps can range from 1 to 49 µm, depending on the connection type (flat-to-flat or conical) and the precision of casting or milling of the prosthetic components [20]. Occlusal forces applied to the healing abutment induce elastic deformation of the abutment screw and micromotion at the implant–abutment interface. This phenomenon is referred to as the micro-pumping effect [21]. Compared to flat-to-flat connections, conical connections demonstrate significantly greater mechanical stability and resistance to microleakage. Owing to the cold-welding effect and frictional fixation, Morse taper connections minimize abutment micromotion, thereby reducing the micro-pumping effect [22]. In cases of subcrestal placement of implants with flat-to-flat connections, bone resorption continues until the implant platform is exposed or the bone level is stabilized 1.5–2.0 mm below the implant–abutment interface. This results in the formation of a crater-like defect that serves as a niche for plaque accumulation and further progression of peri-implantitis [23]. The concept of platform switching, involving the use of an abutment with a smaller diameter than the implant platform, shifts the inflammatory cell infiltrate horizontally toward the implant's central axis and has been shown to significantly reduce vertical bone loss [24]. However, even with platform-switched conical connections, effective bacterial sealing cannot be guaranteed, particularly under immediate loading, when achieving final screw torque is limited by the risk of implant rotation. Under these conditions, the internal chamber of the implant becomes an ideal anaerobic incubator for the most virulent "red complex" pathogens [25]. Hollow spaces within the implant have been shown to harbor up to 10^8 colony-forming units (CFUs) of bacteria [26].

Continuous release of bacterial endotoxins through the microgap induces a chronic inflammatory response in the surrounding tissues. The inflammatory infiltrate, in turn, serves as a source of pro-inflammatory cytokines – interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor alpha (TNF-α) – which stimulate the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) on osteoblasts [27]. Osteoclastogenesis is subsequently activated, with osteoclasts resorbing bone tissue to remove it from the site of infection, thereby establishing the biologic width. Vitamin D exerts anti-inflammatory effects by suppressing the production of IL-1, IL-6, and TNF-α, reducing RANKL expression, and inhibiting osteoclastogenesis. In patients with vitamin D deficiency (<20 ng/mL), the risk of early implant loss increases by 140% [28].

The transmucosal healing abutment is placed into a fresh post-extraction socket or a prepared surgical site. A persistent clinical challenge remains the transition zone between the natural gingival attachment and the peri-implant soft tissue interface. In natural dentition, collagen fibers insert perpendicularly into the root cementum, whereas the titanium surface induces the formation of parallel-oriented fibers [29]. This "biological cuff" is characterized by a reduced fibroblast count and inadequate vascularization. Around zirconia abutments, a structure more closely resembling the natural tooth is formed, with a portion of the collagen fibers oriented perpendicularly [30]. The use of temporary plastic crowns or standard titanium healing abutments with microrough surfaces during immediate loading carries specific risks. Since fibroblast adhesion to polished titanium is reduced, surface roughness promotes biofilm accumulation, occurring as early as 30 minutes after surgery, and bacterial invasion can trigger an inflammatory response, leading to marginal bone resorption and gingival recession [31]. By positioning

the implant platform deep subgingivally (typical in the esthetic zone), complete removal of excess cement becomes virtually impossible. Residual cement acts both as a rough surface promoting biofilm formation and as a toxic agent, eliciting a pronounced inflammatory response when in contact with connective tissue [32]. Therefore, the current gold standard for prevention is the use of screw-retained restorations or customized abutments, in which the cementation margin is positioned at the gingival level.

The role of soft tissue thickness. A vertical gingival thickness of 2.0 mm or less triggers inevitable bone resorption of approximately 1.5 mm, regardless of the implant platform position (supracrestal or epicrestal) [33]. Evidence suggests that thin soft tissues fail to provide adequate vascular supply to the interface region and that peri-implant mucosal ischemia leads to marginal necrosis of the flap, wound dehiscence, and exposure of the cover screw or healing abutment. Compromised vascularization leads to an ineffective immune response, facilitating early pathogen colonization of the implant surface even before the healing process is complete [34]. The temporary crown serves as a matrix for soft-tissue contouring. A concave abutment design allows for increased connective tissue volume, whereas a convex emergence profile results in tissue compression, thinning, and eventual recession [35]. The absence of keratinized mucosa (<2 mm) significantly correlates with higher plaque indices, bleeding on probing, and patient discomfort during oral hygiene procedures [36].

Infectious inflammatory complications (peri-implantitis). Early complications under immediate loading result from a breakdown in the immune system's tolerance to the titanium implant, with bacteria acting merely as triggers, while tissue destruction is primarily mediated by the host's own cells. Bone resorption is an immune-mediated process, and the success of implantation depends on whether the titanium surface is first colonized by host cells (fibroblasts/osteoblasts) or by bacteria [37]. As early as 2 weeks after implantation, the implant environment is colonized by *P. gingivalis* and T. *forsythia*, even in patients with clinically healthy periodontal tissues. The "red complex", including *Porphyromonas gingivalis*, *Tannerella forsythia,* and *Treponema denticola*, is of particular concern. *P. gingivalis* acts as a keystone pathogen; using its enzymes, it does not merely degrade tissues but suppresses the immune system, specifically the complement system, thereby facilitating the unimpeded proliferation of other bacterial species. Furthermore, *P. gingivalis* can invade epithelial cells, thereby evading the effects of antibiotics [25]. Given that the average size of oral pathogens (such as *P. gingivalis* and *T. denticola*) ranges from 0.5 to 1.0 μm, while their toxins and pro-inflammatory mediators are measured in nanometers, even a minimal microgap creates favorable conditions for bidirectional exchange of fluids and bacteria between the internal environment of the implant and the surrounding tissues. Fungal infection represents a critical component of the pathological process that threatens the integrity of the implant-supporting bone. Modern clinical *Candida* strains, isolated from patients with oral diseases, exhibit high resistance to conventional antifungal agents. This complicates the management of peri-implant infectious complications and necessitates the development of novel antimycotics [38].

DISCUSSION

An analysis of the literature on the biomechanical causes of complications in dental implantation performed according to immediate loading protocols indicates that there are no clear data on the correlation between radiographic bone density and its metabolic status, a consideration essential for predicting a "stability dip." Therefore, the development of an index integrating computed tomography parameters with blood biochemical markers, specifically serum alkaline phosphatase and osteocalcin levels, appears promising. Such an approach would facilitate the identification of patients with predominant bone resorption relative to bone formation, thereby justifying their exclusion from the standard loading protocol.

According to the literature, microgaps at the implant–abutment interface represent a persistent source of bacterial leakage, which is further exacerbated by the micro-pumping effect during chewing. Therefore, the development of a protocol for intraoperative sealing of the implant's internal cavity is warranted. However, the optimal choice of sealing material remains unresolved. Accordingly, comparative clinical and microbiological studies are needed to assess the efficacy of silicone or antiseptic-loaded matrices in both sealing the implant chamber and reducing the load of "red complex" periodontal pathogens in the peri-implant sulcus.

Bone loss results from the immune response to foreign bodies and biofilms, leading to osteoimmunology imbalance regulated by the RANKL–OPG (osteoprotegerin) system. Therefore, factors such as hypovitaminosis, vitamin D deficiency, and metabolic disorders that may compromise osseointegration should be considered. Moreover, the potential use of agents that inhibit proinflammatory cytokine

synthesis and reduce osteoclast activity in the early postoperative period warrants further investigation.

According to current evidence, soft tissue thickness deficiency is critical for marginal bone stability. Conventional augmentation techniques are often traumatic and may increase the risk of infection. The use of platelet-rich fibrin (PRF) membranes requires further investigation to assess the effects of growth factors on the rate of socket healing and the formation of a "biological cuff" around the abutment.

CONCLUSIONS

An analysis of the literature indicates that complications in dental implantation under immediate loading protocols are multifactorial, with biomechanical, biological, and infectious inflammatory factors acting as interrelated pathogenetic links that frequently overlap and exacerbate tissue disintegration. Accounting for these factors can facilitate the development of protocols that incorporate an individualized approach at various stages of implantation.

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CONFLICT OF INTEREST

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CASE STUDY

CONTENTS

Hemorrhagic shock in the late postoperative period after the Nuss method procedure – case study

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ABSTRACT

Aim: The study aims to describe a clinical case and its management in the Hospital Emergency Department of a seventeen-year-old boy with posttraumatic internal hemorrhage into the pleural cavity during the late postoperative period following Nuss surgery.

Materials and Methods: This paper presents a clinical case of a 17-year-old patient who was initially admitted to the Emergency Department (E.D.) of the District Hospital named after John Paul II in Bartoszyce, with a history of surgical treatment of pectus excavatum, after blunt chest trauma and in result hemorrhagic shock.

Conclusions: Although low-energy injuries usually do not cause significant health damage, the overall clinical impression, the ABCDE assessment, and a detailed patient history are crucial. Information about previous surgical procedures can be invaluable in identifying the source of an acute health threat. With access to EBM, the analysis should consider all possible causes - from the most common, through the rarest, to those not described in the medical literature but potentially possible.

KEY WORDS: hemorrhagic shock, nuss method, ED, DCS

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INTRODUCTION

Hemorrhagic shock is a subtype of hypovolemic shock that develops as a consequence of whole blood loss and is responsible for approximately 40% of trauma-related fatalities [1]. Blood extravasation accounts for up to 80% of deaths occurring during surgery in patients with severe traumatic injuries [2] and contributes to nearly 50% of trauma-related deaths within the first 24 hours following injury. In adults, blood loss of less than 750 mL (approximately 15% of the circulating blood volume) typically on physical examination hypotension, tachycardia or altered mental status do not reveal or only minor changes, however the general condition is usually unchanged [3]. Early recognition of hemorrhagic shock is critical, as is the prompt initiation of the damage control surgery (DCS) approach. DCS specifically encompasses, among other measures, rapid control of hemorrhage, restoration of hemostasis, and minimization of secondary tissue injury. This paper presents a clinical case of a 17-year-old patient who was initially admitted to the Emergency Department (E.D.) of the District Hospital named after John Paul II in Bartoszyce, with a history of surgical treatment of pectus excavatum, after blunt chest trauma and in result hemorrhagic shock.

Pectus excavatum (PE) is a deformity that develops due to abnormal attachment of the ribs to the sternum. PE originates from an embryological developmental disorder, with its pathogenesis beginning around the 35th day of gestation, continuing throughout pregnancy, and ultimately concluding with ossification of the ribs during adolescence. As a result, the sternum is pulled inward, creating a characteristic depression in the chest wall. PE is the most common congenital chest wall anomaly, occurring approximately five times more frequently in boys than in girls [4]. A genetic hypothesis has been proposed, as the condition frequently occurs in individuals whose relatives have presented with similar disorders. The problems arising from pectus excavatum are primarily cosmetic in nature; however, in some patients, deformity may cause respiratory difficulties, chest pain, or reduced exercise tolerance. This can lead to emotional and psychological disturbances in patients, particularly in adolescents. The Nuss procedure is the gold standard for the treatment of pectus excavatum. The procedure, developed by Nuss in 1988, is used to correct funnel

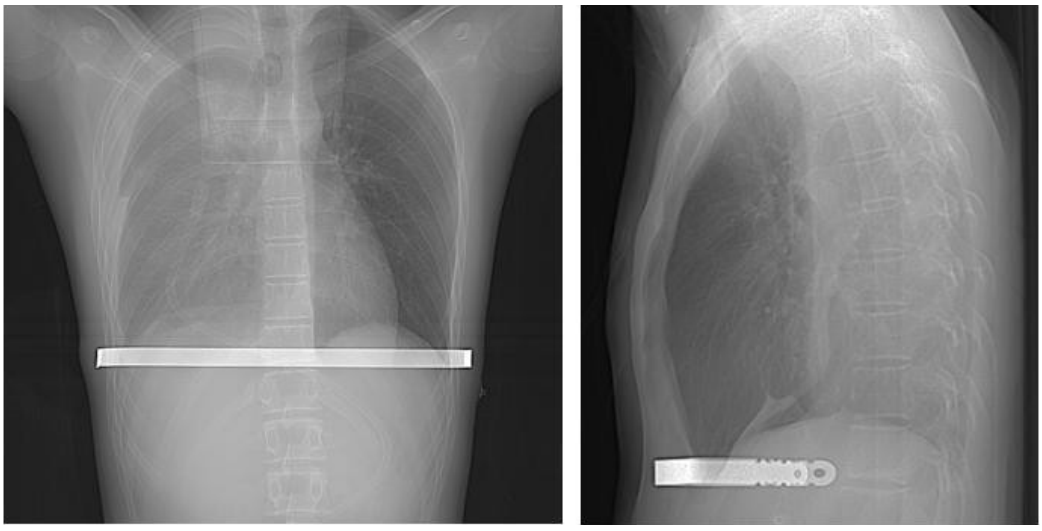

Fig. 1. Nuss bar implanted in the patient's wall chest: A – frontal view, B – lateral view

chest, a deformity of the sternum and adjacent costal cartilages in which these structures are depressed inward. It involves the insertion of a curved metal plate beneath the sternum, which, after rotation within the thoracic cavity, allows correction of the sternal deformity. The corrective plate is introduced through bilateral incisions on the sides of the chest, under thoracoscopic guidance. In Poland, the first Nuss procedure was performed in 1998 by the team led by Professor Janusz Bohosiewicz in Katowice [5]. Figure 1 shows Nuss bar implanted in the patient's wall chest

Surgeries performed at surgical centers specializing in PE treatment yield excellent outcomes. According to various sources, recurrences occur in 2–10% of patients undergoing surgery [6]. Some studies have shown that nearly 95% of patients are satisfied with both the aesthetic appearance and functional aspects. The incidence of complications (both severe and mild) ranges between 15 to 20%. Early postoperative complications (within the first month) include pneumothorax, pleural effusion, pneumonia, hemothorax, pericarditis, and surgical site infection, while late complications (occurring more than a month postoperatively) include: prolonged chest pain and – rarely – overcorrection of the deformity resulting in 'pigeon chest', displacement of the plate or rib fracture, and hypertrophic scarring.

The primary postoperative recommendation is that patients should refrain from engaging in strenuous physical exertion for approximately six weeks. Following this period, they may generally resume aerobic activities, such as football or basketball; however, until the corrective metal bar is removed, participation in high-contact sports, including American football or ice hockey, is contraindicated. Such high-contact activities can be safely resumed only after approximately two years, once the desired shape of the sternum has been achieved [7].

AIM

The study aims to describe a clinical case and its management in the Hospital Emergency Department of a seventeen-year-old boy with posttraumatic internal hemorrhage into the pleural cavity during the late postoperative period following Nuss surgery.

MATERIALS AND METHODS

This paper presents a clinical case of a 17-year-old patient who was initially admitted to the Emergency Department (E.D.) of the District Hospital named after John Paul II in Bartoszyce, with a history of surgical treatment of pectus excavatum, after blunt chest trauma and in result hemorrhagic shock.

CASE REPORT

On 5 May 2024, at 20:45, a seventeen-year-old boy presented to the E.D. with his mother, complaining of breathing difficulties. The boy reported that the problems had occurred while playing with friends approximately 30 minutes before the hospital visit. The patient's mother reported that he had never received long-term medical treatment, was not on any regular medications, and had no known allergies. She also stated that he had eaten dinner at 18:00. On initial assessment of vital signs according to System for Managing Patient Service Modes in the Hospital Emergency Department (TOPSOR) at 20:47, the following values were recorded. The patient was triaged as a yellow priority in Emergency Severity Index (ESI) (Table 1).

At 20:55, a reassessment of the vital signs was performed using the modified Early Warning Score (EWS) on the observation ward (Table 2).

During the initial assessment, pallor of the skin and a forced body position were observed. When asked

Table 1. Vital signs , hour 20:47

Hour	AVPU	BP syst. (mmHg)	BP diast. (mmHg)	Heart rate (/min)	Resp. rate (/min)	SpO2, k (%)	Oxygen l/min	Body. Temp. (°C)	NRS	Priority ESI
20:47	A	113	69	68	16	100	On room air	36,4	0/10	3

Table 2. Vital signs , hour 20:55

Hour	AVPU	BP syst. [mmHg]	BP diast. [mmHg]	Heart rate [/min]	Resp. rate [/min]	SpO^2 [%]	Oxygen [l/min]	Body Temp. [°C]	NRS	Glycemia [mg%]
20:55	A	110	73	65	16	99	On room air	36,4	0/10	137

Table 3. Blood count, hour 21:02

Hour	Leukocyttes [10^3/µl]	Neutrocytes [10^3/µl]	Lymphocytes [10^3/µl]	Erythrocytes [10^6/µl]	Hemoglobin [g/dl]	Hematocrit [%]	Platelets [10^3/µl]
21:02	9.60	5.38	3.54	4.35	13.5	39.2	268

Table 4. Vital signs , hour 21:58

Hour	AVPU	BP syst. [mmHg]	BP diast. [mmHg]	Heart rate [/min]	Resp. rate [/min]	SpO_2 k [%]	Oxygen [l/min]	Body. temp. [°C]	NRS
21:58	A	61	34	81	28	100	2	36,4	0/10

Table 5. Blood count, hour 22:00

Hour	Leukocytes [10^3/µl]	Neutrocytes [10^3/µl]	Lymphocytes [10^3/µl]	Erythrocytes [10^6/µl]	Hemoglobin [g/dl]	Hematocrit [%]	Platelets [10^3/µl]
22:00	15.27	12.30	1.82	3.46	10.8	31.4	228

Table 6. Vital signs , hour 22:20

Hour	AVPU	BP syst. [mmHg]	BP diast. [mmHg]	Heart rate [/min]	Resp. rate [/min]	SpO2 k [%]	Oxygen l/min	Body. Temp. [°C]	NRS
22:20	A	100	62	85	22	99	2	36,2	0/10

about previous surgeries or hospitalizations, the mother reported that approximately one year prior, her son underwent surgery to correct his funnel-shaped chest. When asked about the possible cause of the injury, the mother explained that during play, a friend standing behind him squeezed her son tightly in the chest area.

On physical examination:

A – Airways patent and unobstructed

B – Breathing: respiratory rate 16/min, unlabored, no accessory muscle use; SpO_2 99%/ FiO_2 0.21; auscultation reveals normal, symmetrical vesicular breath sounds

C – Steady heart rate, 72/min with normal tension, skin pale and

dry; capillary refill 3 seconds; jugular veins normally filled.

D – no abnormalities, GCS 15

E – abdomen soft, no pathological resistance, no signs of peritonitis, no

visible external injuries; normothermic

An intravenous line was secured, and blood samples were taken for additional investigations, including full blood count with smear, CRP, creatinine, urea, ALT, AST, serum amylase, and troponin. The patient was started on analgesic therapy and stress ulcer prophylaxis. An ECG showed a regular sinus rhythm at 80/min, with an intermediate cardiac axis and repolarisation abnormalities present. An eFAST ultrasound was performed, which revealed inconclusive findings in the right pleural cavity. A chest X-ray was planned. Initial full blood count results showed no abnormalities (Table 3).

At the X-ray department, the patient suddenly felt faint upon being positioned upright; his condition improved rapidly when placed in the supine position. Oxygen was administered via nasal cannula at a flow rate of 2 L/min. A decision was made to forgo the chest X-ray and to extend the diagnostics to include a contrast-enhanced chest CT scan, which revealed an excessive amount of free fluid in the right pleural cavity and metal artefacts from the chest stabilizer.

Given the above situation, the patient was moved to the resuscitation and treatment area, and a reassessment of the parameters according to the EWS scale was performed (Table 4).

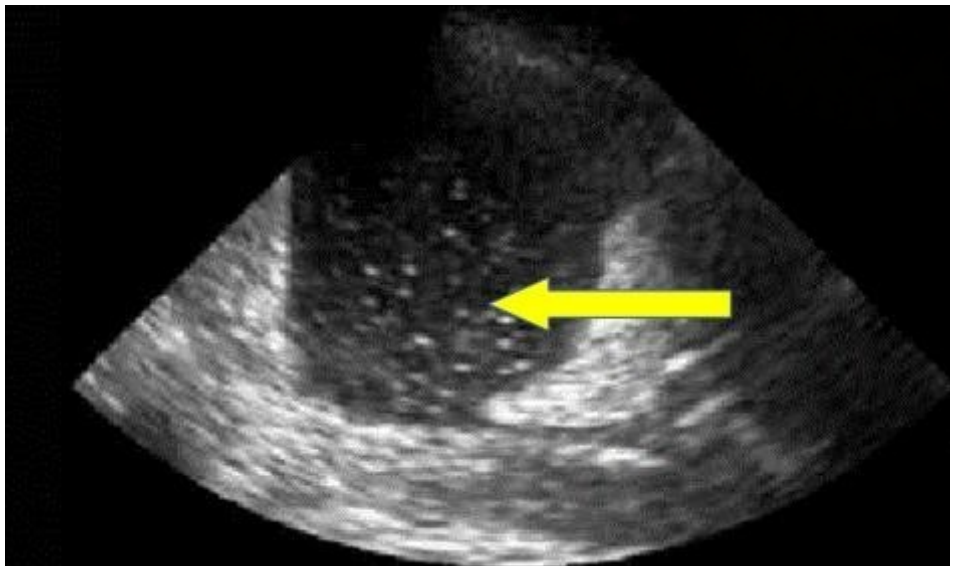

Fig. 2. Swirling sign in right pleural cavity (yellow arrow indicates visible movement of blood particles within a fluid collection)
Source: Own materials

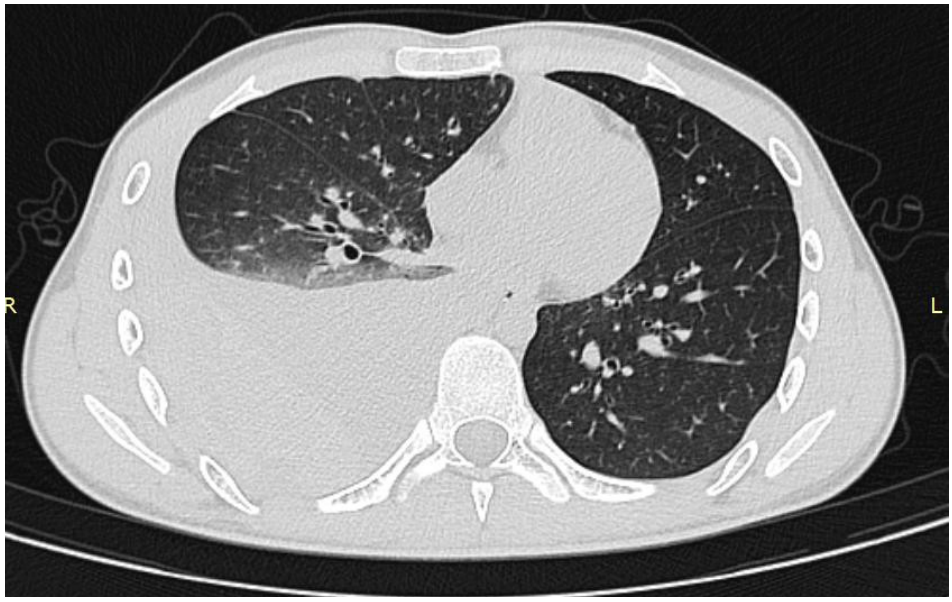

Fig. 3. CT of the chest with right hemothorax
Source: Own materials

Hypovolemic shock was suspected. Fluid resuscitation was initiated with 250 ml aliquots of warm balanced crystalloids (38°C), tranexamic acid 1 g i.v. over 10 minutes, followed by 1 g over 8 hours via continuous infusion, and warmed Ringer's lactate solution. Blood was taken for blood group determination and compatibility testing, and 2 units of PRBCs and 2 units of fresh frozen plasma were ordered. A repeat full blood count was also requested. Thermal comfort was ensured by maintaining body temperature above 36 °C. A consultation with the on-call general surgeon was also requested. Due to the presence of free fluid in the pleural cavity on chest CT and suspicion of active bleeding, a repeat bedside ultrasound was performed, revealing a swirl sign suggestive of the above diagnosis.

A diagnostic thoracentesis of the right pleural cavity was performed under ultrasound guidance, yielding bright red aspirate.

On follow-up blood count, features of anemia were noted (Table 5).

Due to the unavailability of O Rh (-) blood in the blood bank, 1 unit of group-compatible PRBCs and 1 unit of FFP were transfused, resulting in the following vital signs according to the modified EWS (Table 6)

During the transfusion of blood products, a telephone consultation was held with a physician from the Department of Pediatric Surgery and Urology at the Provincial Specialist Children's Hospital in Olsztyn. After presenting the clinical condition and the results of additional tests, it was decided to refrain from inserting a right pleural cavity drain and to transfer the patient as a matter of urgency. The estimated transport time was 60 minutes. While awaiting transport, a repeat full blood count was requested, and transfusion of another unit of PRBCs was started, which was continued during transport. At 23:26, the patient, in a moderately severe but stable general condition, was transported to the receiving hospital by interhospital transfer under the supervision of the on-call physician.

During transport, the chest CT report was received: a significant amount of heterogeneous fluid (blood) was present in the right pleural cavity, measuring up to 75 mm from the diaphragm towards the lung apex. Administration of contrast revealed active extravasation into the pleural space from the region of the anterior chest wall just above the diaphragm (possible injury to the intercostal artery? diaphragmatic artery? internal thoracic artery?). Accurate assessment was limited by metallic artefacts from the stabilizer. Posttraumatic diaphragmatic injury could not be excluded. There was a slight leftward mediastinal shift due to pressure from the hematoma. The CT report was communicated to the receiving physician at WSDD (Fig. 3).

Further information was obtained after an official request to the WSSD in Olsztyn with the written consent of the patient's mother.

The patient, in a critically stable condition, was admitted to the Pediatric Emergency Department in Olsztyn (PED). Based on the diagnostic evaluation performed at the Bartoszyce ED, he was urgently indicated for an exploratory thoracotomy. During this procedure, the right pleura was opened in the intercostal region, and a plate was removed, revealing a bleeding internal thoracic artery. After securing the patient, hemostasis was achieved, blood clots were removed from the right pleural cavity (approximately 800 mL), and a size 24 chest drain was inserted below the incision line. Bleeding on the left side was also excluded. During the procedure, the patient required transfusion of 4 units of packed red blood cells (PRBC) and 2 units of fresh frozen plasma (FFP). In addition, 1500 IU of Beriplex (prothrombin complex concentrate), 2 g of Riastap (human fibrinogen), and 4 mg of Novoseven (recombinant activated factor VII) were administered, and circulatory support was provided with a norepinephrine infusion. The patient was admitted to the Pediatric Intensive Care Unit (PICU) in a stable, severe condition with Richmond Agitation Sedation Scale (RASS) agitation/sedation score of 4. A postoperative chest X-ray showed signs of bilateral pneumothorax. Suction drainage to the left pleural cavity was performed. Intravenous antibiotic therapy was initiated and modified based on the clinical condition and laboratory test results. Due to anemia, thrombocytopenia and circulatory disorders, blood transfusions and blood products were administered repeatedly. Anticoagulant prophylaxis was introduced.

On the third day after admission, a repeat chest CT scan was performed, which revealed no evidence of contrast extravasation, bilateral pneumothorax, a small amount of fluid in the pleural cavities, and consolidation of the pulmonary parenchyma in the lower lobe of the right lung. During the hospitalization in the PICU, gradual clinical improvement was observed, allowing discontinuation of amine vasopressors and analgosedation. Following extubating, respiratory support was temporarily provided via high-flow nasal cannula (HFNC).

One week after admission, the patient, with stabilized respiration, was transferred to the Pediatric Surgical and Urological Ward, where the chest drains were removed. Conservative management was continued, not requiring surgical intervention. Following follow-up imaging and laboratory tests, which revealed no abnormalities, the patient was discharged home in good general condition, with recommendations for ongoing outpatient care.

DISCUSSION

COMPLICATIONS

Complications following the Nuss procedure occur with varying frequency, ranging from minimal to severe. One large retrospective study [8] reported that the most common complications include bar displacement, pneumothorax, wound infection, pleural effusion, and chronic pain. From other analyses:

- The overall complication rate ranges from 2 % and 27 % [9]
- In adult patients, a significantly higher risk of complications was observed, including bar displacement, bleeding, increased pain, and prolonged hospitalization [10]
- The 2004 study showed that in a group of patients (n=335), postoperative complications accounted for 16.1 %. Among early complications (within 30 days), the most frequent were pneumothorax (6.9 %), serous fluid accumulation (3.3 %), and bar displacement (2.4 %). Among late complications (beyond 30 days), pericardial effusion (1.5 %), bar displacement (1.2 %), and pleural hematoma (0.9 %) were observed [11].

BAR DISPLACEMENT AND REOPERATIONS

The percentage of bar displacement cases after the Nuss procedure is relatively low, and various data can be found in the literature. The outcome is largely determined by surgical technique, patient age, the use of stabilizers, and double bars [12].

- In a 2023 analysis, Muzammil Akhtar, Daniel Razick demonstrated that bar displacement occurred in 4.5% of patients in a group of 1135 people [6] .
- The authors also analyzed this group and reported that only 2.4% of the patients underwent reoperation.
- The literature suggests that early displacement of

the bar (within the first 3- 6 months postoperatively) may occur more frequently, primarily among patients who did not follow guidance to limit strenuous physical activity or avoid injury. At a later stage, the risk of displacement significantly decreases.

BAR FRACTURE

Bar fracture after Nuss surgery is a relatively rare but very serious mechanical complication requiring reoperation

- In a 2006 analysis, Andre Hebra, M.D., and Jeffrey P. Jacobs reported that, in a group of patients (n=30), fracture of the bar stabilizer accounted for 3% of all complications [13]
- The situation is so rare that in later publications, the complication is not listed as a separate item
- One of the most dangerous consequences of a bar fracture is injury to major vessels such as the intercostal arteries, the internal thoracic artery

SHOCK

Shock after Nuss surgery is a very infrequent but very serious complication. It is most often related to the perioperative or early postoperative period. Depending on the cause, the following types can be distinguished:

- Hemorrhagic – due to bleeding from cardiac or vascular injury
- Septic – due to postoperative infection
- Anaphylactic – due to an allergic reaction to a medical device – bar

Based on available studies and meta-analyses, it can be concluded that shock after the Nuss procedure occurs extremely rarely and is not a typical complication of this surgery.

- In the meta-analysis by Kanagaratnam A. (2016) covering (n=1432) patients, including 912 who underwent the Nuss procedure, shock was not listed as a major complication [14].
- In other meta-analysis by Hamza Rshaidat, Eliyahu Gorgov (2024) covering (n=2843) reported that, postoperative hemorrhagic complications occurred at very rarely [15]
  - 0.86% in pediatric patients
  - 3% in adult patients

In this same study, female patients had higher hemorrhagic complications (≈6%) than males (≈0.97%)

- In next analysis Turkan Dubus (2024) compared complications between pediatric (n=53) and adult (n=37) groups. She noticed only one hemothorax in each group in postoperative early complications and neither in postoperative late complications [16].

However, isolated cases of shock as a late complication of the Nuss procedure requiring surgical intervention have been reported.

- A teenager was admitted to the hospital two months after the Nuss procedure with symptoms of shock and cardiac tamponade due to damage to the ascending aorta caused by a displaced rod [17].
- Chieh-Wen Lin, Ke-Chi Chen (2011) found history of 13-year-old boy who developed late-onset bilateral hemothorax with hypovolemic shock 5 months after the Nuss procedure. They stressed that, this is the first case of the late-onset life-threatening bilateral hemothorax with hypovolemic shock ever reported [18].

MORTALITY

The Nuss procedure is widely considered a safe and effective surgical "gold standard" for correcting pectus excavatum. The overall incidence of minor and major complications that have been reported ranges from a low of 2% to a high of 27%. The Clavien-Dindo severity grading system classifies complications into 5 grades with grade 4 being life-threatening complications and grade 5 being death of the patient. Most of the complications associated with the Nuss repair are fortunately grade 1-3 and either cause minimal to no harm to the patient, or with grade 3 complications require some surgical or radiographic intervention. For grade 4 and 5 complications with the Nuss repair , the true incidence is not well-established and there is only estimated mortality risk <0.1% overall (very rare) [19].

CONCLUSIONS

Although low-energy injuries usually do not cause significant health damage, the overall clinical impression, the ABCDE assessment, and a detailed patient history are crucial. Information about previous surgical procedures can be invaluable in identifying the source of an acute health threat. With access to Evidence Base Medicine EBM), the analysis should consider all possible causes - from the most common, through the rarest, to those not described in the medical literature but potentially possible. In hospital care, especially at the E.D. level, continuous reassessment of the patient's general condition and the use of bedside methods—such as POCUS ultrasound—facilitate early recognition of shock, determination of etiology, monitoring of dynamics, and evaluation of response to treatment. Early and appropriate anti-shock management increases the likelihood of perioperative survival, which is essential for definitively controlling the source of bleeding, particularly in situations that exceed the routine capabilities of a given hospital and require long-distance transport.

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CONFLICT OF INTEREST

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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VARIA

CONTENTS

Current performance as an indicator of the foreign students' KROK-2 license examination results

Anna M. Antonenko, Andrii A. Borysenko, Ihor M. Pelo, Alina A. Slipets, Natalia V. Velyka, Serhii T. Omelchuk, Serhii V. Bilous, Mykola V. Kondratiuk
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ABSTRACT

Aim: The aim of the work is to comprehensively assess the impact of indicators of current success of foreign students on the results of passing the licensing exam KROK-2 and its component - the subtest "Hygiene, Public Health".

Materials and Methods: A single-center retrospective quantitative study was conducted, during which a dataset of foreign students (n=70) with depersonalized records of current performance for the 3rd and 6th years, ECTS scales, traditional grades, the final result of KROK-2 and the subtest "Hygiene, Public Health" (2025) was analyzed. Data processing and modeling were carried out in the Python.

Results: Current success in the 6th year is statistically related to the result of KROK-2, but explains a limited proportion of the variation in the result ($R^2 \approx 0.13$-0.21). Indicators of the 3rd year provide moderate incremental value; the most informative is PC_3% (independent association in the extended model). ECTS (6th year) acts as a suitable risk stratifier: categories D/E are associated with a decrease in the expected result from STEP-2 by approximately 11 percentage points compared to the reference C. Traditional assessments have a clear linear gradient: in the 6th year ≈+8.9 p.p./point, in the 3rd year – ≈+7.3 p.p./point.

Conclusions: The Hygiene, Public health subtest is poorly predicted by overall grades in the 6th year of study, highlighting the need for subject-specific interventions. Multicollinearity between components of the 6th year current control is high; the use of robust and regularized approaches (HC3, PCA/PC1, Ridge, residualization) confirmed the robustness of key findings under alternative specifications.

KEY WORDS: KROK-2, targeted training, hygienic disease prevention

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INTRODUCTION

In the context of European integration and globalization of medical education, Ukraine occupies a prominent place in the educational market and consistently attracts foreign students to study in domestic medical higher education institutions (HEIs) [9]. At the same time, the full-scale war and the consequences of the pandemic have posed unprecedented challenges to the continuity and quality of training, the organization of the practical component, and the adaptation of international applicants to the changed educational environment [2,5,6,8,11-13,16]. Under these conditions, standardized quality indicators, primarily the licensed integrated exams KROK, which are mandatory elements of state certification and tools for external control of educational achievements, acquire special importance [14,15].

International and local studies emphasize the role of formative assessment, systematic educational work, and feedback as factors that increase students' readiness for final exams [1,3,4]. Current academic performance (weekly/module assessments, semester summaries), its aggregated representations (traditional grades, ECTS scale), and disciplinary results can serve as early risk markers and practical guidelines for mentoring interventions even before the KROK-2 is compiled. For international students who simultaneously overcome language, cultural, and organizational barriers, these markers acquire additional weight as indicators of successful academic adaptation [6,10].

The disciplines "Hygiene and Ecology" and "Hygienic Prevention of Diseases" play a key role in the formation of preventive thinking of a future doctor, which is especially relevant given the significant proportion of diseases associated with behavioral and environmental factors [7]. Along with the transition to mixed formats and distance learning technologies, there is a need to empirically verify the extent to which current learning indicators (for the 3rd and 6th years), ECTS scales and traditional assessments are associated with the results

of the "Hygiene, Public Health" subtest and the result of KROK-2 in real wartime conditions [8,11–13,16]. That is why the analysis of departmental data on foreign students, combining descriptive statistics, correlations and regression modeling, is timely and practically significant for improving intra-course monitoring, targeted support and planning of preparation for the licensing exam [14,15].

AIM

The purpose of the work is to comprehensively assess the impact of current performance indicators of foreign students on the results of the KROK-2 licensing exam and its component - the "Hygiene, Public Health" subtest, and on this basis to offer practical guidelines for early risk identification and targeted training.

MATERIALS AND METHODS

A single-center retrospective quantitative study was conducted, during which a dataset of foreign students (n=70, only those students who studied continuously and were in the 3rd year in 2021/2022 academic year and in the 6th year in 2024/2025 academic year) with depersonalized records of current performance for the 3rd and 6th years, ECTS scales, traditional grades, the final result of KROK-2 and the subtest "Hygiene, Public Health" (2025) was analyzed. The results of KROK-2 (KROK_2) and the subtest "Hygiene, Public Health " (H_PH) are presented in percentages; current performance assessments (0-80) (CPA_3 and CPA_6), final control (0-120) (FC_3 and FC_6), overall result for disciplines (0-200) (D_3 and D_6) were in points; the ECTS scale was reflected by categorical variables in the form of letter designations "A", "B", "C", "D", "E", "F" and "FX"; interpretation of traditional assessment (T_O_3 and T_O_6) - in a five-point scale.

To unify the scales, the point scores were converted into percentages: scores for current study / 80×100 (CPA_3_% and CPA _6_%); scores for final control / 120×100 (FC_3_% and FC_6_%); score for discipline / 200×100 (D_3_% and D_6_%). ECTS were coded as dummy variables (reference – "C") and additionally as an ordinal variable (A=5 … E=1) for ranked tests. Traditional scores were considered as a numerical variable for a linear trend.

To control the quality of manually entered data, identify omissions, and check the robustness of conclusions to possible errors, we conducted a sensitivity analysis of the consistency of the integral assessment of the discipline. First, the consistency of the assessments in each course $\Delta = D - (CPA + FC)$ was checked, and then the results of the models were compared in three specifications: (1) Original – using the original value of D, (2) Recomputed – replacing D with the arithmetic sum of CPA + FC, (3) Drop($|\Delta|>1$) – excluding records where the absolute deviation exceeded 1 point (the threshold value was chosen taking into account the typical "graininess" of the assessment and possible rounding). Comparison of the β coefficients, their HC3-robust errors, R^2, and p-values between these three approaches allowed us to assess whether the key associations (in particular D_% → KROK_2) persist regardless of the source of potential data inconsistency. The stability of estimates across all scenarios is interpreted as the robustness of the results to inconsistencies in the construction of the variable D.

The primary characterization of continuous variables was carried out using descriptive statistics methods with the presentation of the mean, standard deviation and 95% confidence intervals calculated by the t-method. Linear relationships between indicators were assessed using Pearson correlation, and rank or potentially insensitive to deviations from normality - Spearman correlations. The associations of the STEP_2 result with the current indicators of the 6th and 3rd years, as well as with the results of the H_PH, were analyzed separately, which allowed comparing the general and subject-specific success profiles.

To quantify the contribution of predictors to the variation in KROK_2, ordinary least squares (OLS) models were used using HC3-robust standard errors, which ensures the correctness of inference under conditions of potential heteroscedasticity and deviations from normality of residuals. The report provided unbiased estimates of coefficients (β), p-values, as well as coefficients of determination R^2 and adjusted R^2, which characterize the explanatory power of the models taking into account their complexity.

1) Basic model (A):
2) Aggregated model (B):
3) Advanced (Basic model + CPA_3 та FC_3):

The added value of the 3rd year was assessed by an F-test comparing nested models (Extended vs Basic) on the same subsample.

4) Basic model "3rd year only":

To assess the role of ECTS (3rd and 6th years), we combined three approaches that reflect different aspects of the relationship. First, the ordinal (monotonic) relationship was determined by the Spearman coefficient, having previously coded the categories as A=5 … E=1. Second, to test for between-group differences without assuming normality, the Kruskal-Wallis test was used. Third, for quantitative interpretation, OLS models were created with dam-predictors for levels A, B, D, E (reference – C), where the coefficient estimates are in-

terpreted as absolute differences in the mean KROK-2 (in percentage points) relative to category C. All linear models were reported with HC3-robust standard errors, which increases the correctness of the inference in the presence of potential heteroscedasticity; additionally, 95% CI (confidence interval) and two-sided p-values (α=0.05) were given. Particular emphasis is placed on practically significant thresholds – primarily categories D/E as indicators of reduced expected results.

The impact of traditional grades (5-point scale; 3rd and 6th years) was analyzed in two complementary planes. The main quantitative assessment was provided using an OLS model with a linear trend, where the slope (β) is interpreted as a change in KROK_2 in percentage points for each +1 point of the traditional grade; robustness was provided by HC3-SE, 95% CI and p were reported. Spearman correlation (rank relationship) and Kruskal-Wallis test (comparison of distributions between discrete levels of 2–5 points) were used as a non-functional check of the linearity assumption and to confirm monotonicity. This combination allows to identify both a general trend (linear effect) and nonlinear deviations or asymmetries between individual assessment categories.

The correctness of the inference in the regression models was checked using a standard battery of diagnostics. The normality of the distribution of the residuals of each OLS model was assessed using the Shapiro–Wilk test with accompanying visualization on QQ plots; for the basic variables, the Shapiro–Wilk and D'Agostino–Pearson tests were additionally used. Homoscedasticity was checked using the Broisch–Pagan test; in case of deviations or doubts about the fulfillment of the assumptions, further inference was performed based on HC3-robust standard errors (with presentation of β-coefficients, 95% CI and p-values).

Multicollinearity between predictors was assessed comprehensively: by VIF (interpreting values >10 as high), pairwise correlations and condition number of the design matrix. Given the significant correlation between the components of the current success of the 6th year (CPA_6%, FC_6%), a number of robust checks were performed: (I) construction of the PCA composite PC1 as a "general index of current success" to avoid instability of estimates in multidimensional space; (II) use of RidgeCV on standardized predictors as a sensitivity analysis to strong collinearity (with selection of the socialization parameter by cross-validation). Additionally, to separate the unique contribution of indicators, residualization was used (the effect of FC_6% on CPA_6%) and the results were interpreted together with partial R^2 indicators and standardized β-scores. The combination of these procedures ensured the consistency of conclusions under different model specifications and increased their methodological robustness.

All statistical tests were performed two-sided at a significance level of α = 0.05. Given the identified non-normality of the distribution of residuals and potential heterogeneity of variances in key models, inference was performed based on HC3-robust standard errors, which ensures the correctness of the estimation of p-values and confidence intervals for deviations from classical OLS assumptions.

Data processing and modeling were performed in Python (Google Colab / locally) using pandas, numpy, scipy, statsmodels (OLS, HC3, diagnostic tests), scikit-learn (PCA, RidgeCV) and matplotlib for visualization. For reproducibility, all summary tables (descriptive statistics, correlation matrices, coefficient estimates, VIF, sensitivity analysis and diagnostic results).

RESULTS

The analysis included 70 6th-year foreign students. The generalized characteristics (%, 95% CI) showed the following average values: CPA_6% – 72.39 (70.61-74.17), FC_6% – 72.45 (70.77-74.13), D_6% – 72.41 (70.68-74.13), H_PH – 78.01 (75.00-81.02), KROK_2 – 80.28 (77.10-83.46). The obtained confidence intervals indicate moderate variability of indicators and relative homogeneity of the sample in terms of success results. Checking the internal consistency of the variable D with its components (CPA+FC) revealed only single deviations (maximum |Δ|=3-4 points), which were not systemic. To control for the possible impact of these biases on the association estimates, the results were further tested within a sensitivity analysis with alternative specifications.

Complex correlation analysis showed that the closest relationship with the result of KROK_2 has the subject-specific indicator H_PH (Pearson r = 0.809; Spearman r = 0.789), which is consistent with the concept of convergent validity between the integrated result and the profile subtest. Among the indicators of success of the 6th year, moderate linear associations with KROK_2 were found: for CPA_6% r = 0.367, D_6% r = 0.358 and FC_6% r = 0.334. For the 3rd year, a moderate relationship was established between D_3% and KROK_2 (r = 0.334), which indicates the informativeness of early indicators for indicative screening. At the same time, the internal correlations between the components of the current success of the 6th year were extremely high (r(CPA_6%, FC_6%)≈0.98), which indicates significant multicollinearity and necessitates careful interpretation of multifactor models and the use of robust/collinearity-resistant approaches in further analysis.

In the basic model (Model A) KROK_2 = 38.08 + 1.54·CPA_6% - 0.95·FC_6%, R^2=0.147 (adjusted 0.122) was obtained; according to HC3 errors, the model is generally significant (p=0.041), the contribution of CPA_6% has the character of a threshold trend (β=1.54; p=0.054), while FC_6% does not reach significance (p=0.211). Diagnostics indicate pronounced multicollinearity between predictors (VIF≈21 for both); the residuals deviate from normality (Shapiro–Wilk p<0.001), and the test for homoscedasticity is borderline (Broisch-Pagan test p≈0.08). Partial contributions confirm the dominance of CPA_6% (partial R^2=0.035) over FC_6% (0.012).

The aggregated model (Model B) KROK_2 = 32.49 + 0.66·D_6 % gives R^2=0.128 (adjusted 0.115), with a statistically significant coefficient for D_6% for HC3 (p=0.024), which supports the presence of a linear association of the integral success indicator with the exam result.

The extended model (6th+3rd years) KROK_2 = 14.84 + 1.46·CPA_6% - 0.91·FC_6% + 0.11·CPA_3% + 0.32F_3% improves the explanatory power to R^2=0.213 (adjusted 0.164); while FC_3% retains an independent and statistically significant association (β=0.32; p=0.001 by HC3), while the effects of the 6th year indicators decrease (for CPA_6% p=0.053; for FC_6% p=0.202). Comparison of nested models (Extended vs Basic) on the same subsample shows a trend towards incremental predictive value of the 3rd year indicators (F=2.709; p=0.074).

The "3rd year only" model KROK_2 = 40.57 + 0.29·CPA_3 % + 0.35·FC_3% demonstrates R^2=0.116 (adjusted 0.089); for HC3, FC_3% remains statistically significant (p<0.001), while CPA_3% is on the verge of significance (p≈0.057). Taken together, the results indicate that 6th year indicators are related to KROK_2, however, adding information for 3rd year (primarily FC_3%) provides a moderate increase in explainability against the background of high collinearity between the components of current 6th year success.

Analysis of ECTS scales showed a monotonic relationship with the KROK-2 score in the 6th year (Spearman r=0.270; p=0.024). In the linear model with dami-coding (reference – C), categories D and E were associated with significantly lower KROK-2 scores (β≈-10.87; p=0.009 and β≈-11.99; p=0.020, respectively), with a cumulative explanatory power of R^2≈0.157; however, A/B did not differ from C at a statistically significant level. For the 3rd year, the ordinal relationship was weaker (Spearman r=0.245; p=0.041) and corresponded to the low explanatory power in OLS (R^2≈0.071). Therefore, ECTS 6th year provides a practically useful risk stratification, where categories D/E can be considered as threshold indicators of reduced expected KROK-2 outcomes.

Traditional assessments demonstrated a clear linear trend: in the 6th year, each additional point corresponded to an increase of ≈+8.91 pp (percentage points) in the KROK-2 (p=0.0013; R^2=0.142), while in the 3rd year – ≈+7.32 pp/point (p=0.039; R^2=0.061). Nonparametric tests (Spearman, Kruskal-Wallis) confirmed the monotonicity and consistency of these findings. Taken together, this suggests that aggregated learning indicators (ECTS, 5-point scale) are convenient for interpretation and can serve as operational thresholds for early identification of students at risk before taking the STEP-2.

In models with HC3-robust errors, the basic specification H_PH ≈ CPA_6% + FC_6% demonstrated low predictive power (adjusted R^2≈0.03), and the predictor coefficients did not reach statistical significance. Adding ECTS_6 did not provide a noticeable increase in model quality, while including T_O_6 gave only a threshold effect (adjusted R^2≈0.06; p≈0.05 for traditional assessment). The full model with simultaneous consideration of ECTS_6 and T_O_6 retained a low level of predictive power (adjusted R^2≈0.058), which is consistent with the conclusion about the limited predictive power of generalized course metrics for this subtest. The results obtained indicate that H_PH is more sensitive to subject-specific training (targeted training in the hygienic direction, practicing test strategies, language component) and requires targeted educational interventions, and not just increasing general indicators of current success.

In order to check the robustness of the conclusions to possible errors in calculating the integral assessment of the discipline, three scenarios were compared: Original (initial D), Recomputed (replacing D with the arithmetic sum of CPA+FC) and Drop(|Δ|>1) (exclusion of records with absolute deviation |Δ|=|D-(CPA+FC)|>1). For the 6th year, the correlations with KROK _2 remained close: r≈0.358 (Original), 0.350 (Recomputed) and 0.278 (Drop|Δ|>1); for the 3rd year, r≈0.334/0.335/0.339, respectively. Thus, the qualitative conclusions did not change regardless of the method of constructing the variable, which indicates the robustness of the obtained associations; the moderate decrease in r in the 6th year dropout scenario is mainly due to a decrease in the sample size (n) rather than a systemic change in the relationship. In practice, this means that small differences in accounting for D do not distort the interpretation of the dependence on KROK_2.

Extremely high multicollinearity was recorded between the predictors of the current control of the 6th year: the pairwise correlation of CPA_6%-FC_6 % is r=0.976, and VIF ≈ 21 for both variables, which theoretically causes inflation of standard errors and instability of estimates in multivariate OLS models. The use of

PCA with the construction of the PC1 composite (as a "generalized index of current success") gave $R^2 \approx 0.125$; $p=0.023$ in the model KROK_2 ≈ FC1, confirming the presence of an integral relationship between total academic success and exam result. Additionally, RidgeCV on standardized predictors (optimal regularization parameter $\alpha \approx 178$) stabilized the weights and retained positive coefficients for both components CPA_6, FC_6), which is consistent with the expected common direction of effects. Residualization analysis (projection of FC_6% onto CPA_6%) showed that the unique contribution of CPA_6% remains statistically significant ($p=0.018$), while the unique effect of FC_6% does not reach significance, a result that conceptually reproduces the findings of multivariate modeling with HC3 errors. Taken together, these approaches confirm that under conditions of strong collinearity, the interpretation of individual β-coefficients requires caution, and instead the use of aggregated indicators (such as D_6 % or PC1) and regularized/robust methods are justified for stable assessment of predictive relationships.

DISCUSSION

The results show that the indicators of the current control of the 6th year are statistically related to the result of the KROK-2, but explain a limited proportion of the variation of the result ($R^2 \approx 0.13–0.21$). The addition of indicators of the 3rd year, primarily FC_3%, provides a threshold/moderate incremental predictive value, which makes them relevant for early screening. The ECTS_6 scale provides a practically useful stratification: categories D/E are associated with a decrease in the expected KROK-2 by approximately 11 points, while traditional assessments give an interpreted linear gradient (≈+9 points for each point in the 6th year). At the same time, H_PH is poorly predicted by generalized assessments, which emphasizes the need for subject-oriented educational interventions. The high collinearity found between the components of current 6th year performance requires caution when interpreting individual coefficients; however, the use of robust approaches (HC3, PCA, Ridge, residualization) confirmed the stability of key findings under different specifications.

Our results confirm the hypothesis of the connection between current success and the result of the licensing exam, but at the same time indicate its limited scale. The explanatory power of the models based on the 6th year grades was low ($R^2 \approx 0.13–0.21$), which is consistent with the idea of the multifactorial nature of the KROK-2 results: in addition to academic scores, it is influenced by language competence, test strategies, interdisciplinary integration of knowledge and contextual factors of learning. High convergent validity with H_PH ($r \approx 0.81$) indicates that subject-specific indicators can better reflect readiness for individual components of the exam than aggregated indicators of success indicators.

An important practical conclusion is the role of ECTS and traditional grades as convenient "threshold" indicators. D/E categories in the 6th year were associated with a decrease in expected KROK-2 by approximately 11 points, and each additional point on the 5-point scale corresponded to an increase of ≈9 points. This creates operational guidelines for early identification of at-risk groups and targeting of mentoring interventions, which is consistent with the literature on the effectiveness of formative assessment and timely feedback. [1, 4].

Adding 3rd year data provided a threshold/moderate incremental benefit (primarily FC_3%), i.e. early academic performance already signals future performance, although it does not dominate 6th year metrics. This supports the use of screening in junior years for preventive support: systemic consultations, test strategy training, individual preparation plans.

At the same time, the models for H_PH showed low explainability even after adding ECTS and traditional grades. This reinforces the thesis that subject-oriented measures are needed to improve the subtest results: work with typical errors, training in hygiene disciplines, language support and regular short knowledge sections with detailed feedback.

A significant methodological challenge was multicollinearity between components of the current 6th year control ($r \approx 0.98$; VIF≈21). The use of HC3-robust errors, PCA-composite (PC1), regularization (RidgeCV) and residualization confirmed the robustness of the key findings, but reminded us to be cautious in interpreting individual β-coefficients. In such situations, it is advisable to focus on aggregated indicators (e.g., D or PC1) and on the consistency of results across multiple specifications, rather than on the target importance of individual predictors.

Sensitivity analysis of the consistency of D = CPA + FC showed the stability of the findings under different scenarios (Original / Recomputed / Drop$|\Delta|>1$), and the decrease in correlation after dropout is explained by the reduction of the sample. This reduces the risk of systematic distortion due to small discrepancies in scoring.

Limitations of the study include a single-center retrospective design and a relatively small sample size (n=70), which limits generalizability. Diagnostics revealed deviations from normality of residuals and marginal homoscedasticity; we minimized their impact by HC3 estimation, but causal inferences remain limited.

Also, extraneous variables (English language proficiency, participation in additional preparatory courses, attendance/absence in classes, self-study time) that potentially explain some of the unaccounted variation were not taken into account.

Practical implications: (1) implement regular subject-specific training to improve H_PH results; (2) use ECTS D/E thresholds and the gradient of traditional grades to prioritize support; (3) monitor early indicators (especially FC_3%) as a trigger for mentoring activities; (4) favor aggregated or regularized models in reporting and internal monitoring.

Directions for further research: multicenter validation on larger cohorts; expansion of predictors (language level, attendance, time on the educational platform, practical OSCE metrics); analysis of tests to identify their complexity; comparison of alternative models (ordinal regression for ECTS, gradient boosting with cross-validation) with an emphasis on reproducibility and practical interpretability.

Overall, the results support the role of ongoing monitoring as a useful, but not exhaustive, indicator of success in KROK-2 and highlight the need to combine general academic metrics with targeted subject-specific interventions and a broader set of predictors of learning behavior. This opens the way for more personalized training trajectories and for systematic optimization of the educational process in the department.

CONCLUSIONS

Current performance in the 6th year is statistically related to the KROK-2 score, but explains a limited proportion of the variation in the result ($R^2 \approx 0.13$-0.21).

The 3rd year indicators provide moderate incremental value; the most informative is the FC_3% (independent association in the extended model).

ECTS (6th year) acts as a suitable risk stratifier: categories D/E are associated with a decrease in the expected result from KROK-2 by approximately 11 percentage points compared to the reference C.

Traditional grades have a clear linear gradient: in the 6th year ≈+8.9 p.p./point, in the 3rd year – ≈+7.3 p.p./point.

The subtest "Hygiene, Public health" is weakly predicted by the overall grades in the 6th year of study, which emphasizes the need for subject-oriented interventions.

Multicollinearity between components of the 6th year current control is high; the use of robust and regularized approaches (HC3, PCA/PC1, Ridge, residualization) confirmed the robustness of key findings under alternative specifications.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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