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Clinical audit of newborn infants admitted in neonatal intensive care unit of a teaching hospital: A retrospective study

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Abstract. Understanding the causes of neonatal intensive care unit admission and their association with the neonatal outcome may help organising better patient care services. The objective was to investigate the causes and outcome of the newborn infants admitted in the neonatal intensive care unit. This was a retrospective study that analysed demographic and clinical data on newborns infants and their mothers obtained from medical records of all the neonatal intensive care unit admissions from 2018-2020. As a result, out of 400 neonatal admissions, majority (61.7%) of the newborn infants were aged <72 hours with male preponderance (63%) and presented with respiratory distress (59%) and jaundice (75%). Almost all outborn infants were found hypothermic on admission. Proportion of the patients presenting at the age of 4 days or more was significantly higher in the infants delivered inborn. Majority of mothers were aged 18-35 years (91.5%), multigravida (55.5%), and had >4 antenatal care visits (57.5%). Overall, a total of 252 (63%) infants recovered, 44 (11%) died and 104 (26%) left against the medical advice. The recovery rate was significantly higher (81.8%) in the inborn infants. Respiratory distress and jaundice in the newborn infants were the common clinical conditions on admission. High rate of left against the medical advice was a cause of concern and needed further studies to find out the actual reasons. The study highlighted the need for early risk identification, improved neonatal transport, targeted maternal care, strategies to reduce cases of leaving against medical advice, and enhanced in-hospital delivery services to improve neonatal outcomes and optimise neonatal intensive care unit

Keywords: neonatal mortality rate; respiratory distress syndrome; neonatal jaundice; maternal risk factors; neonates and mothers

Introduction

This retrospective clinical audit of neonates admitted to the Neonatal Intensive Care Unit (NICU) provides critical insights into the demographic, clinical, and perinatal factors influencing neonatal health outcomes in a teaching

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hospital setting. By analysing maternal and neonatal parameters, including delivery details, pregnancy complications, and neonatal interventions, the study highlights differences between inborn and outborn infants, shedding light on potential gaps in care for referred neonates. This research is particularly relevant in resource-limited settings, where understanding these factors is essential for reducing neonatal morbidity and mortality rates.

Neonatal admissions to the NICU form a crucial part of perinatal healthcare systems, reflecting the burden of neonatal morbidity and mortality. Neonatal mortality, defined as the death of a newborn within the first 28 days of life, is a critical indicator of community health. It provides insight into the accessibility and quality of maternal and neonatal healthcare services, and socio-economic disparities in different regions. Alarmingly, approximately 75% of neonatal deaths occur within the first week of life, with nearly 25% happening in the first 24 hours after birth. V.G. Chavan *et al.* [1] noted that these figures underscore the importance of timely and effective perinatal care to reduce neonatal mortality.

Globally, neonatal mortality remains a pressing public health concern, particularly in low- and middle-income countries. In 2023, an estimated 2.3 million newborns died within the first 28 days of life, translating to approximately 6,300 deaths per day and accounting for about 47% of all under-five deaths [2]. Although the global neonatal mortality rate declined from 37 deaths per 1,000 live births in 1990 to 17 per 1,000 in 2023, this reduction has lagged behind progress made in lowering post-neonatal under-five mortality rates. Since 2015, the pace of improvement has notably slowed, threatening the achievement of Sustainable Development Goal target 3.2, which aims to reduce neonatal mortality to at least 12 per 1,000 live births by 2030 [3]. Alarmingly, current trends indicate that 65 countries are not on track to meet this target without accelerated and targeted interventions.

In India, neonatal mortality has significantly declined between 2010 and 2021, reflecting the impact of sustained public health interventions. According to the Sample Registration System Statistical Report 2021, the national Neonatal Mortality Rate (NMR) dropped to 19 per 1,000 live births, down from 44 in 2010 – a 57% reduction. Rural areas saw a decline from 48 to 21, while urban areas improved from 29 to 12 per 1,000 live births. Additionally, six states and union territories, including Kerala, Delhi, Tamil Nadu, and Maharashtra, have already achieved the Sustainable Development Goal target of reducing NMR to 12 or below. These improvements are largely attributed to initiatives like Janani Suraksha Yojana (JSY), Janani Shishu Suraksha Karyakram (JSSK), and the National Health Mission (NHM), which have enhanced maternal and neonatal care services [4].

However, disparities in neonatal mortality persist across regions and states in India. P.S. Salve *et al.* [5] noted significant variations in NMR among Indian states. For instance, Jammu & Kashmir reported the highest NMR at 2,182 deaths per 100,000 live births, followed by Madhya Pradesh (1,743), Haryana (1,592), Rajasthan (1,564), Assam (1,507), Odisha (1,458), and Meghalaya (1,438). In contrast, Maharashtra had a significantly lower NMR of 711 deaths per 100,000 live births. P.S. Salve *et al.* emphasised that these disparities reflect differences in healthcare access, infrastructure, and quality between urban and rural areas, necessitating region-specific interventions.

A significant portion of neonatal deaths is preventable. M.J. Gondwe *et al.* [6] highlighted that preterm birth complications, infections, and intrapartum-related events like birth asphyxia are among the most common causes of neonatal mortality. They noted that addressing these causes requires identifying modifiable factors – those that, if addressed differently, could have prevented adverse outcomes. M.J. Gondwe *et al.* further emphasised the importance of strengthening antenatal care, ensuring skilled birth attendance, and implementing effective infection control measures to improve neonatal outcomes.

Clinical audits have emerged as a vital tool in identifying modifiable factors and improving neonatal care. R. Rashid *et al.* [7] highlighted the importance of analysing adverse events in NICUs to identify gaps in care and implement evidence-based interventions. Clinical audits not only promote accountability but also support continuous improvement in healthcare delivery. R. Rashid *et al.* noted that these audits are particularly valuable in resource-limited settings, where neonatal care often faces challenges such as inadequate infrastructure and workforce shortages. It is worth to mention studies by W. Khasawneh *et al.* [8], P.K. Panda & P.K. Panda [9] and A. Deka *et al.* [10]. W. Khasawneh *et al.* reported most of the inborn cases (92.2%), and only 7.8% were outborn. P.K. Panda & P.K. Panda had 58% of their cases as outborn. The burden of outborn babies in the study by A. Deka *et al.* was 39.5%. These findings underscore the critical role of clinical audits in enhancing neonatal outcomes, particularly by addressing systemic weaknesses and guiding targeted interventions based on the specific context and patient demographics of each healthcare setting.

Addressing neonatal mortality requires a multifaceted approach, including strengthening healthcare infrastructure, training healthcare workers, and ensuring equitable access to essential services. Hence, the purpose of the study was to investigate the causes and outcome of the neonates admitted in the NICU and suggest measures to improve the quality of new-born care with the help of a retrospective study at a tertiary care teaching hospital in North India.

Materials and Methods

The present retrospective study was conducted on 400 neonates admitted in the NICU, Department of Paediatrics, Era University, Lucknow. Demographic and clinical data of the newborn infants and their mothers were extracted from the medical records of all NICU admissions from 2018-2020. The Institutional Ethics Committee gave waiver from obtaining the informed consent [11]. Clinical information was collected from the written records (maternal age, gravida, type of delivery, presence of meconium,

induced or spontaneous labour, and pregnancy complications). NICU records provided additional information about newborn infants (APGAR score, gender, age at admission, birth weight, resuscitation required, intervention given). Complete clinical and delivery details of the mothers and resuscitation and treatment history of the newborn infants was not available in the out-born infants delivered in other health facilities and referred to NICU. The inborn and outborn infants were identified. The data collected was subjected to analysis using SPSS 21.0 software suite. Chi-square, Independent samples t-tests and paired t-tests were used to compare the data. The p-value of less than 0.05 indicated a statistically significant difference.

Results and Discussion

Out of 400 admissions the majority of neonates were aged <72 hours (61.7%), were male (63%), and presented with respiratory distress (59%) and jaundice (75%). Proportion of those presenting at age 4 days or more was significantly higher (51%) in the inborn infants compared to outborn babies (34%) ($p = 0.017$). Among different other presenting signs and symptoms, inborns had significantly higher presentation with meconium aspiration (21.5%) and vomiting (4.5%) as compared to outborns (11.2 and 0.6% respectively) ($p = 0.012$ and $p = 0.008$). Majority of mothers were aged 18-35 years (91.5%), multigravida (55.5%), and had >4 antenatal care (ANC) visits (57.5%). The proportion of primigravida mothers was significantly higher in the outborn (63%) compared to the inborn infants (56.8%) ($p < 0.001$). Most common maternal blood group was B Rh+ (34.7%). A total of 50 (12.5%) women were Rh negative and 10% required Anti-D screening. There was a significant difference between inborn and outborn groups with respect to the maternal medical history of diabetes which was significantly higher in the outborns (8.9%) compared to that in inborn (2.2%) (Table 1).

Table 1. Maternal characteristics

Characteristics	Inborn n = 88 (%)	Outborns n = 312 (%)	Total n = 400 (%)	Statistical significance
Age				
<18 years	0	6 (2.5)	6 (1.5)	$X^2 = 1.046$; $p = 0.593$
18-35 years	84 (48.8)	282 (90.3)	366 (91.5)	
>35 years	4 (4.5)	24 (7.6)	28 (7)	
Gravida				
Primi	50 (56.8)	197 (63.1)	222 (55.5)	$X^2 = 28.22$; $p < 0.001$
Multi	38 (43.2)	115 (36.9)	178 (44.5)	
ANC visits				
≤3	30 (34)	140 (35)	170 (42.5)	$X^2 = 3.265$; $p = 0.071$
≥4	58 (65)	172 (43)	230 (57.5)	
Maternal blood group				
A	18 (20.4)	71 (22.7)	89 (22.2)	$X^2 = 1.748$; $p = 0.0626$
B	35 (39.7)	104 (33.3)	139 (34.7)	
AB	10 (11)	48 (15.3)	58 (14.5)	
O	25 (28.4)	89 (28.5)	114 (28.5)	
Maternal Rh status				
Positive	78 (88)	272 (87.1)	350 (87.5)	$X^2 = 0.133$; $p = 0.715$
Negative	10 (11.3)	40 (12.8)	50 (12.5)	
Anti – D				
Required	10 (11.3)	30 (9.6)	40 (10)	$X^2 = 3.047$; $p = 0.218$
Not required	78 (88)	272 (87.1)	350 (87.5)	
Not known	0	10 (3.2)	10 (2.5)	
Maternal medical history				
Hypertension	4 (4.5)	7 (2.2)	11 (2.7)	$X^2 = 1.360$; $p = 0.244$
Diabetes mellitus	2 (2.2)	28 (8.9)	30 (7.5)	$X^2 = 4.444$; $p = 0.035$
Bronchial asthma	0	1 (0.3)	1 (0.2)	$X^2 = 0.283$; $p = 0.595$
Tuberculosis	0	1 (0.3)	1 (0.2)	$X^2 = 0.283$; $p = 0.595$
Hypothyroidism	1 (1.2)	4 (1.2)	5 (1.2)	$X^2 = 0.018$; $p = 0.9135$
UTI	1(1.2)	4 (1.2)	5 (1.2)	$X^2 = 0.018$; $p = 0.9135$
Fever	3 (3.4)	6 (1.9)	9 (2.2)	$X^2 = 0.6892$; $p = 0.4064$

Source: compiled by the authors

Majority of cases were outborn (78%), did not require induction (83.8%), had vertex presentation (90%), had spontaneous delivery (52%), clear or unknown meconium status (82%). A significant difference between the two groups was observed for liquour characteristics and infection only. Stained/unknown meconium colour and infection rates were significantly higher in outborn compared to inborn ($p < 0.05$) (Fig. 1).

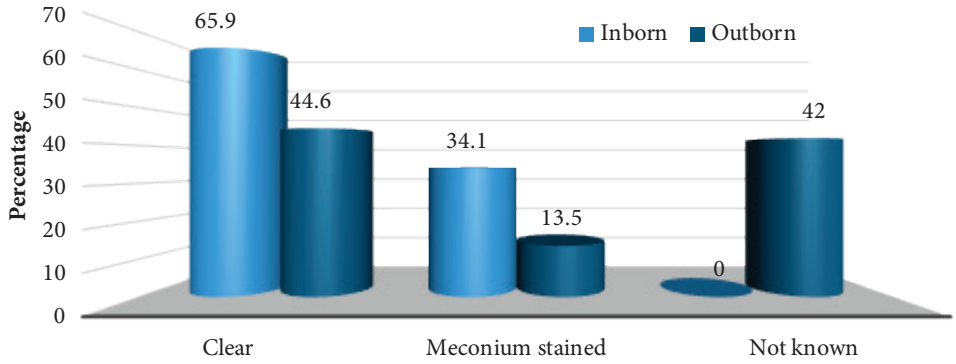

Figure 1. Comparison of liquor status during delivery/labour of inborn & outborn neonates
Source: compiled by the authors

Majority of neonates were born at term (58%), did not have any congenital defect (95.8%), had birth weight <2,500 g (53%), APGAR score in 7-10 or unknown range (75.3%). Proportion of those with birth weight <2,500 g was significantly higher in inborn (61.3%) as compared to that in outborn (42.9%) ($p = 0.023$). APGAR Score was not known in 40.7% outborn infants. Proportion of those on top feed only was also significantly higher in outborn (23.3%) as compared to inborn (2.2%) neonates ($p < 0.001$) In the study, Kangaroo mother care (KMC) was provided to 18 inborn neonates (20.4%) and 94 outborn neonates (23.5%), totalling 112 cases (28%) across the cohort of 400 neonates. The difference in KMC implementation between inborn and outborn groups was not statistically significant (Chi-square = 3.186; $p = 0.074$), indicating comparable rates of KMC practice between both groups. (Table 2; Fig. 2).

Table 2. Neonatal characteristics

Characteristics	Inborn n = 88 (%)	Outborns n = 312 (%)	Total n = 400 (%)	Statistical significance
Gestational age				
Term (37-41 wks)	61 (69.3)	171 (54.8)	232 (58)	$X^2 = 7.201$; $p = 0.066$
Preterm (<37 wks)	20 (22.7)	118 (37.8)	138 (34.5)	
Ext. preterm (28 wks)	5 (5.6)	18 (5.7)	23 (5.7)	
Post-term (≥42 wks)	2 (2.2)	5 (1.6)	7 (1.7)	
Birth weight				
<1,000 g	1 (1.1)	8 (2.5)	9 (2.25)	$X^2 = 9.554$; $p = 0.023$
1,000-1,499 g	9 (10.2)	42 (13.4)	51 (12.7)	
1,500-2,499 g	24 (27.2)	128 (41)	152 (38)	
>2,499 g	54 (61.3)	134 (42.9)	188 (47)	
APGAR				
<7	23 (26.1)	76 (24.3)	99 (24.7)	$X^2 = 59.819$; $p < 0.001$
7-10	65 (73.9)	109 (34.9)	174 (43.5)	
Not known	0	127 (40.7)	127 (31.7)	
Baby ABO group				
A	30 (34)	82 (26.2)	112 (28)	$X^2 = 5.774$; $p = 0.213$
B	33 (37.5)	127 (40.7)	160 (40)	
AB	4 (4.5)	38 (12.1)	42 (10.5)	
O	21 (23.8)	65 (20.8)	86 (21.5)	
Rh incompatibility	5 (5.6)	17 (5.4)	22 (5.5)	$X^2 = 0.007$; $p = 0.123$
Temperature				
Normothermic	58 (65.9)	230 (73.7)	288 (72)	$X^2 = 3.064$; $p = 0.215$
Hypothermic	27 (30.6)	75 (24)	102 (25.5)	
Hyperthermic	3 (3.4)	7 (2.2)	10 (2.5)	
Resuscitation				
Required	24 (27.2)	93 (29.8)	117 (29.2)	$X^2 = 6.572$; $p = 0.010$
Not required	64 (72.7)	219 (70.1)	283 (70.7)	
Infant morbidity profile				
Juandice	68 (77.2)	243 (77.8)	311 (77.7)	$X^2 = 0.015$; $p = 0.903$
Respiratory distress	30 (34)	206 (66)	236 (59)	$X^2 = 7.563$; $p = 0.001$
Seizures	39 (44.3)	117 (37.5)	156 (39)	$X^2 = 1.134$; $p = 0.247$

Continued Table 2

Characteristics	Inborn n = 88 (%)	Outborns n = 312 (%)	Total n = 400 (%)	Statistical significance
Neonatal sepsis	53 (60.2)	214 (68.5)	267 (66.7)	$X^2 = 2.163$; p = 0.141
Congenital defect	3 (3.4)	14 (4.4)	17 (4.2)	$X^2 = 0.196$; p = 0.066
Culture report				
Blood culture	48 (54.4)	202 (64.5)	250 (62)	$X^2 = 3.046$; p = 0.081
CSF culture	28 (31.8)	97 (31)	125 (31.2)	$X^2 = 0.017$; p = 0.896
Feed				
Exclusive breast feeding	66 (75)	150 (48)	216 (53)	$X^2 = 3.176$; p = 0.075
Topfeed only	2 (2.2)	73 (23.3)	75 (18.7)	$X^2 = 20.11$; p < 0.001
Mixed feed	9 (9)	29 (9.2)	38 (9.5)	$X^2 = 0.003$; p = 0.953

Source: compiled by the authors

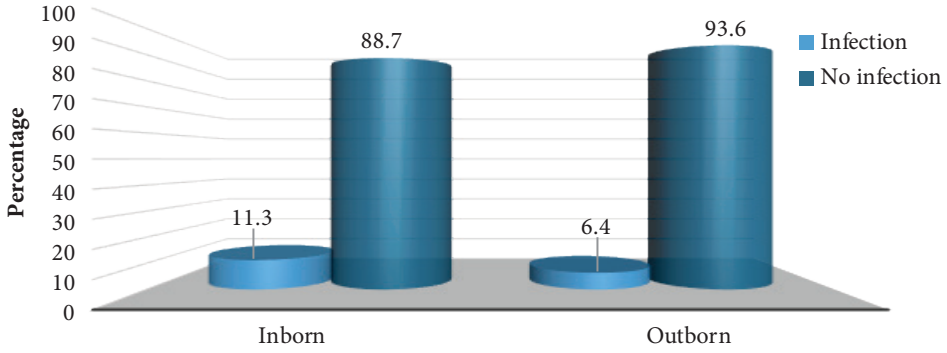

Figure 2. Comparison of infection during delivery/labour (neonatal sepsis and intrapartum maternal infections) of inborn & outborn neonates

Source: compiled by the authors

Phototherapy was required in 76% of cases and exchange transfusion was done in 18 (4.5%) cases. Oxygen therapy in the form of hood, nasal cannula, Continuous Positive Airway Pressure (CPAP) and mechanical ventilator was done in 21.7, 27.7% and 29% neonates respectively. Oxygen requirement by hood or ventilator, antibiotic need, blood product transfusion, Fresh Frozen Plasma (FFP), Packed Red Blood Cells (PRBC) and platelet need were significantly higher in outborn compared to inborn babies. Compared to inborns, outborn neonates had a significantly higher proportion of those diagnosed with Respiratory Distress Syndrome (RDS – 38.2%) and Hypoxic-Ischemic Encephalopathy (HIE – 19%) and a significantly lower proportion of those diagnosed with meconium aspiration syndrome. Overall, a total of 252 (63%) recovered, 44 (11%) died and 104 (26%) left against medical advice. Proportion of those recovering was significantly higher in inborn (81.8%) as compared to outborn (57.7%) cases (p < 0.001).

In the present study, the majority of admitted neonates were males (63%). A dominance of males in NICU is a general feature reported in almost all the studies. In their study, D. Kumar & S. Gupta [12] had 60.4% males, which is close to that in the present study. In the study of R.S. Sinha *et al.* [13], 64% of neonates admitted to the NICU were males. P.K. Panda & P.K. Panda [9] in their study had a marginally higher proportion of males (52%) as compared to that of females (48%). In the study by V. Anurekha *et al.* [14], 55.5% were males. However, K. Maheswari & N. Sharma [15] found that the majority (53.1%) of neonates are females.

The present study found significantly higher proportion of inborn neonates presented after 72 hours of birth (52%) as compared to outborn neonates (35%). As far as other studies are concerned, they usually attribute delays in admission to NICU to be associated with poor NICU outcomes and often state in special context with outborn NICU admissions [16]. S.M. Abdel-Aziz *et al.* [17] reported all the inborn NICU admissions to take place within 24 hours of birth compared to 93.1% of outborn admissions to take place after 24 hours of birth and found this difference to be statistically significant too. This is contrary to the findings of the present study, where the most common cause of NICU admission was neonatal jaundice (75%) followed by respiratory distress (59%), seizures (39%), etc. Compared to the present study, A. Deka *et al.* [10] reported sepsis to be the most important cause of NICU admission (21.6%) followed by jaundice (19.0%), respiratory distress (16.8%), birth asphyxia (11.7%), and meconium aspiration syndrome (7.9%). G.S. Shah *et al.* [18] also reported sepsis as major cause.

The present study found the proportion of male sex, refusal to feed, and respiratory distress to be significantly higher in outborn than in the inborn group. Simultaneously, meconium aspiration and vomiting were substantially higher in inborn as compared to outborn admissions. Similar to the present study, V. Anurekha *et al.* [14] also had

higher proportion of males in outborn (57%) as compared to inborn (54%). Similar to the present study, P.K. Panda & P.K. Panda [9] did not find a substantial difference between inborn and outborn groups for various indications for admission. S.M. Abdel-Aziz *et al.* [17] reported the same findings. W. Khasawneh *et al.* [8], reported as many as 23.5% NICU babies were born to mothers with age >35 years. Primipara mothers contributed to only 24% of total NICU admissions in their study. C.R. Iyer *et al.* [19] in their study, found majority of mothers to be aged 21-30 years (73%). However, in their study primigravida (49.5%) women were close to the present study. A dominance of those paying 4 or more ANC visits (70.5%) was also reported by R.S. Sinha *et al.* [13]. Compared to the present study, K. Maheswari & N. Sharma [15] reported the prevalence of cumulative maternal complications in 60.4% of infants admitted to NICU. Their study also found these complications to be related to NICU mortality. However, S.M. Abdel-Aziz *et al.* [17] reported maternal complications like diabetes to be higher in inborn compared to outborn preterm and full-term neonates admitted to NICU.

In the present study, majority of pregnancies did not require induction for labour (83.8%), had vertex presentation (90%), were spontaneous deliveries (52%), and had clear or unknown liquor status (82%). Compared to the present study, S.M. Abdel-Aziz *et al.* [17] dominated those born through caesarean section (81.9% in preterm and 82.6% in full-term group) but did not describe much about the other labour characteristics. In the present study, most neonates were born at term (58%) and did not have congenital defects (95.8%). Significantly higher proportion of outborn as compared to inborn neonates had birth weight <2.5 kg, unknown APGAR, resuscitation need, and lack of exclusive breastfeeding.

In the study by V. Anurekha *et al.* [14], substantial difference between inborn and outborn groups was observed with respect to neonatal jaundice, which was reported in 2.22 times higher number of inborn as compared to outborn neonates. Differences in neonatal morbidity profiles between inborn and outborn groups were also observed by A. Deka *et al.* [10], who reported the major cause of admission in inborn neonates to be jaundice (22%) followed by RDS (18.8%) and sepsis (18.6%), whereas in outborn neonates sepsis (30.09%) was the predominant cause. Compared to the present study, V. Anurekha *et al.* [14] reported the majority of newborns to be >2.5 kg (51.1%) and preterm (54.63%). S.M. Abdel-Aziz *et al.* [17] also reported the predominance of preterm births (56.78%). A. Deka *et al.* [10] in their study, not only had majority of neonates with birth weight >2.5 kg (52.27%), but also did not find any substantial difference between inborn and outborn groups with respect to birth weight.

Antibiotic overuse were observed in present study, as neonatal sepsis was suspected in only 60.2% of cases, whereas antibiotic use was reported in as high as 89% of cases. This is an alarming issue. It seems that antibiotics were used as prophylaxis in the present study, which cannot be termed as a good clinical practice and indicates a need for methodological improvement. However, S. Mandal [20] reported a much higher use of antibiotics (38.8%) compared to the proportion of neonatal sepsis (16.6%) cases. In the present study, mortality rate was 2.84 times higher in outborn (12.8%) compared to inborn (4.5%) neonates. Compared to the present study, A. Deka *et al.* [10] reported recovery, referral, leave against medical advice (LAMA), and death in 82.3, 1.14, 4.17, and 12.37% cases, respectively. They also found the mortality rate to be more than twice in outborn (18.0%) compared to inborn (8.7%) neonates.

D. Kumar & S. Gupta [12] reported mortality and LAMA in 3.26 and 3.26% of patients only. However, in the present study, it was reflected in terms of a high LAMA rate. In the study by R.S. Sinha *et al.* [13] LAMA rate was only 9.5%, still they considered it to be higher. Mortality rate in their study was only 0.9%. Though, P.K. Panda & P.K. Panda [9] reported mortality rate of 11% which is close to that in the present study yet in their study LAMA rate was only 9% and discharge after recovery was achieved in 75%. A slightly higher mortality rate (12.7%) was reported by C.R. Iyer *et al.* [19], who also had a high LAMA rate (17.5%). However, they did not find a significant difference in mortality rate between inborn (13.2%) and outborn (12.2%) groups. In their study referral/LAMA rate was only 9.51%. The mortality rate was also unaffected by place of birth. V. Anurekha *et al.* [14] in their study, did not report any LAMA as they had already excluded them from the study. However, after excluding LAMA, the mortality rate in their study (11.28%) was similar to present study.

The present study provided valuable insights and highlighted several differences in the clinical profile of inborn and outborn neonates admitted to NICU. It was shown that outborn neonates often reach in a more severe condition than the inborn neonates and have higher interventional needs. Moreover, outborn neonates' mortality rate (2.84 times) is more than twice that of inborn neonates. Apart from highlighting these differences, the study also introspected some gaps in service, primarily highlighted in terms of high antibiotic use and a high LAMA rate.

Conclusions

The present retrospective study on 400 neonates admitted to the NICU at Era University, Lucknow, provides critical insights into neonatal outcomes and their association with maternal and perinatal factors. Among the neonates, 61.7% were aged less than 72 hours, 63% were male, and the primary presentations included respiratory distress (59%) and jaundice (75%). Outborn neonates constituted the majority (78%), with significantly higher rates of infection, top feeding (23.3 vs 2.2%, $p < 0.001$), and hypothermia (30.6 vs 24%, $p = 0.215$) compared to inborn neonates. Inborn infants showed a higher proportion of low birth weight (<2,500 g) cases (61.3 vs 42.9%, $p = 0.023$) and better recovery rates (81.8 vs 57.7%, $p < 0.001$). Maternal factors also played a significant role in neonatal outcomes. The

majority of mothers (91.5%) were aged 18-35 years, and multigravida mothers constituted 55.5% of the cohort. Primigravida mothers were more prevalent among outborn neonates (63 vs 56.8%, $p < 0.001$). The prevalence of diabetes mellitus among mothers was significantly higher in the outborn group (8.9 vs 2.2%, $p = 0.035$). Most mothers had adequate antenatal care (≥4 visits in 57.5%), and maternal blood group B Rh+ was the most common (34.7%). Clinical interventions and outcomes also showed notable differences between inborn and outborn neonates. Oxygen therapy, including CPAP and mechanical ventilation, was required in 7.5 and 29% of neonates, respectively, with outborn neonates showing a significantly higher requirement. Phototherapy was administered to 76% of neonates, and exchange transfusion was needed in 4.5% of cases. The incidence of RDS and HIE was significantly higher in outborns, whereas meconium aspiration syndrome was more frequent in inborns. Despite the higher morbidity in outborn neonates, 63% of all neonates recovered, with the inborn group showing a significantly higher recovery rate. In conclusion, inborn neonates demonstrated better clinical outcomes compared to outborn neonates, underscoring the importance of institutional deliveries and comprehensive antenatal care to improve neonatal outcomes. Enhanced infrastructure and care protocols in referral centers are critical to addressing the disparities observed in outborn neonates, particularly concerning morbidity and recovery rates. Future research should be supported by systematic improvements of infrastructure, staff training, and enhancing inter-personal communication alongside the establishment of evidence-based antibiotic use guidelines.

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Клінічний аудит новонароджених, госпіталізованих у відділення інтенсивної терапії новонароджених навчальної лікарні: ретроспективне дослідження

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Анотація. Розуміння причин госпіталізації новонароджених до відділення інтенсивної терапії новонароджених і їхнього зв'язку з неонатальними результатами може допомогти в організації кращих послуг для пацієнтів. Метою було дослідити причини та результати лікування новонароджених, госпіталізованих до відділення інтенсивної терапії новонароджених. Було проведене ретроспективне дослідження, у якому проаналізовано демографічні та клінічні дані новонароджених і їхніх матерів, що отримані з медичних записів усіх випадків госпіталізації до відділення інтенсивної терапії новонароджених за період 2018-2020 років. У результаті, із 400 госпіталізованих новонароджених більшість (61,7 %) були віком менше 72 годин, із переважанням хлопчиків (63 %), і мали респіраторний дистрес (59 %) та жовтяницю (75 %). Майже всі діти, народжені поза лікарнею, мали гіпотермію під час надходження. Частка пацієнтів, які надходили у віці 4 днів і більше, була значно вищою серед дітей, народжених у лікарні. Більшість матерів були у віці 18-35 років (91,5 %), були вагітними раніше (55,5 %) і мали понад чотири антенатальні огляди (57,5 %). Загалом 252 (63 %) немовлята одужали, 44 (11 %) померли, а 104 (26 %) залишили лікарню всупереч медичним рекомендаціям. Рівень одужання був значно вищим (81,8 %) серед дітей, народжених у лікарні. Респіраторний дистрес і жовтяниця були найпоширенішими клінічними станами при надходженні. Високий рівень випадків, коли пацієнт залишав лікарню всупереч медичним рекомендаціям, викликав занепокоєння і потребує подальших досліджень для з'ясування реальних причин. Дослідження підкреслило необхідність раннього виявлення ризиків, поліпшення транспортування новонароджених, цілеспрямованої допомоги матерям, стратегій щодо зменшення випадків виписки проти медичних рекомендацій та покращення послуг з пологів у лікарнях для поліпшення показників новонароджених та оптимізації роботи відділень інтенсивної терапії новонароджених

Ключові слова: рівень смертності новонароджених; респіраторний дистрес-синдром; жовтяниця новонароджених; фактори ризику для матерів; новонароджені та матері

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Seizure syndrome in stroke and traumatic brain injury: Incidence and treatment outcomes in the intensive care unit

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Abstract. Seizure syndrome is one of the most common and complex neurological complications in patients with acute brain injuries, particularly following stroke and traumatic brain injury. This complication significantly worsens clinical outcomes, increases the risk of mortality, and prolongs patients' length of stay in the intensive care unit. This study aimed to determine the frequency of seizure syndrome in patients with stroke and traumatic brain injury, to analyse the main causes of its development, and to evaluate the efficacy and safety of antiepileptic therapy in order to improve treatment approaches and clinical outcomes. A comprehensive literature review was conducted using 50 relevant scientific publications from PubMed, Scopus, Web of Science and Google Scholar databases. The selection was carried out in accordance with PRISMA guidelines and included studies with clearly defined patient populations with stroke or traumatic brain injury, data on seizure frequency, antiepileptic drug use, and statistically analysed results published in peer reviewed journals. Published studies indicated that seizure syndrome occurs in approximately 15%-30% of patients after stroke and in 20%-40% of individuals with traumatic brain injury. Early seizures (within the first seven days) are consistently reported in approximately 7% of patients after stroke and are associated with a poorer prognosis. The literature review identified multifactorial pathophysiological mechanisms, including primary structural brain tissue damage, secondary metabolic disorders, neuroinflammation, and an imbalance of neurotransmitter systems. Data from numerous studies demonstrated a high efficacy of modern antiepileptic therapy – particularly levetiracetam and lorazepam – estimated at 70%-85% with a favourable safety profile. The binding of levetiracetam to synaptic vesicle protein 2A is widely regarded as a key mechanism of seizure control, while lorazepam remains a first-line drug for the acute management of seizures due to its enhancement of GABAergic transmission

Keywords: antiepileptic therapy; levetiracetam; lorazepam; neurological complications; neuroinflammation

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Introduction

Seizure syndrome is one of the most common and clinically challenging neurological complications in patients with acute brain injuries, particularly following stroke and traumatic brain injury (TBI). Its occurrence significantly worsens the clinical course, increases the risk of mortality and long-term disability, and prolongs stays in the intensive care unit. The relevance of this issue stems from the heterogeneity of pathophysiological mechanisms underlying seizures in different types of brain injury and the persistent difficulty in selecting optimal anticonvulsant therapy, which remains limited by insufficient evidence and variability in clinical practice.

The problem of seizure syndrome in acute brain injuries, particularly following stroke and TBI, has been widely examined in the scientific literature. In the review by M. Galovic *et al.* [1], the authors summarised current findings on the epidemiology, biomarkers, and management of seizures and poststroke epilepsy. Their research emphasised the high incidence of early and late seizures, the central role of cortical structural injury, and the importance of emerging prognostic markers for identifying patients at risk of developing epilepsy after stroke. In a multicentre matched study conducted by C. Ferreira-Atuesta *et al.* [2], the authors analysed the frequency of seizures following ischaemic stroke and the clinical factors associated with their occurrence. They demonstrated that seizures are more common in patients with severe neurological deficits, cortical involvement, and haemorrhagic transformation, underscoring the need for individualised monitoring and preventive measures in high-risk patient groups. A systematic review and meta-analysis by A. Nandan *et al.* [3] synthesised data on the incidence of post-stroke seizures and epilepsy, as well as the principal risk factors contributing to their development.

The researchers reported a significantly increased risk of seizures in patients with cortical lesions, haemorrhagic strokes, and younger age, providing important insights for clinical risk stratification. Complementing these findings, S. Misra *et al.* [4] conducted a further large systematic review and meta-analysis that evaluated the outcomes of patients experiencing post-stroke seizures. Their results demonstrated clear associations between seizures and higher mortality, poorer functional outcomes, and an increased need for long-term rehabilitation services. A separate research focus has addressed the development of tools for predicting post-stroke epilepsy. In the study by Z. Wang *et al.* [5], the authors developed an automated deep learning model for predicting poststroke epilepsy in patients with intracerebral haemorrhage using non-contrast computed tomography imaging. Their study demonstrated the potential of artificial intelligence to enable early identification of high-risk patients, allowing more targeted application of anticonvulsant therapy and more efficient resource allocation in intensive care settings. The rehabilitation phase has also attracted considerable attention. In the study by M. Scarpino *et al.* [6], researchers assessed stroke-related epilepsy during inpatient post-stroke rehabilitation. They found that post-stroke epilepsy significantly complicates the rehabilitation process, reduces functional independence, and prolongs the duration of hospitalisation. These observations highlighted that seizures are clinically relevant not only in the acute stage but throughout the recovery period.

The effectiveness and safety of anticonvulsant therapy in acute brain injury settings were investigated in the PEACH randomised, double-blind, placebo-controlled phase III trial by L. PeterDerex *et al.* [7]. This study evaluated prophylactic levetiracetam for seizure prevention during the acute phase of intracerebral haemorrhage. The trial provided important data regarding the potential benefits and limitations of routine prophylactic therapy, as well as the drug's safety profile in critically ill neurological patients. Ukrainian clinical practice is guided by medical and technological documents approved by the Order of the Ministry of Health of Ukraine No. 276 [8], which outline standardised approaches to the diagnosis and treatment of epilepsy. Although these guidelines are not specific to seizures in stroke or TBI, they provide a regulatory framework for the use of modern anticonvulsant medications – such as levetiracetam and lorazepam – and may be adapted for seizure management in intensive care units.

Collectively, the reviewed studies demonstrated substantial advances in understanding the epidemiology, risk factors, and outcomes of seizure syndrome following stroke, as well as ongoing efforts to develop predictive models and evaluate anticonvulsant therapy. However, the majority of studies have focused primarily on stroke populations. Research addressing seizure syndrome specifically in patients with TBI and within intensive care settings remains comparatively limited, highlighting the need for further investigation to optimise diagnostic strategies and therapeutic interventions for this patient group. Accordingly, the aim of the present study was to determine the frequency and principal risk factors for the development of seizure syndrome in stroke and TBI, as well as to evaluate the efficacy and safety of anticonvulsant therapy in intensive care settings, to identify patterns of seizure development and the most promising treatment approaches for improving patient outcomes.

Materials and Methods

To conduct the literature review, 50 relevant peer-reviewed publications were selected addressing the incidence of seizure syndrome, its aetiological factors, and the effectiveness of anticonvulsant therapy in patients with stroke and TBI. The review also included an analysis of current international and national clinical guidelines [8-11] on the management of patients with stroke and acute TBI. The literature search was performed using major international scientometric databases – PubMed, Scopus, Web of Science, and Google Scholar – and applied English-language keywords, including "seizure syndrome", "stroke", "traumatic brain injury", "antiepileptic therapy", "intensive care", "epileptic complications", "anticonvulsant efficacy", and "seizure

incidence in stroke and TBI". No strict time limits were imposed; however, priority was given to studies published between 2015 and 2025 to ensure topical relevance.

The selection of publications followed the PRISMA methodology [12] (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), including the removal of duplicates, initial screening of titles and abstracts, and subsequent full-text evaluation. A PRISMA flow diagram was not included, which represents a methodological limitation of the review. Inclusion criteria comprised defined patient populations with stroke or TBI; data on the incidence of seizure syndrome; information on anticonvulsant therapy and its effectiveness; statistically analysed outcomes; and publication in peer-reviewed journals. Exclusion criteria included review articles, case reports, studies with insufficient sample sizes or inadequate statistical reporting, and publications unrelated to seizure syndrome in stroke or TBI. The quality of sources was evaluated based on study design (RCTs, cohort studies, and retrospective analyses), sample size, availability of control groups, clarity of statistical methodology, and adherence to ethical standards. Studies with low levels of evidence or significant methodological shortcomings were excluded. The temporal distribution of publications was also considered to ensure the chronological relevance and representativeness of the included material.

Results and Discussion

Incidence and predictors of seizure syndrome following stroke and TBI. In a review by S. Neri *et al.* [13], it was reported that early seizures develop in approximately 3.3%-3.9% of patients following an acute cerebrovascular event, with notably lower rates observed in lacunar stroke (0.9%). Post-stroke epilepsy occurs in approximately 7% of patients after stroke, accounting for 30%-49% of all new-onset seizures in individuals aged over 65 years. The authors emphasised that stroke severity and extent, cortical involvement, and haemorrhagic transformation are the principal predictors of seizure occurrence, with haemorrhagic stroke almost doubling the risk of post-stroke epilepsy. In addition, younger age at stroke onset was identified as a significant predictor of post-stroke epilepsy in this population. Similar conclusions were drawn by S. Ma *et al.* [14], who demonstrated in their meta-analysis of 18 case-control studies involving 13,289 patients that cortical involvement [OR 5.00, 95% CI (2.85-8.74)], cerebral infarction with haemorrhagic transformation [OR 2.77, 95% CI (1.87-4.11)], and intracerebral haemorrhage [OR = 1.83, 95% CI (1.13-2.97)] were significantly associated with early-onset seizures after stroke. In a Ukrainian cohort study, O. Kauk *et al.* [15] reported that inflammatory biomarkers – particularly elevated C-reactive protein (CRP) (>10 mg/L, peaking at 24-48 hours) and IL-6 (>20 pg/mL, peaking at 2-6 hours) – correlate with larger infarct size, blood-brain barrier disruption, and early neurological deterioration in intensive care patients with ischaemic stroke. These findings indirectly support a mechanistic link between post-stroke neuroinflammation and an increased risk of seizures. Y. Bondarenko *et al.* [16] further noted that both ischaemic and haemorrhagic strokes with cortical involvement frequently lead to neurological complications that adversely affect cognitive recovery in intensive care settings.

Across these studies, a consistent pattern emerges: seizures following stroke occur more frequently in haemorrhagic subtypes, cortical infarctions, and cases characterised by a pronounced inflammatory response. Early seizures (<7 days) repeatedly appear to be a marker of more severe brain injury and a predictor of poorer functional outcomes. The reviewed data collectively support the understanding that the development of seizures reflects both the extent of structural brain damage and the degree of neuroinflammatory activation in acute cerebrovascular disease.

M. Pease *et al.* [17] examined patients with severe TBI and found that post-traumatic epilepsy develops in approximately 25%-32% of cases, with higher rates among individuals exhibiting cortical contusions, intracranial haematomas, and severe impairment on the GCS at admission. Their findings indicated that seizures serve as a marker of diffuse and penetrating neuronal injury. A complementary analysis by H.F. Sødal *et al.* [18] demonstrated that early post-traumatic seizures occur in 5.6% of hospitalised TBI patients, with risk factors including alcohol abuse (OR 3.6, 95% CI 2.3-5.7), moderate and severe brain injury (OR 2.2, 95% CI 1.3-3.8 and OR 2.1, 95% CI 1.2-3.6, respectively), brain contusion (OR 1.6, 95% CI 1.0-2.4), and subdural haematoma (OR 1.6, 95% CI 1.0-2.6). In an extensive review, S. Fordington & M. Manford [19] described a sequence of acute pathological processes following traumatic injury, including necrosis, microhaemorrhage, axonal injury, apoptosis, demyelination, microgliosis, inflammation, and oxidative stress. Later phases of neurodegeneration, regeneration, revascularisation, and neural remodelling were shown to contribute to circuit alterations that ultimately result in post-traumatic epilepsy. R.E. Teneralli *et al.* [20] demonstrated that among 205,183 individuals with newly diagnosed epilepsy, those who developed drug-resistant epilepsy within one year (4.1%) already exhibited a substantially higher burden of physical and psychiatric comorbidities – such as pain syndromes, headache, neuropathy, musculoskeletal disorders, traumatic brain injury, depression, anxiety, bipolar disorder, suicidal ideation, substance dependence, and sleep disorders – even prior to the initial epilepsy diagnosis. These findings suggest that broader systemic vulnerability may predispose certain patients to more severe seizure phenotypes.

Post-traumatic seizures represent a dynamic continuum in which the extent of structural brain disruption interacts with an individual's physiological resilience to shape long-term neurological trajectories. Across studies, a consistent pattern emerges: epileptogenesis following traumatic brain injury is a multifactorial process driven by acute tissue damage, inflammatory activation, and subsequent network remodelling. Within this framework, clinical outcomes range from isolated early seizures to persistent or drug-resistant epilepsy, highlighting not only

the severity of the initial injury but also the systemic vulnerability that predisposes some patients to more severe seizure phenotypes.

Early seizures in acute neurological injury. A comparative analysis of the reviewed literature demonstrated that seizure syndrome occurs more frequently after TBI than after stroke, although both conditions share overlapping risk factors such as cortical involvement and intracerebral haemorrhage. Early seizures consistently emerge as a negative prognostic indicator across both groups. Moreover, the reviewed studies collectively identified brain oedema, inflammatory activation, and metabolic instability as common mechanisms contributing to early seizure development. While stroke-specific literature emphasised the roles of haemorrhagic transformation and neuroinflammation, TBI-focused studies highlighted mechanical tissue disruption and haematoma-related cortical irritation. Despite methodological differences, the overall evidence converges on the conclusion that seizure incidence in intensive care settings reflects the severity of structural brain damage and systemic physiological stress following acute neurological injury.

The findings of this review underscore the multifactorial nature of seizure syndrome following acute brain injury, demonstrating that both structural and molecular factors contribute to seizure vulnerability. T. Okada *et al.* [21] highlighted that blood-brain barrier (BBB) disruption after stroke plays a central role in generating cortical hyperexcitability through inflammatory activation, ionic dysregulation, and impaired metabolic homeostasis. This concept aligns with evidence that early seizures often coincide with peak BBB permeability and neuroinflammatory cascades, a relationship further supported by clinical risk-stratification data in hypoxic-ischaemic brain injury described by D.S. Mankovskyi [22]. These mechanisms help explain the observed associations between haemorrhagic stroke, cortical involvement, and an increased incidence of seizures. Management strategies for acute seizures must also be informed by evidence-based clinical guidelines. F. Rosenow & J. Weber [23], in the S2k guideline for status epilepticus, underscore the necessity of prompt benzodiazepine administration, continuous electroencephalography (EEG) monitoring, and timely escalation to second-line antiseizure medications when required. Their recommendations emphasised that early recognition and intervention significantly reduce morbidity and mortality, particularly in neurocritical care settings. The importance of EEG monitoring is further supported by F. Misirocchi *et al.* [24], who demonstrated that ICU-based EEG services improve outcomes in patients with status epilepticus by enabling earlier detection of subclinical seizures. Pharmacological therapy remains central to seizure control in stroke and TBI populations. T. Hakami [25] reported that over the past three decades, approximately 20 newer-generation (second- and third-generation) antiseizure drugs (ASDs), characterised by diverse mechanisms of action and pharmacokinetic profiles, have been introduced into clinical practice. This development has expanded the therapeutic armamentarium of epilepsy and broadened the selection of ASDs to better match individual patient characteristics. J.A. French *et al.* [26] and A.M. Kanner *et al.* [27] observed that newer ASDs do not differ significantly in seizure control compared with older agents, but some demonstrate improved tolerability, particularly with fewer neurotoxic adverse effects. For focal-onset seizures, lamotrigine is considered the first-line drug of choice [25]. Other widely used first-line agents include levetiracetam and zonisamide; however, findings from the SANAD II trial reported by A. Marson *et al.* [28] suggested that these drugs are inferior to lamotrigine with respect to time to 12 month remission. Medication selection must therefore consider seizure type, patient characteristics, adverse-effect profile, potential drug-drug interactions, and cost. Despite the expansion of therapeutic options, drug-resistant epilepsy occurs in approximately 25%-30% of patients, and treatment with first-generation ASDs fails in 30%-40% of individuals due to intolerable adverse effects, as noted by T. Hakami [25]. A. Bayat *et al.* [29] found that genetic testing significantly influences treatment decisions in childhood-onset epilepsies, enabling optimised medication selection and avoidance of ineffective therapies. Similarly, B. Castellotti *et al.* [30] demonstrated that next-generation sequencing facilitates personalised therapeutic planning based on channelopathy-specific or metabolism-related gene mutations. These findings are reinforced by A. Balaji *et al.* [31], who highlighted the emerging role of genome-based therapeutics in drugresistant epilepsies. Non-pharmacological therapies also play an important role in the management of refractory seizures. J.H. Na *et al.* [32] reported high clinical efficacy and acceptable safety of the ketogenic diet in patients with genetically confirmed drug-resistant epilepsy, suggesting its potential application in selected adult and paediatric populations following acute brain injury. Advanced neurostimulation techniques offer additional therapeutic benefits: E. Sharma *et al.* [33] showed that responsive neurostimulation results in significant seizure reduction in patients with refractory mesial temporal lobe epilepsy, underscoring the potential applicability of neuromodulation strategies in carefully selected survivors of TBI or stroke.

Taken together, the reviewed findings indicate that seizure syndrome following acute brain injury arises from a dynamic interplay of structural lesions, BBB dysfunction, excitatory-inhibitory imbalance, and systemic metabolic disturbances. Early seizures consistently function as a marker of severe neurological damage and are associated with poorer functional outcomes. Evidence clearly demonstrates that prompt EEG-based diagnosis, appropriate selection of antiseizure medication, and individualised treatment strategies improve prognosis, reduce complications, and shorten the duration of ICU stays. Overall, the integrated data confirm that optimising seizure management requires a multimodal approach, combining rapid pharmacological intervention, continuous monitoring, and precision medicine tools, such as genetic testing.

Main causes of seizure syndrome. Seizure syndrome is a complex manifestation of neuronal hyperexcitability, characterised by sudden, paroxysmal disturbances in motor, sensory, or cognitive function. S. Wu & D.R. Nordli [34] described seizure semiology as the clinical manifestation of a seizure generated by activation of the symptomatogenic zone. Semiology represents a major component of epilepsy evaluation, providing important information for seizure classification and assisting in seizure localisation. The site of seizure onset and the network of seizure propagation contribute to semiology, together with age, underlying pathophysiology, and the rate of electrical discharge propagation, all of which influence the clinical presentation of seizures. Importantly, seizure syndrome is not a discrete disease entity but rather reflects impaired neuronal regulation caused by organic, metabolic, infectious, or toxic factors. R.E. Stirling *et al.* [35] noted that epilepsy is characterised by unpredictable, recurrent seizures that vary widely between individuals. Advances in seizure forecasting, including machine-learning approaches and the investigation of non-EEG biomarkers, have substantially improved the understanding of seizure patterns. Most individuals exhibit circadian rhythms of seizure occurrence, with many also experiencing longer multiday cycles. M.Y. Xu [36] highlighted that ischaemic and haemorrhagic strokes induce neuronal injury, excitotoxicity, and inflammatory activation, thereby creating epileptogenic zones, particularly within cortical regions. R. Brondani *et al.* [37] further demonstrated that large-territory strokes, especially malignant middle cerebral artery infarctions requiring decompressive hemicraniectomy, markedly increase seizure risk as a result of extensive cortical disruption. In their retrospective cohort of 36 patients with a mean follow-up of 1,086 days, seizures occurred in 22 patients (61.1%, 95% CI 45.17%-77.03%), with 13 patients (36.1%) developing seizures within the first week after stroke. Among the 34 patients who survived the acute phase, 19 (55.9%, 95% CI 39.21%-72.59%) developed post-stroke epilepsy. Notably, no specific risk factors – including age, sex, stroke laterality, vascular risk profile, haemorrhagic transformation, or timing of craniectomy – distinguished patients who developed seizures from those who remained seizure-free, suggesting that stroke volume and the extent of cortical ischaemia are the primary determinants of epileptogenesis in this population. The authors emphasised that seizures may occur particularly early in patients not receiving anticonvulsant prophylaxis. MRI-based investigations, such as the systematic review by F.P. Mariajoseph *et al.* [38], demonstrated seizure-associated cortical abnormalities, supporting the concept that structural and metabolic stressors exacerbate cortical instability after stroke. TBI constitutes another major aetiological category. According to R.E. Stirling *et al.* [35], recent advances in seizure forecasting have shown substantial progress, including improvements in predictive algorithms using machine learning and exploration of non-EEG measures of seizure susceptibility, such as physiological biomarkers, behavioural changes, environmental factors, and cyclical seizure patterns. Investigations into periodicities in individual seizure activity have demonstrated that over 90% of individuals exhibit circadian seizure rhythms, with many also experiencing multiday, weekly, or longer cycles. Potential indicators of seizure susceptibility include stress levels, heart rate, and sleep quality, all of which can be captured non-invasively over extended periods. Applications of seizure-forecasting technologies include improving quality of life, guiding treatment planning and medication titration, optimising presurgical monitoring, and directing future scientific research. A. Szűcs *et al.* [39] observed that reflex mechanisms may also contribute to seizure development in certain TBI patients, particularly when cortical circuits become hypersensitised after injury. These findings help explain why prolonged impaired consciousness and mass-effect lesions substantially increase seizure susceptibility. Metabolic disturbances constitute an additional major group of seizure triggers. Hyponatraemia, hypocalcaemia, and disturbances in glucose homeostasis alter neuronal membrane stability and intracellular signalling, thereby facilitating uncontrolled depolarisation. S. Wu & D.R. Nordli [34] described motor semiology as a major component of epilepsy evaluation, providing essential information for seizure classification and localisation. Typical motor seizures include tonic, clonic, tonic-clonic, myoclonic, atonic seizures, epileptic spasms, automatisms, and hyperkinetic seizures. Beyond "positive" motor signs, negative motor phenomena, such as atonic seizures and Todd's paralysis, are crucial for seizure analysis. Several motor signs – including version, unilateral dystonia, and asymmetric clonic termination – have significant clinical value in seizure lateralisation. The study reviewed the localisation value and underlying pathophysiology using updated evidence from intracranial electroencephalographic recordings, particularly stereoelectroencephalography. Overall, seizure syndrome arises from the convergence of primary structural damage, secondary metabolic instability, and inflammatory alterations within the neuronal microenvironment. Across the reviewed studies, a consistent theme is that cortical involvement – whether resulting from stroke, TBI, infection, or metabolic crisis – plays a central role in seizure generation. These findings underscore the importance of early diagnostic evaluation, including neuroimaging and electrophysiological monitoring, to guide timely therapeutic intervention and prevent progression to chronic epilepsy. Figure 1 illustrated a fundamental transition, whereby acute brain injury initiates a convergent pathophysiological process in which structural disruption, neuroinflammation, metabolic failure, and an imbalance between excitatory and inhibitory signalling collectively drive the brain towards network hyperexcitability.

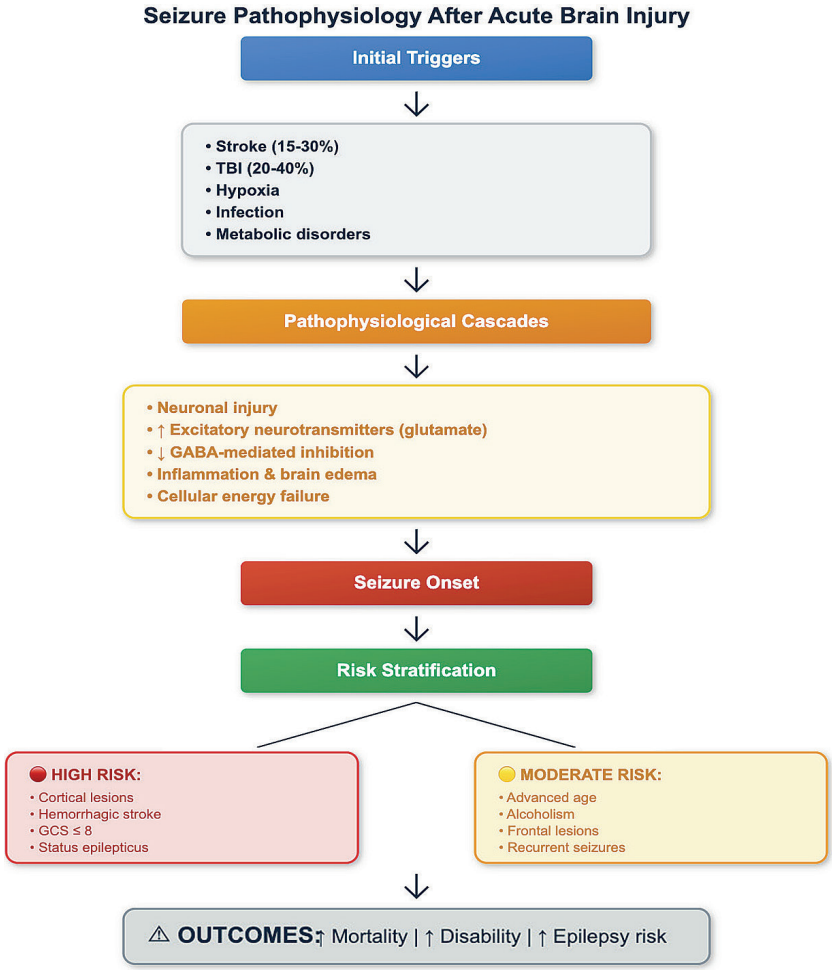

Figure 1. Post-acute brain injury seizure pathophysiology flowchart
Source: prepared by the authors during the course of the research

Although stroke and traumatic brain injury share several mechanistic determinants, their seizure profiles diverge according to the depth and spatial distribution of cortical involvement. Early seizures consistently signal profound circuit destabilisation and strongly predict adverse neurological and survival outcomes. Cortical lesions and intracranial haemorrhage remain the most robust predictors of seizure emergence across cohorts. These insights highlight the need for precise early detection, risk-based stratification, and timely targeted intervention to prevent progression towards chronic epileptogenesis and long-term disability.

Therapeutic approaches to seizure management. Anticonvulsant therapy is central to the management of seizure syndrome, as it controls abnormal cortical electrical activity, as noted by E. Perucca *et al.* [40] and G.J. Sills & M.A. Rogawski [41]. According to these studies, seizures arise from an imbalance between excitatory and inhibitory systems, whereby excessive glutamatergic activity, combined with insufficient inhibition mediated by γ-aminobutyric acid, leads to neuronal hyperexcitability. In addition, anticonvulsant drugs reduce neuronal excitability and stabilise synaptic transmission by acting on multiple mechanisms within the pathophysiological cascade of epileptogenesis.

Levetiracetam is a widely used modern anticonvulsant that acts by binding to the synaptic vesicle protein SV2A, thereby reducing glutamatergic neurotransmission while producing minimal sedative effects. I.J. Contreras-García *et al.* [42] and B.A. Lynch *et al.* [43] reported that levetiracetam's mechanism involves high-affinity binding to SV2A, with rapid penetration of the blood-brain barrier. For acute seizures and status epilepticus, E. Trinka *et al.* [44] and T. Glauser *et al.* [45] identified lorazepam as the first-line treatment, owing to its rapid and sustained enhancement of GABA-A-mediated inhibition. Intranasal midazolam provides a rapid and practical alternative when IV access is unavailable. R. Kienitz *et al.* [46] reported that in 20%-30% of drugresistant cases, neurosurgical approaches – such as vagus nerve stimulation, deep brain stimulation, and resective surgery – represent

viable therapeutic options. T. Xue *et al.* [47] and F.V. Gouveia *et al.* [48] corroborated these findings, emphasising their relevance for carefully selected patients. G.W. Culler & B.C. Jobst [49] further underscored the clinical applicability of these interventions. A. Jiang *et al.* [50] additionally noted that ketogenic therapy may be considered as an alternative metabolic strategy. Pharmacogenetics enables treatment individualisation based on mutations in ion-channel and synaptic protein genes, and genetic targeting is increasingly recognised as an essential component of precision epileptology. K. Borowicz-Reutt *et al.* [51] and A.A. Shaimardanova *et al.* [52] highlighted the importance of pharmacogenetics in tailoring treatment strategies based on mutations in SCN1A, KCNQ2, PCDH19, and other ion-channel and synaptic protein genes. Their conclusions emphasised the role of gene-targeted diagnostics in modern precision epileptology. These molecular data may predict drug responsiveness, guide the avoidance of ineffective or potentially harmful medications, and help identify candidates for emerging gene-directed therapies. Therapy should be individualised according to seizure type, age, aetiology, comorbidities, and EEG findings. Equally important is the role of systematic monitoring of treatment effectiveness and safety. Patient management includes regular clinical assessment of seizure frequency and characteristics, monitoring of laboratory parameters, and EEG surveillance, including video-EEG for the detection of subclinical seizures. Where necessary, dose titration or switching to alternative anticonvulsants is undertaken, taking into account pharmacokinetic and pharmacodynamic parameters, as summarised in Table 1.

Table 1. Therapeutic approach to seizure syndrome

Aspect	Medication/method	Mechanism/use	Notes
Core therapy	Anticonvulsants	Suppress cortical hyperexcitability; balance excitatory (glutamate) and inhibitory (GABA) signalling	Foundation of treatment
Primary drug	Levetiracetam	Binds SV2A protein; reduces glutamate release	Minimal adverse effects; rapid CNS penetration
Acute seizures	Lorazepam (IV)	Enhances GABA-A-mediated inhibition via Cl^- channel potentiation	Onset: 2-3 min; duration up to 12 h
Emergency (field use)	Midazolam (intranasal)	Rapid absorption via the nasal mucosa	Suitable for paediatric use and when IV access is unavailable
Refractory epilepsy	Vagus nerve stimulation, brain surgery, ketogenic diet	Modulates neuronal excitability or cerebral metabolism	Used in 20%-30% of drugresistant cases
Genetic targeting	Gene-based diagnostics	Analysis of SCN1A, KCNQ2, PCDH19 mutations	Enables personalised medicine
Drug selection factors	–	Seizure type, age, aetiology, EEG findings	Tailored therapeutic strategy
Monitoring	EEG, clinical assessment	Detection of subclinical seizures; monitoring of drug effects	Therapy adjusted according to response and safety

Source: prepared by the authors during the course of the research

Effective seizure management following acute brain injury requires an integrated, multimodal strategy capable of providing both rapid stabilisation and sustained modulation of neuronal network activity. Immediate control with lorazepam or intranasal midazolam forms the foundation of emergency management, while levetiracetam supports longer-term synaptic stability through targeted SV2A interaction. In pharmacoresistant cases, deeper modulation of neuronal and metabolic dysregulation may be achieved through vagus nerve stimulation, surgical resection of epileptogenic zones, or ketogenic metabolic therapy, with genetic approaches offering additional precision by accounting for individual ion-channel and synaptic abnormalities. Taken together, the combination of pharmacological and non-pharmacological interventions facilitates restoration of network stability, reduction of seizure recurrence, and mitigation of long-term neurological complications.

Recommendations for optimising the management of seizure syndromes. Optimisation strategies are based on current international clinical guidelines issued by the ILAE, AAN, and NICE [27, 53, 54] and are aimed at ensuring maximal therapeutic efficacy and patient safety. A key determinant of successful management is the early detection of seizure syndromes, achieved through comprehensive clinical monitoring, the use of standardised consciousness assessment scales (e.g. the Glasgow Coma Scale), neuroimaging modalities (CT or MRI), and EEG. EEG represents an essential diagnostic modality, enabling the identification of subclinical seizures, focal epileptiform discharges, and non-convulsive status epilepticus – conditions that frequently occur in the absence of overt motor manifestations – thereby rendering the timely initiation of targeted antiseizure therapy critically important.

Individualised treatment constitutes a core principle of modern antiseizure management. The selection of antiepileptic drugs should be guided by seizure aetiology, age-related patient characteristics, comorbid conditions, potential drug-drug interactions, the presence of hepatic or renal impairment, and – when available – the results of pharmacogenetic testing. For example, in seizures associated with structural brain pathology (including ischaemic stroke, traumatic brain injury, tumours, or congenital

malformations), the use of agents with favourable blood-brain barrier penetration and central nervous system bioavailability, such as levetiracetam, lamotrigine, or topiramate, is considered appropriate In cases of acute symptomatic seizures (e.g. hypoglycaemia, hyponatraemia, or encephalitis), the prompt administration of benzodiazepines (lorazepam or midazolam), either intravenously or intranasally, is recommended to achieve rapid seizure termination. In pharmacoresistant epilepsy or refractory status epilepticus, alternative therapeutic approaches are warranted. These include continuous infusions of propofol or thiopental under strict monitoring of vital parameters, implementation of a ketogenic diet (particularly within paediatric practice), neurosurgical interventions (such as focal cortical resection), or the implantation of vagus nerve stimulation devices. The field of personalised medicine has undergone substantial development, with increasing attention to genetic polymorphisms (e.g. SCN1A, KCNQ2, GABRA1), which may assist in predicting drug responsiveness and reducing the risk of adverse drug reactions. Systematic monitoring of antiseizure therapy effectiveness is a critical component of high-quality patient care. This process encompasses not only clinical evaluation of seizure frequency, duration, and semiology, but also assessment of treatment tolerability, psycho-emotional status, and cognitive function. The use of video-EEG monitoring facilitates the detection of both clinically apparent and subclinical epileptic activity, enables objective evaluation of therapeutic response, and assists in identifying potential adverse effects, including behavioural changes, sedation, cognitive impairment, or hepatotoxicity. Dose adjustments should be informed by pharmacokinetic parameters – half-life, bioavailability, plasma protein binding, and individual metabolic characteristics. In patients with hepatic or renal dysfunction, therapeutic drug monitoring in serum is recommended to prevent drug accumulation and toxicity. Overall, effective seizure management following acute brain injury relies on an integrated, individualised strategy combining early diagnosis, continuous EEG surveillance, and aetiology-driven selection of antiseizure therapies (Table 2).

Table 2. Recommendations for optimising seizure management

Area	Key recommendation	Details/examples
Early detection and diagnosis	Ensure prompt seizure identification	Clinical observation, Glasgow Coma Scale assessment, CT/MRI, and EEG for detection of subclinical seizures and non-convulsive status epilepticus.
EEG monitoring	Central diagnostic modality	Identification of epileptiform discharges and subclinical activity; supports early initiation of targeted therapy.
Individualised therapy	Tailor treatment to patient-specific factors	Consider seizure aetiology, age, comorbidities, drug interactions, hepatic/renal function, and pharmacogenetic data.
Drug selection by aetiology	Align drug properties with the seizure cause	Structural lesions: lipophilic agents such as levetiracetam, lamotrigine or topiramate.
Acute symptomatic seizures	Use rapid-acting agents in emergency situations	In hypoglycaemia, hyponatraemia, or encephalitis: administer intravenous or intranasal benzodiazepines (lorazepam, midazolam).
Refractory epilepsy/ status epilepticus	Escalate to advanced therapeutic options	Use propofol or thiopental under intensive monitoring; consider a ketogenic diet, surgical resection, or vagus nerve stimulation.
Personalised medicine	Integrate genetic testing into clinical decision-making	Genes such as SCN1A, KCNQ2, and GABRA1 influence drug efficacy and the risk of adverse effects.
Therapeutic monitoring	Ensure continuous assessment of treatment effectiveness	Monitor seizure frequency and duration, treatment tolerability, cognitive function, and psychological status.
Video-EEG monitoring	Gold standard for longitudinal assessment	Captures both clinical and subclinical seizures and facilitates evaluation of drug effectiveness and adverse effects (e.g. sedation, behavioural changes).
Dose adjustment	Adjust dosing based on pharmacokinetic principles	Consider half-life, bioavailability, plasma protein binding, hepatic and renal function, and perform therapeutic drug monitoring when indicated.

Source: prepared by the authors during the course of the research

Current approaches focus on individualising therapeutic interventions, taking into account the patient's metabolic, pharmacokinetic, and genetic characteristics, with advanced strategies such as ketogenic metabolic modulation, targeted neurosurgical interventions, and vagus nerve neurostimulation playing a key role in the management of refractory cases. Continuous evaluation of network biomarkers, treatment response, and adaptive dose adjustment enhances both safety and therapeutic precision. Collectively, these elements constitute a next-generation, evidence-based framework aimed not merely at seizure suppression, but at modulating pathological network dynamics to improve long-term neurological outcomes and minimise chronic complications.

Conclusions

This comprehensive review of 54 scientific sources demonstrated that seizure syndrome represents a frequent and clinically significant complication of acute brain injury, occurring in 15%-30% of stroke patients and 20%-40% of individuals with traumatic brain injury. The findings consistently indicated that early seizures, particularly those occurring within the first seven days, serve as strong predictors of unfavourable clinical outcomes, including increased mortality, greater functional disability, and prolonged intensive care unit stays. Across the reviewed literature, cortical involvement, intracerebral haemorrhage, and severe neurological impairment (e.g. low Glasgow Coma Scale

scores) repeatedly emerged as the most prominent risk factors. Neuroinflammation, excitatory-inhibitory neurotransmitter imbalance, cellular energy failure, and cerebral oedema constitute the core pathophysiological mechanisms underlying seizure development in both stroke and TBI populations. The analysis further confirmed that levetiracetam and lorazepam represent the most effective and safest antiseizure medications in acute care settings, owing to their favourable pharmacokinetic profiles and low systemic toxicity. Evidence also highlighted the critical importance of continuous EEG monitoring for detecting subclinical seizure activity, guiding therapeutic adjustments, and preventing secondary neurological deterioration.

Optimisation of treatment was shown to depend strongly on personalised management strategies that take into account comorbidities, drug-drug interactions, renal and hepatic function, and the aetiological subtype of brain injury. Moreover, non-pharmacological interventions, including neurostimulation, ketogenic dietary therapy, and surgical approaches, play an important role in the management of refractory seizure syndromes, although their application remains limited to carefully selected patient populations. A key conclusion of this review is that early identification and risk stratification of vulnerable patients significantly improve clinical outcomes by enabling the timely initiation of targeted therapy. The findings further underscore the need for unified clinical protocols in intensive care settings to standardise management, enhance diagnostic accuracy, and reduce variability in treatment outcomes across healthcare systems. Future research should prioritise the advancement of precision-medicine approaches and the development of reliable biomarkers for seizure prediction and therapeutic response assessment. In addition, long-term prospective studies are required to elucidate the cognitive and functional consequences of seizure syndrome following stroke and traumatic brain injury.

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Conflict of Interest

No financial or personal relationships are declared that could potentially influence the objectivity or integrity of this research.

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Судомний синдром при інсульті та черепно-мозковій травмі: частота і результати лікування у відділенні інтенсивної терапії

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Анотація. Судомний синдром є однією з найпоширеніших і найскладніших неврологічних ускладнень у пацієнтів з гострими ушкодженнями головного мозку, зокрема після інсульту та черепно-мозкової травми. Це ускладнення суттєво погіршує клінічні результати, підвищує ризик смертності та подовжує перебування пацієнтів у відділенні інтенсивної терапії. Метою дослідження було визначити частоту судомного синдрому у пацієнтів з інсультом і черепно-мозковою травмою, проаналізувати основні причини його розвитку та оцінити ефективність і безпечність протисудомної терапії для вдосконалення підходів до лікування й поліпшення клінічних результатів. Проведено комплексний огляд літератури з використанням 50 релевантних наукових джерел із баз даних PubMed, Scopus, Web of Science та Google Scholar. Відбір здійснювався згідно з принципами PRISMA і включав дослідження з чітко визначеними популяціями пацієнтів з інсультом або черепно-мозковою травмою, даними щодо частоти судом, використанням протисудомних препаратів та статистично опрацьованими результатами, опублікованими в рецензованих виданнях. Опубліковані дослідження свідчили, що судомний синдром виникає приблизно у 15-30 % пацієнтів після інсульту та у 20-40 % осіб із черепно-мозковою травмою. Ранні судоми (протягом перших 7 днів) стабільно реєструються приблизно у 7 % пацієнтів після інсульту і асоціюються з гіршим прогнозом. Огляд літератури описав мультифакторні патофізіологічні механізми, що включають первинне структурне ушкодження мозкової тканини, вторинні метаболічні порушення, нейрозапалення та дисбаланс нейромедіаторних систем. Дані численних досліджень демонстрували високу ефективність сучасної протисудомної терапії – зокрема леветирацетаму та лоразепаму – на рівні 70-85 % із сприятливим профілем безпечності. Зв'язування леветирацетаму з білком синаптичних везикул 2A часто називають ключовим механізмом контролю судом, тоді як лоразепам залишається препаратом першої лінії для невідкладного купірування судом завдяки посиленню ГАМК-ергічної нейротрансмісії

Ключові слова: протисудомна терапія; леветирацетам; лоразепам; неврологічні ускладнення; запалення

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Combined therapy for intestinal dysbiosis as a strategy for the treatment of lipid metabolism disorders

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Abstract. Dyslipidaemia, a major contributor to cardiovascular diseases, necessitates multifaceted treatment strategies. This study aimed to evaluate the effectiveness of combined therapy – including probiotics, lifestyle modifications, and dietary recommendations – in managing dyslipidaemia and its impact on gut microbiota composition. A total of 168 patients with dyslipidaemia were enrolled and categorised according to cardiovascular risk using the SCORE scale. Group 1 included low-risk patients, while Group 2 comprised moderate-risk patients who also received simvastatin. Both groups were prescribed a 12-week probiotic regimen containing *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12, alongside recommendations for physical activity and cholesterollowering diets. Comprehensive assessments of lipid profiles and gut microbiota composition were conducted before and after treatment. The results demonstrated significant improvements in lipid profiles in both groups. Total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels decreased, while high-density lipoprotein cholesterol levels increased. Statistical analysis showed that the differences in the percentage reductions of lipid profile parameters between the two groups were not statistically significant ($p > 0.05$), confirming the potential impact of probiotic therapy on lipid levels. Analysis of the gut microbiota revealed an increase in Bacteroidetes, and reductions in Firmicutes and Actinobacteria, along with a notable improvement in the Firmicutes/Bacteroidetes ratio, indicating restored microbial balance. This study highlighted the potential of probiotics as an effective adjunct in the management of dyslipidaemia, capable of complementing – or even reducing dependence on – statin therapy. The findings support the integration of microbiota-targeted therapies into personalised treatment strategies for dyslipidaemia

Keywords: microbial composition; dyslipidaemia; probiotics; *Bifidobacterium lactis* BB-12; *Lactobacillus acidophilus* LA-5

Introduction

The rising prevalence of metabolic disorders – particularly dyslipidaemia – poses a significant public health challenge worldwide. Alterations in the gut microbiota have emerged as a critical factor influencing lipid metabolism. An imbalanced intestinal microbial community may trigger inflammatory processes and disrupt the regulation of cholesterol and triglycerides, thereby increasing the risk of cardiovascular diseases. In this context, exploring novel therapeutic approaches – including probiotic interventions to restore microbial equilibrium and improve lipid profiles – has become increasingly important. Addressing these issues is essential not only for advancing scientific understanding but also for developing effective strategies to reduce the burden of metabolic diseases.

Data from the study by E. Pavlidou *et al.* [1] demonstrated that gut microbiota composition plays a crucial role in cardiovascular health, particularly through its influence on lipid metabolism and inflammatory responses. The review of clinical evidence indicated that specific probiotic and prebiotic interventions may reduce serum cholesterol

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levels, lower blood pressure, and exert antiinflammatory effects. These results support the potential of microbiota-targeted therapies as a promising strategy for the prevention and management of cardiovascular disease. This is also consistent with the findings of the review conducted by A. Oniszczuk *et al*. [2], who emphasised that modulation of the gut microbiota through the use of probiotics and prebiotics may support cardiovascular health by regulating blood lipids, reducing inflammation, and improving endothelial function.

Findings from the research conducted by K. Banach *et al*. [3] demonstrated that the consumption of probiotic yoghurt enriched with *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12 could improve lipid profiles in obese individuals. In their randomised controlled trial, the authors reported significant reductions in total cholesterol and improvements in the LDL/HDL ratio when the probiotic product was administered under an energy-restricted diet. Their detailed evaluation highlighted that both the dosage and duration of probiotic administration were critical factors influencing the observed metabolic benefits.

Results from the investigation by P. Markowiak-Kopeć & K. Śliżewska [4] revealed that probiotics play a crucial role in enhancing the production of short-chain fatty acids (SCFAs). The study's data showed that increased SCFA production was closely associated with reduced inflammatory responses and improved regulation of lipid metabolism. According to the authors, this mechanism may represent a key pathway through which probiotics alleviate dyslipidaemia, thereby providing a foundation for the development of probiotic-based metabolic therapies.

In the study by S.A. Palaniyandi *et al*. [5], the authors reported that specific cholesterollowering *Lactobacillus fermentum* strains exhibit promising probiotic characteristics *in vitro*. Their research provided evidence that these probiotics can directly influence cholesterol metabolism, suggesting a potential role for such microorganisms in reducing cholesterol levels *in vivo*. Data from A. Khare & S. Gaur [6] further underscored the cholesterol-lowering effects of various *Lactobacillus* species, demonstrating that these bacteria can contribute to improved lipid profiles by modulating bile acid metabolism and enhancing lipid excretion. Their comprehensive analysis highlighted the potential of probiotic interventions to restore metabolic homeostasis [7].

Evidence obtained by S. Wongrattanapipat *et al*. [8] highlighted the complexity of probiotic effects on metabolism by selecting potential probiotics with cholesterol-lowering properties for yoghurt production. Their *in vitro* assays identified strains capable of significantly reducing cholesterol levels, thereby supporting the concept that probiotic supplementation can be an effective tool in managing dyslipidaemia. Despite some promising results, certain findings remain contested. For instance, in a double-blind, randomised, placebo-controlled clinical trial, M. Noori *et al*. [9] demonstrated that although probiotic-enriched kefir containing *Lactobacillus helveticus* and *Bifidobacterium longum* did not significantly alter lipid profiles in elderly individuals over an eightweek period, it resulted in notable improvements in the atherogenic plasma index and Castelli's risk index.

Thus, more detailed studies are needed to determine the most effective strategies for incorporating these probiotic strains into treatments aimed at improving lipid metabolism. This study aimed to investigate the potential impact of specific probiotic strains on lipid metabolism in patients with dyslipidaemia.

Materials and Methods

The study included 168 patients diagnosed with dyslipidaemia types IIa, IIb, and IV according to the Fredrickson classification, who were either hospitalised or treated on an outpatient basis. This study did not account for the specific types of dyslipidaemia when assessing treatment outcomes but instead focused on the overall dynamics of lipid profile reduction across the combined patient group. All patients received care in the therapeutic department of Saint Pantaleon Hospital under the First Territorial Medical Association of Lviv, the therapeutic department of Agency Truskavetskurort LLC, or outpatient clinics No. 1 and No. 2 of the Intersono private medical centre, which also serve as clinical bases for the Department of Therapy No. 1, Medical Diagnostics, Hematology, and Transfusiology at Danylo Halytsky Lviv National Medical University. The study Group comprised 75 men and 93 women, aged 21 to 69 years, with a mean age of 45.03 ± 2.67 years.

All participants were informed about the protection of their anonymity, and the intended use of their data, and provided informed consent for its use in scientific research. This information was presented in accordance with current ethical standards and regulations governing medical research involving human subjects. The study was conducted in accordance with the main provisions of the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects [10], the Council of Europe's Convention on Human Rights and Biomedicine [11], and Order No. 690 of the Ministry of Health of Ukraine [12]. The inclusion criteria for participation were: a confirmed diagnosis of dyslipidaemia based on clinical, laboratory, and instrumental methods; and the patient's signed informed consent to participate in the study.

Patient assessments were conducted using a range of methods. General clinical methods included the evaluation of medical history, collection of anamnesis, and general physical examination, including the measurement of anthropometric parameters. Biochemical methods were used to assess total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) levels before and after the prescribed therapy. These analyses were performed using enzymatic colourimetric methods with reagent kits supplied by Human GmbH (Germany), on a BioChem

FC-360 analyser (High Technology Inc., USA). Instrumental methods included ultrasound examination of the abdominal organs and the head and neck vessels, performed using the LOGIQ P10 XDclear device. Bacteriological methods involved quantitative real-time polymerase chain reaction (qRT-PCR) analysis of the intestinal microbiota using primers targeting the 16S rRNA gene, specific for Firmicutes, Actinobacteria, and Bacteroidetes, as well as universal primers. The procedure was carried out using a Rotor-Gene 6000 (QIAGEN, Germany). Given the current absence (as of 20232024) of universally established normal ranges for bacterial phyla – due to variability influenced by the region of residence, dietary patterns, and individual habits – this study included 45 practically healthy volunteers aged 18 to 59 years, to assess the typical microbiota composition in the region of study (Lviv) (Table 1).

Table 1. Microbiota composition in healthy volunteers (n = 45)

	M ± m
Bacteroidetes, %	43.8 ± 1.4
Firmicutes, %	35.26 ± 0.89
Actinobacteria, %	7.38 ± 0.41
Others, %	14.22 ± 0.11
Firmicutes/Bacteroidetes ratio	3.55 ± 0.4

Source: compiled by the author

A total of 168 patients with dyslipidaemia were divided into two subgroups based on individual cardiovascular risk, calculated using the SCORE scale [13] to estimate the 10-year risk of fatal cardiovascular events and stratified according to LDL cholesterol levels as follows: <2.6 mmol/L, 2.6 to <3.0 mmol/L, and ≥3.0 mmol/L. The classification was performed in accordance with the guidelines of the European Society of Cardiology and the European Atherosclerosis Society [14], as well as the Recommendations for the Diagnosis and Treatment of Dyslipidaemia issued by the Ukrainian Association of Cardiologists [15]. In Group 1, LDL cholesterol levels were up to 3.0 mmol/L, and patients were categorised as having low or moderate risk according to the SCORE scale. No atherosclerotic changes were detected in the carotid arteries based on ultrasound findings. Group 2 comprised patients with LDL cholesterol levels exceeding 3.0 mmol/L, moderate SCORE-based cardiovascular risk, and/or atherosclerotic changes observed on carotid ultrasound. These patients were prescribed simvastatin at a dose of 10 mg once daily (in the evening), as recommended by a cardiologist. The study design did not include a comparison Group receiving statin therapy without concomitant probiotic supplementation.

Both groups received lifestyle modification recommendations, including engaging in 150 minutes of moderate-intensity physical activity per week, spread over 3 to 5 sessions, incorporating both aerobic and strength training. In addition, dietary guidance aimed at lowering cholesterol levels was provided. Therapy for correcting gut microbiota was prescribed in both groups. This included administration of a probiotic containing *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12 at a total concentration of 2×10^9 CFU, with 1×10^9 CFU per capsule, at a dosage of two capsules per day, for 12 weeks. Patients who failed to adhere to the prescribed medication regimen or who were unable to continue treatment due to unforeseen circumstances or newly developed comorbidities were excluded from the study. As a result, the final number of patients who completed the course of treatment was 112: Group 1 (n = 65) – patients with dyslipidaemia classified as lowrisk according to the SCORE scale; Group 2 (n = 47) – patients with dyslipidaemia classified as moderaterisk according to the SCORE scale. Data were analysed using Statistica 11.0 for Windows. Results are presented as mean ± standard error (M ± m). Student's t-test was used for comparing means, with significance defined as $p < 0.05$. Non-parametric methods were employed for variables that did not follow a normal distribution. Correlation analysis was conducted to explore relationships among parameters.

Results

Firstly, the composition of the gut microbiota was assessed in patients with dyslipidaemia and a control Group comprising individuals without lipid metabolism disorders (Table 2).

Table 2. Gut microbiota composition in patients with dyslipidaemia (n = 168) and the control Group (n = 86)

	Dyslipidaemia group (n = 168)	Control group (n = 86)	p-value
Bacteroidetes	35.17 ± 1.36	45.66 ± 1.18	<0.05
Firmicutes	45.26 ± 2.34	33.93 ± 1.63	<0.05
Actinobacteria	12.37 ± 0.39	8.9 ± 1.5	<0.05
Firmicutes/ Bacteroidetes ratio	3.52 ± 0.4	4.57 ± 1.48	>0.05

Note: $p < 0.05$ – statistically significant
Source: compiled by the author

Based on the obtained data, a significant difference was observed in the levels of nearly all phylotypes identified in the microbiota of patients with dyslipidaemia compared to those without. However, an important observation was that

the indicators among individuals without dyslipidaemia showed minimal variation from those of healthy volunteers, who served as the control sample. The gut microbiota composition was also analysed in both patient groups, categorised according to their SCORE risk levels, who were subsequently prescribed probiotic therapy (Table 3).

Table 3. Gut microbiota composition in patients with dyslipidaemia, grouped by SCORE risk classification

	Group 1 (n = 65)	Group 2 (n = 47)
Bacteroidetes	38.5 ± 0.78	35.01 ± 1.19
Firmicutes	42.14 ± 1.67	46.05 ± 0.89
Actinobacteria	10.76 ± 1.49	12.95 ± 0.81
Firmicutes/ Bacteroidetes ratio	3.8 ± 0.51	2.9 ± 1.65

Source: compiled by the authors

After 12 weeks of treatment with the probiotics *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12, the gut microbiota composition was re-assessed in both groups of patients (Figs. 1, 2).

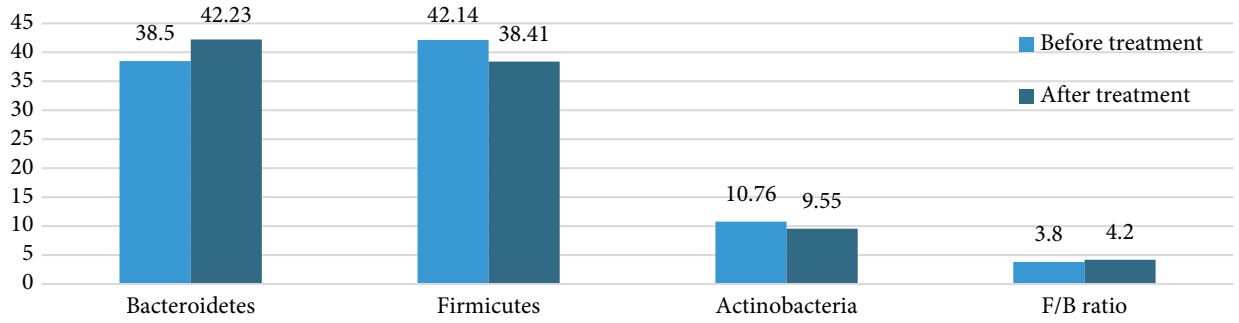

Figure 1. Gut microbiota composition in Group 1 patients with dyslipidaemia before and after combined therapy
Source: compiled by the author

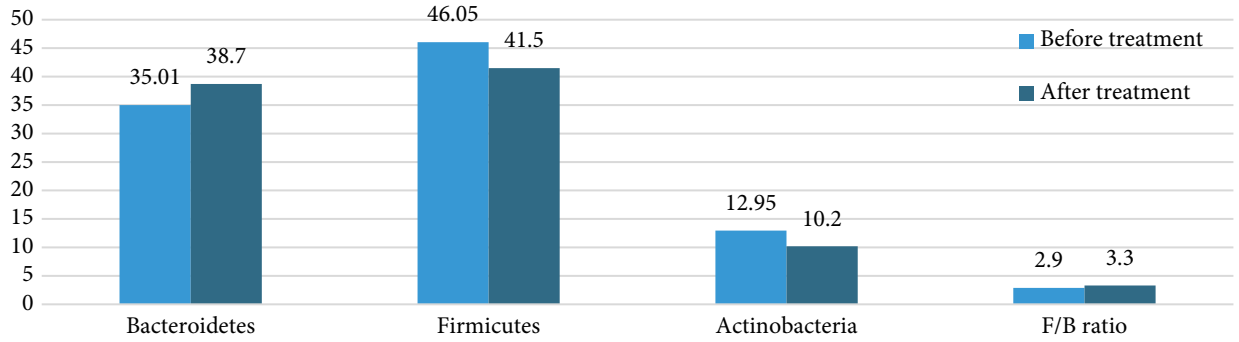

Figure 2. Gut microbiota composition in Group 2 patients with dyslipidaemia before and after combined therapy
Source: compiled by the author

As shown in Figure 1, patients in Group 1 demonstrated an increase in Bacteroidetes levels, a decrease in Firmicutes and Actinobacteria, and a shift in the Firmicutes/Bacteroidetes ratio during probiotic administration. In Group 2, an increase in Bacteroidetes was also observed, accompanied by a marked decrease in Firmicutes and Actinobacteria, as well as corresponding changes in the Firmicutes/Bacteroidetes ratio (Fig. 2). Since the primary aim of the study was to explore the interrelationship and potential influence of gut microbiota modulation on the development of dyslipidaemia, it was essential to evaluate lipid profile parameters both before and after treatment (Tables 4, 5).

Table 4. Lipid profile parameters in Group 1 patients with dyslipidaemia before and after combined treatment

Group 1	Before treatment (n = 65)	After treatment (n = 65)	p-value
TC, mmol/L	6.4 ± 0.22	5.4 ± 0.28	<0.05
TG, mmol/L	1.6 ± 0.34	1.4 ± 0.12	>0.05
HDL-C, mmol/L	1.36 ± 0.04	1.72 ± 0.02	<0.05

Continued Table 4

Group 1	Before treatment (n = 65)	After treatment (n = 65)	p-value
LDL-C, mmol/L	3.88 ± 0.19	2.8 ± 0.23	<0.05
VLDL, mmol/L	0.9 ± 0.28	0.77 ± 0.07	>0.05

Note: $p < 0.05$ – statistically significant
Source: compiled by the author

Table 5. Lipid profile parameters in Group 2 patients with dyslipidaemia before and after combined treatment

Group 2	Before treatment (n = 47)	After treatment (n = 47)	p-value
TC, mmol/L	6.8 ± 0.35	5.2 ± 0.08	<0.05
TG, mmol/L	2.3 ± 0.36	1.6 ± 0.16	<0.05
HDL-C, mmol/L	1.25 ± 0.21	1.68 ± 0.56	>0.05
LDL-C, mmol/L	3.99 ± 0.18	3.1 ± 0.23	>0.05
VLDL, mmol/L	1.09 ± 0.16	1.01 ± 0.07	>0.05

Note: $p < 0.05$ – statistically significant
Source: compiled by the author

The assessment of probiotic therapy, combined with lifestyle modifications and dietary recommendations, significantly improved TC levels in Group 1 patients, from 6.4 ± 0.22 to 5.4 ± 0.28 mmol/L. A statistically significant reduction in LDL-C was also observed following treatment. Furthermore, HDL-C levels increased by 26%. According to Table 5, the mean total cholesterol level in Group 2 decreased significantly after combined therapy, from 6.8 ± 0.35 mmol/L to 5.2 ± 0.08 mmol/L ($p < 0.05$). The TG were also nearly halved, decreasing from 2.3 ± 0.36 mmol/L to 1.6 ± 0.16 mmol/L post-treatment. A statistically significant increase in HDL-C levels was recorded, rising from 1.25 ± 0.21 mmol/L to 1.68 ± 0.56 mmol/L ($p < 0.05$). In light of these findings, the percentage changes in lipid profile parameters across both groups were evaluated (Figs. 3, 4).

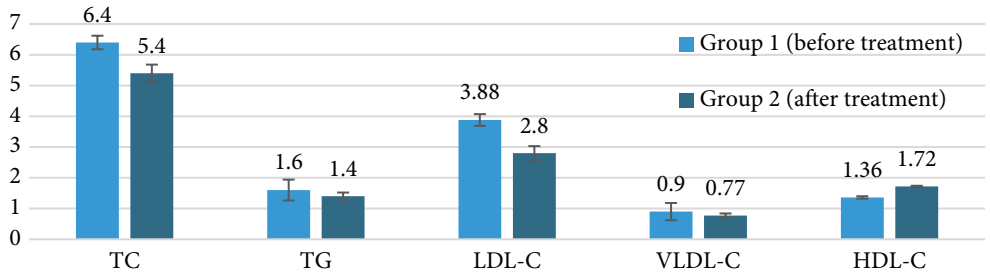

Figure 3. Lipid profile changes after combined therapy in Group 1 patients with dyslipidaemia
Source: compiled by the author

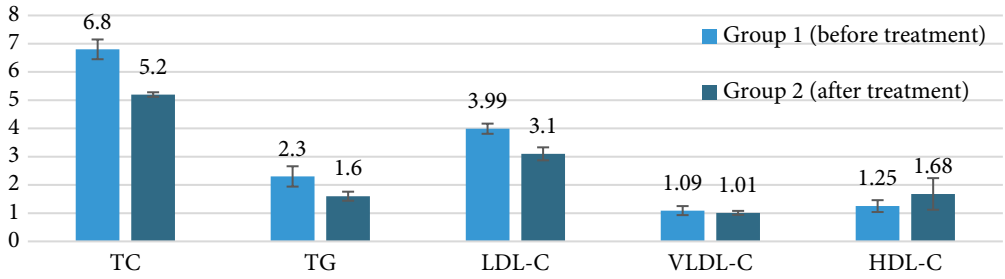

Figure 4. Lipid profile changes after combined therapy in Group 2 patients with dyslipidaemia
Source: compiled by the author

When comparing the lipid profile dynamics presented in Figures 3 and 4, statistical analysis revealed that, despite the addition of simvastatin to probiotic therapy in Group 2, the percentage changes in lipid parameters did not differ significantly between the two groups ($p > 0.05$). In Group 1, TC decreased by 15.6% (from 6.4 ± 0.22 to 5.4 ± 0.28 mmol/L, $p < 0.05$), and LDL-C decreased by 27.8% (from 3.88 ± 0.19 to 2.8 ± 0.23 mmol/L, $p < 0.05$). In Group 2, TC was reduced by 23.5% (from 6.8 ± 0.35 to 5.2 ± 0.08 mmol/L, $p < 0.05$), and LDL-C by 22.3% (from 3.99 ± 0.18 to 3.1 ± 0.23 mmol/L, $p > 0.05$). Although the absolute percentage reductions in TC and LDL-C appeared somewhat greater in Group 2, inter-Group comparisons did not yield statistically significant differences ($p > 0.05$ for

both parameters). Additionally, HDL-C levels increased by 26.5% in Group 1 (from 1.36 ± 0.04 to 1.72 ± 0.02 mmol/L, $p < 0.05$) and by 34.4% in Group 2 (from 1.25 ± 0.21 to 1.68 ± 0.56 mmol/L, $p > 0.05$), with no significant difference observed between groups ($p > 0.05$).

Discussion

The results of this study provide strong evidence supporting the effectiveness of combined therapy – consisting of probiotic supplementation with *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12, alongside lifestyle modifications and dietary recommendations – in improving lipid metabolism in patients with dyslipidaemia. Notably, the administration of these probiotic strains resulted in significant improvements in TC, LDL-C, and TG levels, while also inducing a marked increase in HDL-C. Importantly, these benefits were observed in both low- and moderate-risk groups, as classified by the SCORE scale, indicating that probiotic therapy may exert a direct effect on lipid metabolism irrespective of concurrent pharmacological interventions.

S. Zhou *et al.* [16] provided compelling evidence linking gut microbial metabolism with variations in circulating non-HDL cholesterol levels, thereby reinforcing the concept that modifications in microbiota can lead to measurable improvements in lipid profiles. In a complementary approach, C. Yan *et al.* [17] investigated the interplay between gut microbiota, systemic inflammation, and LDL-C using multiomics techniques, suggesting that inflammatory mediators may partly account for the benefits observed with probiotic interventions. The underlying mechanisms indicate that improvements in lipid parameters may result from both direct microbial modulation and indirect anti-inflammatory effects.

B. Flaig *et al.* [18] reviewed the potential of targeted gut microbiota therapy as an innovative strategy for managing dyslipidaemia. Their conclusions align with the present findings, particularly the observation that percentage reductions in lipid parameters were comparable between patients receiving probiotics alone and those receiving a combination of probiotics and simvastatin. This suggests that, for certain patients, probiotics might complement – or even reduce the need for – conventional statin therapy.

Further supporting the link between gut microbial activity and cardiovascular risk, M. Canyelles *et al.* [19] examined the role of microbiota-derived metabolites, such as TMAO, in promoting atherosclerotic disease. Although the present study did not directly measure TMAO levels, the observed improvements in lipid profiles and favourable shifts in microbial composition may indirectly contribute to a reduced cardiovascular risk by altering the profile of microbial metabolites. E.M. Brown *et al.* [20] further reviewed how the gut microbiota influences lipid metabolism and host physiology, reinforcing the concept that microbiome-targeted interventions can yield systemic benefits extending beyond lipid regulation alone.

Regarding improvements in lipid profiles, the data showed that both study groups experienced reductions in TC, LDL-C, and TG, alongside an increase in HDL-C. Notably, although Group 2 received simvastatin in addition to probiotics, the percentage reductions in lipid parameters were similar to those in Group 1, which received probiotic therapy alone. This finding suggests that probiotics may exert a direct and robust effect on lipid metabolism, potentially reducing reliance on statins in selected patient populations. The substantial increase in HDL-C and reduction in LDL-C in both groups underscore the potential of these probiotic strains to enhance cardiovascular health – likely through mechanisms involving bile acid metabolism modulation, enhanced lipid excretion, and the attenuation of systemic inflammation.

It is worth noting that J. Roessler *et al.* [21] demonstrated that modulation of the gut microbiota significantly influences both cholesterol and glucose metabolism, with potential implications for the prevention and management of atherosclerotic cardiovascular disease. Their findings further support the concept that microbiome-targeted interventions – such as probiotic supplementation – can exert systemic metabolic benefits beyond lipid-lowering, thereby reinforcing the therapeutic potential of such strategies in patients with dyslipidaemia. Moreover, Y. Duan *et al.* [22] conducted a metaanalysis on the therapeutic effects of probiotic interventions in obese or overweight adolescents, demonstrating that probiotic supplementation can significantly improve metabolic parameters, including lipid profiles. Their findings suggest that modulating the gut microbiota plays a crucial role in enhancing cholesterol regulation, which aligns with the observation that probiotic therapy can favourably alter lipid metabolism.

In conclusion, the combination of probiotic therapy with lifestyle and dietary modifications presents a promising approach to the management of dyslipidaemia and associated cardiovascular risks. The findings of the present study, together with those of other researchers such as D.J. Kenny *et al.* [23], L. Lei *et al.* [24], and B.A. Kappel *et al.* [25] contribute to the growing body of evidence that targeting the gut microbiota can significantly impact lipid metabolism. This study also underscored the need for personalised treatment strategies that integrate both microbiometargeted therapies and traditional pharmacological interventions.

Conclusions

The results of this study demonstrated that combined therapy – including the use of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12, alongside lifestyle modifications and dietary adjustments – is an effective strategy for managing dyslipidaemia. This approach significantly improved the lipid profile of patients by reducing TC, LDL-C, and TG levels while increasing HDLC. These changes were accompanied by measurable alterations in the gut microbiota, with a notable increase in Bacteroidetes and reductions in Firmicutes and Actinobacteria, suggesting a strong interrelationship between gut microbiota composition and the regulation of lipid metabolism.

A key finding was that the improvements in lipid profile in the Group receiving both probiotics and simvastatin were comparable to those observed in the Group receiving probiotics alone, highlighting the potential for probiotics to complement – or even reduce the need for – pharmacological interventions in selected cases. This underscored the value of incorporating gut microbiome-targeted therapies into comprehensive dyslipidaemia management strategies. The study also revealed that, in patients with dyslipidaemia, the Firmicutes/Bacteroidetes ratio significantly improved following treatment, indicating restored microbial balance as a potential mechanism contributing to lipid metabolism correction. These findings were particularly relevant in regions with distinctive dietary patterns and microbiota characteristics, where tailored interventions could optimise health outcomes.

Future research should focus on exploring the long-term effects of probiotic therapy on cardiovascular health, identifying the most effective strains and dosages, and investigating potential synergies between probiotics and pharmacological treatments. Expanding such studies to include larger and more diverse populations will help to refine the therapeutic potential of gut microbiota modulation in dyslipidaemia. In conclusion, this study confirms the effectiveness of combined therapy in improving both lipid profiles and gut microbiota health, supporting its role as a cornerstone in personalised dyslipidaemia management strategies.

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Conflict of Interest
None.

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Комбінована терапія лікування дисбіозу кишківника як один зі шляхів корекції порушень ліпідного обміну

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Анотація. Дисліпідемія, яка є основним чинником розвитку серцево-судинних захворювань, вимагає багатокомпонентних підходів до лікування. Метою даного дослідження було оцінити ефективність комбінованої терапії, яка включає пробіотики, зміни способу життя та дієтичні рекомендації, у корекції дисліпідемії та її вплив на склад мікробіому кишківника. У дослідженні взяли участь 168 пацієнтів із дисліпідемією, яких розподілили на групи відповідно до рівня серцево-судинного ризику за шкалою SCORE. До першої групи увійшли пацієнти з низьким ризиком, тоді як друга група складалася з пацієнтів із помірним ризиком, які додатково отримували симвастатин. Обом групам було призначено 12-тижневий курс пробіотиків, що містив *Lactobacillus acidophilus* LA-5 та *Bifidobacterium lactis* BB-12, а також рекомендовано фізичну активність і дієту для зниження рівня холестерину. Перед і після лікування було проведено комплексну оцінку ліпідного профілю та складу мікробіому кишківника. Результати показали значне покращення ліпідного профілю в обох групах. Рівні загального холестерину, ліпопротеїнів низької щільності та тригліцеридів зменшилися, тоді як рівень ліпопротеїнів високої щільності зріс. Статистичний аналіз показав, що різниця у відсотковому зниженні параметрів ліпідограми між обома групами не була статистично значущою ($p > 0,05$), що підтверджує можливий вплив пробіотичної терапії на рівень показників ліпідограми. Аналіз мікробіому кишківника виявив збільшення рівня бактерій типу Bacteroidetes та зменшення рівня Firmicutes і Actinobacteria, а також суттєве покращення індексу Firmicutes/Bacteroidetes, що свідчить про відновлення мікробного балансу. Дане дослідження підтверджує ефективність пробіотиків як важливого додаткового методу лікування дисліпідемії, здатного доповнити або навіть зменшити необхідність у застосуванні статинів. Отримані результати підтримують інтеграцію терапій, спрямованих на мікробіом, у персоналізовані стратегії лікування дисліпідемії

Ключові слова: мікробіом; дисліпідемія; пробіотики; *Bifidobacterium lactis* BB-12; *Lactobacillus acidophilus* LA-5

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Situs inversus totalis with ileal neuroendocrine tumour: A diagnostic and surgical challenge

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Abstract. Small intestine neuroendocrine tumours are becoming increasingly widespread, despite being a relatively rare condition. While abdominal computed tomography scans during routine check-up often detect small intestine neuroendocrine tumours, many cases are still diagnosed unexpectedly during emergency surgery. This subject is relevant because two rare conditions, *situs inversus totalis* and intestinal malrotation, can also affect the abdominal region thus misleading the diagnosis. The purpose of this study was to highlight the case of an acute onset of small bowel obstruction caused by neuroendocrine tumour with concomitant presence of *situs inversus totalis*. *Situs inversus totalis* usually stays asymptomatic, being discovered by chance during imaging, or manifests itself early in neonatal period with obstructive features or in old age as acute intestinal obstruction. However, this case is unique as the coexistence of *situs inversus totalis*

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and small intestine neuroendocrine tumours causing acute intestinal obstruction has not been previously reported in medical literature. The patient was diagnosed with a case of *situs inversus totalis*; when evaluated for cause of recurrent intestinal obstruction, the patient developed a fresh episode of acute intestinal obstruction while check-up was still ongoing and had to be taken up for emergency exploratory laparotomy. Patient underwent segmental small bowel resection and stapled side-to-side anastomosis. The real cause for recurrent obstructions was neuroendocrine tumour and not preoperatively presumed *situs inversus totalis*. Despite significant advancements in the treatment and management of small intestine neuroendocrine tumours, which improved patient outcomes, diagnosing these tumours continues to be a substantial challenge. This study aids in keeping a broader mindset on practical grounds, while concluding cause for intestinal obstruction in cases of multiple presumptions

Keywords: intestinal obstruction; adenocarcinoma; malrotation; abdominal tuberculosis; small intestine; emergency surgery

Introduction

Situs inversus totalis (SIT) is an uncommon congenital anomaly characterised by the complete mirror-image reversal of thoracic and abdominal organs. While it may stay asymptomatic throughout life, the reversed anatomical orientation introduces substantial challenges when clinical intervention is necessary, particularly in emergency and oncological settings. Diagnostic modalities and operative procedures, which are largely standardised to normal anatomical configurations, must be reoriented in SIT cases, making surgical planning and intraoperative navigation more complex. These challenges become especially pronounced when SIT coexists with rare neoplasms such as ileal neuroendocrine tumours (NETs). Considering the low incidence of both conditions individually, their concurrent presence is exceedingly rare and poorly represented in clinical literature. As a result, clinicians often rely on sparse case reports and limited surgical experience, which increases the risk of diagnostic delays, misinterpretation of imaging, and intraoperative errors.

Several reports have emphasised the significant diagnostic and surgical challenges posed by SIT, particularly when coexisting with intra-abdominal pathologies. K. Ramavathu *et al.* [1] highlighted that SIT is often discovered incidentally during imaging for unrelated symptoms, with reversed organ orientation leading to misinterpretation on radiographs and delayed diagnoses. A. Said *et al.* [2] reinforced the value of clinical vigilance, describing a diabetic patient in whom SIT was only recognised during evaluation for hypovolemic shock, while G. Deshimo *et al.* [3] and J. Huss-Bawab & L. Szymanski [4] demonstrated how atypical presentations complicated emergency assessments and postnatal evaluations, especially in the presence of other congenital anomalies. S. Karki *et al.* [5] reported a case of SIT, incidentally diagnosed during imaging for urinary symptoms, highlighting diagnostic challenges due to its asymptomatic nature and emphasising the need for thorough imaging to avoid potential surgical or procedural errors stemming from unrecognised reversed anatomy.

Surgically, the condition demands considerable adaptation. A. Tofigh *et al.* [6] reported altered operative approaches in SIT patients with acute abdominal conditions, emphasising the need for modified techniques and extended operative time. K. Eitler *et al.* [7] further noted that laparoscopic procedures, including transplantation and endoscopic retrograde cholangiopancreatography, required customised preoperative planning and highlighted the role of genetic factors influencing laterality, reinforcing the complexity of operative interventions in SIT. Together, these findings underscored the critical need for heightened awareness, detailed imaging, and surgical preparedness when managing patients with SIT.

Despite growing documentation of SIT-related surgical difficulties, the literature is still sparse regarding standardised guidelines for oncologic management in such patients. Particularly underrepresented are studies focusing on ileal neuroendocrine tumours in the context of SIT. Neuroendocrine tumours themselves are rare and often slow-growing, frequently presenting with vague or nonspecific symptoms that further complicate diagnosis when the anatomy is reversed. The altered lymphatic drainage and vascular architecture in SIT patients add additional layers of complexity to oncologic staging, yet systematic studies exploring these variables are still limited. Thus, the purpose of this study was to present a rare case of ileal neuroendocrine tumour in a patient with SIT, emphasising the diagnostic dilemmas and surgical challenges encountered during clinical management.

Materials and Methods

This case report described the clinical management of a rare and complex presentation of SIT with an ileal neuroendocrine tumour at a tertiary care centre in Pune, Maharashtra, specifically within the Department of Surgery at the Armed Forces Medical College (AFMC). The patient, a middle-aged adult, presented with nonspecific symptoms including intermittent abdominal pain and vague gastrointestinal discomfort. Initial clinical evaluation was confounded by the altered anatomical layout characteristic of SIT, which significantly deviated from expected symptom localisation and clinical findings. This led to delays in establishing a definitive diagnosis. A comprehensive review was conducted, including the patient's medical history, findings from physical examination, results from laboratory investigations, imaging studies, intraoperative observations, and postoperative outcomes.

Non-invasive imaging methods such as abdominal ultrasonography and computed tomography (CT) were employed initially; however, interpretation was complicated by the mirror-image reversal of abdominal and thoracic organs. While CT scans eventually aided in identifying the mass, the diagnostic process was prolonged due to anatomical disorientation. Endoscopic and nuclear imaging modalities, such as somatostatin receptor scintigraphy (Octreoscan) and Ga-68 DOTATATE PET-CT, albeit ideal for early detection of neuroendocrine tumours, were not utilised in the early stages due to limited access, delayed referral, and the absence of clinical suspicion of a neuroendocrine neoplasm in a patient with atypical anatomical presentation. These factors, combined with non-specific symptoms and an initial focus on more widespread abdominal pathologies, contributed to the delayed diagnosis.

After establishing the diagnosis, surgical intervention was planned and conducted by the primary author of this report, who led a multidisciplinary surgical team familiar with anatomical variants. The surgical approach required careful preoperative planning and intraoperative navigation, accounting for reversed vascular and intestinal structures. Intraoperatively, the ileal mass was identified and resected with appropriate margins, followed by lymphadenectomy and meticulous anatomic orientation to prevent iatrogenic injury. The procedure was performed successfully without intraoperative complications. Data for this case report was collected retrospectively from the patient's medical records, surgical notes, histopathology reports, and radiological archives. To ensure completeness and contextual understanding, additional qualitative information was obtained through structured interviews with the patient and their immediate family members. These interviews provided crucial insights into the patient's symptom timeline, diagnostic delays, and psychosocial concerns.

Prior to the publication of this case, informed written consent was obtained from the patient, including permission to use relevant clinical, surgical, and imaging data, ensuring that patient identity stays confidential throughout the report. The study was conducted in strict accordance with the ethical standards of the Institutional Ethics Committee of the Armed Forces Medical College (AFMC), Pune, which follows guidelines aligned with national and international ethical norms for biomedical research. These standards emphasise respect for patient autonomy, non-maleficence, beneficence, and justice, and are consistent with the principles outlined in the Declaration of Helsinki [8], which governs ethical conduct in research involving human subjects. Patient confidentiality was rigorously maintained throughout the study. No identifying information was disclosed, and all clinical images, if included, were anonymised.

Results and Discussion

Female patient, aged 42, symptomatic with recurrent episodes of abdominal pain, vomiting, and constipation for past 2 months, August 2024-September 2024, presented to emergency department on 13 October 2024, with aggravation of symptoms since past 3 days and obstipation for 1 day. History of significant weight loss (10 kg over 2 months) was present. On examination, the patient was dehydrated and tachycardic; abdomen was distended with well-healed Pfannenstiel scar of lower segment caesarean section, 15 years prior; a globular soft mass (approx. 14×12 cm) felt on the left side of periumbilical region, clinically suggestive of clumped small bowel loops. Chest X-ray was suggestive of dextrocardia with gastric shadow towards left side. Contrast enhanced computed tomography (CECT) (Abdomen + Pelvis) revealed SIT with dilated jejunal and ileal loops reaching up to 4 cm in diameter. Focal wall thickening and enhancement was seen at transition point in distal ileum 20 cm from Ileo-caecal junction. Bowel loops distal to transition point were collapsed (Fig. 1).

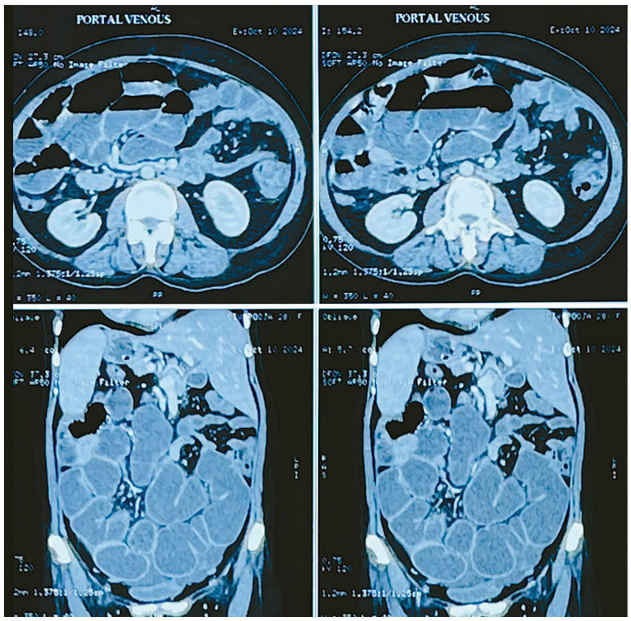

Figure 1. CECT showing dilated small bowel loops, liver in left hypochondrium, and spleen in right hypochondrium
Source: original photo by the authors of this study

Considering significant weight loss with recurrent episodes of abdominal pain, palpable soft abdominal mass, intestinal obstruction was suspected to be caused by abdominal tuberculosis. Patient was taken up for emergency exploratory laparotomy due to worsening clinical status and feculent nasogastric output. Intraoperative findings revealed extensive congenital adhesions throughout the abdomen with peritoneal cavity completely encased by thick fibrous tissue layer and dense interbowel adhesions between small and large bowel. Liver with gall bladder and caecum with appendix were visualised in left hypogastrium and left iliac fossa, respectively. Two tight strictures, causing complete obstruction, proximal to ICJ (ileocecal junction) at approximately 20 cm and 40 cm each, with multiple enlarged lymph nodes were visualised in the corresponding mesentery segment (Fig. 2).

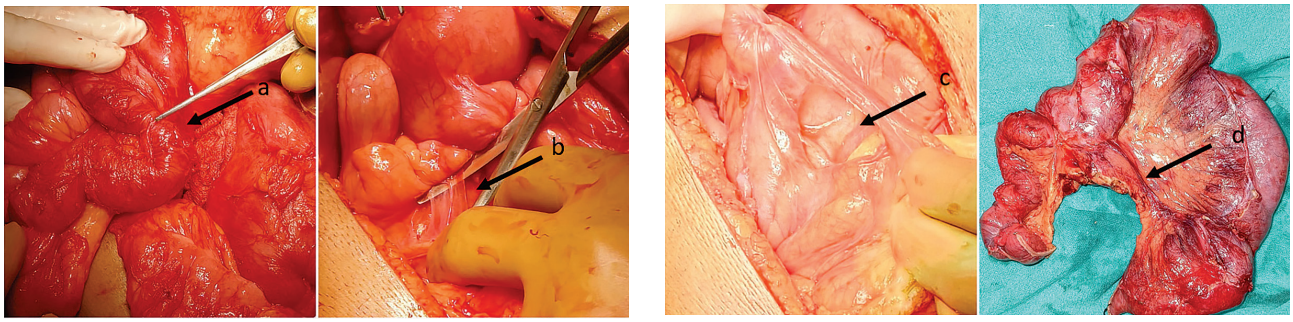

Figure 2. Intra op images
Note: a – strictured ileal segment; b – interbowel dense adhesions; c, d – ileal strictures and mesenteric lymphadenopathy
Source: original photo by the authors of this study

The patient was managed with adhesiolysis, resection of strictured ileal segment, and side-to-side stapled ileo-ileal anastomosis. Post-operatively, the patient recovered well. Histopathology examination report of resected ileal segment revealed well-differentiated NET, grade I (pT3N1) with positive lympho-vascular and perineural invasion. Synaptophysin and chromogranin were found to be positive. Tumour size was noted to be 1.5 cm. 1 Mitosis/2 mm^2 was also noted. Ki-67 proliferative index was <2%. 2/19 lymph nodes were found positive for the tumour (Fig. 3). Post operative Ga-68 DOTANOC PET-CT showed no focal uptake in post operative site. No abnormal Somatostatin receptor expression seen (Fig. 4).

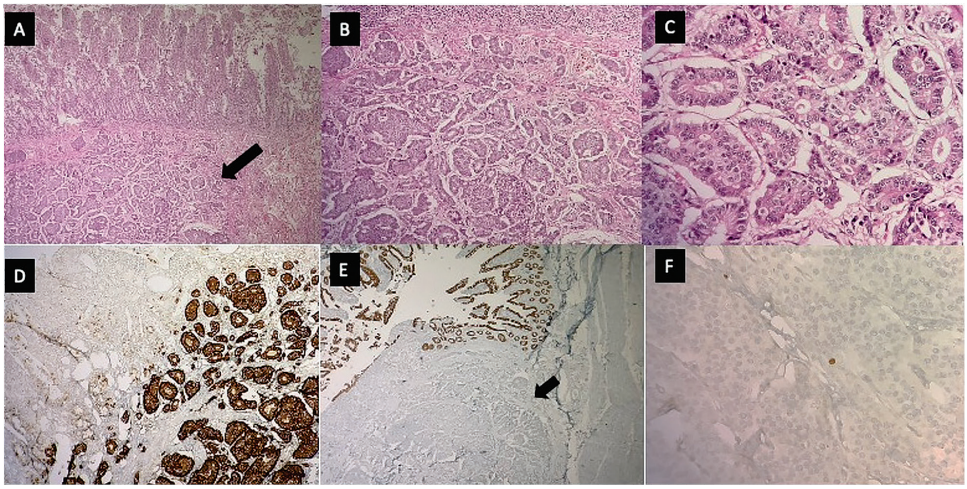

Figure 3. Photomicrographs showing tumour in submucosa (A, B), salt and pepper chromatic pattern (C), positive for synaptophysin (D), negative for CK20 (E), low Ki-67 index (F)
Source: original photo by the authors of this study

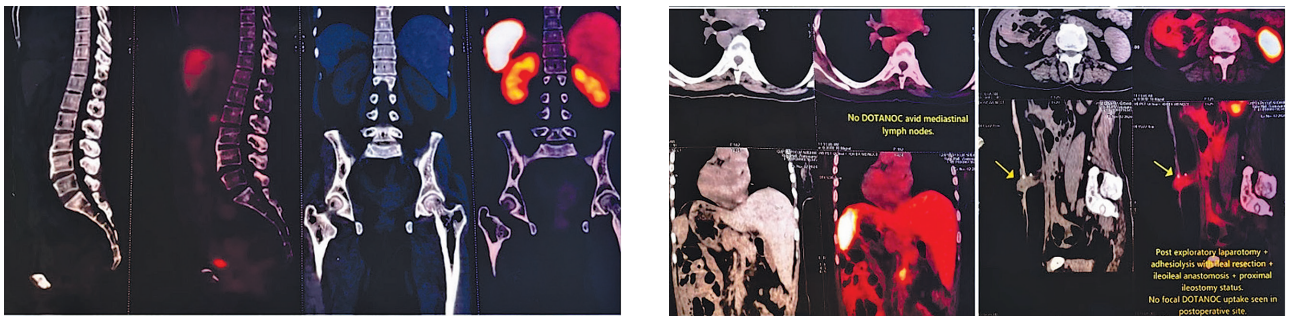

Figure 4. Postoperative Ga-68 DOTANOC PET-CT image showing no focal uptake or abnormal SSTR (somatostatin receptor) expression
Source: original photo by the authors of this study

Patient was discharged in a stable condition with no postoperative complications or requirement of adjuvant therapy. Small intestine neuroendocrine tumours (SI-NETs) are rare and slow-growing, often presenting diagnostic challenges due to nonspecific symptoms and limited anatomical access. Originating from Kulchitsky cells, which migrate from neural crest cells, SI-NETs can cause intestinal obstruction through peritumoural fibrosis, tumour invasion, or desmoplastic reactions, leading to bowel kinking, scarring, or ischemia. Prognostic factors for SI-NETs include age (<50 years), tumour size (<2 cm), duodenal location, TNM staging (T2/N0), and complete surgical resection. Mitotic activity and Ki-67 proliferation index are also reliable prognostic indicators. Tumour size significantly affects the frequency of metastatic disease at diagnosis. This case report of a 42-year-old female with SIT and SI-NETs causing small bowel obstruction underscores the unique diagnostic and surgical considerations involved, while reinforcing existing literature on the topic.

The clinical presentation of recurrent abdominal pain, vomiting, weight loss, and eventual intestinal obstruction in this case aligns with nonspecific symptoms commonly reported in SI-NET cases [9]. Weight loss, which is a frequent finding, was significant in present case similar to the 60-year-old male patient reported by L. Daraghmeh *et al.* [10], who experienced appetite loss and significant weight loss before diagnosis. Unlike most cases, patient's anatomical variation and SIT posed an additional diagnostic challenge, particularly when interpreting imaging studies. The diagnostic journey for SI-NETs often involves multiple imaging modalities and, occasionally, exploratory surgery when non-invasive methods fail to provide clarity. Here, CECT identified small bowel obstruction with ileal strictures but could not conclusively determine the underlying pathology. This is comparable with findings by C. Leal *et al.* [9], who emphasised the limited utility of non-invasive diagnostics in analogous cases, often necessitating surgical exploration. L. Daraghmeh *et al.* [10] noted that diagnosis was only confirmed postoperatively through biopsy and histopathological analysis.

Intraoperatively, the extensive adhesions and dense fibrosis encountered in the current case intra-operatively were suggestive of chronic inflammation, mimicking features of abdominal tuberculosis, as noted in other reports of obstructive SI-NETs [11-13]. The strictures and lymphadenopathy identified during surgery are typical for SI-NETs, as also described by H. Behi *et al.* [11], and highlight the need for meticulous surgical resection and lymph node dissection to achieve optimum disease control. Histopathological examination revealed a well-differentiated NET (Grade I) with lympho-vascular and perineural invasion. This aligns with the report by M. Basendowah *et al.* [13] of analogous histological features in patients with intestinal obstruction caused by jejuno-ileal NETs. The low Ki-67 index (<2%) observed in this case also corroborates findings from most well-differentiated NET cases, which generally exhibit low proliferative activity and better prognostic outcomes.

Postoperative management of SI-NETs often involves functional imaging and biochemical monitoring to rule out residual or metastatic disease. The Ga-68 DOTANOC PET-CT revealed no abnormal SSTR expression, indicating the absence of active disease. This imaging modality is critical for staging and surveillance in SI-NET patients, as highlighted in reviews by H. Behi *et al.* [11] and E. Kaçmaz *et al.* [12], who emphasised its sensitivity for detecting recurrence and metastases. Surgery continues to be the primary treatment for SI-NETs, particularly in cases with obstructive symptoms. The side-to-side ileo-ileal anastomosis performed intra-operatively is consistent with established surgical approaches, which have been shown to result in low complications and favourable outcomes for well-differentiated tumours [13]. However, as demonstrated by E. Kaçmaz *et al.* [12], outcomes can vary with hospital volume and surgical expertise, reinforcing the significance of treatment in specialised centres.

This case highlighted the value of considering SI-NETs as a potential diagnosis in patients presenting with recurrent unexplained abdominal pain, weight loss, or intestinal obstruction, regardless of age or unusual presentations, such as SIT. While studies like those by C. Leal *et al.* [9] and L. Daraghmeh *et al.* [10] predominantly report cases in older adults, the relatively younger age of patient in present case emphasised the broad age spectrum of SNET presentation. Several case reports have described small bowel NETs with varied clinical presentations, findings, and histopathological evaluations. C. Leal *et al.* [9] reported a 31-year-old woman presented to the hospital with symptoms of nausea, vomiting, and acute severe abdominal pain, which developed suddenly. Imaging revealed a 2 cm mass in the small intestine and a 3 cm mass in the mesentery, with histopathological examination confirming invasive, well-differentiated Grade 1 NET. In an analogous report, L. Daraghmeh *et al.* [10] discussed a 60-year-old male with one month of severe postprandial epigastric pain, loss of appetite, and weight loss. Examination identified multiple palpable masses approximately 1×2 cm each, located 35 cm from the ileocecal valve, with 30 cm of ischemic small bowel 70 cm from the valve; histopathology confirmed a well-differentiated Grade 1 NET.

C. Leal *et al.* [9] described a 76-year-old male with abdominal distension, flatulence, irregular bowel habits, weight loss, and intermittent partial intestinal obstruction. Imaging revealed severe dilation of the small intestine, with a clear transition point located 60 cm from the ileocecal valve, indicating a potential obstruction. Further examination of the tissue revealed a well-differentiated, Grade 1 NET in the ileum, measuring 2 cm in diameter. H. Behi *et al.* [11] presented a rephrased version: a 75-year-old man presented with severe abdominal pain, vomiting, and bowel obstruction. Imaging studies revealed that the obstruction was caused by an ileo-ileal intussusception, where a portion of the ileum had telescoped into another section, in the distal ileum, approximately 80 cm from the ileocecal valve. The intussusception was associated with a neoplastic lesion

and enlarged, hardened mesenteric lymph nodes. Histopathological examination confirmed the presence of an invasive, well-differentiated Grade 1 NET. M. Basendowah *et al.* [13] documented a case of a 75-year-old man who experienced recurring paraumbilical colicky pain, vomiting, abdominal distension, and changes in bowel habits over a period of six months. Further investigation revealed two masses in the mid-ileum, attached to the small bowel mesentery, located 2.5 meters from the duodenojejunal flexure. These masses were identified as Grade 1, well-differentiated NETs originating in the mid-ileum.

The case contributes to the existing literature by highlighting the crucial need for clinicians to consider NETs in the differential diagnosis of patients presenting with acute abdominal symptoms, thereby maintaining a high index of suspicion [14, 15]. The involvement of mesenteric vessels, as seen in the case, was reported by E. Swafford & D. Magge [16], reinforcing the potential for NETs to complicate mesenteric blood flow and lead to ischemia. F. Butz *et al.* [17] underlined the critical role of surgical management in small bowel NETs. This case reflects these findings, demonstrating successful resection with clear margins, which continues to be a cornerstone of treatment. However, controversies persist regarding surgical approaches in patients with metastatic disease. K. Søreide *et al.* [18] argue for resection even in cases of liver metastases, provided patients are fit for surgery, while Y. Peng *et al.* [19] advocate for personalised management strategies based on survival prediction models. The case calls within the paradigm of primary tumour resection in nonmetastatic disease, supporting the value of individualised treatment planning.

The study highlighted the overlap in imaging and clinical features between NETs and other rare conditions, such as Castleman disease or intussusception secondary to malignancy. K.A. Manjesh *et al.* [20] also underscored the diagnostic complexity and the need for multidisciplinary input. The CECT imaging accurately identified the underlying pathology, emphasising the role of advanced imaging modalities in early diagnosis. L. Chang *et al.* [21] provided insights into prognostic factors for NETs, identifying tumour grade, stage, and surgical intervention as significant determinants of survival. The case aligns with this evidence, illustrating favourable outcomes following early intervention and complete tumour resection. According to E. Bosch *et al.* [22], the growing use of endoscopic and minimally invasive techniques marks a significant breakthrough in the diagnosis and treatment of small bowel NETs. Although these methods were not employed, they hold promise for enhancing preoperative localisation and managing rare complications, such as variceal bleeding or obstruction, ultimately leading to improved patient outcomes.

These cases highlighted the varied presentations of small bowel NETs, ranging from acute pain to chronic symptoms like bowel obstruction and weight loss. Despite differing presentations, the histopathological findings consistently indicate well-differentiated Grade 1 tumours, underscoring the significance of thorough evaluation for prompt diagnosis and management.

Conclusions

The case illustrated the significance of considering alternative or coexisting causes in patients with known congenital anomalies presenting with intestinal obstruction. It emphasised the need for thorough evaluation, as rare pathologies such as SI-NET may be masked by more apparent but incidental conditions like SIT. The presented publication was a report of unique and unprecedented case of SIT in a patient with a SI-NET located in the distal ileum, who presented with the rare symptom of acute intestinal obstruction due to bowel stricturing caused by the primary tumour. This case highlighted the role of maintaining a high index of suspicion for NETs in patients, particularly younger individuals, presenting with small bowel obstruction. Due to the growing incidence of NETs in small intestine, possibility of keeping it as a differential diagnosis, apart from widespread causes of obstruction like abdominal TB, postoperative adhesions, Ladd's band associated with malrotation of gut in SIT, is crucial in leading to complete evaluation, correct definitive surgical management, faster postoperative recovery, and decreased morbidity. Due to the rarity of such cases, documenting the clinical presentation, diagnostic process, and management approach provides valuable insights that can inform future treatment strategies and diagnostic protocols for patients with SIT. This case report can enhance healthcare professionals' ability to deliver accurate and prompt care, ultimately improving patient outcomes. It also promotes further research and invention of better diagnostic modalities that will help in distinguishing the real insult from other concomitant factors present at the same time.

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Conflict of Interest

The authors of this study declare no conflict of interest.

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Situs inversus totalis з нейроендокринною пухлиною клубової кишки: діагностична та хірургічна проблема

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Анотація. Нейроендокринні пухлини тонкої кишки набувають все більшого поширення, незважаючи на те, що є відносно рідкісним захворюванням. Хоча комп'ютерна томографія черевної порожнини під час планових обстежень часто виявляє нейроендокринні пухлини тонкої кишки, багато випадків все ще діагностуються несподівано під час невідкладної хірургічної операції. Ця тема є актуальною, оскільки два рідкісні стани, *situs inversus totalis* і мальротація кишечника, також можуть впливати на абдомінальну ділянку, тим самим вводячи в оману при постановці діагнозу. Метою цього дослідження було висвітлити випадок гострого нападу непрохідності тонкої кишки, спричиненої нейроендокринною пухлиною з супутньою наявністю *situs inversus totalis*. Зазвичай *situs inversus totalis* протікає безсимптомно, будучи випадково виявленим під час візуалізації, або проявляється в ранньому неонатальному періоді з обструктивними ознаками, або в похилому віці у вигляді гострої кишкової непрохідності. Однак цей випадок є унікальним, оскільки в медичній літературі раніше не повідомлялося про співіснування *situs inversus totalis* і нейроендокринних пухлин тонкої кишки, що спричиняють гостру кишкову непрохідність. У пацієнта був діагностований *situs inversus totalis*; під час обстеження на предмет рецидивуючої кишкової непрохідності у нього розвинувся новий епізод гострої кишкової непрохідності і його довелося госпіталізувати для проведення екстреної діагностичної лапаротомії. Пацієнту виконали сегментарну резекцію тонкої кишки та наклали степлерний анастомоз «бік у бік». Справжньою причиною рецидивуючої непрохідності була нейроендокринна пухлина, а не передопераційна підозра на *situs inversus totalis*. Незважаючи на значний прогрес у лікуванні та веденні нейроендокринних пухлини тонкої кишки, який покращив результати лікування пацієнтів, їхня діагностика й надалі залишається значною проблемою. Це дослідження допомагає підтримувати більш широке мислення на практичному рівні, одночасно встановлюючи причину кишкової непрохідності у випадках, коли є кілька припущень

Ключові слова: кишкова непрохідність; аденокарцинома; мальротація; туберкульоз черевної порожнини; тонка кишка; невідкладна хірургія

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Clinical, forensic aspects of oral lesions during tobacco use

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Abstract. Tobacco use provokes the development of numerous pathological changes in the oral mucosa, from inflammatory processes to precancerous conditions and malignant neoplasms, which requires a comprehensive forensic medical assessment to establish cause-and-effect relationships in the investigation of cases of occupational pathology and compensation for harm to health. The study was aimed at summarising existing scientific data on the clinical and forensic features of oral lesions caused by tobacco use and determining the role of dentists in conducting forensic examinations of such cases. A systematic analysis of the literature was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 methodology, with a search in international databases. The results of the analysis revealed three categories of lesions with maximum forensic value: erythroplakia (severe dysplasia/carcinoma *in situ*), leukoplakia with epithelial dysplasia, and submucosal fibrosis with stromal hyalinisation due to the high risk of malignancy. An aetiological relationship was established between tobacco use and the pathogenesis of lesions through biochemical (decrease in glutathione and albumin), immunohistochemical (expression of SERPINA6, SERPINF1, p16), and molecular (microRNA-21) markers. Epidemiological data showed mucosal lesions in 60.1% of tobacco users, with submucous fibrosis (110 cases, 27.5%) and leukoplakia (102 cases, 25.5%) dominating the 400 surveyed. A gradation system for assessing the severity from mild (5-15% disability) to severe (40-100%, persistent dysfunction) was developed, which provides a legal qualification of lesions. Comparative analysis showed a functional differentiation of the competencies of forensic medical experts and dentists, which justified the need for interdisciplinary integration in the examination of tobacco-related lesions. Morphological characteristics of lesions of forensic significance were systematised, and criteria for assessing the severity of tobacco-associated mucosal changes were determined. The necessity of integrating dental knowledge into forensic medical practice and involving dentists in conducting an expert examination in cases of oral pathology associated with tobacco use was substantiated

Keywords: mucosa; pathological changes; nicotine stomatitis; malignant transformation; interdisciplinary integration; functional disorders

Introduction

Tobacco use remains a substantial medical and social problem, as it leads to the development of a wide range of pathological changes in the oral mucosa, from inflammatory processes to precancerous conditions and malignant neoplasms. Timely detection and objective forensic medical assessment of such lesions is of particular importance for establishing cause-and-effect relationships in the investigation of cases of occupational pathology, accidents, and cases of compensation for health damage.

The prevalence and morphological variability of oral mucosal lesions associated with tobacco use became the object of a comprehensive research in clinical and pathological analysis at the training hospital. P.P. Domadiya *et al.* [1] focused on a combination of histological, immunohistochemical, and imaging diagnostic methods, especially in cases of malignant transformations. It was established that more than half of the 200 patients examined had malignant neoplasms, and the verified lesion in the form of

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squamous cell carcinoma was most often localised in the buccal region and directly correlated with chronic tobacco use. Non-specific white lesions of the oral cavity induced by chewing tobacco use were the subject of clinical observation in an adolescent patient. A.M. Buendia *et al.* [2] described a case where mucosal changes were misinterpreted as a consequence of orthodontic treatment, although further analysis revealed that the source of the pathology was tobacco addiction. The authors proved that the severity of damage is directly dependent on the intensity and duration of tobacco use. The spread of precancerous changes in the oral mucosa among high-risk groups was investigated as part of an epidemiological survey of representatives of the Malayali tribe in India. D.L. Francis [3] uncovered that almost half of the participants (49.8%) had morphological signs of precancerous or cancerous transformations, with prolonged tobacco use in various forms being the main cause. Clinical lesions were most often localised in the buccal and labial areas.

In the work of clinicians from India, an examination of almost 200 cases of changes in the oral mucosa was conducted with verification of morphological diagnoses. R.R. Mahapatra *et al.* [4] analysed the relationship between lesion localisation, tobacco use, and histological characteristics, focusing on the area of the gingival complex. The main attention was paid to the morphotypes of epithelial changes – hyperkeratosis and benign neoplasms, which can be predictors of malignant transformations. The authors pointed out the lack of early diagnosis programs and the lack of integration of clinical and socio-behavioural data, which makes it difficult to conduct a forensic assessment of the likely genesis of such lesions. The spread of new forms of tobacco use, in particular, tobacco heating systems, has made it necessary to evaluate their impact on the oral cavity in a clinical context. A study conducted by M. Ilchyshyn *et al.* [5] analysed the dental status of 75 people, some of whom used tobacco heating systems (GLO, IQOS). A considerable decrease in the resistance of hard tissues of teeth to caries and a deterioration in the hygiene index in this group were established. The prevalence of inflammatory and dystrophic-inflammatory lesions of periodontal tissues increased with increasing duration of tobacco use. The researchers stress the need for further clinical trials and standardisation of dental support for people who use heated tobacco products.

The growing prevalence of smoking among adolescents and young people in Ukraine has made it necessary to research biophysical changes in oral fluid as markers of impaired homeostasis of the oral mucosa. I.S. Lisetska & M.M. Rozhko [6] analysed saliva properties in 114 people aged 15-24 years, divided into four groups by type of tobacco use (traditional cigarettes, e-cigarettes, tobacco heating systems, and the control group). Smokers of all types presented a decrease in buffer capacity, salivation rate, and increased saliva viscosity. These changes are interpreted as a decrease in the functional reserves of the oral cavity, which, according to the authors, may indicate a potential role of oral fluid as an early diagnostic indicator. The problem of the effect of tobacco heating systems on periodontal tissues was considered in an analytical publication by I.D. Kiiun & O.M. Šoltys [7]. The paper summarised data on the prevalence of smoking in Ukraine, particularly the growing popularity of electronic cigarettes and tobacco heating systems. The authors underline the presence of toxic components in the aerosol of these devices (metals, aldehydes, flavourings), which can cause dysfunction of oral cells. The search for correlations between the type of tobacco use, the age of patients, and the condition of the oral mucosa was presented in the study by R. Moroka *et al.* [8]. An online survey was conducted among 1,113 people of different ages, where the types of tobacco products consumed, features of hygienic behaviour, and the presence of visually noticeable mucosal lesions were recorded. Strong correlations were found between smoking experience ($r = 0.79$), consumption intensity ($r = 0.75$), and oral hygiene disorders ($r = 0.71$) with the development of pathological changes in the oral cavity.

The analysis of the publications indicated that the vast majority of works concentrate exclusively on the clinical and morphological aspects of tobacco-related lesions without integrating forensic approaches to the assessment of cause-and-effect relationships, legal qualification of the severity of injuries, and determining the role of dental specialists in conducting an expert examination. Despite the large number of studies devoted to oral lesions in tobacco use, the papers aimed at a comprehensive investigation of the clinical and forensic aspects of this problem and the role of dentists in conducting forensic medical examinations were not enough, which led to the need for an ongoing systematic review of the literature to summarise the available scientific data on the integration of dental knowledge into forensic practice. The purpose of the study was to summarise scientific data on the clinical-forensic aspects of oral lesions during tobacco use and determine the role of dentists in conducting forensic medical examinations. Objectives of the study: systematise the clinical and morphological features of oral lesions associated with tobacco use, which have forensic medical significance; determine the criteria for forensic medical assessment of the severity of lesions of the oral mucosa during tobacco use; justify the need to involve dentists in conducting a forensic medical examination in cases of oral pathology associated with tobacco use.

Materials and Methods

The current study was conducted in the format of a systematic literature review in accordance with the methodological recommendations of PRISMA 2020 [9]. The search and analysis of scientific sources was conducted during January-September 2025 in the international databases PubMed/MEDLINE, Scopus, Web of Science Core Collection, Google Scholar, Cochrane Library and OpenGrey. In addition, a manual search was performed in the literature lists of relevant review articles to identify potentially missed studies. The search strategy was based on a combination of

key terms in three thematic blocks: tobacco use ("tobacco use", "smoking", "smokeless tobacco", "heated tobacco products"), oral cavity lesions ("oral cavity lesions", "leukoplakia", "erythroplakia", "oral submucous fibrosis", "oral cancer"), and forensic aspects ("forensic medicine", "forensic odontology", "medicolegal aspects"). Search queries were generated using Boolean and/or operators and MeSH thesauri. The time frame covered publications from 2019 to 2025, with the possibility of including earlier fundamental works. Language restrictions were not applied.

Inclusion criteria included the availability of data on the clinical and morphological characteristics of tobacco-related oral mucosal lesions, forensic aspects of assessment, or the role of dentists in examination. The review included original studies, clinical observations, systematic reviews, meta-analyses, and regulatory documents. The exclusion criteria covered publications without full-text access, with an insufficient description of the methodology, work on exclusively systemic effects of tobacco without analysing local changes in the oral cavity. Initially, 387 sources were identified. The final sample consisted of 61 literature sources, of which 53 were scientific publications (original studies, systematic reviews, clinical observations) and 9 were regulatory documents (international guidelines, forensic standards, classifications). A detailed source selection process is shown in Figure 1.

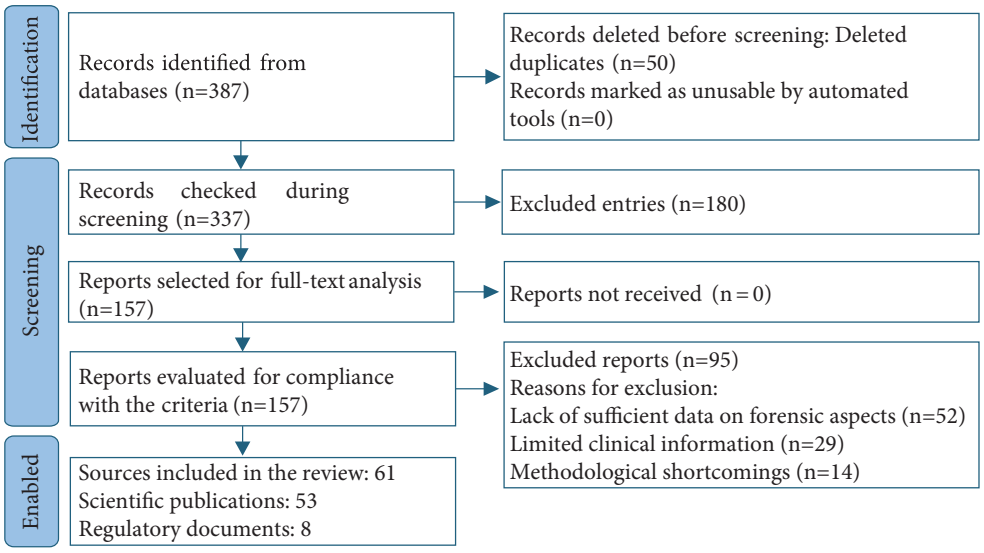

Figure 1. PRISMA flowchart

Source: compiled by the author

A standardised form was used to extract the data, including bibliographic information, study design characteristics, sample size, diagnostic methods, clinical and morphological characteristics of lesions, forensic evaluation criteria, and conclusions about the role of dentists. The quality of the studies was evaluated on the Newcastle-Ottawa Scale [10] for observation studies and AMSTAR-2 [11] for systematic reviews. The risk assessment for systematic errors considered the completeness of documentation, the validity of methods and conclusions. Content analysis was used for regulatory documents. Data synthesis was conducted in the format of a narrative review with thematic grouping in accordance with the objectives of the study: systematisation of clinical and morphological characteristics of lesions, generalisation of forensic medical evaluation criteria according to Order of the Ministry of Health of Ukraine No. 6 [12] and justification of the role of dentists in the examination. For quantitative data, descriptive statistical processing was performed with the calculation of prevalence percentages and correlation coefficients.

No meta-analysis was performed due to the high heterogeneity of the studies. The interpretation was based on a comparison of clinical characteristics with forensic criteria for qualifying the severity of injuries, which allowed establishing the compliance of pathological conditions with legally defined categories of harm to health. The results of a systematic review directly contribute to the achievement of the goal through the formation of an evidence base on the clinical, forensic, and medical aspects of tobacco-associated lesions. The systematisation of characteristics allowed the identification of diagnostic markers to establish causal relationships, the generalisation of criteria provided a standardised approach to severity qualification, and the justification of the role of dentists provided the basis for the development of interdisciplinary protocols for assessing oral pathology.

Results and Discussion

Clinical and morphological characteristics of tobacco-associated oral lesions with forensic significance. In patients with chronic tobacco use, persistent clinical and morphological patterns of oral mucosal lesions were observed, which differed depending on the form of use and had different levels of forensic relevance. According to

J. Öhman *et al*. [13], erythroplakia manifested itself as a bright, well-defined erythematous plaque with a "velvety" surface, pathohistologically dominated by severe epithelial dysplasia, carcinoma *in situ*, or early invasive squamous cell carcinoma; the transformational potential of this nosology was higher than that of leukoplakia, which made it a priority to document the severity of the lesion in forensic assessment. For leukoplakia in smokers, as evidenced by A.E. Şerban *et al*. [14], the clinical picture included homogeneous and non-homogeneous white plaques with localisation on the buccal mucosa, dorsal surface of the tongue and retromolar region; hyper-and parakeratosis, acanthosis, variable dysplasia with impaired cell stratification and polarity, and increased density of lymphoplasmocytic infiltration of its plate were microscopically recorded. The presence of dysplasia and a non-homogeneous phenotype increased the risk of malignant transformation, which gave such foci more forensic weight in terms of qualifying harm to health. With prolonged chewing of tobacco (often in combination with areca), submucosal fibrosis was formed. X. Cai *et al*. [15] described gradual restriction of mouth opening, dense fibrous strands in the buccal areas, pallor, and rigidity of the mucosa; histologically dominated by hyalinisation of subepithelial connective tissue, dense collagen bundles with reduced vascularity, epithelial atrophy with loss of epithelial processes and areas of superficial parakeratosis. The combination of clinical signs of rigidity, long-term chewing habits, and described morphological markers was considered informative for forensic confirmation of long-term harmful effects and assessment of persistent changes, including due to the established risk of developing squamous cell carcinoma.

Hookah smoking was characterised by nicotine stomatitis of the hard palate. H. Dashti & D. Sundaram [16] recorded a dose-dependent relationship in water smokers between the number of "bowls" and smoking hours and the appearance of multiple white keratotic fields with punctured erythematous openings of the excretory ducts of small salivary glands; hyperkeratosis and metaplasia of the ductal epithelium with periductal inflammation were histologically noted. This combination of thermal action and tobacco aerosol created a reproducible macro- and micro-pattern suitable for forensic interpretation of the duration and intensity of exposure. In addition, when using snus/nicotine sachets, local "tobacco pockets" with a whitish-grey keratous area at the site of laying were recorded; pathohistologically described thickening of the stratum corneum, acanthosis, vacuolisation of cells, subepithelial lymphocytic-plasmocytic infiltrate and areas of micronecrosis, which corresponded to chronic mechanical and chemical damage to the mucosa [17]. Although these foci usually regressed after discontinuation of exposure, the presence of cytological changes and persistent hyperkeratosis formed the basis for careful fixation and dynamic control. Summarising, lesions with a proven high risk of transformation and specific micromorphological criteria were of the greatest forensic importance for documenting the severity of the lesion: erythroplakia with a predominance of severe dysplasia/carcinoma *in situ*, leukoplakia with epithelial dysplasia and non-homogeneous phenotype, and submucosal fibrosis with hyalinisation and collagen rearrangement of the stroma. For hookahsmokers, nicotine stomatitis with a characteristic "cobblestone" pattern and metaplasia of the ductal epithelium served as a marker of intense thermal exposure and chronic irritation, which complemented a comprehensive forensic assessment.

In 2019-2025, a number of studies were published that contributed to the deepening of scientific understanding of the differential diagnosis of precancerous and cancerous lesions of the oral cavity caused by tobacco, with an emphasis on the causal relationship between tobacco use and the development of pathological changes in the context of forensic medical examination. Research efforts have focused on exploring biochemical, immunohistochemical, and molecular genetic markers that can verify early-stage carcinogenesis. A. Nimbal *et al*. [18] determined that blood glutathione and serum albumin levels were statistically significantly reduced in smokeless tobacco users with precancerous and especially cancerous lesions compared to the control group. These indicators were considered as reliable biomarkers for early diagnosis and prediction of the course of malignant transformations, while total serum protein was poorly associated with these processes. Another study, conducted by V. Mohanty *et al*. [19] focused on proteomic changes in the serum of patients with oral squamous cell carcinoma, accounting for the type of tobacco use. Increased expression of serpin family proteins, in particular, SERPINA6 and SERPINF1, was found in patients who used tobacco in the form of chewable products. These proteins have been proposed as potential serum biomarkers for screening individuals at high risk of developing oral squamous cell carcinoma.

From the standpoint of immunohistochemistry, A.E. Fares & A.M. Kamel [20] analysed the expression of P16 and CD34 proteins in gum tissue in smokers. P16 expression was found to be greatly elevated, indicating activation of apoptotic mechanisms in response to tobacco loading. However, the increase in CD34, a marker of angiogenesis, did not reach statistical significance, which called into question the prognostic value of this indicator for predicting malignant transformation in such conditions. At the molecular genetic level, D. Vageli *et al*. [21], in a pilot study, established considerable overexpression of miRNA-21 in the saliva and serum of patients with oral squamous cell carcinoma, especially among smokers. They proposed a panel of multiple microRNAs (miR-21, miR-136, miR-3928, miR-29b) that could potentially be used as a non-invasive index for early diagnosis of oral squamous cell carcinoma and assessment of the risk of smoking-related tumours. Consequently, the research base has demonstrated a clear causal relationship between tobacco use and the development of precancerous and malignant changes in the oral cavity, which is important for forensic examination. Biomarkers, in particular, GSH, SERPINA6, P16, CD34,

and microRNA molecules, have been proposed as tools for verifying the mechanisms of carcinogenesis and establishing an aetiological relationship with the tobacco factor.

In clinical practice and forensic medical examination, several classification systems were used to assess tobacco-related lesions of the oral mucosa, which allowed for both diagnosing the stage of the pathological process and conducting an objective assessment of the degree of harm to health. These included the World Health Organisation's (WHO) classification of potentially malignant oral conditions, the dysplasia gradation system, and the TNM classification (Tumour, Node, Metastasis) used for staging oral cancer. According to S. Gupta & P.M. Shrestha [22], in a population-based study in Nepal, a diagnosis of tobacco-induced lesions was conducted considering the WHO classification to assess potentially malignant disorders of the oral mucosa, where among the lesions, the highest proportion were varieties of leukoplakia. Histological examination revealed that moderate to severe dysplasia was most often recorded in patients aged 41 to 80 years, which was of direct importance for assessing the stage of precancerous changes and justifying serious harm to health in the forensic context.

A study by Y. Abbas *et al.* [23], tobacco lesions were also evaluated in accordance with international guidelines, including criteria for dysplastic changes and nosological boundaries between leukoplakia, erythroplakia, palatal keratosis, and malignancies. Systematic detection of such conditions was important for further gradation according to the TNM system, especially in cases of verified squamous cell carcinoma of the oral cavity, which was recorded in 2.75% of cases. As part of a retrospective analysis conducted by M. Alshayeb *et al.* [24], used the WHO classification to register leukoplakia, palatine keratosis, submucous fibrosis, and other lesions. Data from patients' medical records were systematised, accounting for the topography of the lesions and the probability of their progression, which provided for further applying the TNM classification in confirming malignant transformation, which is crucial for forensic determination of the severity of damage. In a study by J. Sidhu *et al.* [25], the severity of tobacco-induced lesions was graded according to the clinical aspect and the potential progression to dysplasia of varying degrees, for which morphological criteria consistent with the WHO histopathological scale were used. As a result, it was revealed that the predominant forms were hyperkeratosis and leukoplakia, which, in the context of frequent tobacco use, were subject to dispensary observation, and when confirming dysplastic changes – pathoanatomical analysis to assess the risk of malignant degeneration, which is the basis for establishing moderate or severe harm to health. Thus, interrelated classification systems have been used in clinical practice and Forensic Medicine – WHO for malignant diseases of the oral mucosa, TNM for assessing the prevalence of oral cancer, and the histopathological scale of dysplasia. Their simultaneous use helped to reliably assess the severity of tobacco-induced lesions and establish the legal qualification of harm to health in accordance with forensic criteria.

Epidemiological studies on tobacco-related oral diseases have confirmed their prevalence in different socio-demographic groups and regions, focusing on significant gender, age, and social differences that are important for forensic assessment and prevention policy formation. A study by L. Jacob *et al.* [26] established that among the urban population in southern India, 24.19% of adults used tobacco in any form, and 60.1% of users experienced oral mucosal lesions, with a predominance of conditions such as "smoker's palate", especially in patients over 45 who used tobacco for more than 20 years. This emphasised the importance of anamnestic factors (duration and type of consumption) in predicting health damage and the possibility of a legal assessment of the severity of these injuries. According to the results of an epidemiological study among adolescents in 133 countries conducted by M.A. Nazir *et al.* [27], the prevalence of tobacco use in the 13-15 age group was 19.33%, with a predominance among boys, especially in high-income countries, where rates reached 24.76% among boys and 19.4% among girls. The most common lesions in this age group were gingivitis (72.8%), gingival bleeding (51.2%), and bad breath (39.6%), indicating systemic harm caused by tobacco in the early stages of life and the importance of early intervention in prevention.

In a regional study in the city of Jammu (India), Y. Abbas *et al.* [23] determined that oral submucous fibrosis (110 cases) and leukoplakia (102 cases) were the most common clinical manifestations among 400 tobacco-dependent individuals examined, indicating a high proportion of precancerous lesions among tobacco users, mainly smokeless-type (63.5%). This allowed extrapolating the results for clinical and forensic interpretation of the severity of injuries. In the study by A. Das *et al.* [28], concerning the structure of tobacco lesions among migrant construction workers in Chennai, determined that 84.8% of respondents used tobacco in smokeless form, and the prevalence of oral mucosal lesions was 36.8%, among which leukoplakia (8.6%) and oral submucous fibrosis (7.8%) were the most common. The more vulnerable group was men aged 28-38 years, which indicated the age-specific nature of clinical manifestations and their potential forensic significance in determining the severity of harm to health. Thus, the data showed a high level of prevalence of tobacco-related oral lesions, considerable regional differences, and gender-age imbalances, which created the basis for medical and social assessment, planning of preventive measures, and legal qualification of injuries in forensic medical examination.

Analysis of the clinical and morphological characteristics of tobacco-associated oral lesions indicated the formation of specific patterns of mucosal damage depending on the form of tobacco use. Erythroplakia with a predominance of severe dysplasia or carcinoma *in situ*, leukoplakia with epithelial dysplasia and non-homogeneous phenotype, along with submucosal fibrosis with hyalinisation and collagen restructuring of the stroma, are of the highest forensic significance for documenting the severity of harm to health due to the proven high risk of malignant

transformation and specific micromorphological criteria. Current studies have demonstrated a clear causal relationship between tobacco use and the development of precancerous and malignant changes, confirmed by biochemical, immunohistochemical, and molecular genetic markers. Reduced levels of glutathione and serum albumin, increased expression of serpin family proteins (SERPINA6, SERPINF1), P16 protein, and overexpression of miRNA-21 have been proposed as potential biomarkers for verifying the mechanisms of carcinogenesis and establishing an aetiological association with tobacco factor in forensic medical examination. Simultaneous application of the WHO classification for potentially malignant oral conditions, the histopathological dysplasia gradation scale, and the TNM classification provides an objective assessment of the degree of harm to health. Epidemiological data confirm the high prevalence of tobacco-related lesions with considerable regional, gender, and age imbalances, which actualises the need for early diagnosis, the formation of preventive policies and the unification of approaches to the legal qualification of injuries in forensic medical practice.

Criteria for assessing the severity of injuries caused by tobacco use and methodology of expert research. In the period from 2019 to 2025, the issue of legal regulation of forensic medical examination of the severity of injuries in cases of oral pathologies, including tobacco-related lesions, was examined in the context of harmonisation of national and international approaches. In Ukraine, the main document regulating the procedure for such an examination remained Order of the Ministry of Health of Ukraine No. 6 [12]. In international practice, World Health Organization [29, 30] documents, and J. Payne-James & R.M. Jones [31] and J. Payne-James *et al.* [32] served as guidelines. However, the specific nature of tobacco-related lesions, such as leukoplakia, nicotine stomatitis, black hairy tongue, erythroplakia, and premalignant or malignant conditions of the mucous membrane, required additional detail, which was not always provided by existing regulations.

In international practice, the importance of adapting clinical protocols to the specifics of tobacco damage has been recognised. For instance, A. Ralho *et al.* [33] indicated high attachment loss index scores, elevated levels of pro-inflammatory cytokines, and frequent manifestations of hyperplastic candidiasis and nicotine stomatitis in e-cigarette users, which required an extension of damage classifications to these conditions as part of peer review. In addition, F.R. Lozano [34] underscored that international hygiene and dental societies, including the European Network for the Prevention of Smoking and Tobacco, actively promoted changes in damage assessment standards in connection with new forms of tobacco addiction. It has been proposed to include conditions such as refractory periodontitis, hyperplastic mucosal changes, and delayed healing after dental interventions as important clinical criteria that should be considered in the forensic examination of the severity of injuries. Thus, the current regulatory documents in Ukraine mostly disregarded the specific features of tobacco-related damage, and international practice demonstrated a desire to integrate clinical, biochemical and forensic criteria that would reflect the complex nature of injuries caused by tobacco use. A classification of the severity of tobacco-associated lesions has been developed, which is presented in Table 1 to systematise approaches to forensic medical assessment.

Table 1. Forensic classification of the severity of tobacco-related oral lesions with documentation criteria

Severity of the injury	Nosological form	Clinical criteria	Morphological features	Duration of the health disorder	Disability	Legal qualification
Mild	Nicotine stomatitis	Hyperemia, papillary hyperplasia of the palate	Hyperkeratosis, acanthosis, dilated excretory ducts	Up to 21 days	5-10%	Minor leasure
	Tobacco leukoplakia (flat form)	White spots, not removed, asymptomatic	Hyperkeratosis without dysplasia	21-60 days	10-15%	Minor leasure
Medium	Leukoplakia with mild/moderate dysplasia	Rough plaques, discomfort, localisation on the tongue, cheeks	Epithelial dysplasia (lower/middle third), cellular atypia	60-180 days	15-30%	Moderate bodily injury
	Submucosal fibrosis of the oral cavity	Restricted mouth opening, burning sensation, pale fibrous strands	Collagen hyalinisation, vascular reduction	>180 days	30-40%	Moderate bodily harm
Heavy	Leukoplakia with severe dysplasia	Foci of red-white plaques, painful	Lesions of the entire thickness of the epithelium, pathological mitoses	Persistent loss of function	40-60%	Grievous bodily harm
	Oral cancer *in situ*	Visually similar to dysplasia, minimal symptoms	Loss of architecture, without invasion	Threat to life	60-80%	Serious bodily harm (threat to life)
	Invasive squamous cell carcinoma	Tumour, ulcer, bleeding, dysphagia	Invasion of underlying tissues, lymphovascular invasion	Permanent loss of function, life-threatening	80-100%	Serious bodily harm (life-threatening)

Source: created by the author based on Y. Abbas *et al.* [23], F.M. Zahran *et al.* [35], M. Aroquiadasse *et al.* [36]

Analysis of the proposed classification table allowed tracing a clear correlation between the severity of tobacco-associated oral lesions, the nature of histopathological changes, the duration of health disorders, and the legal qualification of injuries. A gradual gradation was observed-from reversible superficial inflammatory and hyperkeratotic processes to severe proliferative changes with high oncogenic potential. A direct relationship was established between the morphological complexity of the pathology and the need for advanced verification methods, including immunohistochemistry and molecular diagnostics. Therewith, a clear distinction between moderate and severe severity of injuries was reflected not only in the biological aggressiveness of the process but also in the level of potential disability and threat to life. Methodologically, the table recorded the priority of biopsy and morphological assessment as the main source of forensic evidence, accounting for objective clinical indicators (for example, restriction of mouth opening or persistence of symptoms), which emphasised the evidence-based validity of an interdisciplinary approach in the qualification of injuries.

In the complex forensic medical examination of tobacco-associated oral lesions, a multicomponent methodology was used, which included clinical-anatomical research, pathomorphological analysis, toxicological studies, immunohistochemical, and molecular genetic methods. The clinical and anatomical stage was based on detailed fixation of the localisation, morphology, duration, and progression of the pathological process, in particular, in malignant lesions, such as squamous cell carcinoma. Pathomorphological analysis of biopsies allowed determining the degree of differentiation of tumours, revealing, for example, highly differentiated squamous cell carcinoma, which was a common morphological form of damage in chronic tobacco users, as shown by A. Guddur *et al.* [37]. The toxicological examination was based on determining the levels of cotinine – the main metabolite of nicotine – in biological fluids (blood serum, saliva). The enzyme-linked immunosorbent assay technique was widely used to quantify this biomarker. The results of V. Mayank & C.Z. Pardeshi [38] pointed to a considerable increase in cotinine levels in chronic tobacco smokers, which correlated with the duration and frequency of tobacco use, along with the development of malignancies, thus confirming the role of cotinine as an evidence-based marker of tobacco exposure. Immunohistochemical methods have detected the expression of carcinogenesis-related proteins, such as p53, Ki-67, or cell cycle proteins. Such approaches provided for assessing the proliferative activity of tissues and the potential for malignant transformation, which supplemented the pathomorphological picture. In addition, molecular genetic studies, in particular, polymerase chain reaction, were used to detect mutations or epigenetic changes in critical oncogenes and suppressor genes that could be induced by components of tobacco smoke, although such methods remained mainly within the framework of scientific research and were not always implemented in routine forensic practice.

In terms of evidence, the determination of cotinine levels had the greatest forensic value as an objective and quantitative marker of tobacco exposure, suitable for both lifetime and post-mortem studies. This was confirmed by the high sensitivity and specificity of the method, particularly when using saliva as an alternative non-invasive biomaterial [39]. It was also established that a decrease in the total antioxidant capacity of saliva at elevated cotinine levels had diagnostic value for the destructive effect of tobacco on oral tissues, enabling the integration of a biochemical approach to a comprehensive assessment [40]. Thus, the most evidence-based approach in terms of establishing a causal relationship between tobacco use and oral lesions was a combined approach that included pathomorphological analysis in combination with toxicological determination of cotinine, while immunohistochemical and molecular genetic methods played an auxiliary role in stratifying the malignant potential of the detected lesions. In forensic medical practice, the establishment of a causal relationship between tobacco use and the development of severe oral lesions was accompanied by a number of methodological and epistemological difficulties. One of the main problems was the presence of a latent period between the onset of tobacco use and the appearance of clinically significant lesions. As shown in the study by M. Gabhane *et al.* [41], the majority of patients with a verified diagnosis of oral squamous cell carcinoma had a history of tobacco use for over 10 years, and the frequency of consumption was also high. This prolonged latent period complicated the determination of the time limit between exposure and the development of pathology, especially in cases where other risk factors existed in parallel.

The multifactorial aetiology of precancerous and cancerous conditions was instrumental in the complication of causal analysis. Specifically, R.K. Kommalapati *et al.* [42] proved that in South Indian industrial workers, the proportion of people with oral lesions was statistically associated not only with tobacco but also with background exposure to occupational hazards and likely low socio-economic status. This multiplicity of factors made it impossible to establish the isolation effect of only one agent – tobacco – without thorough stratification of all cofactors. A separate aspect was the individual genetic predisposition to the transformation of normal epithelium into dysplastic. Y. Shahi *et al.* [43] determined that carriers of interleukin-6 gene variants (IL-6 – 596 G/A and – 572 G/C) who were simultaneously smokers had a remarkably higher risk of developing precancerous lesions compared to individuals without such polymorphisms. Thus, even with the same dose and duration of tobacco use, the degree of carcinogenic effect varied depending on the genetic profile, which significantly complicated the assessment of the weight of tobacco as a separate aetiological factor.

Expert conclusions in such cases were based on a combination of several parameters: the duration and intensity of tobacco use, the nature of the lesion (morphological verification), the exclusion of other significant risk factors, and

consideration of the existing scientific base on the carcinogenicity of tobacco components. E.A. Saeed *et al.* [44] noted that even when multiple factors (tobacco, alcohol, age, localisation) were identified, smokeless tobacco remained the leading aetiological factor if there was a chronic nature of consumption, confirmed by anamnesis and biological markers. The emphasis was placed on typical histopathological changes characteristic of tobacco lesions, which supported the logic of the expert opinion on the causal link. Thus, the establishment of a causal relationship required a comprehensive approach with mandatory consideration of the time of exposure, the degree of consumption, the presence of concomitant risk factors, and the individual biological reactivity of the patient.

The analysis of forensic criteria for evaluating tobacco-related oral lesions indicates the insufficiency of the current Ukrainian regulatory documents for adequate qualification of specific pathologies caused by both traditional and alternative forms of tobacco use. The proposed classification demonstrated a clear gradation from mild reversible processes to severe proliferative changes with high oncogenic potential, establishing a direct correlation between the morphological complexity of the pathology, the duration of the health disorder, and the legal qualification of injuries. The comprehensive methodology of expert research covered pathomorphological diagnostics, toxicological determination of cotinine as an objective biomarker of tobacco exposure, immunohistochemical and molecular genetic methods, while the combined approach has the greatest evidentiary value for establishing a causal relationship. Expert assessment is complicated by a long latent period, multifactorial aetiology, and individual genetic predisposition, which requires a comprehensive analysis considering the duration and intensity of exposure, morphological verification, and exclusion of other substantial risk factors.

The role of the dentist in the forensic examination of tobacco-related lesions and interdisciplinary integration. In order to conduct a high-quality forensic medical examination of oral cavity lesions associated with tobacco use, a dentist must possess a range of specific professional knowledge, clinical competencies, and diagnostic skills. First, a deep knowledge of the anatomy of the maxillofacial region, specifically, the oral mucosa, is necessary, which allows accurately localising lesions, determining their boundaries and relationship with nearby structures. According to B.W. Chaffee *et al.* [45], the dentist's anatomical awareness is crucial for identifying pathologies such as periodontitis, leukoplakia, lesions associated with tobacco use and the latest tobacco products, since they most often leave specific signs in the oral cavity. Along with this, pathomorphological training of the dentist helps differentiate different forms of mucosal lesions, from benign to potentially malignant, and, importantly, in the context of forensic medical examination, determine the aetiological role of the tobacco factor. R. Jayaram *et al.* [46] accentuated that the visual and histopathological characteristics of tobacco-induced lesions can only be identified at an early stage with proper professional training, which is crucial for the correct legal interpretation of causal relationships. In addition, knowledge in the field of oncostomatology plays a central role, as tobacco is the main aetiological factor in the development of malignant neoplasms in the oral cavity.

Finally, professional training in the field of clinical diagnosis of changes in the oral cavity associated with tobacco use seriously increases the level of objectivity of the expert opinion. As a study by B. Chandrashekar *et al.* [47] presented, the integration of specialised training modules into dental training seriously improved their ability to recognise the clinical manifestations of tobacco lesions and increased their confidence in making an expert diagnosis. This is significant in the context of forensic cases, where the correct interpretation of the clinical picture has the weight of legal evidence. Thus, the involvement of a dentist as a specialist or expert in the process of forensic medical examination of oral lesions during tobacco use provides a deeper understanding of pathological changes, allows for establishing an accurate diagnosis, tracing the causal relationship between tobacco exposure and clinical manifestations, and remarkably increases the completeness and objectivity of the expert opinion. Comparative characteristics of the competencies of forensic experts and dentists in the context of tobacco-related oral lesions research are key to determining the role of each specialist in the examination process and forming an interdisciplinary approach. Table 2 presented data based on current scientific sources highlighting the interaction between forensic and dental professionals in the assessment, diagnosis, and management of tobacco-related pathologies.

Table 2. Comparative analysis of the competencies of a forensic medical expert and a dentist in the examination of tobacco-related oral lesions

Peer review parameters	Competence of a forensic medical expert	Competence of a dentist	Common areas of competence	Limitations of the forensic expert	Limitations of the dentist	Advantages of an interdisciplinary approach
Clinical interpretation of tobacco lesions	Determination of time limits of damage, legal examination of pathological changes	Assessment of functional dental consequences and precancerous changes	Visual diagnosis, recording of lesions, collection of medical history	May not recognise the initial mucosal changes caused by tobacco	Does not always have legal qualifications in relation to personal injuries	Unified interpretation of clinical manifestations, in view of the legal context

Continued Table 2

Peer review parameters	Competence of a forensic medical expert	Competence of a dentist	Common areas of competence	Limitations of the forensic expert	Limitations of the dentist	Advantages of an interdisciplinary approach
Applying special methods	Postmortem histology, toxicology (nicotine, cotinine), forensic imaging	Lifetime biopsy, stomatoscopy, fluorescence	Joint use of biopsy, cytology, digital imaging	Unavailability of dental equipment	Lack of experience in forensic protocols	Combined use of intravital and postmortem methods
Interpretation of morphology	Identification of post-traumatic changes, determination of the nature of damage	Detection of dysplasia, degree of malignancy	Joint histopathological assessment	Limited knowledge of the specifics of the oral epithelium	Lack of practice in assessing post-mortem changes	Morphological consensus in the case of disputable lesions

Source: created by the author based on R. Jayaram *et al.* [46], A. Shatara & C.E. Lahham [48], P.J. Ford & A.M. Rich [49]

Analysis of Table 2 revealed systematic differentiation between the roles of forensic medical experts and dentists in the examination of tobacco-related oral lesions. The most noteworthy aspect was the asymmetry of competencies, which is due to the different methodological bases of both specialities: the forensic medical expert is focused on the legal qualification of injuries and the time characteristics of the pathological process, while the dentist focuses on the biological mechanisms of damage and functional consequences. This dichotomy creates gaps in diagnostic coverage that can only be addressed through interdisciplinary interaction. It is in common areas – such as histopathological interpretation, fixation of morphological changes, and documentation of the clinical picture – that both specialists are able to complement each other. The expert activity of each of them has certain limits: dental specialisation does not cover the legal aspects of expertise, and forensic medicine does not provide full details of the functional state of the oral cavity organs. Thus, the table reflects not only the competence distribution but also the need for standardised collaborative assessment algorithms, especially in the face of expert contradictions or complex oncological pathology.

The International and Ukrainian experience of involving dentists in conducting forensic medical examinations in cases related to oral pathology demonstrated the gradual institutionalisation of forensic odontology as an interdisciplinary field that requires specialised legal training in addition to clinical expertise. In the study by V. Osmolian *et al.* [50], the criminal and procedural legislation of Ukraine, Georgia, Poland, and the Czech Republic was analysed to identify legal prerequisites for involving dentists in forensic examinations. The authors concluded that, despite the existence of international legal acts ratified by these countries, the regulatory framework remains insufficiently specified, specifically, in terms of determining the limits of competence of a dentist in the framework of forensic medical research. It was recommended to amend the guidelines and training programmes regulating the participation of dentists in the collection of evidence in criminal proceedings.

In the world practice, as evidenced by the results of the study by S. Indu *et al.* [51], the role of forensic odontological units in the structure of military medical services has increased. The paper substantiates the need to create specialised laboratories for forensic odontology in the armed forces, which would allow effective identification of dead servicemen based on dental records, including in conditions of mass losses. The authors focused on the uniqueness of the dentoalveolar apparatus as a bioidentification indicator, which is able to persist in extreme conditions longer than other tissues. The issues of training and training of specialists were covered by S. Pavičin *et al.* [52], who analysed the practice of maintaining dental records and the level of awareness of Croatian dentists about the forensic odontological potential of these records. It was determined that only a third of respondents were aware of the legal norms for maintaining medical records, and a considerable part of them did not understand the potential legal value of their own clinical practice for identifying individuals. The authors concluded that it is necessary to integrate forensic odontology into basic dental education.

In the context of interdisciplinary cooperation, coordination by forensic institutions with the participation of dentists, forensic experts, anthropologists, and criminologists has proved to be an effective model. As noted by M. Hachem *et al.* [53], it was this integration that ensured the full use of the potential of dental impressions, bite analysis, and age assessment for identification. The authors emphasised the importance of standardising techniques such as creating digital jaw models, computerised bite analysis, and dental profiling. Current areas of development were also described by G.V. Lacasella *et al.* [54], summarising the latest technologies used in forensic odontology, including the use of artificial intelligence for image analysis, three-dimensional jaw modelling, digital identification, and molecular diagnostics using tooth enamel and pulp. The authors accentuated that the effectiveness of these instruments depended largely on inter-agency cooperation and the existence of unified legal and procedural standards, which currently remained fragmented. Thus, International and

Ukrainian practice demonstrated that the effectiveness of involving dentists in forensic medical examinations largely depended on the availability of legislative regulations, professional training, and institutional support in the form of specialised units, in addition to the technological integration within the framework of multidisciplinary expert models.

In forensic practice, there were a number of clinical scenarios in which the participation of a dentist as an expert was mandatory or appropriate within the framework of a commission examination. Firstly, such situations included cases of diagnostic uncertainty between dental pathologies and other lesions of the maxillofacial region, cases of severe oral injuries, suspicions of medical negligence or errors during dental treatment, and the need to identify individuals by dental remains in cases of mass disasters. As noted by H.U. Brauer & A. Bartols [55], in German court practice, the dentist was a key expert in civil lawsuits concerning the quality of dental care, in particular, in assessing the compliance of the treatment performed with clinical standards and the existence of a causal relationship between the doctor's actions and complications. The authors emphasised that in difficult cases, the expert should conduct a systematic review of the literature, and not just rely on their own clinical practice.

In cases of differential diagnosis, especially when it came to distinguishing between tobacco-related pathologies, such as leukoplakia or precancerous conditions of the oral mucosa, the involvement of the dentist allowed verifying the diagnosis and assessing the likely aetiology of lesions. As indicated by M. Daoudian [56], the detection of such diseases had not only clinical but also legal importance since it could be associated with claims against health care providers for late diagnosis or improper informing of the patient about the risks. In the context of criminal proceedings, the involvement of dentists was mandatory when analysing bites that remained on the victim's body in cases of physical violence, rape, or self-defence. As noted by M. Hachem *et al.* [53], such examinations provided for the comparison of dental fingerprint samples with suspects, and in some cases isolated DNA from saliva left in wounds. Thereby, dentists who were proficient in 3D modelling, digital morphometry, and computer bite analysis played an important role.

Another area of mandatory involvement of dental specialists was the identification of bodies by dental remains in cases of mass disasters or significant destruction of soft tissues. As described in a study by S. Jain *et al.* [57], in such situations, even in the absence of complete dental documentation, it was possible to perform facial profiling based on the morphological characteristics of the teeth, the presence of dentures or implants, which greatly improved the accuracy of identification. The legal mechanisms for initiating the participation of a dentist as an expert were based on procedural rules that provided for the appointment of a forensic examination if special knowledge was needed. As displayed by the findings of S. Farooq *et al.* [58], even in jurisdictions where judicial odontology was not systematically integrated into expert practice, dentists could be involved on the basis of a petition from the parties or a court order, as well as on the basis of special contracts with expert institutions. The authors noted the need to unify the procedures for training expert dentists and develop standards for professional opinion, which would increase the legitimacy of such examinations in court. Consequently, dental examination was important in the context of clinical identifications, legal disputes about the quality of medical care, analysis of injuries, as well as in criminal proceedings with the lack of other biological material. Legal mechanisms provided for to initiating such involvement on the basis of an expert request or on the initiative of a court in the presence of a clinically significant dental component in the case.

In the context of the development of forensic dental examination of tobacco-related oral lesions in Ukraine, the need to create specialised training programmes for dentists who should conduct expert activities was shown. As noted by B. Chandrashekar *et al.* [47], the development of the Tobacco Counseling Training Module greatly improved the level of knowledge, diagnostic skills of tobacco-related lesions, and confidence in communication with patients. The effectiveness of implementing such modules in the educational process was confirmed by a marked improvement in results on all key educational indicators. O. Oyapero *et al.* [59] presented a policy proposal for the integration of tobacco cessation interventions into the dental care system, where special attention was paid to the reform of training programs, the introduction of certification of specialists in the treatment of tobacco addiction, and improving the skills of dentists in the field of evidence-based approaches to prevention. Financial incentive mechanisms for dentists' participation in the tobacco control system are proposed, which can be adapted in the Ukrainian context, accounting for the needs of institutionalising the role of the dentist in forensic medical examination. In turn, the analysis of Ukrainian legislation conducted by I. Demchenko [60] demonstrated that although Ukraine has made progress in implementing the provisions of the WHO Framework Convention on Tobacco Control, legislative gaps still prevented a clear definition of the role of specific health professionals in implementing tobacco control interventions. This analysis highlighted the need to define primary health care standards for tobacco addiction, paying attention to the role of dentists.

S.A. Trofimets [61], within the framework of methodological recommendations for the examination of tobacco raw materials, outlined the potential of using spectral analysis, gas chromatography, and mass spectrometry as tools for assessing the quality and safety of tobacco products, which, if adapted to the dental context, can ensure the objectivity of expert conclusions. The proposed technologies can be part of a comprehensive approach to the development of standardised protocols for the examination of oral lesions. In conclusion, the prospects for the development

of forensic dental examination of tobacco-related lesions in Ukraine included the need to create specialised training modules, improve the regulatory framework, introduce the latest diagnostic technologies, harmonise with international approaches to assessing the state of the oral cavity and integrate dentists into the system of expert medicine.

The analysis of the role of a dentist in the forensic medical examination of tobacco-related oral lesions indicates the need to possess specific professional competencies, covering deep knowledge of the anatomy of the maxillofacial region, pathomorphological training, oncostomatological examination, and clinical diagnostics of tobacco lesions. The comparative characteristics of competencies demonstrated a systematic differentiation between a forensic expert focused on legal qualifications and time characteristics of the pathological process, and a dentist focused on biological mechanisms of damage and functional consequences, which determines the need for interdisciplinary integration to eliminate diagnostic gaps. International experience has shown the gradual institutionalisation of forensic odontology with the mandatory involvement of dentists in cases of diagnostic uncertainty, severe oral injuries, suspected medical negligence, bite analysis in criminal proceedings, and identification of persons by dental remains, but the Ukrainian regulatory framework remains insufficiently specified regarding the limits of competence of a dentist in forensic medical research. Prospects for the development of forensic dental expertise in Ukraine include the creation of specialised training modules, improvement of the regulatory framework with a clear definition of expert opinion standards, introduction of digital imaging and molecular diagnostics technologies, and systematic integration of dentists into multidisciplinary expert models with unification of procedures based on international standards of forensic odontology.

Conclusions

A systematic review of 61 scientific sources confirmed the complex nature of tobacco-associated oral lesions and justified the need to integrate dental knowledge into forensic medical practice. Systematisation of clinical and morphological characteristics revealed priority nosological forms for forensic expert practice: erythroplakia as the most aggressive form with the dominance of severe dysplastic changes in the epithelium and carcinoma *in situ*, leukoplastic lesions with a heterogeneous clinical picture and enhanced malignancy potential of heterogeneous varieties, oral submucosal fibrosis with specific structural changes in the form of hyalinisation of collagen fibres and epithelial atrophy. The causal relationship of tobacco exposure with the pathogenesis of lesions was confirmed through a system of diagnostic markers: biochemical link (glutathione and blood albumin depression), immunohistochemical indicators (activation of SERPINA6, SERPINF1, p16) and molecular level (miRNA-21 elevation). According to the results of population studies, the frequency of mucosal pathologies in tobacco-dependent individuals is 60.1%, and in a cohort of 400 patients, the leading positions were occupied by fibrous transformations of the submucosal layer (27.5% or 110 observations) and leukoplastic changes (25.5% or 102 observations), while invasive carcinomas were verified in 2.75% of the subjects.

A multi-level system of expert qualification was created that differentiates lesions by severity: the initial level is characterised by a 5-15% functional deficit and a 21-60-day recovery period, the intermediate level is characterised by 15-40% labour loss for 60-180 days, and the critical level is characterised by 40-100% loss of functionality with irreversible masticatory disorders, which allows legally adequately classifying injuries in accordance with national legislation. It was proven that the optimal expert strategy is an integrative method that combines morphological verification with toxicological detection of cotinine as a specific bioindicator of tobacco exposure, supplemented by immunohistochemical and molecular genetic profiling to predict malignancy. Critical methodological barriers of expertise were identified: extensive latent interval between the initiation of tobacco use and the manifestation of clinically relevant lesions (decade or more), aetiological polyfactoricity with the need to eliminate competitive professional exposures, genetic heterogeneity with respect to dysplastic susceptibility, which determines a multiparametric analytical algorithm with quantification of exposure characteristics.

The comparative characteristics of professional capabilities demonstrated functional asymmetry: forensic experts specialise in the legal interpretation of traumatic consequences and temporal parameters of the pathological process, while dentists focus on the biopopathogenetic links of the lesion and functional-anatomical deficits, which generates expert gaps in a monodisciplinary approach and argues for multispecialty collaboration. The requirement of unification of protocols of primary documentation of dental status with detailed registration of morphological descriptors of lesions (chromatic characteristics, linear parameters, textural features, topographic localisation) and exposure history (quantitative, temporal, formal attributes of tobacco use) for constructing an evidence-based causal communication platform was substantiated. Promising areas include conducting prospective clinical studies to form national standards for the expert assessment of tobacco-related lesions and developing molecular genetic markers to objectify the causal relationships between tobacco exposure and pathological changes in the oral mucosa.

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Conflict of Interest

None.

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Клініко-судово-медичні аспекти уражень порожнини рота при тютюнокористуванні

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Анотація. Тютюнокористування провокує розвиток численних патологічних змін слизової оболонки ротової порожнини, від запальних процесів до передракових станів та злоякісних новоутворень, що потребує комплексної судово-медичної оцінки для встановлення причинно-наслідкових зв'язків при розслідуванні випадків професійної патології та компенсації шкоди здоров'ю. Дослідження було спрямоване на узагальнення існуючих наукових даних щодо клініко-судово-експертних особливостей уражень ротової порожнини, спричинених вживанням тютюну, та визначення ролі стоматологів у проведенні судово-медичних експертиз таких випадків. Здійснено систематичний аналіз літератури відповідно до методології Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 з пошуком у міжнародних базах даних. Результати аналізу виявили три категорії уражень з максимальною судово-експертною цінністю: еритроплакію (тяжка дисплазія/карцинома *in situ*), лейкоплакію з епітеліальною дисплазією та підслизовий фіброз з гіалінізацією строми через високий ризик малігнізації. Встановлено етіологічний зв'язок між тютюнокористуванням та патогенезом уражень через біохімічні (зниження глутатіону та альбуміну), імуногістохімічні (експресія SERPINA6, SERPINF1, p16) та молекулярні (мікроРНК-21) маркери. Епідеміологічні дані продемонстрували ураження слизової у 60,1 % користувачів тютюну, при цьому серед 400 обстежених домінували субмукозний фіброз (110 випадків, 27,5 %) та лейкоплакія (102 випадки, 25,5 %). Розроблено градаційну систему оцінки тяжкості від легкого ступеня (5-15 % втрата працездатності) до тяжкого (40-100 %, стійка дисфункція), що забезпечує юридичну кваліфікацію тілесних ушкоджень. Компаративний аналіз засвідчив функціональне розмежування компетенцій судово-медичних експертів та стоматологів, що обґрунтувало потребу міждисциплінарної інтеграції при експертизі тютюнасоційованих уражень. Систематизовано морфологічні характеристики уражень, що мають судово-експертне значення, та визначено критерії оцінки тяжкості тютюнасоційованих змін слизової оболонки. Обґрунтовано необхідність інтеграції стоматологічних знань у судово-медичну практику та залучення лікарів-стоматологів до проведення експертизи у випадках патології порожнини рота, пов'язаної з тютюнокористуванням

Ключові слова: слизова оболонка; патологічні зміни; нікотиновий стоматит; злоякісна трансформація; міждисциплінарна інтеграція; функціональні порушення

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Evaluation of p16 expression in carcinomas of the uterine cervix diagnosed only on cervical biopsies

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Abstract. The use of differing diagnostic terms by pathologists in their histopathological reports relating to tumours of the uterine cervix can affect the clinical decision-making of treating physicians or surgeons. A retrospective cross-sectional study was conducted with the aim of evaluating p16 expression in all cervical carcinomas diagnosed solely through cervical biopsies, following a review of their previous diagnoses. Any association between p16 expression and the age of the patient or previous diagnosis was also examined. The mean, median and mode ages in the study were 53 ± 12.4, 60, and 65 years, respectively. In 70 out of 393 cervical biopsies (17.8%) were diagnosed cervical carcinomas. A significant inconsistency in the use of diagnostic terminology by pathologists was observed. Of the 53 cases submitted for p16 immunostaining, 50 cases (94.3%) were p16 positive, and 3 (5.7%) were negative. Moreover, 88.7% of cases were reclassified as squamous cell carcinoma, human papillomavirus-associated, 5.7% as squamous cell carcinoma, human papillomavirus-independent, and 5.7% as Adenocarcinoma, human papillomavirus-associated of the uterine cervix. A mean age at diagnosis of 65 years was not significantly associated; however, the previous categories of large cell non-keratinising and keratinising squamous cell carcinoma showed the highest p16 positivity ($p < 0.001$). The inclusion of p16 status in pathological reports would not only promote uniformity in histopathological reporting but also assist physicians and surgeons in determining the appropriate treatment approach, predictive value, and prognosis

Keywords: human papillomavirus; immunohistochemistry; squamous cell; haematoxylin; eosin

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Introduction

p16INK4A, also known as p16, is considered a surrogate marker of human papillomavirus (HPV) infections in the uterine cervix and other anatomical sites. Two HPV subtypes – 16 and 18 are primarily responsible for pre-cancerous and cancerous lesions of the uterine cervix. Early and accurate diagnosis of cervical tumours offers substantial benefits to patients in terms of timely treatment initiation and improved prognosis. However, it is not uncommon for pathologists to use inconsistent nomenclature or provide incomplete histopathological reports, which can hinder physicians and surgeons in initiating the most appropriate treatment regimen. Multiple factors may contribute to this avoidable situation.

In 2022, cervical cancer ranked fourth in both incidence and mortality among cancers affecting females worldwide. It was the leading cause of cancer-related deaths in women in 37 countries, particularly in sub-Saharan Africa, South America, and South-Eastern Asia [1]. Cervical cancer is most prevalent among women aged 40 to 60 years. However, S. Neumeyer *et al.* [2], in a German study, reported that the incidence in women over 65 years, who constituted 30% of all cases, may be underestimated due to the lack of hysterectomy correction in the data. The World Health Organization (WHO) [3] emphasised the role of HPV infection and has classified squamous cell carcinomas of the uterine cervix primarily into HPV-associated and HPV-independent types. Squamous cell carcinoma, not otherwise specified (NOS), remains a separate entity. Similarly, adenocarcinomas are mainly grouped as HPV-associated or HPV-independent types of the uterine cervix.

There are limited studies available that have examined the different histopathological diagnoses offered by pathologists in biopsy-diagnosed cervical carcinoma cases, based on the latest WHO guidelines. A.K. Höhn *et al.* [4] noted that the 2020 WHO classification of tumours of the lower female genital tract is designed to distinguish between HPV-associated and HPV-independent squamous cell carcinoma. Histopathological morphology alone cannot differentiate between these two forms; therefore, p16 immunohistochemistry is recommended. P.D. Chaganti *et al.* [5], in India, studied 124 cases of cervical cancer diagnosed through cervical biopsies and other hysterectomy specimens, and performed p16 immunohistochemistry on 40 cases only. Based on the results of p16 IHC, they concluded that 90% of the 40 cases were HPV-associated, and 10% were HPV-independent. W.K. Cho *et al.* [6] also applied the 2020 WHO classification to retrospectively review 365 patients with endocervical adenocarcinoma who had undergone hysterectomy, and compared the HPV-associated carcinomas with HPV-independent types in terms of tumour characteristics, patterns of recurrence, and survival outcomes. They observed that 75.3% of cases were HPV-associated, while 24.7% were HPV-independent adenocarcinomas, which were found to have a poorer prognosis and lower survival rates.

Several studies have investigated mixed pre-cancerous and cancerous lesions of the cervix. F.S. Medeiros *et al.* [7], in their study, collected a total of 94 cervical biopsies (62 from cervical lesions and 32 from adjacent areas near the lesions) from 62 Brazilian women and subjected them to p16 IHC. Two pathologists classified the lesions blindly as benign, LSIL, or HSIL. They noted that the intensity of p16 positivity increased with the severity of the lesions: 5% of LSIL and 37% of HSIL cases showed high p16 expression. Z. Zuberi *et al.* [8] also conducted a study in which they collected 145 cervical biopsies from lesions ranging from benign to malignant in Tanzania, to assess p16 and topoisomerase II-alpha (TOP2A) protein expression. Ninety-five biopsies were from malignant lesions. Two independent pathologists also reviewed the slides. They found p16 to be strongly positive in all 83 SCC (100%) and in six adenocarcinomas. R. Ebisch *et al.* [9], in their study, noted that combining H&E and p16 staining in 326 colposcopic cervical biopsy specimens (from lesions ranging from CIN I to carcinoma) led to a change in diagnosis in 27.3% of cases (n = 89), with a decrease in the number of CIN I and CIN II diagnoses, and an increase in the number of CIN III and cancer diagnoses, compared to standard H&E-based CIN diagnosis alone.

As can be seen from the above, there are few studies that have specifically analysed p16 expression in cervical carcinomas diagnosed solely on biopsy specimens. Most existing studies have investigated mixed cervical lesions, ranging from normal to malignant, and some included hysterectomy specimens as well. No studies were identified in which the previous diagnoses of cervical carcinomas were revised and reclassified according to the 2020 WHO Female Genital Tumours (FGT) guidelines, and correlated with HPV association status using p16 IHC. There was a need for a study to address this gap. The present study aimed to analyse the results of p16 immunohistochemistry following a review and reclassification of all previously biopsy-diagnosed cervical carcinomas in accordance with the latest WHO guidelines.

Materials and Methods

A retrospective, descriptive cross-sectional study was conducted to evaluate p16 expression in all cases of cervical carcinoma diagnosed from cervical biopsies received at the Department of Pathology of a tertiary care centre in Eastern part of India over a five-year period, from January 2017 to 31 December 2021. The study was approved by the Institutional Ethics Committee (Application number 155BH/05/IEC/2022) and adhered to the principles of the Declaration of Helsinki [10]. No patient intervention of any kind was involved in the study. Relevant data, including age and previous final diagnosis, were retrieved from histopathological reports and respective histopathology registers. All cases of cervical carcinoma diagnosed on cervical biopsy during the study period were included, provided that Formalin-fixed, paraffin-embedded (FFPE) blocks with adequate residual

tissue were traceable. Cases with untraceable FFPE blocks or blocks with insufficient residual tissue were excluded.

The total number of cervical biopsies received during the study period was recorded, along with the total number of cervical carcinoma cases diagnosed by various pathologists. All H&E-stained slides for each diagnosed case, along with the corresponding FFPE blocks, were retrieved and assessed for staining quality and intensity. In cases where slides were poorly stained or faded, fresh sections were prepared and stained with haematoxylin and eosin. All slides were reviewed independently by two pathologists; in cases of disagreement, a third pathologist provided a final opinion. FFPE blocks with sufficient residual tissue were selected. New 1-2 sections, each 3-4 microns thick, were cut from each block and mounted on poly-L-lysine-coated slides. For p16 immunostaining, both positive and negative controls were used in each batch. A mouse anti-human p16INK4A monoclonal antibody (Clone MX007, manufactured by Vitro Master Diagnostic, Sevilla, Spain) was used with an indirect detection system. p16 expression was considered positive when moderate to strong nuclear and cytoplasmic staining (block positivity) was observed in more than 10% of epithelial cells, and negative when no staining or weak staining was present in less than 10% of epithelial cells. A multi-head Olympus BX53 microscope (Japan) was used to examine both H&E and immunohistochemically stained slides.

Revised histological diagnoses were made in accordance with the 2020 WHO Classification of Female Genital Tumours [3], based on p16 expression, and recorded as either squamous cell carcinoma, HPV-associated or HPV-independent, or adenocarcinoma, HPV-associated or HPV-independent of the uterine cervix, for each case studied. All data were entered into a Microsoft Excel spreadsheet. SPSS software was used for data analysis and statistical testing. Chi-square tests were applied to examine any association between age, previous diagnosis, and p16 expression. A p-value less than 0.05 was considered statistically significant.

Results and Discussion

The 5th edition of the WHO Classification of Tumours [3] was published nearly six years after the previous 4th edition in 2014 [11]. Numerous changes were introduced across nearly all organ systems, with some of the most significant involving the classification of carcinomas of the uterine cervix, vagina, and vulva, due to substantial advances in the understanding of these neoplasms. The WHO has recommended the adoption of a p16-based classification of squamous lesions of the cervix, vagina, and vulva, as well as glandular lesions of the cervix, distinguishing between HPV-associated and HPV-independent carcinomas. The pathogenesis of cervical carcinoma involves the inactivation of the p53 and retinoblastoma protein (pRb) tumour suppressor genes by the HPV E6 and E7 oncoproteins, respectively. Expression of the E7 viral oncoprotein leads to inactivation of pRb, which in turn increases the activity of E2F transcription factors, driving the cell into the S-phase of the cell cycle and resulting in increased p16 expression [12-14].

Of the 17,142 cervical biopsy samples received between January 2017 and December 2021, only 393 were cervical biopsies (i.e. diagnostic samples from the cervix). Of these, 70 were diagnosed as cervical carcinomas. Only 53 FFPE blocks with sufficient residual tissue could be located for analysis. Therefore, the detection rate of cervical carcinoma in this study over a five-year period was approximately 0.17%. Most patients (70%) were over 50 years of age, with squamous cell carcinoma being the most common histological type, accounting for 94% of cases. The mean age (± standard deviation) was 53 ± 12.4 years, with median and mode values of 60 and 65 years, respectively. Different reporting pathologists were involved throughout the study period, which may have contributed to the use of varying diagnostic terminology in the final histopathological reports. There was notable inconsistency in the diagnostic terms used, with a lack of standardisation across pathologists. The previous histopathological diagnoses are summarised in the pie chart shown in Figure 1. p16 immunostaining was performed on 53 cases, as only these had traceable FFPE blocks with adequate residual tissue. The results of the immunostaining are presented in Table 1.

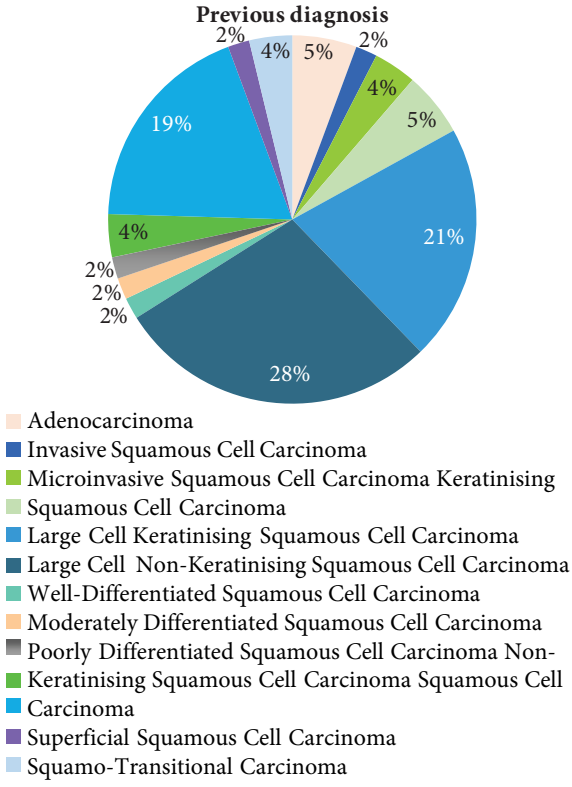

Figure 1. Pie chart showing the distribution of cases according to previous diagnoses

Note: Large Cell Non-Keratinising Squamous Cell Carcinoma (28%), Large Cell Keratinising Squamous Cell Carcinoma (21%), and Squamous Cell Carcinoma (19%) were the most frequently noted categories

Source: compiled by the authors

Table 1. Results of p16 immunostaining

Previous diagnosis	Number of cases (n = 53)	Age ≤50 yr	Age >50 yr	p16 positive	p16 negative	Revised diagnosis according to WHO FGT 2020
SCC	10 (18.8%)	–	10 (27.02%)	7 (14%)	3 (6.7%)	SCC HPV-associated (89%) SCC, HPV-independent (6%) Adenocarcinoma, HPV-associated (5%) (p < 0.001)
Superficial SCC	1 (1.8%)	1 (6.25%)	–	1 (2%)	0	
Invasive SCC	1 (1.8%)	–	1 (2.7%)	1 (2%)	0	
Microinvasive SCC	2 (3.7%)	–	2 (5.4%)	2 (4%)	0	
KSCC	3 (5.6%)	–	3 (8.1%)	3 (6%)	0	
LCKSCC	11 (20.7%)	6 (37.5%)	5 (13.5%)	**11 (22%)**	0	
LCNK SCC	15 (28.3%)	5 (31.25%)	10 (27.02%)	**15 (30%)**	0	
NKSCC	2 (3.7%)	–	2 (5.4%)	2 (4%)	0	
WDSCC	1 (1.8%)	1 (6.25%)		1 (2%)	0	
MDSCC	1 (1.8%)	–	1 (2.7%)	1 (2%)	0	
PDNKSCC	1 (1.8%)	–	1 (2.7%)	1 (2%)	0	
Adenocarcinoma	3 (5.6%)	3 (18.7%)	–	3 (6%)	0	
Squamo-Transitional carcinoma	2 (3.7%)	–	2 (5.4%)	2 (4%)	0	
Total	**53**	**16 (30.1%)**	**37 (69.8%)**	**50 (94.3%)**	**3 (6.7%)**	

Note: SCC – Squamous Cell Carcinoma; KSCC – Keratinising SCC; LCKSCC – Large Cell Keratinising SCC; LCNKSCC – Large Cell Non-Keratinising SCC; NKSCC – Non-Keratinising SCC; WDSCC – Well-Differentiated SCC; MDSCC – Moderately Differentiated SCC; PDNKSCC – Poorly Differentiated Non-Keratinising SCC
Source: compiled by the authors

More than 90% of patients in both age groups – those under and over 50 years – showed p16 expression, while fewer than 5% were p16-negative. Overall, p16 expression was observed in 50 cases (94.3%). All 53 cases were re-classified based on p16 status as squamous cell carcinoma, HPV-associated or HPV-independent of the uterine cervix, and adenocarcinoma, HPV-associated or HPV-independent of the uterine cervix. Eighty-nine per cent of cervical carcinomas diagnosed during the study period were classified as squamous cell carcinoma, HPV-associated, followed by squamous cell carcinoma, HPV-independent (6%) and adenocarcinoma, HPV-associated (5%).

Chi-square tests were performed to evaluate associations between age, previous diagnoses, and p16 expression. While no significant association was observed between age at diagnosis and p16 expression, the highest positivity was noted at 65 years of age (p = 0.483). In contrast, cases previously diagnosed as large cell keratinising and non-keratinising SCC showed the highest p16 positivity, which was statistically significant (p < 0.001). Photomicrographs of different cervical carcinoma subtypes with corresponding p16 immunostaining are shown in Figures 2-4.

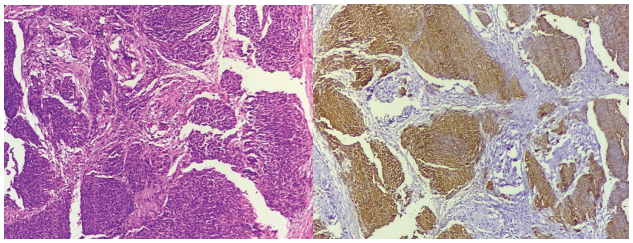

Figure 2. p16-positive in squamous cell carcinomas, HPV-associated, of the uterine cervix (H&E, 10x and IHC, 10x)
Source: compiled by the authors

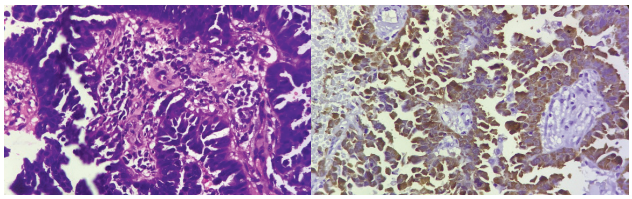

Figure 3. p16-positive in adenocarcinoma, HPV-associated, of the uterine cervix (H&E, 20x and IHC, 20x)
Source: compiled by the authors

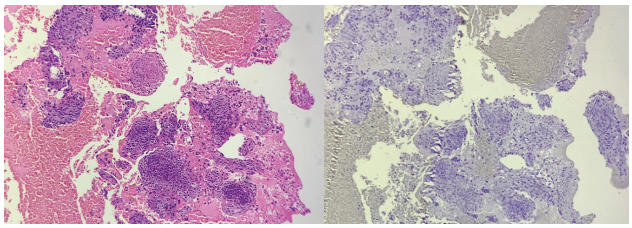

Figure 4. p16-negative in squamous cell carcinoma, HPV-independent, of the uterine cervix (H&E, 10x and IHC, 10x)
Source: compiled by the authors

Most patients in this study were older than 50 years, with a mean age of 53 years. This finding is consistent with the study by C.M. Gnade *et al.* [15], who investigated whether there has been an increase in the age at diagnosis over time, highlighting a shifting age pattern of cervical cancer between 1986 and 2016. They observed that the age at diagnosis increased by 0.2 years per calendar year. In their cohort of 1,019 patients, the average age at diagnosis was 43.7 years in 1986, rising to 49.5 years in 2016. However, among women over 65 years, no significant change was reported over time. Similarly, I. Nicolás *et al.* [16] found a

mean age of 50 years in their study involving 194 women. It is worth noting that while most HPV infections resolve spontaneously, a small proportion may persist and progress to cervical cancer. Although cervical cancer most commonly affects older women, it can also occur in younger age groups. The youngest patient in the current study was 29 years old, and two patients were under the age of 30. B. Gravdal *et al.* [17] used data from the Norwegian Cancer Registry to examine cervical cancer incidence in women from the 1950s onwards. They found that the incidence of cervical cancer among women under 30 years had almost tripled since the 1950s. Among 21,160 cases of cervical cancer (1953-2013), 5.3% were diagnosed in women under 30 years. One possible explanation for this increase is improved detection as a result of expanded cervical screening programmes.

In this study, the majority of patients (89%) showed p16 expression. While age at diagnosis was not significantly associated with p16 status, the highest rate of p16 positivity was observed in women around the age of 65 years. This finding is in line with the study by R.M. Ismail *et al.* [18], who, in their analysis of 95 women with cervical cancer, also found no significant age association with p16 expression. However, they did observe low or absent p16 expression in older women over 60 years of age. In the present study, the higher age at diagnosis among older women could be explained by the possibility that they had been harbouring HPV infections for many years, during which precancerous lesions such as CIN went unnoticed. These women may have been missed by routine screening programmes due to a lack of awareness or due to limited accessibility to healthcare services, especially for the early detection of pre-cancerous cervical lesions.

Regarding diagnosis, it was observed in this study that pathologists used a wide variety of diagnostic terms over time. Despite the availability of the WHO FGT 2014 classification [11] and the more recent WHO FGT 2020 classification [3], which were supposed to guide reporting during most of the study period, Figure 1 clearly indicates a lack of consistency and uniformity in diagnostic terminology among the reporting pathologists. Unlike the FGT 2020, the FGT 2014 classification did not mandate the use of p16 immunohistochemistry, which may explain the absence of p16 testing in the majority of cases diagnosed prior to 2020. In laboratories – particularly those in low- and middleincome countries – the availability of advanced diagnostic tests may be limited. Furthermore, the knowledge and training of pathologists can play a major role in such inconsistencies. In the tertiary care centre where this study was conducted (in Eastern India), the hospital was able to provide p16 testing for all confirmed cervical carcinoma cases at no additional cost to the patients. However, this may not be the case in other facilities, where the p16 test may be either unavailable or financially inaccessible. In such settings, diagnosis relies solely on H&E morphology, potentially contributing to non-uniform and inconsistent reporting.

The most commonly assigned previous diagnosis was large cell non-keratinising squamous cell carcinoma (28%), followed by large cell keratinising squamous cell carcinoma (21%), and squamous cell carcinoma (19%). Diagnostic terms such as well-differentiated, moderately differentiated, and poorly differentiated SCC, or simply SCC, were frequently used. It is evident that this wide range of diagnostic terminology may lead to confusion and a lack of clinical utility for physicians and surgeons when planning patient care and management. Moreover, these diagnoses were not entirely consistent with the 2014 WHO criteria, which categorised cervical tumours into epithelial, mesenchymal, and mixed epithelial and mesenchymal types [9]. Among epithelial tumours, three main categories were identified: squamous tumours, glandular tumours, and other epithelial tumours. The squamous category included squamous cell carcinoma, NOS, its morphological variants, early invasive SCC, CIN III, and SCC *in situ*. Similarly, glandular tumours included adenocarcinoma and its variants, early invasive adenocarcinoma, and adenocarcinoma *in situ*. The term "large cell" was typically used in reference to neuroendocrine tumours.

No studies were identified that examined the association between previous histopathological diagnoses and the revised classification of carcinomas as either HPV-associated or HPV-independent. It is reiterated that, in the present study, cases previously diagnosed with large cell non-keratinising SCC and large cell keratinising SCC showed the highest p16 positivity, which was statistically significantly associated ($p < 0.001$). The finding of 89% p16 positivity in the current study is consistent with findings from several other studies. B. Vedula *et al.* [19] conducted a study assessing p16 expression in both dysplastic and neoplastic cervical lesions in 86 cases over two years, reporting 88% p16 positivity. Similarly, I. Nicolás *et al.* [16] observed 96% p16 positivity in a cohort of 194 cervical cancer patients, in a study evaluating clinicopathological features, HPV genotype correlations, and the prognostic role of p16. R.M. Ismail *et al.* [18] also reported p16 expression in 80% of their 95 patients.

Chemotherapy, immunotherapy, and radiotherapy remain effective treatment modalities for HPV-related cervical carcinomas [20]. Both S. da Mata *et al.* [21] and I. Nicolás *et al.* [16] found that p16 expression is associated with improved survival, although it is not an independent prognostic factor. In the case of p16-positive cervical adenocarcinomas, M. Ishikawa *et al.* [22], in a study involving 82 patients, reported a favourable prognosis and improved overall survival. Conversely, as noted by B. Xing *et al.* [23] and J.-E. Lee *et al.* [24], patients with HPV-negative tumours are more likely to present at an advanced FIGO stage, with a consequently poorer prognosis.

This study observed a lack of consistent implementation of the WHO classification of tumours of the uterine cervix by pathologists over the study period. A significant association was found between previously diagnosed cases of large cell keratinising and non-keratinising squamous cell carcinoma and p16 positivity, whereas age at

diagnosis showed no significant correlation with p16 expression. Among the 53 reclassified cases, 89% were identified as HPV-associated squamous cell carcinoma, 6% as HPV-independent squamous cell carcinoma, and 5% as HPV-associated adenocarcinoma of the uterine cervix.

Conclusions

It can be concluded that in this single-centre study, in which all biopsy-diagnosed cervical carcinoma cases were re-evaluated and reclassified in accordance with the latest WHO guidelines, a wide variation in diagnostic terminology was noted among pathologists over the study period. Some reports included comprehensive information, including p16 status, while others did not. This inconsistency in histopathological reporting may be attributed to several factors, the most prominent being the lack of mandatory p16 IHC in all confirmed cervical carcinoma cases. Additional contributing factors could include insufficient training of reporting pathologists and limited availability of testing kits. p16 immunohistochemistry performed on all 53 cases demonstrated a high positivity rate of 89%, which is comparable to that reported in the literature. While age was not significantly associated with p16 expression, the highest rate of positivity was observed in patients over 65 years of age. This study also investigated the relationship between prior diagnoses and revised classification, finding that cases previously identified as large cell keratinising or non-keratinising squamous cell carcinoma were predominantly p16-positive, with a significant correlation. There is scope for further research, including multicentre studies, to assess how pathologists across different regions or countries classify cervical carcinomas and how their diagnoses align with p16 IHC or HPV genotyping results.

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Conflict of Interest

None.

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Оцінка експресії гену p16 у карциномах шийки матки, діагностованих лише на основі біопсії шийки матки

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Анотація. Використання патологами різних діагностичних термінів у своїх гістопатологічних звітах щодо пухлин шийки матки може впливати на клінічні рішення лікарів або хірургів, які проводять лікування. Було проведено ретроспективне поперечне дослідження з метою оцінки експресії p16 у всіх випадках раку шийки матки, діагностованих виключно за допомогою біопсії шийки матки, після перегляду попередніх діагнозів. Також було досліджено будь-який зв'язок між експресією p16 та віком пацієнтки або попереднім діагнозом. Середній, медіанний та модальний вік у дослідженні становили відповідно 53 ± 12,4, 60 та 65 років. У 70 з 393 біопсій шийки матки (17,8 %) було діагностовано рак шийки матки. Було виявлено значну невідповідність у використанні діагностичної термінології патологами. З 53 випадків, поданих для імунофарбування p16, 50 випадків (94,3 %) були p16-позитивними, а 3 (5,7 %) – негативними. Більше того, 88,7 % випадків було перекласифіковано як плоскоклітинний рак, асоційований з вірусом папіломи людини, 5,7 % – як плоскоклітинний рак, незалежний від вірусу папіломи людини, і 5,7 % – як аденокарцинома шийки матки, асоційована з вірусом папіломи людини. Середній вік на момент діагностики 65 років не мав істотного значення; проте попередні категорії великоклітинного некератинізуючого та кератинізуючого плоскоклітинного раку показали найвищу p16-позитивність ($p < 0,001$). Включення статусу p16 до патологічних звітів не тільки сприятиме уніфікації гістопатологічних звітів, але й допоможе лікарям і хірургам у визначенні відповідного підходу до лікування, прогностичної цінності

Ключові слова: вірус папіломи людини; імуногістохімія; плоскоклітинний; гематоксилін; еозин

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Predictive modelling of clinical outcomes in acute tonsillitis based on microbiome analysis and machine learning algorithms

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Abstract. Acute tonsillitis is a common disease with high clinical variability. Traditional approaches based on clinical scores (e.g., Centor) are often insufficient for accurately predicting individual outcomes. The aim of the study was to determine the significance of integrating clinical parameters and oropharyngeal microbial composition data to construct a predictive model for disease duration and symptom severity using the random forest method. Fifty-two patients with acute tonsillitis were examined. Bacteriological analysis of oropharyngeal swabs, clinical assessment using the Centor score, and rapid testing for streptococcal and viral infections were performed. Random forest and linear discriminant analysis models were constructed and compared. The random forest model demonstrated higher accuracy compared to linear discriminant analysis, especially for predicting pain intensity (overall accuracy 81.8% vs 55.0%). For disease duration, the accuracy of the random forest was 72.7% vs 75.0% for linear discriminant analysis. Feature importance analysis revealed that integrating microbiome indices (pathogen/commensal ratio – Pathogen_ratio) with the Centor clinical score significantly improved predictive ability. Disease duration was associated with bacterial aetiology (positive streptococcal test) and smoking status, while pain intensity correlated with microbial dysbiosis parameters. The combination of clinical and microbiological data in machine learning models improves the accuracy of disease progression prediction and can be used to develop personalised treatment approaches

Keywords: random forest model; oropharyngeal microbiome; Centor score; rapid diagnosis; clinical prognosis; group A streptococcus; viral antigens

Introduction

Acute tonsillitis remains one of the most common infectious diseases in the world, placing a significant burden on healthcare systems. The clinical course of this disease is highly variable, making it difficult to predict its duration and severity on an individual basis. Traditional diagnostic approaches are often insufficient for accurate risk assessment. The introduction of machine learning methods for analysing complex clinical and microbiome data opens up new opportunities for creating accurate prediction tools, which will optimise treatment and improve outcomes for patients.

The relevance of an integrated approach combining microbiome research and machine learning methods was

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confirmed by a number of recent studies. In particular, a review by S.M. Pukhlik & P.O. Zaporozhchenko [1] proved that the state of the microbiome of the lymphoid tissue of the pharynx is a decisive factor determining the susceptibility to chronic inflammation of the tonsils and the severity of its course. Accordingly, a study conducted by S.V. Bobruk [2] substantiated approaches to rational antibiotic therapy, demonstrating the ineffectiveness of empirical selection due to the spread of β-lactam strains. Continuing this theme, S. Wu *et al.* [3] performed 16S rRNA sequencing, identifying specific marker taxa for chronic tonsillitis (*Fusobacterium, Peptostreptococcus*) and hypertrophy (commensal *Streptococcaceae*). Similarly, in their work, H. Xu *et al.* [4] demonstrated that the tonsil microbiome in children is more diverse and contains unique pathobionts, such as *Fusobacterium nucleatum* and *Porphyromonas endodontalis*, associated with clinical manifestations. This confirmed that the tonsil microbiome may play an independent and possibly more important role in pathogenesis than the overall oral microbiota. D.R. Katundu *et al.* [5] made a significant contribution to understanding the underlying processes. Their work, focused on studying bacterial colonisation of the surface and core of the tonsils, found that children with recurrent tonsillitis had a significantly higher frequency of detection of group A β-haemolytic streptococci (GAS) both on the surface (68%) and in the core (44%) of the tonsils. This directly indicated that deep colonisation of GAS in the crypts is a key factor contributing to the persistence of inflammation and recurrence. In addition to microbiome research, significant progress has been made in the application of machine learning. In a retrospective cohort study, Z. Jin *et al.* [6] conducted a comprehensive comparison of machine learning algorithms for differentiating bacterial and viral pharyngitis based on haematological markers. Among the seven algorithms tested, the Random Forest (RF) model demonstrated the highest predictive accuracy. An important result was that the analysis of feature importance revealed the ratio of neutrophils to lymphocytes as one of the most powerful predictors. E.Y. Alqaissi *et al.* [7] systematised the use of machine learning algorithms (RF, Support Vector Machine, Artificial Neural Network) for the diagnosis of infections in a review, emphasising their ability to detect nonlinear relationships between clinical variables that are not accessible to traditional statistics.

The effectiveness of RF has been confirmed in studies of various pathologies. For example, X. Zhou *et al.* [8] successfully used this algorithm to differentiate between COVID-19 and *Mycoplasma pneumoniae* infection. At the same time, Y. Xiong *et al.* [9] demonstrated its superiority in predicting the severity of COVID-19 through comparative analysis. W. Hong *et al.* [10] came to similar conclusions by applying RF to predict severe acute pancreatitis. The versatility of this method is also demonstrated by ecological and epidemiological studies. A striking example was the work of F. Cappelli *et al.* [11], in which RF was used to analyse the impact of a complex of environmental factors on mortality from cardiorespiratory diseases. The model confirmed the priority impact of PM2.5 particles and accurately ranked the factors, justifying environmental policy. Returning to medical practice, an important contribution by W. Zhao *et al.* [12] was the creation of an RF model for predicting infections caused by multidrug-resistant microorganisms. The model identified and ranked key clinical predictors, such as length of hospitalisation and previous antibiotic therapy, allowing early identification of high-risk patients. Concluding the review, the work of X. Yang *et al.* [13] was considered, which confirmed the high effectiveness of RF in predicting respiratory diseases based on a large set of clinical and laboratory data, demonstrating exceptional accuracy, significantly higher than other algorithms.

Thus, the current literature indicates the promise of integrating oropharyngeal microbiome data and machine learning methods. However, despite the wide range of studies cited, the issue of predicting the course of acute tonsillitis based on such data remains understudied. The aim of the study was to evaluate the prognostic value of integrating clinical and microbiological data for predicting the course of acute tonsillitis using the RF method.

Materials and Methods

The study examined 52 patients aged 17-30 years with clinical signs of acute inflammation of the palatine tonsils. Inclusion in the study was based on the following criteria: confirmed diagnosis of acute tonsillitis according to current clinical guidelines, age over 17 years, presentation within 72 hours of symptom onset, absence of complications, signed informed consent. Exclusion criteria were: chronic or recurrent tonsillitis, immunodeficiencies (including human immunodeficiency virus), oncological pathology, autoimmune, mental, severe concomitant diseases, pregnancy/lactation, allergy to antibiotics, resistance to basic therapy, recent surgery or systemic therapy with glucocorticosteroids, as well as refusal to participate. The diagnosis was established in accordance with the Unified clinical protocol for primary, secondary (specialised) and tertiary (highly specialised) medical care Tonsillitis [14] using the Centor and Centor/McIsaac scores. Pain intensity was assessed using a visual analogue score divided into three classes: severe pain (7-10 cm), moderate pain (4-6 cm), and mild pain (1-3 cm). All patients provided written informed consent in accordance with the protocol (No. 81, 03.04.2025) approved by the Bioethics Committee of the I. Ya. Horbachevsky Ternopil National Medical University. The study also complied with the requirements of the Declaration of Helsinki [15] and Order of the Ministry of Health of Ukraine No. 690 [16].

The study material was oropharyngeal swabs collected with sterile swabs for rapid tests and bacteriological analysis. Pathogens were detected using immunochromatographic tests (for *Streptococcus* group A ("Ecotest", China)), influenza A/B viruses, adenoviruses, SARS-CoV-2, RSV ("MedBioAlliance", Ukraine)), as well as the classic bacteriological method. Rapid tests were performed by doctors, and the

results were evaluated visually. Bacteriological studies were performed on nutrient media appropriate for the type of microorganisms. Isolates were identified using standard microbiological methods, including evaluation of colony morphology, haemolytic activity on blood agar (5% sheep erythrocytes, "Sanimed-M", Kharkiv), and a series of biochemical tests (catalase, coagulase, lecithinase for Gram-positive cocci; Simmons citrate, indole, motility for Gram-negative rods), and novobiocin (5 μg) for differentiation of staphylococci, in accordance with standard microbiological protocols using reagents ("Ukrmediasnab LLC", Dnipro; "Pharmactiv", Kyiv). Microorganism counts were determined by colony enumeration and expressed as colony-forming units (CFU) per mL [17]. Microsoft Office 2016 (Microsoft, USA) and Python 3.11 (Python Software Foundation; scikit-learn, pandas, numpy libraries; Colab.research (Google Colaboratory)), Statistics Kingdom (Australia) were used for data analysis. Statistical data processing was performed using Python 3.11 software with the specified libraries.

The statistical significance of the differences was tested using the t-test (for two groups) and analysis of variance (ANOVA) (for three groups). ROC (Receiver Operating Characteristic) analysis was used to assess the predictive ability of individual clinical and microbiological predictors. The area under the Curve (AUC) was selected as the main indicator of model effectiveness. The AUC values were interpreted according to the generally accepted score: 0.9-1.0 – excellent quality, 0.8-0.9 – very good, 0.7-0.8 – good, 0.6-0.7 – low, 0.5-0.6 – random prediction level. The following were used as independent variables (predictors): the level of β-haemolytic streptococci group A, the microbial imbalance index (Strep_balance), pathogen load index (Pathogen_ratio), level of each of the detected bacteria, rapid test results for viral infection (Viral_Test) and beta-haemolytic *streptococcus* (Strept_test), clinical assessment using the Centor score (Centor_Score), smoking. Formulas for calculating indices:

The microbial imbalance index (Strep_balance) is calculated using the following formula:

$$\text{Strep}_{\text{balance}} = LN\left[\frac{(\beta\text{ - haemolytic streptococci} + 1)}{(\alpha\text{ - haemolytic streptococci} + 1)}\right], \quad (1)$$

where *LN* – natural logarithm. The addition of one is used to avoid uncertainty when dividing by zero.

The pathogen load index (Pathogen_ratio) is defined as the proportion of pathogenic bacteria species out of the total number of bacteria in the sample and is expressed as a percentage:

$$\text{Pathogen}_{\text{ratio}} = (\Sigma \text{ pathogens} \div \Sigma \text{ all bacteria}) \times 100, \quad (2)$$

where the group of pathogens included the following species: β-hemolytic *Streptococcus* spp., *S. aureus*, *K. pneumoniae* and *Enterobacter* spp.

The effectiveness of the models was assessed by precision, recall, and F1-measure. The results were considered statistically significant at $p < 0.05$. The classification of patients with mild pain (1-3 points) proved to be the most difficult due to the small number of observations in the test sample ($n = 1$). The limitations of this study were its single-centre design and sample size, which may affect the generalisability of the models.

Results and Discussion

Bacteriological examination of throat swabs from 52 patients with acute tonsillitis confirmed the complex polymicrobial composition of the microbiota. In total, isolates of aerobic and facultative anaerobic microorganisms belonging to 11 families of bacteria and fungi were identified (Table 1). The microbial spectrum included both Gram-positive (*Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Rothia* spp., *Bacillus* spp.), and Gram-negative microorganisms (*Neisseria* spp., *Moraxella* spp., *Haemophilus* spp., *Klebsiella* spp., *Serratia* spp., *Enterobacter* spp.), as well as yeast-like fungi of the genus *Candida*. Among the morphological forms, cocci-like bacteria (*Streptococcus* spp., *Staphylococcus* spp., *Neisseria* spp., *Moraxella* spp.), prevailed, while rod-shaped forms were represented by *Corynebacterium* spp., *Bacillus* spp., *Haemophilus* spp., *Klebsiella* spp., *Serratia* spp. and *Enterobacter* spp.

Table 1. Taxonomic classification of detected microorganisms

Species / Genus	Family	Frequency of detection (%)
β-hemolytic *Streptococcus* spp.	Streptococcaceae	11.5
α-hemolytic *Streptococcus* spp.	Streptococcaceae	76.9
γ-hemolytic *Streptococcus* spp.	Streptococcaceae	17.3
Corynebacterium spp.	Corynebacteriaceae	60.0
Rothia spp.	Micrococcaceae	7.7
Neisseria spp.	Neisseriaceae	40.4
Haemophilus spp.	Pasteurellaceae	5.7
Staphylococcus aureus	Staphylococcaceae	32.7
Coagulase-negative *Staphylococcus* spp.	Staphylococcaceae	7.7
Moraxella spp.	Moraxellaceae	5.7
Serratia spp.	Yersiniaceae	1.9
Klebsiella pneumoniae	Enterobacteriaceae	3.8
Enterobacter spp.	Enterobacteriaceae	1.9
Candida spp.	Saccharomycetaceae	1.9
Haemophilus spp.	Pasteurellaceae	5.7

Source: created by the authors based on research

The bacterial population was dominated by α-haemolytic streptococci (76.9% of isolates), which were characterised by high colonisation density (from 10^7 to 10^9 CFU/ml). *Neisseria* spp. (40.4%) and *Corynebacterium* spp. (28.9%) also had a high prevalence. β-haemolytic streptococci of group A, which are key aetiological agents of acute tonsillitis, were detected in 11.5% of patients, mostly with a high level of colonisation (>10^5 CFU/ml). Among other clinically significant isolates, *S. aureus* was detected in 32.7% of cases, and coagulase-negative staphylococci in 7.7%. Rarer components of the microbiota were *Rothia* spp., *Moraxella* spp., *Haemophilus* spp., *Klebsiella pneumoniae*, *Serratia* spp. and *Enterobacter* spp. (frequency <10%), which, however, had potential clinical significance at high colonisation densities. Yeast-like fungi of the genus *Candida* were isolated in 1.9% of cases. According to the Centor clinical score, most patients (59.6%, n = 31) scored 3 points, indicating a moderate probability of bacterial aetiology. In 23.1% (n = 12) there were 2 points, in 15.4% (n = 8) – 4 points, and only in one patient (1.9%) – 1 point. Immunochromatographic rapid tests confirmed viral aetiology in 67.3% of cases (n = 35), while a positive result for streptococcal infection was found in only 9.6% (n = 5). ROC analysis was performed to determine the diagnostic and prognostic value of individual predictors of pain intensity and disease duration. The results showed that predicting pain intensity based on individual factors is difficult (Fig. 1).

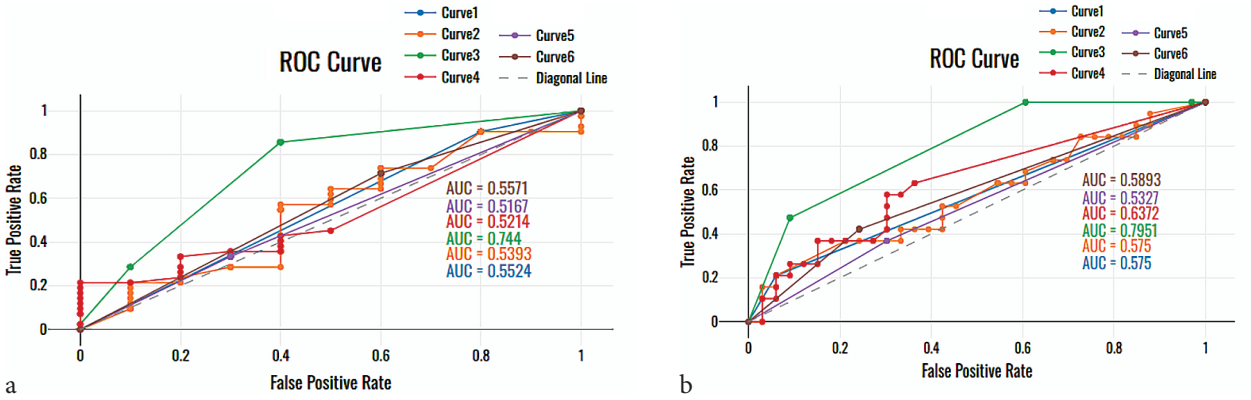

Figure 1. ROC curves for assessing the predictive ability of the studied features

Note: a – ROC curves for the pain intensity factor in relation to predictors; b – ROC curves for the duration factor in relation to predictors. Curve 1 (blue) – β-haemolytic *Streptococcus* spp., Curve 2 (orange) – Strep_balance, Curve 3 (green) – Centor_Score, Curve 4 (red) – Pathogen_ratio, Curve 5 (purple) – *S. aureus*, Curve 6 (brown) – Viral_Test

Source: created by the authors based on research and generated automatically in the Statistics Kingdom programme

ROC analysis showed limited predictive ability of individual predictors for pain intensity (Fig. 1a). The highest AUC was observed for the Centor score (0.74), but the result is not statistically significant (p = 0.225). All other predictors, including pathogenic bacteria (β-hemolytic *Streptococcus* spp., *S. aureus*) and calculated indices (Strep_balance, Pathogen_ratio), as well as the results of rapid tests for the detection of viruses (Viral_Test), showed AUC values in the range of 0.52-0.56, which corresponds to the level of random prediction (p > 0.05). This indicates the limited ability of individual factors to predict the severity of pain syndrome. Analysis of predictors of disease duration demonstrated higher discriminatory ability (Fig. 1b). The best individual predictor was the Centor score (AUC = 0.80, p < 0.001), which corresponds to a good level of accuracy. The second most important predictor is Pathogen_ratio (AUC = 0.64), but its result did not reach statistical significance (p = 0.073). Other predictors (β-haemolytic streptococci, Strep_balance, *S. aureus* and Viral_Test) showed moderate predictive ability with AUC values in the range of 0.53-0.59, and the results are not statistically significant. Thus, the Centor score is the only statistically significant individual predictor, but only for the duration of the disease. None of the predictors studied, including the Centor score, showed statistically significant predictive ability for predicting pain intensity.

The RF model demonstrated high overall accuracy in classifying the clinical manifestations of acute tonsillitis. For predicting the duration of the disease (1-5 days vs 6-9 days), the accuracy was 72.7%. The best results were obtained for patients with a short course (Precision = 0.83, Recall = 0.71, F1-score = 0.77). For a longer course, the accuracy was lower (0.60), reflecting the complexity of differentiating this category. In the case of pain intensity (classes 1-3), after applying SMOTE (Synthetic Minority Over-sampling Technique) to balance the classes, the model achieved an overall accuracy of 81.8%. For the "severe pain" class (7-10 points), ideal indicators were obtained (Precision, Recall, F1 = 1.00), for "moderate" – Precision and Recall = 0.88. Compared to linear discriminant analysis (LDA), which had slightly higher accuracy in predicting duration (75.0% vs 72.7%), RF significantly exceeded it in predicting pain (81.8% vs 55.0%). This demonstrates the advantages of nonlinear ensemble methods for working with microbiome data and clinical parameters when classes are unevenly distributed (Table 2).

Table 2. Comparison of model effectiveness

Metric	Duration prediction		Pain prediction	
	LDA	RF	LDA	RF
Accuracy	75.0%	72.7%	55.0%	81.8%
Best AUC (single predictor)	0.8 (Centor)	-	0.74 (Centor)	-
Precision (Class 1)	0.73	0.83	0.50	1.00
Recall (Class 1)	0.80	0.71	0.67	1.00
F1-score (Class 1)	0.76	0.77	0.57	1.00
Precision (Class 2)	0.78	0.60	0.50	0.88
Recall (Class 2)	0.70	0.75	0.33	0.88
F1-score (Class 2)	0.74	0.67	0.40	0.88
Precision (Class 3)	-	-	0.62	0.00*
Recall (Class 3)	-	-	0.67	0.00*
F1-score (Class 3)	-	-	0.64	0.00*
Number of signs in the top	5	15	5	10
The most important signs	Strep_balance	Centor_Score	Strep_balance	Centor_Score
	Strep_Test	*Neisseria* spp.	Centor_Score	Strep_balance_calc
	Beta-hemolytic	Viral_Test	Pathogen_ratio	Pathogen_ratio_calc

Note: *For class 3 (pain), there was only 1 sample in the test set, which complicated classification; the best single predictor according to the results of ROC analysis (the highest AUC for both clinical outcomes studied (duration of illness and pain intensity) was the Centor Score (Centor_Score)
Source: created by the authors based on the study

According to the analysis of the importance of signs (Table 2; Fig. 2), Centor_Score, which reflects the overall clinical picture, remained the leading predictor in both models. However, the specificity of other signs differed.

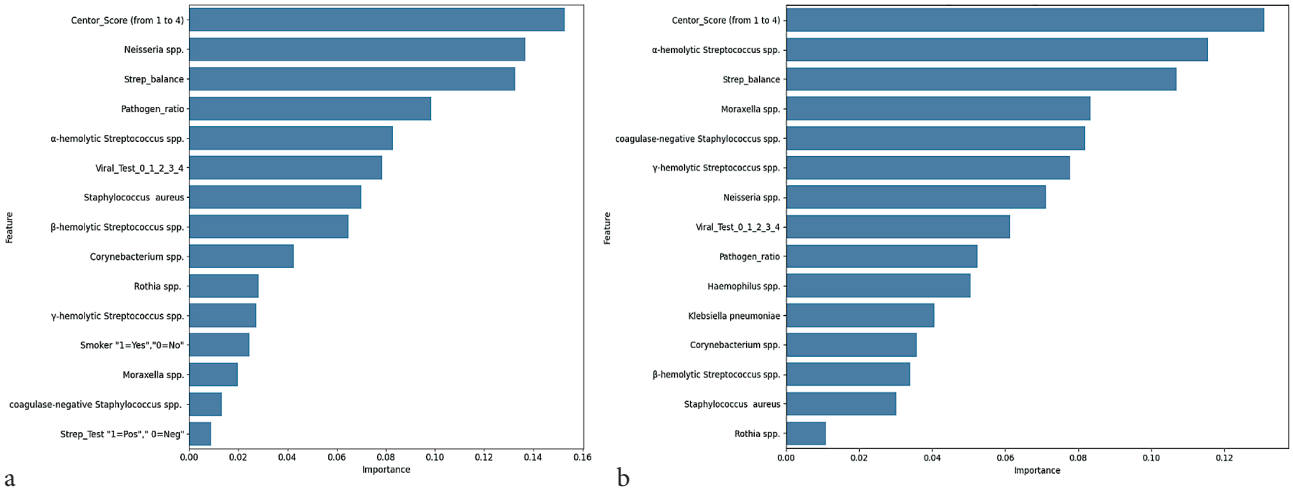

Figure 2. Graph showing the importance of symptoms
Note: a – for duration of illness; b – for intensity of pain
Source: created by the authors based on research and generated automatically by Python 3.11 Colab.research (Google Colaboratory)

To predict the duration of the disease (Fig. 2a), the model identified the Centor clinical score (Centor_Score) as the most important predictor. This confirmed that the overall severity of clinical symptoms is the main factor determining the duration of the disease. Among the microbiome factors, the presence of *Neisseria* spp. and β-haemolytic streptococcus made a significant contribution to the prognosis. Clinical and anamnestic factors such as a positive rapid strep test (Strep_Test) and smoking (Smoking) were also among the most important features for this model. This may indicate that bacterial aetiology (positive test) and factors that impair local immunity (smoking) are associated with a prolonged recovery period. To predict the intensity of pain syndrome (Fig. 2b), the model also identified Centor_Score as the most powerful predictor. However, unlike the model for duration, microbiome indices played a much greater role here: the ratio of pathogens (Pathogen_ratio) and the balance of streptococci (Strep_balance). This indi-

cated that the subjective sensation of pain correlates more strongly with the overall microbial load and imbalance of the oropharyngeal microbiota than with individual clinical or anamnestic factors.

As shown in Table 2, the RF model outperformed LDA in predicting pain intensity, showing significantly higher accuracy (81.8% vs 55.0%). This demonstrates the advantage of the ensemble nonlinear method for analysing complex relationships between microbiome and clinical data, especially when these relationships are not linear. At the same time, both models showed similar and high effectiveness in predicting disease duration (72.7% for RF and 75.0% for LDA). This may indicate that the relationships between features important for predicting duration are more linear and easier to interpret using linear methods. It is important to note that the RF model required more features to achieve optimal performance (15 features for duration and 10 for pain versus 5 features in LDA for both cases), confirming its ability to detect and utilise more complex and less obvious dependencies in the data.

The results of this study, which demonstrated the ability of microbiome indices and the RF algorithm to predict the course of acute tonsillitis, were confirmed and contextualised in a number of scientific studies. The significant advantage of RF in predicting pain intensity compared to LDA (81.8% vs 55.0%) can be explained by a fundamental difference in approaches. As demonstrated by E.J. El Hachem *et al.* [18], methods such as LDA are effective for identifying latent structures and clustering, but they may be limited in modelling complex nonlinear relationships. In contrast, RF's ability to detect such relationships is confirmed by its successful application in various fields. For example, the effectiveness of RF in the field of infectious diseases was confirmed in the work of J. Wang *et al.* [19], where this algorithm not only predicted the probability of critical condition in COVID-19 patients with high accuracy, but also, thanks to feature importance analysis, identified key prognostic factors such as age and lactate dehydrogenase levels. This ability to simultaneously provide an accurate prediction and ensure its interpretation makes RF particularly valuable for clinical studies aimed at revealing pathogenetic mechanisms.

The versatility of RF as a tool for building predictive models was confirmed in the work of M.S. Sharif *et al.* [20]. They used it to create a model that assesses the health risks of regular travel based on data from wearable sensors. This example showed that RF is effective not only in controlled laboratory conditions, but also for predicting complex biological outcomes based on indirect signs. In addition, given the prevalence of infectious diseases, the key task is not only treatment, but also prediction and control of their spread. The study by T.S. Thapelo *et al.* [21] clearly demonstrated this potential: they used an "informed" RF algorithm to model complex relationships between epidemiological data, government policies, and population mobility. Their work confirmed that RF is an extremely flexible tool capable of detecting complex, non-linear patterns in real data.

The importance of microbiome factors revealed in the authors' study was consistent with fundamental work in the field of otolaryngology. The study by J. Galli *et al.* [22] directly pointed to a key mechanism, proving that the formation of *Haemophilus influenzae* biofilm on the tonsils is a critical factor in the development of recurrent adenotonsillitis. This emphasised that not only the presence but also the structure (biofilm) and behaviour of the microbial community determine the clinical outcome. Thus, the authors' approach to analysing the microbiome, aimed at assessing its functional state through the Pathogen_ratio index, is entirely justified, as it allows the pathogenic potential of the microbial community to be assessed. The work of F.J. García Callejo *et al.* [23], devoted to the treatment of abscesses, provided clinical confirmation of this concept. They demonstrated that such serious complications often arise precisely because of the inability of standard therapy to penetrate the deep crypts of the tonsils and eradicate the microbial biofilm. This highlights the limitations of an approach based solely on superficial clinical assessments.

A systematic review by J. Aalbers *et al.* [24] confirmed the role of the Centor score as a useful tool for the initial assessment of the likelihood of streptococcal pharyngitis in adults. However, subsequent studies, notably that of J. Jääskeläinen *et al.* [25], which found a weak correlation between the Centor score and rapid strep test results in children, clearly demonstrated its limitations, particularly in terms of accuracy. Bridging the gap between clinical convenience and diagnostic accuracy requires the development of new, objective prognostic tools. Thus, the authors' study proposed using microbiome composition data for prognosis, which is a logical step in overcoming these limitations. Thus, this approach does not negate the usefulness of Centor, but significantly complements it.

From a clinical practice perspective, the authors' predictive models can be directly applied to patient stratification. A systematic review by O. Guntinas-Lichius *et al.* [26] emphasised the importance of an individualised approach to the treatment of recurrent acute tonsillitis, which is entirely consistent with the authors' objective. The introduction of models capable of identifying patients at risk of prolonged course or severe pain in advance will allow doctors to prescribe more intensive monitoring or symptomatic therapy based on the data [27]. This approach also resonated with the conclusions of A. Osiejewska *et al.* [28], who in their review focused on the comprehensive management of acute tonsillopharyngitis, including adequate pain relief. Microbiome indices showed low predictive ability when analysed separately (AUC < 0.65), but became key predictors in the comprehensive RF model. This phenomenon can be explained by synergistic effects and nonlinear interactions between different components of the microbiota. A study by R.P. Dickson *et al.* [29] confirmed that combinations of bacterial taxa, rather than individual species, demonstrated the highest predictive ability in respiratory infections, which explains the effectiveness of machine learning in identifying complex microbial associations.

The proposed model for predicting pain intensity based on microbiome markers and the RF algorithm demonstrated high predictive value. This approach allows the identification of patients at high risk of severe acute tonsillitis in the early stages. The identified changes in the microbial biocenosis of the tonsils are not only a consequence but also an important pathogenetic factor of the disease. The use of machine learning, in particular RF, provides the necessary sensitivity to detect these complex relationships in biological data. Thus, the results of the study confirm the need to integrate microbiome analysis into clinical algorithms for the development of truly personalised and preventive strategies for the treatment of tonsillitis.

Conclusions

The study demonstrated that the oropharyngeal microbiome is a valuable source of prognostic biomarkers for the clinical course of acute tonsillitis. It was found that machine learning, in particular the RF algorithm, can effectively use complex microbiome data to predict individual outcomes. The key finding was that the Pathogen_ratio index demonstrated moderate predictive ability for disease duration (AUC = 0.64) and, together with the Strep_balance index, was one of the key predictors of pain intensity according to the RF model. The marked advantage of RF over LDA in predicting pain intensity (81.8% accuracy vs 55.0%) highlighted the critical role of nonlinear interactions between clinical and microbial variables in the formation of subjective symptoms. For predicting duration, both models showed high and similar effectiveness (72.7% for RF and 75.0% for LDA), indicating a more linear nature of these relationships. Feature importance analysis revealed clear prognostic determinants: disease duration was primarily associated with aetiological and anamnestic factors, with a positive strep test (Strep_Test) and smoking status (Smoking) among the most important predictors. In contrast, pain intensity was more closely associated with microbiome indices (Pathogen_ratio, Strep_balance) and overall inflammatory burden, as reflected by the Centor score, which was the leading predictor in both models (AUC = 0.80 for duration). An important result was not only the confirmation of the fundamental role of the Centor score, but also the demonstration of its effective integration with microbiome data in a single machine learning model. This paves the way for the development of more accurate clinical decision support tools for risk stratification. For example, the RF model achieved perfect scores (Precision, Recall, F1 = 1.00) in identifying patients with the highest pain intensity ("severe pain", 7-10 points), which could potentially guide more personalised treatment strategies, such as enhanced symptomatic therapy for this group. Thus, the results experimentally confirmed the prognostic value of the oropharyngeal microbiome and provided a scientific basis for integrating machine learning and microbiological data into clinical practice to improve the treatment outcomes of acute tonsillitis.

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Conflict of Interest

None.

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Прогностичне моделювання клінічних результатів при гострому тонзиліті на основі аналізу мікробіоти та алгоритмів машинного навчання

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Анотація. Гострий тонзиліт є поширеним захворюванням з високою клінічною варіабельністю. Традиційні підходи на основі клінічних шкал (наприклад, Centor) часто недостатні для точного прогнозування індивідуального перебігу. Мета дослідження – визначити значущість інтеграції клінічних параметрів і даних мікробного складу ротоглотки для побудови прогностичної моделі щодо тривалості захворювання та тяжкості симптомів за допомогою методу випадкового лісу. Обстежено 52 пацієнтів з гострим тонзилітом. Виконано бактеріологічний аналіз мазків з ротоглотки, клінічну оцінку за шкалою Centor та експрес-тестування на стрептокок та вірусні інфекції. Побудовано та порівняно моделі випадкового лісу та лінійного дискримінантного аналізу. Модель випадкового лісу продемонструвала вищу точність у порівнянні з лінійним дискримінантним аналізом, особливо для прогнозування інтенсивності болю (загальна точність 81,8 % проти 55,0 %). Для тривалості захворювання точність випадкового лісу склала 72,7 % проти 75,0 % для лінійного дискримінантного аналізу. Аналіз важливості ознак виявив, що інтеграція мікробіомних індексів (співвідношення патоген/коменсал – Pathogen_ratio) з клінічною шкалою Centor значно підвищує прогностичну здатність. Тривалість хвороби асоціювалася з бактеріальною етіологією (позитивний стрептококовий тест) та статусом куріння, тоді як інтенсивність болю корелювала з параметрами мікробного дисбіозу. Комбінація клінічних та мікробіологічних даних у моделях машинного навчання дозволяє покращити точність прогнозування перебігу захворювання та може бути використана для розробки персоналізованих підходів до лікування

Ключові слова: модель випадкового лісу; мікробіом ротоглотки; шкала Centor; експрес-діагностика; клінічний прогноз; стрептокок групи А; вірусні антигени

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MTHFR and *MTRR* polymorphisms associations with unexplained female infertility

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Abstract. Polymorphic alleles in genes of folate metabolism are associated with such failures of female infertility like impaired ovulation or fertilisation; however, the data about the single gene polymorphisms in genes which code the enzymes of folate metabolism are controversial. This study aimed to analyse the correlation of polymorphic variants C677T (Ala222Val), A1298C (Glu429Ala) of *MTHFR* gene and A66G (Ile22Met) of *MTRR* gene with the oocytes' maturation and early embryo development in women with unexplained infertility. DNA extraction was performed with extraction kits, real-time polymerase chain reaction was applied for single nucleotide polymorphisms determinations, gonadotropin-releasing hormone antagonists were used for controlled ovarian stimulation, obtained oocytes were fertilised by the method of intracytoplasmic sperm injection, early embryo development *in vitro* was analysed according to the Istanbul

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Consensus, statistical hypotheses were tested at significance levels of 0.05 and 0.01. The part of good-quality cleavage stage embryos was statistically lower for infertile patients-carriers with mutant allele A1298C of *MTHFR* gene in genotype ($\chi 2_{crit.}$ = 18.0361, P = 0.000022). A presence of mutant allele A66G of *MTRR* gene led to decrease in the number of mature MII oocytes in women with unexplained infertility ($\chi 2_{crit.}$ = 11.1469, P = 0.000842). No correlations of studied polymorphisms of *MTHFR* and *MTRR* genes with total number of received oocytes, fertilisation rate and blastocysts formation rate were found out in studied group. Examination of polymorphic variants in genes of folate metabolism C677T (Ala222Val), A1298C (Glu429Ala) of *MTHFR* gene and A66G (Ile22Met) of *MTRR* gene could be included to the tests necessary for women with unexplained infertility

Keywords: single nucleotide polymorphism; folate cycle; oocytes; embryo development; *in vitro* fertilisation

Introduction

According to recent studies, female factors are the cause of infertility in approximately 35% of women who have difficulty conceiving, with the most common etiological factors being ovulation disorders, which usually manifest as irregular or absent menstruation [1]. At the same time, in 10-20% of cases, the cause of infertility cannot be determined, which indicates the presence of unexplored mechanisms of reproductive disorders. In this regard, it is particularly important to study the underlying molecular causes of disorders in the development and maturation of oocytes, as well as to investigate the decisive role of maternal effects and genetic determinants associated with unexplained female infertility.

The enzymes of the folate cycle may have a significant impact on the processes of oogenesis, embryogenesis and endometrium implantation. However, the presented in the literature data about the correlation of polymorphic alleles of genes *MTHFR* (methylenetetrahydrofolate reductase) and *MTRR* (methionine synthase reductase) with the outcome of *in vitro* fertilisation (IVF) cycles are ambiguous. For example, H. Ishitani *et al.* [2] showed that *MTHFR* polymorphisms reduced the blastocyst rate but did not correlate with other stages of embryo development. Nevertheless, the possibility that folate cycle is of major importance during the period before the 8- to 16-cell stage for embryo quality that is not assessed by developmental failure was not excluded in mentioned study. In turn Y.R. Ko *et al.* [3] proved that good-quality embryo rate was higher for *MTHFR* 677CT than those for 677CC and 677TT (40.0% vs 29.4%, p = 0.001 and 40.0% vs 33.3%, p = 0.025, respectively). Besides that, the authors demonstrated that the oocyte maturation rate was significantly lower in 677TT than in 677CC 1298AA/677CC 1298AC and 677CC 1298CC/677CT 1298AA/677CT 1298AC (71.4% vs 76.7%, p = 0.012 and 71.4% vs 75.7%, p = 0.029, respectively). However, no differences were observed in the transplantable embryo rate between *MTHFR* genotypes. H. Zeng *et al.* [4] demonstrated the association of *MTHFR* 677TT with decreased number of good-quality embryos and decreased cumulative live birth rate. But the correlation of *MTHFR* C677T genotypes (677CC, 677CT, 677TT) with the clinical pregnancy rate, miscarriage rate and live birth rate at the first embryo transfer cycle was not proved in this work. The literature provided data on the impact of polymorphic variants of genes which code folate cycle enzyme on female reproductive function and pregnancy outcome. According to the results mentioned in literature, heterozygous polymorphism for the C/T allele of the *MTHFR* 677 gene and an increased frequency of the A/C allele of the *MTHFR* 1298 gene are associated with early pregnancy loss. O.V. Trokhymovych *et al.* [5] in their work mentioned that in women with early pregnancy loss and adenomyosis, a significant increase in the frequency (50%) of the heterozygous polymorphism for the C/T allele of the *MTHFR* 677 gene and a decrease in the frequency of detection of neutral A/A allele and increased frequency (80%) of *MTHFR* 1298 gene allele polymorphism (A/C and C/C) were observed. From the other hand S. Jose [6] summarised the results of different researches and demonstrated that data about the relationship between the *MTHFR A*298C polymorphism and recurrent pregnancy loss risk are still debatable and unclear. In turn Y. Zhang *et al.* [7] demonstrated that the combination of genotypes 677TT and 1298CC of the *MTHFR* gene and 66GG of the *MTRR* gene significantly increase the risk of recurrent pregnancy loss (RPL). In mentioned investigation it was found out that the *MTHFR* c.677C > T heterozygote was associated with lower RPL risk, while the *MTHFR* c.1298A > C variant and *MTRR* c.66A > G heterozygote were correlated with higher RPL risk. In addition, T.L. Arkhypkina *et al.* [8] considered the role of polymorphic variants of folate cycle genes as candidate genes for the development of polycystic ovary syndrome in women of reproductive age. The number of studies demonstrated that the presence of the *MTHFR* A1298C mutation directly correlates with an increase in homocysteine concentration and the risk of polycystic ovary syndrome.

However, the question of the association of single nucleotide polymorphisms of the *MTHFR* and *MTRR* genes both with early embryo development and quality of female gametes requires detailed study. The aim of the present study was to analyse the relationship of polymorphic variants in genes of folate metabolism – C677T (Ala222Val), A1298C (Glu429Ala) of *MTHFR* gene and A66G (Ile22Met) of *MTRR* gene with quality of oocyte and embryo development in IVF cycles in women with unexplained infertility (UI).

Materials and Methods

Between January 2023 and December 2024, 26 couples with the UI underwent infertility treatment using the assisted reproduction techniques in the Reproduction Centre

"Clinic of Professor O.M. Feskov" (Kharkiv, Ukraine). Only couples with normal male karyotype 46,XY and normal female karyotype 46,XX were included to the Experimental Group. Couples with the female-partner aged more than 40 y.o. excluded from the study. Data collection, laboratory examinations and cycles of *in vitro* fertilisation were carried out at the Clinic of Professor O.M. Feskov (Centre of Human Reproduction, Sana-Med Ltd.). All participants gave their informed consent to participate in the study on condition that the data would remain anonymous, which was done. Design of the study was approved by the Institutional Review Board, protocol No. 3 dated 2022 December 20. 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 Declaration of Helsinki [9].

In mentioned group with UI the age of female partners was 31.8 ± 3.3 years old, the pattern age was 35.6 ± 3.8 years old. All the men had normal spermogram indices according to the World Health Organization [10]. The correlation between polymorphic variants in genes of folate metabolism C677T (Ala222Val), A1298C (Glu429Ala) of *MTHFR* gene and A66G (Ile22Met) of *MTRR* gene in female partners and the oocytes maturation, fertilisation rate, the part of good-quality cleavage stage embryos and blastocysts formation rate in IVF cycles for the group with UI was analysed. The Control Group was formed by 25 egg donors with the middle age 28.1 ± 5.2 y.o. Donors' eggs were fertilised by donor sperm. The age of the sperm donors was 33.7 ± 5.2 y.o The frequency of alleles and genotypes of studied polymorphisms for *MTHFR* and *MTRR* genes were calculated both for infertile women and for egg donors. DNA extraction was performed with standard kits (Macherey- Nagel, NucleoSpin® Blood, Germany) [11]. Single nucleotide polymorphysms (SNPs) were determined by the technique of real-time polymerase chain reaction (PCR) with the ABI PRISM 7500 real-time PCR system (USA) and Applied BioSystem kits (USA). Primers with the following nucleotide sequence were used: gene *MTHFR*, C677T (rs1801133): GAAAAGCTGCGTGATGATGAAATCG[G/A] CTCCCGCAGACACCTTCTCCTTCAA; gene *MTHFR*, A1298C (rs1801131): AAGAACGAAGACTTCAAAGACACTT[G/T]CTTCACTGGTCAGCTCCTCCCCCCA; gene *MTRR* (rs1801394): AGGCAAAGGCCATCGCAGAAGAAAT[A/G]TGTGAGCAAGC TGTGGTACATGGAT.

Controlled ovulation stimulation (COS) of patients in couples and egg donors was performed using the protocol with gonadotropin-releasing hormone antagonists (ant-GnRH). Fertilisation of the obtained oocytes was done by intracytoplasmic sperm injection (ICSI) technique. Embryo culture beginning from the stage of zygote and to the blastocyst stage was performed in GAIN medium Single-step (Austria) at a temperature of 36.9-37.1°C and a CO2 content of 5.5-5.8% [12]. Cleavage stage embryos and embryos that reached the blastocyst stage were evaluated by morphological characteristics according to D.K. Gardner criteria, mentioned to the Istanbul Consensus [13]. A difference was considered statistically significant if $P<0.05$. To test the associations between genotypes and qualitative variables, the chi-squared test was used [14]. The Apache Open Office 4.0.0 software package (Sana-Light Ltd., Sana-Med Ltd.) was used for calculation.

Thus, this study was designed as a controlled comparative analysis involving couples with unexplained infertility and a control group of egg donors, with clearly defined inclusion and exclusion criteria. A comprehensive methodological approach combined clinical IVF outcomes with molecular genetic analysis of key polymorphic variants in folate metabolism genes in female participants. This design enabled evaluation of the relationship between maternal genetic background and oocyte maturation, fertilisation efficiency, and embryo developmental potential under standardised assisted reproduction techniques conditions.

Results and Discussion

The frequency of alleles and genotypes of studied polymorphisms for *MTHFR* and *MTRR* genes were calculated for women in group with UI and for egg donors in Control Group. For studied SNP C677T of *MTHFR* gene the frequencies of alleles among egg donors were $P_C = 0.71$; $Q_T = 0.29$. In women with UI there were the next the frequencies of alleles for SNP C677T of *MTHFR* gene was $P_C = 0.77$; $Q_T = 0.23$. There were the next frequencies of alleles of polymorphic variant of *MTHFR* gene A1298C were $P_A = 0.62$ and $Q_C = 0.38$ for the patients in UI-group; for Control Group – $P_A = 0.72$ and $Q_C = 0.28$, respectively. As for studied SNP A66G for *MTRR* gene the allele frequencies were $P_A = 0.35$ $Q_G = 0.65$ for UI-Group, $P_A = 0.42$ and $Q_G = 0.58$ in the Control Group respectively. It should be noted that in subpopulations from different regions of Ukraine, genotypes with low-functional alleles prevail. The most studied is the population frequency of polymorphic variants of the *MTHFR* gene C677T. The average frequency of its genotypes C/C, C/T and T/T among practically healthy individuals in Ukrainian population is 46.0, 48.4 and 5.6%, respectively [15]. A.O. Fesai *et al.* [16] showed that the frequency of at least one low-functional allele of the *MTHFR*, *MTRR*, and *MTR* genes in women with repeated miscarriages was 83%. That is, it can be assumed that in every second Ukrainian woman of reproductive age, folates are not converted into an active form and are not absorbed by the body to a sufficient extent.

As a result of COS, totally 306 oocytes were obtained from patients in group with UI. There were 208 oocytes at the MII maturity stage in mentioned experimental group (68.0% of received oocytes). The fertilisation rate in this group, when using the ICSI method, was 83.2% (173 normal zygotes with two pronuclei). In UI-Group the part of good-quality cleavage stage embryos was 60.1% (104 cleavage stage embryos); blastocyst formation rate (BFR) reached 35.8% (62 blastocyst obtained from 173 normal zygotes with two pronuclei). As for the Control Group, there were 455 oocytes in total, and 387 oocytes at the MII

maturity stage (85.1% of obtained oocytes). The fertilisation rate of donor eggs was 81.9% (317 normal zygotes). In Control Group the part of good-quality cleavage stage embryos was 73.8% (234 cleavage stage embryos); BFR reached 55.8% (177 blastocyst obtained from 317 normal zygotes with two pronuclei).

The part of oocytes at the MII maturity stage was statistically lower in women with UI comparing with this one in egg donors (68.0% vs 85.1%, $\chi2_{crit.} = 30.305$, $P < 0.00001$). The part of good-quality cleavage stage embryos was statistically higher in the Control Group than in Experimental Group (73.8% vs 60.1%, $\chi2_{crit.} = 9.1892$, $P = 0.002434$). Good-quality cleavage stage embryo is presented on Figure 1. Oocyte maturation arrest could be a hidden cause of unexplained infertility. Abnormalities of the oocytes' maturation have not been studied enough in reproductive medicine yet. In most cases, oocyte-specific factors underlying infertility remain undetected until the assessment of oocytes retrieved during *in vitro* fertilisation cycles. The obtained results assumed that the process of the oocytes' maturation in patients with unexplained infertility could play a key role in a proper embryo development.

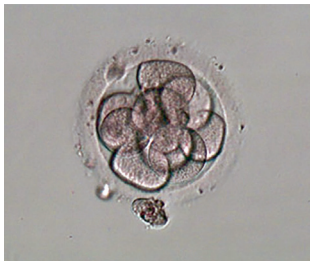

Figure 1. Good-quality cleavage stage embryo according to the Istanbul Consensus *in vitro*
Note: Magnification: x250
Source: compiled by the authors

There was no statistically significant difference in fertilisation rate and blastocyst formation rate between the group of patients with UI and the Control Group. A photo of human blastocyst is shown in Figure 2. The IVF indices like a part of MII mature oocytes and part of good-quality embryos depends on the process of oocytes maturation and oocytes quality. The obtained results demonstrated that donors' oocytes are more competent comparing with the eggs obtained in women with UI.

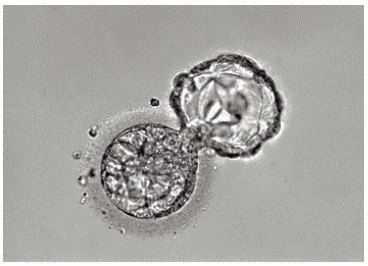

Figure 2. Blastocyst 5AA according to the D.K. Gardner classification *in vitro*
Note: Magnification: x250
Source: compiled by the authors

Based on the SNP C677T of *MTHFR* gene evaluation, there were 16 normal homozygote women with UI and 10 patients were classified as mutant homozygote or heterozygote. As for the laboratory variables, comparing the normal group with the mutant group, no differences were found regarding the total number of oocytes and MII-oocytes retrieved, normal fertilisation rate, good-quality cleavage stage embryos and blastocyst formation rate. Regarding the *MTHFR* polymorphism A1298C, 12 patients presented normal alleles and 14 patients presented mutated alleles. There was no difference in the number of total and mature MII-oocytes, fertilisation rates and BFR between normal genotype-women and the carriers of mutant allele. The part of good-quality cleavage stage embryos was statistically lower in patients with mutant allele in genotype ($\chi2_{crit.} = 18.0361$, $P = 0.000022$). As for studied SNP A66G for *MTRR* gene, there was no any difference in embryo development for normal genotype patients with UI and carriers of mutant allele. From the other hand a presence of mutant allele in mentioned genotype led to decrease in the number of mature MII oocytes in experimental group ($\chi2_{crit.} = 11.1469$, $P = 0.000842$). Obtained results were mentioned in Table 1.

Table 1. Laboratory variables according to presence of single nucleotide polymorphisms in folate-cycle genes

Variable	*MTHFR*, C677T		*MTHFR*, A1298C		*MTRR*, A66G	
	Normal, n = 16	Mutant, n = 10	Normal, n = 12	Mutant, n = 14	Normal, n = 6	Mutant, n = 20
Received oocytes, n	10.7 ± 2.8	11.7 ± 2.7	12.4 ± 3.2	11.2 ± 3.7	10.2 ± 1.7	11.3 ± 2.5
Mature MII oocytes, %	63.9	73.1	66.4	69.4	84.7**	62.8**
Normal fertilisation, %	83.6	82.6	78.8	87.2	83.6	82.9
Good-quality embryos, %	58.7	61.2	78.2*	45,2*	62.7	59.0
Blastocyst formation rate, %	33.7	38.3	41.0	31.6	35.2	36.1
Statistics	*df = 1, $\chi2_{crit.} = 18.0361$, $P = 0.000022$ **df = 1, $\chi2_{crit.} = 11.1469$, $P = 0.000842$					

Note: n – the number of the patients; * – significant; ** – significant; χ2 – Chi-Square test; P – significance level
Source: compiled by the authors

In this study the correlation between oocyte maturation and single nucleotide polymorphisms of *MTHFR* gene C677T and A1298C was not proved. But regarding the *MTHFR* polymorphism A1298C, the part of good-quality cleavage stage embryos was statistically lower in patients with mutant allele in genotype. Nevertheless, Y. Ko *et al.* [3] demonstrated earlier that the oocyte maturation rate was significantly lower in 677TT than in 677CC 1298AA/677CC 1298AC and 677CC 1298CC/677CT 1298AA/677CT 1298AC (71.4% vs 76.7%, p = 0.012 and 71.4% vs 75.7%, p = 0.029, respectively). Besides that, the mentioned authors found out that good-quality embryo rate was higher for *MTHFR* 677CT than those for 677CC and 677TT (40.0% vs 29.4%, p = 0.001). But there was no correlation between genotypes A/A, A/C and C/C for polymorphic variants of the *MTHFR* gene A1298C and good-quality embryo rate in that previous study.

The *MTHFR* gene encodes the 5-methylenetetrahydrofolate reductase enzyme, and thus it relates to folate metabolism. It was not defined the level of homocysteine in this study. But it could be assumed that polymorphisms in *MTHFR* could affect the content of folate and homocysteine. In turn folate deficiency and high concentration of homocysteine (hyperhomocysteinaemia) are considered as factors that increase the risk of infertility [17]. The current study demonstrated the association of *MTHFR* A1298C polymorphism with the decrease of the part of good-quality cleavage stage embryos. Folate deficiency and hyperhomocysteinaemia can affect the development of oocytes. Recent evidence suggests that human embryonic transcription is activated at the one-cell stage. M. Asami *et al.* [18] assumed that the oocyte provides the essential maternal proteins and transcripts needed to initiate embryonic genome activation (also called zygotic genome activation), a process where the embryo switches from maternal control to its own gene expression. The oocyte plays a crucial role in embryo development by storing maternal factors, completing meiosis, and reprogramming the sperm's genetic material. D.F. Albertini [19] demonstrated that oocyte provides the initial cellular machinery and essential components like proteins, mRNAs, and mitochondria needed for early embryogenesis until the embryo's own genome becomes active. Association of *MTHFR* A1298C polymorphism with decrease of good-quality cleavage stage embryos could be explained by the fact that hyperhomocysteinaemia affect the genome of oocytes.

In this investigation, a statistically significant decrease in oocyte maturity was observed in case of the *MTRR* polymorphism A66G. But earlier A.R. Palomares *et al.* [20] assumed that *MTRR* gene seems to have no direct impact on pregnancy consecution after IVF. Besides that, J. Zhou *et al.* [21] suggested that the *MTRR* c.66A > G variant was not significantly associated with the risk of pregnancy loss. From the other hand Y. Zhang *et al.* [7] demonstrated that *MTRR* c.66A > G heterozygote was correlated with higher recurrent pregnancy loss risk (*MTRR* c.66A > G, OR = 1.62, 95% CI = 1.20-2.19, p = 0.002). The *MTRR* gene provides instructions for the methionine synthase reductase enzyme, which affects negatively the folate and methionine cycle by reactivating the methionine synthase enzyme (*MTR*). A common variation in the *MTRR* gene c.66 A > G leads to an aminoacid substitution that marginally reduces the enzyme's biological activity. This change can affect folate metabolism and has been linked to increased homocysteine concentrations [22]. As folate and methionine metabolism is required for transmitting methyl groups for DNA methylation, the association of *MTRR* polymorphism A66G with decrease of part of MII mature oocytes could demonstrate affecting of mentioned polymorphism the process of oocyte maturation.

As literature data about the effect of polymorphic variants in genes of folate metabolism are controversial, the obtained results should be interpreted within the context of heterogeneous clinical and genetic backgrounds of infertile patients. The present findings indicated that polymorphisms of folate-cycle genes may exert a selective influence on oocyte competence and early embryo quality rather than uniformly affecting all stages of assisted reproduction. Such variability in reported associations may reflect differences in the relative contribution of oocyte-dependent mechanisms and metabolic conditions to reproductive outcomes in women with unexplained infertility.

Conclusions

The negative effect of polymorphic variants in genes of folate metabolism C677T, A1298C of *MTHFR* gene and A66G of *MTRR* gene on such IVF parameters as the rate of oocytes maturation and good-quality cleavage stage embryos in IVF cycles in women with unexplained infertility was demonstrated. It was shown that the part of good-quality cleavage stage embryos was statistically lower in patients-carriers with mutant allele A1298C of *MTHFR* gene in genotype (P = 0.000022). It was also observed that the presence of *MTRR* polymorphism A66G in genotype leads to decrease of part of MII mature oocytes in infertile patients (P = 0.000842). Comparative analysis demonstrated that oocyte developmental competence was higher in healthy women than in patients with UI, as reflected by a significantly lower proportion of MII oocytes in the UI group compared with egg donors (P < 0.00001) and a lower proportion of good-quality cleavage stage embryos (P = 0.002434). Association of *MTHFR* A1298C polymorphism with affected oocytes' genome was assumed. The association of polymorphic variants in genes of folate metabolism with the process of blastocyst formation was not proved. The study of the function of *MTHFR* and *MTRR* genes in oogenesis and early embryo development is perspective. Further investigations involving larger and more clinically homogeneous patient cohorts are needed to clarify the contribution of *MTHFR* and *MTRR* polymorphisms to IVF outcomes and early reproductive failures. A comprehensive approach that combines genetic profiling with detailed assessment of oocyte quality and embryo development may

contribute to a deeper understanding of infertility mechanisms affecting different stages of conception.

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Conflict of Interest
None.

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Асоціація поліморфізмів генів *MTHFR* і *MTRR* з жіночим безпліддям невідомого походження

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Анотація. Поліморфні алелі генів фолатного циклу асоціюються з такими причинами жіночого безпліддя, як порушення овуляції або процесу запліднення. Проте дані про поліморфізми окремих генів, що кодують ферменти метаболізму фолатів, є суперечливими. Метою цього дослідження був аналіз кореляції поліморфних варіантів C677T (Ala222Val), A1298C (Glu429Ala) гена *MTHFR* та A66G (Ile22Met) гена *MTRR* з дозріванням ооцитів та раннім розвитком ембріонів у жінок з безпліддям невідомого походження. ДНК виділено за допомогою наборів для екстракції, визначення однонуклеотидних поліморфізмів виконано методом полімеразної ланцюгової реакції у реальному часі, для контрольованої стимуляції яєчників використовували антагоністи гонадотропін-рилізинг-гормону, отримані ооцити запліднювали методом інтрацитоплазматичної ін'єкції сперматозоїдів, особливості раннього розвитку ембріонів *in vitro* проаналізовано відповідно до Стамбульського консенсусу, статистичні гіпотези перевірено на рівнях значущості 0,05 та 0,01. Частка ембріонів морфологічно високої якості на стадії дроблення була статистично нижча для безплідних пацієнток-носіїв поліморфного алеля A1298C гена *MTHFR* у генотипі ($\chi 2_{крит.}$ = 18,0361, P = 0,000022). Наявність мутантного алеля A66G гена *MTRR* призводила до зменшення кількості зрілих ооцитів MII у жінок з безпліддям невідомого походження ($\chi 2_{крит.}$ = 11,1469, P = 0,000842). Не виявлено кореляції досліджуваних поліморфізмів генів *MTHFR* та *MTRR* із загальною кількістю отриманих ооцитів, частотою запліднення та частотою формування бластоцист у досліджуваній групі. Дослідження поліморфних варіантів генів метаболізму фолатів *MTHFR* C677T (Ala222Val), A1298C (Glu429Ala) та *MTRR* A66G (Ile22Met) може бути включено до тестів, необхідних для жінок з безпліддям невідомого походження

Ключові слова: однонуклеотидний поліморфізм; фолатний цикл; ооцити; розвиток ембріонів; запліднення *in vitro*

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Evaluation of the adipose-dependent proinflammatory markers level in patients with acute coronary syndrome

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Abstract. Obesity is a predictor of the development of acute coronary syndrome with ST segment elevation and is realised through increased apoptotic processes. The objective of the study was to evaluate the relationship between body mass index, troponin I as a specific marker of myocardial necrosis and adipose-dependent non-specific markers in patients with acute coronary syndrome with ST segment elevation affected by obesity. An open-label longitudinal comparative cohort study was conducted involving 120 patients with acute coronary syndrome with ST segment elevation, stratified by body mass index into three groups. The levels of troponin I, leptin and caspase-9 (ELISA method) were determined, followed by the use of statistical research methods. In obese patients, a significant increase in the levels of caspase-9 (62.40 ± 3.8 ng/mL) and leptin (57.27 ± 4.1 ng/mL) was found compared to the overweight groups (45.27 ± 2.26 ng/mL and 36.60 ± 2.9 ng/mL) and controls (38.08 ± 2.1 ng/mL and 28.92 ± 2.5 ng/mL; $p < 0.001$). In group 2, there was a nearly linear relationship between leptin and caspase-9 ($r = 0.999$; $p < 0.001$) and a moderate correlation of troponin I with body mass index ($r = 0.632$; $p < 0.001$) and with leptin ($r = 0.316$; $p < 0.05$). With increasing body mass index in patients with ST-elevation myocardial infarction, there is an increased correlation between leptin, caspase-9, and troponin I, which promotes the activation of the adipokine-apoptosis-necrosis sequential cascade. Leptin-dependent activation of apoptosis may be one of the key mechanisms of metabolically mediated myocardial damage. The obtained results support the use of leptin and caspase-9 as additional risk stratification markers in acute coronary syndrome

Keywords: obesity; troponin I; leptin; caspase-9; apoptosis; adipokines; myocardial necrosis; myocardial infarction

Introduction

Acute coronary syndrome (ACS) remains a leading cause of mortality worldwide, with obesity significantly increasing its incidence and severity. Dysfunctional adipose tissue acts as an endocrine organ, producing pro-inflammatory adipokines such as leptin, resistin, caspase-9, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), which accelerate atherosclerosis, promote plaque instability and enhance myocardial ischemic injury. However, the clinical significance of these biomarkers in ACS is not fully defined. Assessing adipose-related inflammatory markers is essential for improving risk stratification and identifying new therapeutic targets in ACS what makes the research relevant.

Obesity is a key factor in the formation of chronic low-level inflammation and remodeling of the endocrine function of adipose tissue, which, due to the imbalance of adipokines (in particular leptin), worsens the course of

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cardiovascular diseases, including acute coronary syndrome with ST segment elevation (STEMI) [1]. According to the study by N.K. Pokrovska [2], adipose tissue is one of the principal regulators of energy balance and a cornerstone of inflammation, energy homeostasis, and atherosclerosis. Endothelial activation is the initial step of endothelial injury and is accompanied by abnormal secretion of pro-inflammatory and pro-thrombotic factors. In the study of O.S. Shchukina [3], particular attention was given to endothelial dysfunction as one of the major pathogenetic mechanisms in coronary artery disease and acute coronary syndromes. Early phases of vascular disease are characterised by endothelial dysfunction; endothelial tissues play an essential role in inflammation, coagulation, and angiogenesis by orchestrating ligand-receptor interactions and secretion of various mediators, including adipose-dependent pro-inflammatory markers. According to A.E. Berezin & A.A. Berezin [4], increased circulating concentrations of high-sensitivity cardiac troponins T (hs-TnT) and I (hs-TnI) serve as diagnostic and prognostic biomarkers of acute coronary syndromes and acute myocardial infarction, as well as independent predictors of cardiovascular risk in the general population. R.A. Byrne's *et al.* [5] guidelines emphasised the leading role of high-sensitivity troponins in the diagnosis and risk stratification of STEMI; however, metabolic and inflammatory alterations associated with obesity may modify the disease phenotype and influence treatment outcomes.

Patients with hypertension and obesity in the study by N.K. Pokrovska [2] demonstrated a more severe clinical course, characterised by higher systolic and pulse blood pressure values ($p < 0.05$), increased left ventricular posterior wall thickness ($p < 0.05$), increased left ventricular myocardial mass and relative wall thickness ($p < 0.05$), and more frequent left atrial enlargement ($p < 0.05$). The study showed that leptin is involved in inflammatory signaling and increases the expression of proinflammatory cytokines in macrophages and T lymphocytes. Regarding cytokines, P.M. Ridker & M. Rane [6] emphasised that, beyond primary prevention, both high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) have long demonstrated predictive value for adverse outcomes in acute coronary ischemia. Notably, IL-6 levels rised locally at the site of plaque rupture. M.A. Matter *et al.* [7] described that, at the molecular level, acute ischemia activates pro-inflammatory pathways (including the IL-6→CRP axis), apoptosis, and cardiomyocyte necrosis, all of which determine the degree of myocardial injury and subsequent remodeling. In A. Demarchi *et al.* [8], among adipokines, leptin attracted particular attention as a marker of fat-dependent changes; its level increased with obesity and was associated with insulin resistance, endothelial dysfunction, activation of immune cells and platelets. Systematic analyses by T. Vilariño-García *et al.* [9] confirmed elevated leptin levels in patients with acute coronary syndrome, especially in those with type 2 diabetes and excess body weight. In non-ST-elevation myocardial infarction, leptin concentrations correlated with inflammatory biomarkers and may have prognostic significance.

Experimental data by M. Wu *et al.* [10] showed that obesity promotes hyperactivation of caspase-9 through mitochondrial dysfunction, oxidative stress, and impaired mitophagy. Therefore, in patients with obesity and STEMI, more pronounced caspase-9-mediated apoptosis is expected, particularly in the context of hyperleptinemia. Simultaneously, the study by H.S. Abd-Alwahab *et al.* [11] confirmed that high-sensitivity cardiac troponins remain the principal markers of myocardial necrosis; however, the relationship between caspase-9 and troponin levels has not been sufficiently explored. Since obesity exacerbates endothelial injury, a stronger association between caspase-9, leptin, and necrosis markers can be anticipated. This justifies evaluating caspase-9 in patients with STEMI, considering BMI and leptinemia as key metabolic modifiers of disease progression.

Objective was to evaluate the associations between body mass index, the specific necrosis marker Troponin I, and the adipose-dependent non-specific markers leptin and caspase-9 in patients with acute coronary syndrome affected by obesity.

Materials and Methods

This study was conducted as an open-label, non-randomised, prospective cohort observational study, involving 120 patients with confirmed acute coronary syndrome with ST-segment elevation. The Municipal Non-profit Enterprise Zaporizhzhia Regional Clinical Hospital of the Zaporizhzhia Regional Council served as the clinical base for the study. The study protocol No. 10 dated 18.02.2025 received approval from the institutional ethics committee. All patients were informed about the study and signed consent to participate in the study. The study was conducted in accordance with European Commission [12] and Declaration of Helsinki [13]. Patients were divided into three groups based on their body mass index (BMI): Group I – 42 overweight patients (BMI 25.0-29.9 kg/m^2); Group II – 34 obese patients (BMI ≥30.0 kg/m^2); Group III (control) – 44 patients with normal body weight (BMI 18.5-24.9 kg/m^2). Eligibility (inclusion) criteria: age 18-90 years; confirmed diagnosis of STEMI in accordance with the Order No. 1936 of the Ministry of Health [14], lack of incapacity or independent ability to sign consent, provision of written informed consent to participate in the study. Exclusion criteria: age <18 or >90 years (all patients were competent and had no guardians); absence of clinical and electrocardiographic signs of STEMI on admission; presence of decompensated chronic renal failure or liver failure; acute surgical pathology of non-cardiac origin; patient refusal to participate in the study, the presence of incapacity or the ability to sign consent independently.

Subjects were assessed for anthropometric data (weight, height), age, gender, laboratory troponin I levels, and underwent laboratory ELISA testing of leptin and caspase-9, which were collected within the first 24 hours

of hospitalisation. The ELISA immunoenzymatic assay using the blinded randomised study methodology included 96 wells for leptin level analysis and 96 wells for caspase-9 analysis. For 120 blood samples of the subjects, patterns were randomly selected separately for leptin and caspase-9 from the entire sample. From one pallet of leptin reagents for 96 wells, 7 were used for calibration of the apparatus, the number of tested materials was 89. The pallet of caspase-9 reagents for 96 wells had 8 for calibration of the apparatus, and the number of results obtained was 88. A statistical analysis was conducted to determine the correlation between BMI, a specific marker of myocardial necrosis, and adipose-dependent nonspecific markers. The first stage of the study was the formation of selection criteria for the subjects, and then, accordingly, their selection. The next stage of the study was the determination of gender, age, and anthropometric data. This was followed by the stage of blood serum collection with centrifugation to obtain plasma. The next stage was the study of patient histories with available laboratory and instrumental characteristics. The next stage of the study was the conduct of enzyme-linked immunosorbent assay with the determination of leptin and caspase-9 from the selected samples. The last stage was mathematical analysis and statistical calculation of the data.

The methods used on research; general clinical with questionnaires, biochemical (troponin I), laboratory ELISA test of leptin (ALPCO, United states of America), caspase-9 (BioVendor, Czech Republic), mathematical and statistical analysis using statistical programs Microsoft Excel 2020, Statistica 13. The statistical methods applied in the study included tests for normality and homogeneity of variances, specifically the Shapiro-Wilk test and Levene's test. Parametric methods comprised one-way analysis of variance (ANOVA), Tukey's post hoc test, and the unpaired t-test for independent samples. Non-parametric comparison methods included the Kruskal-Wallis test, Dunn's post hoc test with Bonferroni correction. Additionally, analyses of frequencies and proportions were performed using Pearson's χ^2 test and Fisher's exact test. Correlation analysis was conducted with determination of Pearson correlation coefficients (r) and/or Spearman's rank correlation coefficients (ρ). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant for all analyses.

Results

In the first stage, a sample structure analysis was conducted. The total number of patients examined was 120, including 76 men (63.3%) and 44 women (36.7%). Within each BMI category, the following gender distribution was found: group 1 (overweight, n = 42): 26 men (61.9%), 16 women (38.1%); group 2 (obese, n = 34): 24 men (70.6%), 10 women (29.4%); group 3 (control, n = 44): 26 men (59.1%), 18 women (40.9%). Thus, in all study groups, a predominance of men over women was observed, and it was most pronounced among obese patients (group II). The distribution of subjects by gender is presented in Figure 1, which clearly demonstrated the male predominance in each group, with a gradual increase in the proportion of men as BMI increases.

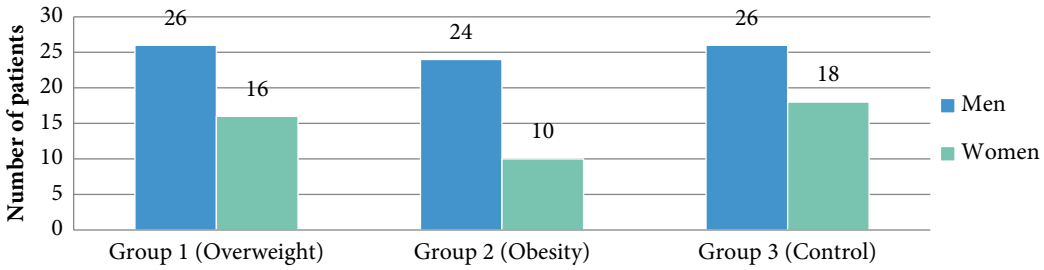

Figure 1. Distribution of patients by gender in the study sample

Source: authors' research

The average BMI in the study population was 27.8 kg/m^2. Men had a slightly higher BMI than women, with a statistically significant difference ($p = 0.038$), supporting the concept of gender-related variability in body weight. BMI differed markedly between the three study groups, reflecting the predefined stratification. Overweight patients had moderately elevated BMI, while the obesity group demonstrated the highest values; both groups significantly exceeded the control group ($p < 0.01$). Median values and nonparametric testing (Kruskal-Wallis, $p < 0.001$) confirmed these differences, with post-hoc comparisons showing consistently higher BMI in groups 1 and 2 relative to normal-weight individuals. BMI mean, median, and standard deviation data are presented in Table 1.

Table 1. Intergroup differences in BMI indicators

Group	Mean BMI ± SD, kg/m^2	Median (IQR), kg/m^2
Group 1 (overweight)	27.73 ± 1.46	28.10 (26.62-28.72)
Group 2 (obesity)	36.14 ± 7.27	33.88 (31.40-37.77)
Group 3 (normal/control)	22.50 ± 1.65	22.84 (21.06-23.94)

Source: authors' research

The average patient age was 64.9 years, with no significant age differences between BMI groups ($p > 0.05$). However, gender-specific analysis showed that men were consistently and significantly younger than women across all BMI categories. In the overweight group, men were approximately 17 years younger than women ($p < 0.01$); in the obesity group – about 13 years younger ($p < 0.02$); and in the control group – nearly 14 years younger ($p < 0.02$). These findings align with established epidemiological data indicating that men typically present with STEMI at a younger age than women. Age-specific differences in STEMI stratification by gender are shown in Figure 2.

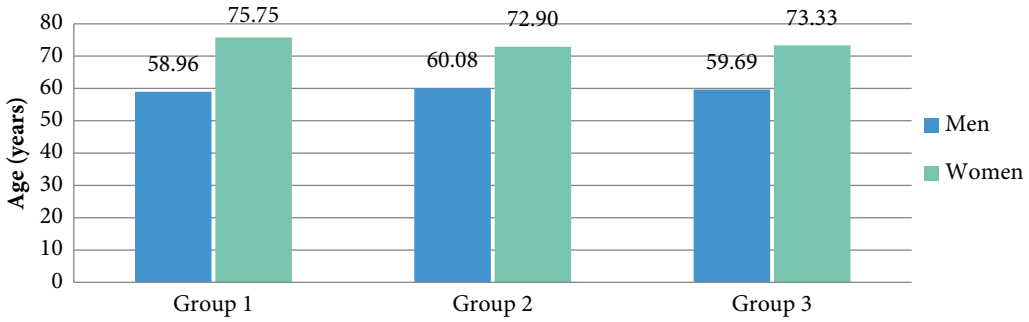

Figure 2. Data on average values by age and gender in the study groups
Source: authors' research

For the following analysis the reliability of data on adipose-dependent inflammatory markers (leptin, caspase 9), troponin I values were assessed as a specific and reference laboratory indicator of cardiomyocyte necrosis in STEMI. Analysis of troponin I concentrations at the time of hospitalisation showed a clear relationship between myocardial damage and the degree of excess body weight. Preliminary testing of the assumptions regarding the normality of distribution using the criterion for the Shapiro-Wilk test in all three BMI-stratified groups demonstrated statistically significant deviations from the normal distribution ($p < 0.01$), which led to the choice of nonparametric approaches for intergroup comparisons. The Kruskal-Wallis pooled test confirmed heterogeneity in troponin I levels between groups ($H = 7.67$; $p = 0.0216$), indicating that increasing BMI is associated with more intense myocardial necrotic damage in the acute phase of STEMI.

Expanded paired analysis using the Mann-Whitney test showed that patients with overweight (group 1) had significantly higher troponin I levels compared with the control group ($p = 0.0177$), whereas patients with obesity (group 2) demonstrated an additional significant increase ($p = 0.0196$). Mean values formed a clear ascending gradient: 2.54 ng/mL in the control group, 3.46 ng/mL in overweight patients, and 4.09 ng/mL in obese individuals. Compared with the control group, troponin I was higher by +0.92 ng/mL in group 1 ($p = 0.018$) and by +1.55 ng/mL in group 2 ($p = 0.006$). Median values showed the same trend (1.90 → 3.00 → 3.50 ng/mL). These findings confirm a progressive increase in myocardial necrotic burden with increasing BMI, consistent with the concept of an "adipo-inflammatory" STEMI phenotype. All quantative data for troponin I were presented in Table 2.

Table 2. Troponin I levels across BMI-stratified groups

Parameter	Group 1 (Overweight)	Group 2 (Obesity)	Group 3 (Control)
Mean ± SD (ng/mL)	3.46 ± 2.17	4.09 ± 4.33	2.54 ± 2.19
Median (ng/mL)	3.00	3.50	1.90
Δ vs Control (ng/mL)	+0.92	+1.55	–
Δ vs Control (%)	+26.59%	+37.00%	–
95% CI (Δ)	0.14-1.70	0.66-2.43	–
p-value vs Control	0.0177	0.0196	–
Overall trend	↑	↑↑	→

Note: CI – confidence interval
Source: authors' research

Determination of leptin levels according to the ELISA study was performed in 89 out of 120 patients (74.2% of the population), caspase-9 – in 88/120 (73.3%). The ranking was carried out in a random order, but the number of studied samples for which leptin and caspase-9 were determined according to their study group does not have a statistically significant discrepancy (the number of caspase-9 samples in group 1 is 85.7%, in group 2 – 67.6%, in group 3 – 68.2%, the number of leptin samples in group 1 is 83.33%, in group 2 – 58.82%, in group 3 – 77.27%). This is taken into account in the further analysis of the obtained results.

Troponin was moderately correlated with both leptin ($\rho = 0.325$; $p = 0.0019$) and caspase-9 ($\rho = 0.299$; $p = 0.0046$) in the overall population. This is consistent with the hypothesis of a more intense "adipo-inflammatory" load and apoptosis at higher BMI, which is associated with a larger area of myocardial damage. Caspase-9 levels demonstrated a clear BMI-dependent gradient. Median values increased from approximately 38 ng/mL in the normal-weight group to 45 ng/mL in the overweight group and exceeded 62 ng/mL in obese patients. ANOVA confirmed highly significant intergroup differences ($F \approx 82$; $p < 0.001$), and Tukey's post-hoc test showed that obese patients had markedly higher caspase-9 levels than both overweight and normal-weight individuals (all $p < 0.001$). Even overweight patients showed significantly elevated values compared with controls ($p < 0.01$), indicating progressive activation of the mitochondrial apoptotic pathway with rising BMI.

Leptin concentrations followed a similar trend, increasing from roughly 29 ng/mL in the normal-weight group to 37 ng/mL in the overweight group and surpassing 57 ng/mL in the obesity group. Intergroup differences were statistically robust ($F \approx 97$; $p < 0.001$). Post-hoc comparisons showed significantly higher leptin levels in obese patients relative to both other groups (all $p < 0.001$), with overweight individuals also differing significantly from controls ($p < 0.01$). Correlation analysis demonstrated strong positive associations between leptin and BMI ($r = 0.876$ in the overweight group; $r = 0.962$ in the obese group; $p < 0.001$), as well as between leptin and caspase-9, reaching almost perfect correlation in the obesity group ($r = 0.999$; $p < 0.001$).

The results demonstrated a consistent increase in leptin and caspase-9 levels as subjects transition from normal body weight to obesity. The highest values for both parameters were found in the obese group, confirming the parallelness of metabolic and apoptosis-mediated disturbances in the progression of metabolic syndrome. Furthermore, the very high correlation between leptin and caspase-9 in group 2 ($r = 0.999$; $p < 0.001$) suggests a direct mechanistic link between hyperleptinemia and caspase activation, potentially contributing to myocardial damage in obese patients. Statistical data for caspase-9 and leptin levels were analysed using ANOVA, pairwise comparison (t-test), and statistical significance testing. The results were shown in Table 3.

Table 3. Statistical measurements of the reliability and significance of caspase-9 and leptin indicators in the study groups

Indicator	Group 1 (overweight) M ± SD	Group 2 (obesity) M ± SD	Group 3 (normal) M ± SD	ANOVA (F)	p-value (ANOVA)	Pairwise comparison (Tukey post-hoc)	p-value
Caspase-9, pcs.	45.27 ± 2.26	62.40 ± 3.80	38.08 ± 2.10	82.47	<0.001	1-3	0.008
						2-3	<0.001
						1-2	<0.001
Leptin, ng/mL	36.60 ± 2.90	57.27 ± 4.10	28.92 ± 2.50	96.83	<0.001	1-3	0.009
						2-3	<0.001
						1-2	<0.001

Source: authors' research

For statistical analysis of intergroup differences, a two-sample t-test for independent populations was used for normal distribution, confirmed by the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. A correlation analysis was conducted between BMI and main laboratory parameters within three stratified groups of patients (overweight, obesity, control). In the obese group, a strong positive correlation relationship was found between troponin I levels and BMI ($r = 0.632$; $t = 5.29$; $p < 0.001$), indicating a significant association between the degree of myocardial damage and excess body weight. In the overweight group, a moderate positive relationship was observed between these indicators ($r = 0.402$; $t = 2.86$; $p < 0.01$), which also indicates a tendency for troponin levels to increase with increasing BMI, although less pronounced than in obese patients.

In the obese group, a significant positive correlation was found between caspase-9 and leptin levels ($r = 0.999$; $p < 0.001$), indicating a close relationship between the activation of mitochondrial apoptosis and the hormonal activity of adipose tissue. A similar, but less pronounced, relationship was also observed between caspase-9 and BMI ($r = 0.976$; $p < 0.001$), consistent with the hypothesis that apoptotic activity increases with increasing body weight. In the overweight group, a high correlation was observed between caspase-9 and leptin ($r = 0.856$; $p < 0.001$), and both parameters had statistically significant associations with BMI ($r = 0.728$ for caspase-9; $r = 0.876$ for leptin; $p < 0.001$). These results indicate that even a moderate increase in BMI is accompanied by activation of apoptosis and endocrine changes in adipose tissue. In the control group with normal BMI, correlations between the studied parameters were weak or absent, which confirms the physiological balance between metabolic and apoptotic processes under conditions of normal body weight.

An assessment of the relationship between markers of apoptosis and myocardial necrosis revealed significant differences between groups. In the obese group, a moderate positive correlation was found between caspase-9 and troponin I levels ($r = 0.217$; $p < 0.05$), which may indicate a synchronous increase in apoptotic activity and cardiomyocyte damage during metabolic overload. The

correlation between leptin and troponin I was also positive ($r = 0.316$; $p < 0.05$), reflecting the potential influence of adipokine dysfunction on the degree of ischemic myocardial damage. The intergroup correlation relationships of leptin, caspase-9, troponin I, and BMI are shown in Figure 3 as heat maps (cross-tabulation).

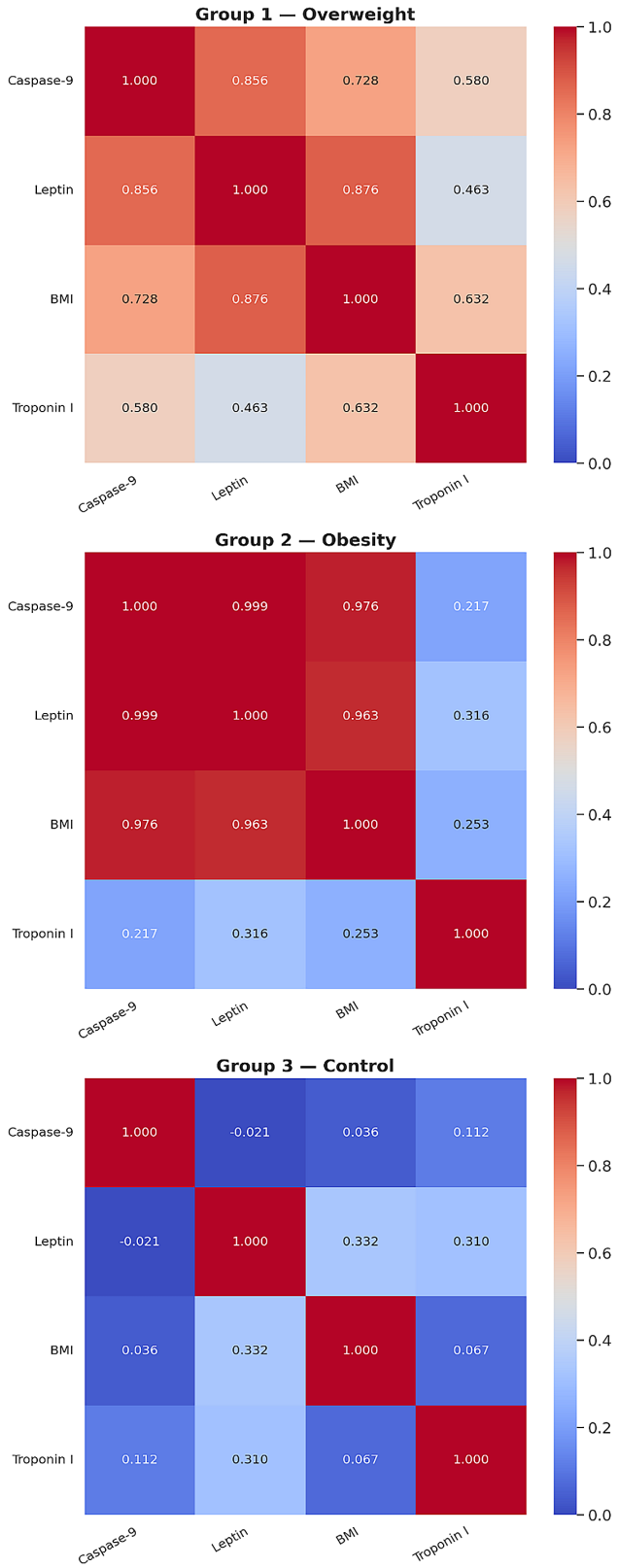

Figure 3. Heat maps of the correlation relationships between laboratory values and BMI
Source: authors' research

In the overweight group, the correlations were more pronounced: for caspase-9 and troponin ($r = 0.580$; $p < 0.01$), and for leptin and troponin ($r = 0.463$; $p < 0.01$). This dependence may reflect the early stages of metabolic imbalance, in which deep apoptotic activation has not yet formed, but a myocardial response to systemic inflammation and hyperleptinemia is already observed. In the control group, correlations between the parameters were weak and statistically insignificant. This indicates the absence of a significant relationship between markers of apoptosis and myocardial damage under conditions of normal body weight and metabolic homeostasis.

Discussion

The results of this study demonstrated that excess body weight and obesity substantially modify the inflammatory, metabolic, and apoptotic profiles of patients with myocardial infarction and ACS, which is consistent with accumulated international evidence. In patients with STEMI and obesity, metabolic inflammation of adipose tissue overlaps with the acute immune response to ischemic myocardial injury. This manifests as hyperleptinemia, activation of monocytes/macrophages, and a potential pro-aggregatory effect on platelets, thereby amplifying thrombosis and microvascular dysfunction [2,3].

In author's cohort, male patients predominated (63.3% overall; up to 70.6% in the obesity group), and men were significantly younger than women across all BMI categories (difference ≈ 13-17 years; $p < 0.02$). This corresponds with epidemiological observations that men more frequently develop STEMI at a younger age, whereas women tend to present at an older age and with a more severe clinical course, as reported by A.A. Dera *et al.* [15]. In their study, regarding sex, no significant differences ($p > 0.05$) were found between leptin concentrations in men and women in Group 1 ($r = 0.74$, $p = 0.139$) or Group 3 ($r = 0.044$, $p = 0.511$). Compared with the control group, women in Group 3 demonstrated a significantly higher BMI ($t = 2.656$; $df = 14$; $p = 0.0188$), Participants with acute myocardial infarction in Groups 2 and 3 also showed a significant difference ($t = 5.370$; $df = 46$; $p < 0.0001$).

The findings of that study are closely aligned with author's study, in which the mean BMI in the cohort was elevated (27.8 kg/m^2), and intergroup differences in BMI were, as expected, significant: the control group corresponded to normal body weight, whereas Groups 1 and 2 demonstrated excess body weight and obesity, respectively ($p < 0.01$ by ANOVA and by the Kruskal-Wallis test). In author's study, leptin levels increased progressively from approximately 28-29 ng/mL in the control group to approximately 36-37 ng/mL in the excess body weight group, and exceeded 57 ng/mL in patients with obesity, with very high statistical significance of intergroup differences ($F \approx 97$; $p < 0.001$) and consistent post hoc results ($p < 0.01$ for all key comparisons). These findings aligned well with the systematic review and meta-analysis by A. Ismaiel *et al.* [1], where leptin levels in patients with acute coronary syndrome were significantly higher than in controls (MD ≈ 10.5 ng/mL; 95% CI 3.67-17.35), particularly in patients with obesity. Similar conclusions were reported in the study by E. Ricottini *et al.* [16], in which patients with hyperleptinemia after percutaneous coronary intervention (PCI) exhibited nearly a twofold higher rate of major adverse cardiovascular events (MACE) compared with the normoleptinemic group (40% vs 21%; hazard ratio or HR 2.3; 95% CI 1.14-4.6). Leptin levels differed significantly across groups stratified by platelet reactivity (PR) ($P = 0.047$). Patients were divided into three PR-based groups defined as low (LPR), normal (NPR), and high (HPR) platelet reactivity. Leptin concentrations were higher in the HPR group (12.61 ± 16.58 ng/mL) compared with the LPR group (7.83 ± 8.87 ng/mL, $P = 0.044$) and the NPR group (7.04 ± 7.03 ng/mL, $P = 0.01$). These findings are consistent with author's data showing the coexistence of leptin-mediated inflammation and ischemic myocardial injury. Summarising the observations of T. Vilariño-García *et al.* [9], leptin is considered not only a marker of energy imbalance but also a pro-inflammatory adipokine that affects endothelial function, cardiac remodeling, and apoptosis. Author's demonstration of a strong relationship between leptin and BMI ($r = 0.876$-0.962; $p < 0.001$), along with its correlation with troponin I, confirmed this concept.

Caspase-9 levels in author's cohort also exhibited a clear BMI-dependent gradient: approximately 38 ng/mL in the control group, ≈45 ng/mL in patients with excess body weight, and >62 ng/mL in those with obesity. ANOVA analysis ($F \approx 82$; $p < 0.001$) and Tukey's post-hoc test confirmed a significant stepwise increase in caspase-9 when transitioning from normal weight to excess weight and obesity (all $p < 0.01$). The caspase-9 elevation observed in author's study fully aligns with current understanding of the intrinsic mitochondrial apoptotic pathway in myocardial ischemia-reperfusion injury, as demonstrated by M. Wu *et al.* [10]. K. Cai *et al.* [17] described caspase-9 activation as a central event in programmed cardiomyocyte death triggered by mitochondrial pore opening, cytochrome c release, and apoptosome formation. Clinically, Y. Liu *et al.* [18] reported that activation of caspase-associated proteins (CARD9) in macrophages enhances post-infarction remodeling, further supporting a multifactorial involvement of apoptosis in myocardial damage. These mechanisms are consistent with author's findings of increased caspase-9 in parallel with elevated troponin I and leptin, as well as their positive correlations. Additional confirmation was provided by A. Demarchi *et al.* [8], who demonstrated that serum leptin levels are directly associated with the inflammatory response during acute myocardial infarction and may play a role in risk stratification.

Author's data demonstrated a clear gradient of myocardial necrosis proportional to BMI: mean troponin I levels increased from 2.54 ng/mL in the control group to 3.46 ng/mL in the excess-weight group and 4.09 ng/mL in the obesity group. Compared with controls, the increase amounted to +0.92 ng/mL in Group 1 (≈ +26.6%) and +1.55 ng/mL in Group 2 (≈ +37%), with statistically significant p-values

($p = 0.018$ and $p = 0.006$, respectively). The strong positive association between BMI and troponin I in obese patients ($r = 0.632$; $p < 0.001$) highlighted that excess adiposity is associated with a greater area of necrosis in STEMI [19]. Similar trends were reported by A.A. Dera *et al.* [15], where patients with acute myocardial infarction and elevated BMI had higher troponin I, leptin, and additional markers of metabolic stress. Also a positive correlation was shown between troponin I (cTnI), creatine kinase MB (CK-MB), leptin, and resistin in patients with acute myocardial infarction. BMI and leptin showed a positive association in Group 3. For instance, among participants in Group 3, leptin showed a stronger correlation with BMI ($r = 125$, $p = 0.01$).

The findings of I. Chernyavska *et al.* [20] demonstrated that in all patient groups, cardiac troponin levels increased significantly both before and after PCI. The data also indicated that troponins begin to appear in the bloodstream 4-10 hours after the onset of acute myocardial infarction, while peak concentrations occur between 12 and 48 hours. The authors additionally reported a significant reduction ($p < 0.05$) in leptin levels during ACS treatment accompanied by parallel weight loss in both men and women. Correlation analysis between waist circumference and leptin levels in both sexes demonstrated a significant association. These results are consistent with author's findings showing elevated leptin levels in patients with ACS – particularly in those with obesity and excess body weight – as well as a positive correlation between BMI and troponin, and between leptin and BMI.

D. Skrypnik *et al.* [21] reported that elevated serum leptin levels are strongly associated with coronary artery disease (CAD). Serum leptin concentration positively correlated with coronary artery disease severity, being higher in patients with stable angina compared with controls, and highest in those with unstable angina. Their results also showed that serum leptin concentrations were higher in the control group (without cardiac rehabilitation) compared with group S, in which patients underwent a 2-week rehabilitation program. In author's study, leptin concentrations showed a similar pattern, increasing from approximately 29 ng/mL in the normal-weight group to 37 ng/mL in the excess body weight group, and exceeding 57 ng/mL in the obesity group. Troponin displayed a moderate positive correlation with leptin ($\rho = 0.325$; $p = 0.0019$).

Consistent with author's detection of higher troponin I levels in patients with overweight and obesity (up to 3.46 and 4.09 ng/mL, respectively), the findings of R.H. Al-Shibli *et al.* [22] also demonstrated markedly elevated troponin I levels in atherosclerotic groups both with and without obesity ($p \leq 0.001$). The authors additionally reported a unidirectional increase in other proinflammatory and pro-oxidant markers – CRP and malondialdehyde – which fully agrees with author's data showing activation of apoptosis and accentuated necrosis with rising BMI. Meanwhile, in the cohort of this study, the authors found a much stronger correlation between leptin and caspase-9 (up to $r = 0.999$ in the obese group) than was described in R.H. Al-Shibli *et al.* study, indicating a more pronounced "adipokine-apoptosis-necrosis" cascade reaction, particularly in conditions of acute myocardial infarction. The findings of R.H. Al-Shibli *et al.* regarding reduced total antioxidant capacity and increased MDA further confirmed the important role of oxidative stress in patients with obesity. Although these parameters were not measured in author's study, the elevated levels of caspase-9 in the overweight and obesity groups (45.27 and 62.40 units/mL, respectively) indirectly indicate activation of mitochondrial apoptosis, which is typically triggered by oxidative stress.

Additionally, results from O. Mayer *et al.* [23] confirmed the adverse prognosis associated with hyperleptinemia. Specifically, leptin concentrations ≥18.9 ng/mL were associated with more than a twofold increase in all-cause mortality (HR 2.10; 95% CI 1.29-3.42), cardiovascular mortality (HR 2.65; $p < 0.001$), and the risk of hospitalisation for heart failure (HR 1.95; $p < 0.020$). In current study, the leptin level in the obesity group averaged 57.27 ± 4.1 ng/mL, markedly exceeding these risk thresholds, and its correlation with troponin I ($r = 0.316$; $p < 0.05$) confirms the likely contribution of a leptin-dependent proinflammatory pathway to worsening the course of STEMI.

Thus, the results obtained in author's study are consistent with international findings and indicate that patients with ACS and obesity exhibit an enhanced interaction between leptin and caspase-9, which may serve as a marker of metabolically driven apoptotic stress in the myocardium. The extremely strong correlation ($r \approx 0.999$) between these biomarkers underscored that leptin-dependent activation of apoptosis may represent one of the central mechanisms of cardiac injury in this patient population. These observations open opportunities for further development of prognostic models and suggest that combined assessment of leptin and caspase-9 may serve as an additional tool for risk stratification in ST-segment elevation myocardial infarction.

Conclusions

The present study demonstrated a clear and statistically significant metabolic-inflammatory pattern linked to excess body weight. It was found that overweight and obese patients exhibited distinctly higher levels of adipose-derived biomarkers compared with normal-weight individuals. BMI differed significantly between groups ($p < 0.01$), confirming the validity of stratification. Although mean age did not differ across BMI categories, men consistently presented with ACS at a significantly younger age, which corresponds to known epidemiological characteristics of the syndrome. Analysis showed a progressive increase in the myocardial necrosis marker troponin I with rising BMI: from 2.54 ng/mL in normal-weight patients to 3.46 ng/mL in overweight and 4.09 ng/mL in obese individuals, with statistically significant differences ($p = 0.0177$ and $p = 0.0196$). Correlation testing proved that troponin I had a strong positive association with BMI in obese patients ($r = 0.632$; $p < 0.001$), supporting the link between excess adipose tissue and increased myocardial injury in ACS.

A central outcome of this research was the demonstration of a highly consistent adipokine-apoptotic response. Leptin increased from approximately 29 ng/mL in normal-weight patients to 37 ng/mL in overweight and exceeded 57 ng/mL in obese subjects ($p < 0.001$). Caspase-9 displayed a similar trend (38 → 45 → 62 ng/mL; $p < 0.001$). Moreover, it was demonstrated that the association between leptin and caspase-9 intensified dramatically with increasing BMI, reaching almost perfect correlation in obese patients ($r = 0.999$; $p < 0.001$). Both markers also correlated positively with troponin I, indicating the existence of an interconnected "adipokine-apoptosis-necrosis" axis in ACS. Overall, the study proved that obesity amplifies myocardial injury in ACS through adipose-related inflammatory and apoptotic mechanisms, characterised by hyperleptinemia, activation of mitochondrial apoptosis (caspase-9), and elevated troponin I levels. These findings highlighted the pathogenic value of adipose-dependent biomarkers in ACS and underscore their potential use for metabolic-inflammatory phenotyping of high-risk patients. Future perspectives include longitudinal evaluation of leptin and caspase-9 as prognostic markers of adverse outcomes, integration of adipokine-apoptotic indicators into ACS risk-stratification models, and exploration of therapeutic approaches targeting adipose-driven inflammation and apoptosis to improve outcomes in overweight and obese ACS patients.

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None.

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Оцінка рівня адипозалежних прозапальних маркерів у пацієнтів із гострим коронарним синдромом

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Анотація. Ожиріння є предиктором розвитку гострого коронарного синдрому з елевацією сегмента ST і реалізується через посилення апоптотичних процесів. Метою дослідження було оцінити взаємозв'язок між індексом маси тіла, тропоніном I як специфічним маркером некрозу міокарда та адипозалежними неспецифічними маркерами у пацієнтів із гострим коронарним синдромом з елевацією сегмента ST, які мають ожиріння. Було проведено відкрите проспективне порівняльне когортне дослідження за участю 120 пацієнтів із гострим коронарним синдромом з елевацією сегмента ST, стратифікованих за індексом маси тіла на три групи. Визначали рівні тропоніну I, лептину та каспази-9 (метод ELISA), після чого проводили статистичний аналіз. У пацієнтів з ожирінням виявлено значуще підвищення рівнів каспази-9 (62,40 ± 3,8 нг/мл) та лептину (57,27 ± 4,1 нг/мл) порівняно з групами з надмірною масою тіла (45,27 ± 2,26 нг/мл і 36,60 ± 2,9 нг/мл відповідно) та контрольною групою (38,08 ± 2,1 нг/мл і 28,92 ± 2,5 нг/мл; $p < 0,001$). У групі 2 виявлено майже лінійний зв'язок між лептином і каспазою-9 ($r = 0,999$; $p < 0,001$), а також помірну кореляцію тропоніну I з індексом маси тіла ($r = 0,632$; $p < 0,001$) та з лептином ($r = 0,316$; $p < 0,05$). Зі зростанням індексу маси тіла у пацієнтів із STEMI посилюється кореляція між лептином, каспазою-9 та тропоніном I, що сприяє активації послідовного каскаду «адипокін – апоптоз – некроз». Лептинзалежна активація апоптозу може бути одним із ключових механізмів метаболічно опосередкованого пошкодження міокарда. Отримані результати обґрунтовують використання лептину та каспази-9 як додаткових маркерів стратифікації ризику при гострому коронарному синдромі

Ключові слова: ожиріння; тропонін I; лептин; каспаза-9; апоптоз; адипокіни; некроз міокарда; інфаркт міокарда

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Human resource management in health care under martial law

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Abstract. The mobilisation and management of medical personnel in active combat zones constitute one of the principal challenges of contemporary armed conflicts, directly affecting the continuity of medical care and the preservation of patients' lives. This study aimed to synthesise existing scientific and practical evidence concerning the organisation of medical staff workflows, approaches to psychological support, and safety measures under conditions of martial law. The methodology involved a literature search in international databases covering publications from 2014 to 2025. The analysis focused on cases from Ukraine, Syria, Israel, and Sudan. The findings indicated that the implementation of Hospital Emergency and Contingency Planning enhances the resilience of health care systems through the formation of mobile teams, clear coordination mechanisms, and personnel reserves, while the 10-1-2 doctrine reduced battlefield mortality from 13% to 3%. The use of mathematical models for staff rotation demonstrated effectiveness in ensuring continuity of medical services. In the field of psychological support, cognitive-behavioural interventions and Eye Movement Desensitisation and Reprocessing therapy, along with group and individual programmes, proved the most effective, reducing distress levels by an average of 23%. Online platforms and international initiatives provided additional opportunities for remote assistance and knowledge exchange. In the domain of physical security, protocols for marking medical facilities, the establishment of protected zones, and training in tactical medicine play a decisive role. The analysis confirmed that the integration of staff mobilisation strategies, psychological support, and legal safeguards is essential for maintaining the functionality of the medical system during armed conflict. The practical significance of the findings lies in their applicability for public administration bodies, medical institutions, and humanitarian organisations seeking to optimise personnel policies, implement support programmes, and strengthen staff safety

Keywords: adaptive staffing strategies; psychological resilience of medical personnel; international safety protocols; rotational work models; traumatic stress in conflict zones; humanitarian resource coordination

Introduction

In contemporary armed conflicts, medical personnel encounter heightened risks, resource shortages, and substantial psychological burdens, which underscores the necessity of studying effective strategies for workforce mobilisation and management. Systematic psychological support, including cognitive-behavioural interventions, group therapy, and online platforms, has become a pivotal factor in preserving staff performance and adaptive capacity. At the same time, there is a need for a comprehensive assessment of the interrelation between staffing, organisational, and legal mechanisms for protecting medical workers, as violations of international humanitarian law and exposure to physical danger continue to pose serious challenges to the continuity of medical care.

Issues of medical personnel management in conflict zones have increasingly attracted scholarly attention due to rising incidents of violence and restrictions on access to patients. G.C. Shen *et al.* [1] conducted an analysis of the historical experience and evolution of medical personnel management in wartime, identifying a trade-off between safety and access, the shifting of risk to local specialists, and the commercialisation of humanitarian

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assistance. These factors consequently lead to the fragmentation of assistance and constrain the practical application of high-reliability principles. The absence of coordination and standardised procedures in humanitarian initiatives further diminishes the quality of medical care in conflict settings. N. Markou-Pappas *et al.* [2] identified a lack of unified operational vision, weak command structures, and limited interagency coordination, as well as challenges in patient routing, staff training, and pharmaceutical logistics. The findings indicated the need for integrated planning and a systemic approach to managing medical resources. Staff preparation and retention emerge as critical determinants of health-system stability in conflictaffected regions. B. Bogale *et al.* [3] demonstrated that effective functioning requires the integration of displaced specialists, upskilling of local personnel, the use of telemedicine, and community support. The results emphasised the importance of comprehensive training and capacitybuilding strategies for strengthening the resilience of medical systems.

The impact of military operations on the health-care system is reflected in a sharp decrease in the overall number of medical consultations and a shift in patient priorities. N.V. Horach *et al.* [4] showed that in 2022, the total number of medical visits in Ukraine declined by nearly 53%, while the demand for specialised care increased. This indicated a transformation in the structure of medical services and a marked reduction in preventive examinations in subsequent years. The economic and social consequences of war directly affect the health-care infrastructure and personnel. M. Dzhus & I. Golovach [5] demonstrated that during the first months of the invasion (February-May 2022), 672 medical facilities in Ukraine were damaged or destroyed, including 115 hospitals that were completely demolished. During the same period, at least 295 attacks on health workers and medical facilities were recorded, resulting in 59 injured and 76 killed. These events severely restricted public access to medical services and interrupted the treatment of individuals with chronic conditions.

The escalation of attacks on medical personnel violates the principles of international humanitarian law and medical neutrality. D. Norcliffe-Brown & A. Green [6] found that since 2010, the number of conflicts and assaults on health workers has risen sharply, leading to more than 900 deaths of medical personnel in 2024. The scale of workforce outflow in Syria and Myanmar illustrated the critical consequences for the availability and quality of medical care. Existing regulatory mechanisms for protecting medical personnel have proved insufficient during wartime. T. Mykhailichenko *et al.* [7] showed that international humanitarian law contains provisions for safeguarding medical workers, but their enforcement is not guaranteed, while martial law enables states to restrict citizens' rights. This creates additional challenges for ensuring the quality control of medicines, managing humanitarian assistance, and administering the healthcare system. Medical professionals also face ethical dilemmas that exacerbate stress and increase the risk of error. G.D. Muhammad *et al.* [8] found that medical personnel are compelled to balance professional duty with personal safety, work beyond their formal qualifications, overcome cultural and gender barriers, and make critical choices between treating the wounded and assisting those who are severely injured. These challenges underscore the importance of preparing staff for both psychological and professional resilience.

In the body of research reviewed, attention has largely focused on broad trends in the management of medical personnel, whereas the effectiveness of specific staffing strategies, rotation models, and integrated coordination protocols has been assessed only to a limited extent. The impact of psychological support on staff resilience in conditions of direct combat has also been examined only fragmentarily, and the long-term consequences for professional and psychological adaptation remain insufficiently investigated. Furthermore, previous studies have been constrained primarily to cases from particular regions and organisations, which limits the generalisability of the findings to other conflict zones. This study aimed to conduct a comprehensive systematisation and critical assessment of scientific evidence concerning the mobilisation, management, psychological support, and legal aspects of protecting medical personnel in the context of armed conflicts and crisis scenarios.

Materials and Methods

As part of this review study, a systematic search and synthesis of scientific publications and reports addressing the protection of medical infrastructure and the management of medical personnel in armed conflicts were conducted. The search was carried out using academic databases and platforms, including ResearchGate, Wiley Online Library, BioMed Central, MDPI, Emerald Insight, Sciendo, Taylor & Francis Online, Springer Link, Cambridge Core, and The Lancet. In addition, thematic reports by the World Health Organization (WHO) [9, 10], which contain relevant data on attacks on health care and the protection of personnel, were included in the analysis.

The search covered materials published up to 2025, with a primary focus on English-language articles and relevant Ukrainian-language publications. To ensure the quality of the review and assess the reliability of the included sources, a critical appraisal of the methodological soundness of primary studies was carried out. The assessment employed the Joanna Briggs Institute critical appraisal checklist [11], adapted to the design of each publication. The sources were independently evaluated against criteria such as adequacy of sampling, clarity of methodological reporting, and validity of conclusions. The resulting assessments were used to improve understanding of the reliability of the data presented in the sources.

The search queries consisted of combinations of keywords and their synonyms in English and Ukrainian, including: "healthcare workers", "medical personnel", "attacks on health care", "conflict", "war", "psychological support", "migration of health workers", "hospital emergency planning",

"tactical medicine", "medical personnel in war", "attacks on healthcare facilities", "migration of medical workers", and "psychological support for medical workers". The queries were adapted to the syntax of each database, using logical operators (AND, OR) and filters by document type (peer-reviewed original studies, systematic reviews, meta-analyses, and review articles).

This review study also examined the functioning of health-care systems in armed conflict environments using the cases of Ukraine, Syria, Israel, and Sudan. The primary focus was on the impact of war on the mobilisation of medical personnel, the organisation of work under shelling and attacks on medical facilities, and the provision of psychological support, legal safeguards, and physical protection for health workers. The analysis examined mechanisms of large-scale emigration among medical personnel, the application of operational and tactical medical protocols – such as timeframes for tactical, resuscitative, and surgical care – and crisis-response systems, including mobile medical units, vaccination campaigns, and measures to ensure the continuity of medical services across diverse conflict settings. This approach enabled a comprehensive evaluation of adaptive and crisis-management mechanisms within health-care systems in different contexts and highlighted the importance of strategic workforce and resource management. In addition, modelling studies aimed at optimising staff management and logistics for the deployment of a field hospital were considered, using the example of the Spanish Simple Triage and Rapid Treatment (START) team, which allowed the assessment of integrated approaches under crisis conditions. The initial search yielded 127 publications. After removing duplicates and irrelevant materials (based on titles and abstracts), the number of sources was reduced to 94. Subsequent full-text analysis resulted in the selection of 41 publications that fully met the inclusion criteria. The inclusion criteria comprised peer-reviewed studies, literature reviews, systematic reviews, and meta-analyses examining the protection of medical facilities and personnel, staff mobilisation and outflow, organisational models of care delivery, measures for strengthening the resilience of health-care systems in conflict zones, and psychological support interventions for personnel. Editorials, commentaries, letters to the editor, materials without full-text access, and publications lacking primary or synthesised data on the specified topics were excluded.

Results and Discussion

Mobilisation and management of medical personnel during wartime. In wartime conditions, medical personnel operate far beyond conventional professional duties, becoming a frontline force not only in providing emergency care but also in upholding human rights. In conflict settings, they face acute resource shortages and are frequently targeted in attacks, necessitating immediate mobilisation and the redistribution of staff. Health workers increasingly assume additional responsibilities, including training less experienced colleagues, participating in vaccination and sanitation activities, and providing psychosocial support to individuals affected by trauma [12]. Human resource management in the health-care sector during crises, including armed conflict, is regarded as a key factor in mitigating adverse consequences and ensuring the continuity of medical services. The analytical review by T. Stroiko *et al.* [13] indicated that Ukraine lost approximately 30% of its jobs – equivalent to 4.8 million positions – following the onset of the full-scale invasion in 2022. Under a scenario of rapid conflict resolution, the labour market could recover up to 3.4 million jobs, whereas further escalation may result in losses of up to 7 million positions, representing 43.5% of total employment. To address the workforce deficit, three strategic approaches have been identified: encouraging higher birth rates and the return of migrants, implementing technological solutions and enhancing labour productivity, and attracting foreign specialists. A combined approach is considered the most effective; however, in practice, 50% of organisations increased salaries, while 39% froze budgets for staff development.

The concept of Hospital Emergency and Contingency Planning (HECP) be T. Wurmb *et al.* [14] provided the principal framework for adapting human resources in crisis situations. It requires clear operational coordination, including the establishment of mobile teams, specialist rotation, and the deployment of reservists and volunteers to compensate for personnel shortages in critical areas. A central element of effective HECP implementation is the presence of an HECP lead, responsible for organising, structuring, and executing decisions related to workload management, priority setting, and the legal protection of staff. Research indicates that the availability of trained personnel reserves and systematic staff rotation substantially enhances the capacity of medical institutions to maintain stable operations, even under conditions of mass casualty influx. This confirms that effective workforce management, supported by regular drills and strategic planning, constitutes a cornerstone of healthsystem resilience. Figure 1 presented consolidated data illustrating attacks on the health-care system and their consequences for life and health.

As shown in Figure 1, the distribution of attacks on health-care facilities across different types of infrastructure, as analysed by H.J. Kim *et al.* [15], highlights key aspects of workforce adaptation within the health-care system during armed conflict and substantiates the need for a comprehensive approach to medical personnel management. The highest rate of injuries occurred among health-care transport vehicles (10.5% of all recorded incidents), highlighting a critical need for rotation of ambulance teams and mobile medical units. Prolonged deployment of the same personnel in high-risk zones leads to cumulative losses, necessitating the mobilisation of additional human resources, including volunteers and reservists, to maintain uninterrupted emergency medical services. Simultaneously, medical supply storage facilities showed the highest mortality rate among staff (10.3%), indicating the need to

redistribute logistical functions across a larger workforce and to establish reserve storage capacities with rotational service teams. Medical institutions, despite representing the largest sample (n = 1,301), exhibit relatively stable injury (3.8%) and mortality rates (1.5%), making them suitable bases for the training and retraining of medical personnel, mobilisation of reserve staff, and coordination of rotation schemes across different segments of the healthcare system. These statistics support the implementation of HECP strategies and active personnel rotation as primary mechanisms for preserving human resources: differentiated risk profiles by facility type allow for optimal allocation of experienced staff, new recruits, and volunteers, ensuring both the maintenance of critical healthcare functions and the minimisation of personnel losses through systematic rotation between zones of varying risk.

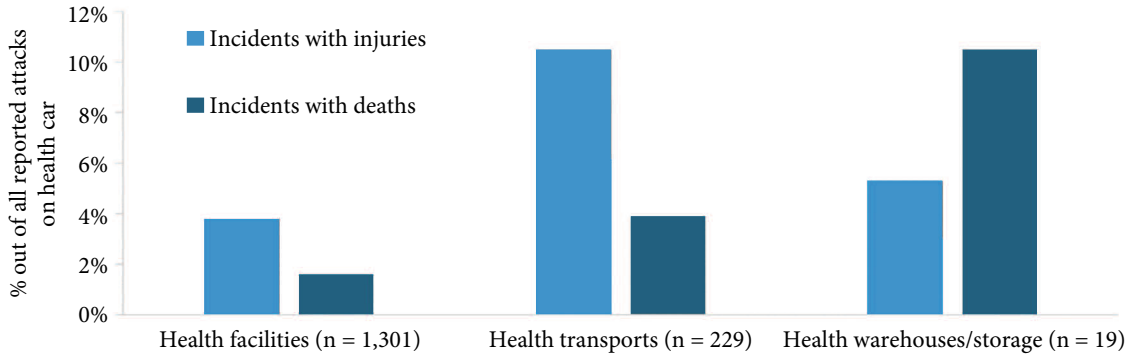

Figure 1. Attacks on medical infrastructure resulting in injury and loss of life, by affected facility type
Source: H.J. Kim *et al.* [15]

According to a review by O. Onvlee *et al.* [16] on human resources for health in conflict settings, armed conflict precipitates an acute shortage of personnel due to both migration and withdrawal from the profession. This is illustrated by the case of Syria, where, since the onset of conflict in 2011, over 70% of healthcare workers were forced to leave the country, and by April 2020, more than 900 healthcare personnel had died, leaving the system understaffed and fragmented. Specifically, in Aleppo and the north-western regions, the number of physicians per 1,000 population fell from 1.5 in 2010 to 0.3 in 2018, while the number of nurses and midwives declined from 1.4 to 0.6 per 1,000 population. In the north-western areas, home to approximately 4.17 million people, only around 1,000 doctors, 1,693 nurses, 358 midwives, and 709 primary care workers remained [17]. Such attrition places immense pressure on those who remain, forcing them to operate under resource constraints and direct threats to their lives. Consequently, new private training institutions have emerged in several countries to address workforce gaps, although the quality of education in these institutions is often low, contributing further to the shortage of qualified personnel. In addition, irregular and inadequate remuneration, coupled with widespread corruption and nepotism, demotivates staff, while a lack of coordination between Ministries of Health and Education limits the capacity for effective training and allocation of professionals [16]. This indicates that armed conflicts generate a critical shortage of medical personnel, placing severe strain on those who remain and increasing the risks associated with delivering effective healthcare. Workforce instability consequently diminishes the healthcare system's capacity to respond to population needs in crisis situations.

In response to these challenges, a range of organisational measures has been implemented. Experience from conflicts in Ukraine, analysed in a descriptive study by H. Tien & A. Beckett [18], demonstrated that the 10-1-2 doctrine (10 minutes for tactical care, 1 hour for resuscitation, 2 hours for surgical intervention) imposes stringent demands on personnel management, requiring high readiness for rapid mobilisation and rotation of medical staff. Implementation of this approach involves the creation of mobile surgical teams and emergency workload redistribution systems, which, for example, reduced battlefield mortality from 13% to 3%. Simultaneously, the loss of guaranteed air superiority complicates the movement of specialists, emphasising the need for a dispersed allocation of human resources, including mobile surgical units. Predicted patient loads, ranging from 24,000 to 51,000 wounded over three weeks of combat, require not only full engagement of military medical personnel but also integration of specialists from civilian hospitals [18]. Thus, military medical support systems demonstrate the necessity not only for technical adaptation but also for comprehensive personnel management capable of ensuring effective coordination, rotation, and mobilisation of medical resources during large-scale conflicts.

The further development of tactical medicine approaches, as illustrated in a study by A. Mohylnyk *et al.* [19] in Israel – where surgical points are located within 200 metres of the front line – enables resuscitation to commence within the first minutes after injury, reducing the average evacuation time from 90 to 40-60 minutes. According to a descriptive study by N. Movlyanova *et al.* [20], similar measures were implemented in Ukraine to enhance the effectiveness of medical care, including the redistribution of

medical personnel and the establishment of mobile surgical points closer to the front line, which shortened average evacuation times. Expanding the scope of pre-hospital care provided by both medical personnel and trained military personnel, alongside the integration of civilian specialists into military medical structures, stabilises wounded patients and supports system functioning under high operational loads. This strategy emphasises the importance of controlled personnel placement, rapid mobilisation, and staff training to stabilise casualties, demonstrating that human resource management in extreme conditions is a decisive factor in the effectiveness of military medical support.

The modelling described by F.J. Martin-Campo *et al.* [21], which focused on optimising personnel management and logistics for the deployment of a field hospital using the example of the Spanish START team in Turkey, demonstrated the efficiency of a flexible approach to staff rotation planning. A mathematical model was developed to select volunteers from an existing list and schedule their rotation according to three key criteria: minimising financial costs, maximising staff availability, and enhancing personnel qualifications. The model enabled the evaluation of different rotation scenarios and their impact on costs and staff availability. For instance, for 160-165 participants, total costs ranged from EUR 288,535 to EUR 296,701, depending on the selected priorities. The application of this model during the mission in Turkey ensured the uninterrupted operation of the field hospital throughout the first three weeks, even under high workload conditions: optimised rotation reduced the average shift duration by 12% and cut flight costs by nearly 8%. The use of a multi-criteria approach allows for simultaneous financial efficiency (through reduced travel costs), consideration of staff availability, and improved quality of medical services by deploying the most qualified personnel. The modelling results emphasise that flexible staff rotation planning is critically important for effective mobilisation and management of human resources in crisis situations, enabling optimal resource allocation and continuity of healthcare delivery.

Ukraine serves as an example of healthcare system adaptation under full-scale war, demonstrated through the mobilisation, rotation, and engagement of medical personnel [22]. By early 2023, significant demographic changes – over 7.7 million people became refugees or internally displaced – led to a redistribution of medical staff: 30,000 healthcare workers joined the Armed Forces of Ukraine or volunteered, 4,500 were internally displaced, and 2,500 left the country. To compensate for the destruction of infrastructure – over 1,200 damaged and 160 completely destroyed medical facilities – hospitals were relocated, and mobile clinics and field hospitals were established. This demonstrates that war compels the healthcare system to adapt rapidly, optimising the allocation and mobilisation of medical personnel to ensure continuity of care. Significant demographic and infrastructural challenges further increase the need for flexible staffing strategies and rapid operational responses.

The organisation of personnel management in conflict settings requires particular attention to psychological and organisational factors [23]. For example, during rocket attacks in Israel in May 2021, 77.4% of medical personnel continued to perform their duties. High work readiness correlated with higher resilience (mean score 28.9 on the Connor-Davidson Resilience Scale, CD-RISC 10), as well as with age (41.7 years) and absence of children under two years old. Attendance was highest among specialist physicians (93.8%) and nurses (81.7%), whereas pharmacy staff reported to work in only 40% of cases. Managerial positions increased the likelihood of attendance by 2.4 times, while limited access to childcare and a high perceived level of danger reduced it. This underscores the importance of organisational measures – such as ensuring security, developing childcare services, and providing psychological support – in retaining human resources.

The example of Sudan demonstrated that mobile medical teams, frequently deployed by the WHO, are capable of delivering high-quality medical care rapidly during emergencies or following significant infrastructure damage. According to M. Ahmed *et al.* [24], they respond to large-scale disease outbreaks, such as cholera and measles, reaching nearly 321,000 people through mobile units. Additionally, they trained 550 local healthcare workers, enhancing community capacity to respond effectively to medical crises, while vaccination programmes contributed to reduced overall mortality and saved the lives of thousands of children. Similarly, in humanitarian and conflict-related crises, healthcare systems must mobilise personnel – including volunteers and reservists – and implement measures to protect and support their wellbeing, given the elevated risks to life and health [25]. Strategic management of medical personnel through mobilisation, training, rational resource allocation, and deployment of mobile teams is therefore essential to maintain continuity of care and strengthen the resilience of healthcare systems in crisis settings.

Existing data reveal substantial variation in the scale of medical workforce attrition during armed conflicts. For instance, T. Gutor *et al.* [22] found that in Ukraine, 30,000 healthcare workers joined the Armed Forces, 4,500 became internally displaced, and 2,500 left the country. In contrast, Y. Bdaiwi *et al.* [17] reported that 50% of healthcare personnel and 95% of doctors in Aleppo had left Syria since 2011. Thus, in contrast to the Ukrainian context, where a substantial proportion of personnel were integrated into internal structures, the conflict in Syria led to mass emigration of staff, highlighting the difference in how war impacts internal mobilisation versus external migration.

Data on the effectiveness of battlefield medical care also reveal varying outcomes depending on organisational measures. A study by A. Mohylnyk *et al.* [19] demonstrated that 60% of deaths from combat injuries occur within three hours of trauma, with up to 50% occurring within the first 60 minutes, and that staff redistribution and the establishment of mobile treatment points reduced the average evacuation time for the wounded from 90 to 40-60 minutes. Similarly,

Y. Bdaiwi *et al.* [17] showed that implementing emergency workload redistribution systems, forming mobile teams, and supporting tactical medicine reduced battlefield mortality from 13% to 3%. This evidence indicated that comprehensive organisational measures combined with tactical medicine significantly improve survival rates and emphasises the importance of a systemic approach integrating rapid response with optimisation of human resources.

In the management of medical personnel during crises, strategies show considerable variation. T. Stroiko *et al.* [13] proposed measures to stimulate birth rates and encourage the return of migrants to replenish the workforce, whereas T. Wurmb *et al.* [14] focused on the HECP programme, which is based on mobilising, rotating, and deploying reserves to ensure the continuity of healthcare services. F.J. Martin-Campo *et al.* [21] demonstrated that flexible staff scheduling optimises costs and maximises personnel availability, delivering better outcomes than rigidly structured operations. In a study by M. Ahmed *et al.* [24] examining Sudan, mobile medical teams, which combine multidisciplinary expertise with rapid deployment, were shown to enhance the efficiency of healthcare delivery in crisis conditions by addressing largescale disease outbreaks and training local healthcare workers. This illustrated the effectiveness of a comprehensive management approach to mobilisation, resource allocation, and strengthening the resilience of healthcare systems in emergency settings. H. Tien & A. Beckett [18] examined the 10-1-2 doctrine, which prioritises tactical, resuscitative, and surgical care within specified timeframes. The evidence indicates that medical personnel management in crisis situations requires adaptive, integrated strategies combining motivation, efficient allocation of labour resources, and organisational innovation.

The highest effectiveness is achieved through integrated approaches that incorporate staff rotation, shift planning, incentives for additional personnel engagement, and the prioritisation of key areas of medical care. Such systematic organisation enables the continuity of healthcare services, optimises the use of available resources, and enhances the overall productivity of medical facilities during emergencies. This approach promotes effective resource management, ensuring maximum adaptability and resilience of healthcare facilities to the challenges arising in crisis situations.

Psychological support for medical personnel in conflict zones. Providing psychological support to medical personnel in conflict zones is critically important, as they face unique challenges that impose a heightened psychological burden. According to an analytical review by O.S. Chaban & O.O. Khaustova [26], up to 30% of individuals in conflict zones experience post-traumatic stress disorder (PTSD), and 90% of these cases involve comorbid conditions. These findings indicated that healthcare workers operating under such conditions require particular attention, as they themselves are at elevated risk. To prevent PTSD and burnout, the World Health Organization has developed the Mental Health Gap Action Programme [27], which includes early diagnosis and intervention. An effective "cognitive blockade" strategy involves engaging in visuospatial tasks, such as playing a 25-minute session of Tetris in the hours immediately following a traumatic event, which has been shown to reduce intrusive memories. Additionally, psychoeducation and stress management training constitute important organisational measures that support staff adaptation to extreme conditions.

Psychological and emotional challenges among healthcare personnel arise from sustained exposure to traumatic events. A study by A.A. Faraj *et al.* [28] found that medical staff working with mass casualties exhibit high levels of anxiety (up to 73%), depression (78%), and stress (68%). The greatest burden was observed among mortuary specialists, who, in addition to constant exposure to death, face added pressures from the emotional responses of relatives, political factors, and strict deadlines for medico-legal procedures. A review of the scientific literature by M.V. Krasnoselskyi *et al.* [29] indicated that chronic stress and personal risk factors contribute to psychological trauma, manifesting as depression (11-47%) and anxiety disorders. One key aspect is the concept of a "shared traumatic reality", in which healthcare workers simultaneously experience trauma while providing care to patients.

Additionally, a study by L. Hamama *et al.* [30], which analysed of 200 nurses at a large public hospital in southern Israel, identified two stress-response profiles: "resilient" (n = 156), characterised by high positive affect, resilience, and a sense of social support, and "reactive" (n = 44), who exhibited significantly higher levels of psychological distress and a greater prevalence of adjustment disorders. Nurses in the reactive group were more likely to have experienced the abduction of a family member (18% vs 4%), were younger on average (M = 38.9 years vs 43.7 years), and rated their own health more poorly (M = 3.01 vs 3.30). Notably, the frequency of providing care to victims of mass-casualty events or active combat zones did not statistically influence the distribution of nurses across profiles, emphasising the importance of individual and family factors in shaping psychological resilience. In a review by V.A. Dobiesz *et al.* [31], interventions aimed at reducing these risks include group therapy, which restores a sense of belonging and mutual support; individual psychological support, focused on processing traumatic experiences; and strengthening social connections both within the team and with external support networks. Despite the limited availability of systemic programmes, there is growing recognition of the importance of healthcare worker wellbeing and the need for targeted preventive measures. This underscored the necessity of comprehensive approaches that integrate psychotherapeutic methods with organisational support and training in stress-resilience skills.

To mitigate such consequences, the importance of systematic monitoring of staff mental health and the implementation of psychological support strategies is emphasised. These strategies include regular psychodiagnostic

assessments, targeted programmes for the prevention of post-traumatic stress disorder and burnout, and the use of resilience-enhancing methods, such as group supervision, access to specialised psychological services, and supportive resources that ensure timely intervention and promote the restoration of professional and emotional wellbeing [28]. Among psychological support and burnout-prevention strategies, cognitive-behavioural therapy, Eye Movement Desensitisation and Reprocessing (EMDR), and stress-management and relaxation programmes are particularly effective. The quality of team functioning and training is also a crucial factor influencing mental health, acting as a protective element [29]. These measures are considered essential for minimising distress and maintaining the capacity of healthcare professionals during prolonged conflict.

The effectiveness of support programmes is supported by the outcomes of implemented initiatives. In Ukraine, the Ukraine Advanced Clinical Trauma Training (ACTT) programme, which combines a series of training sessions, has contributed to increased professional confidence among participants. Such approaches demonstrated a positive impact on healthcare workers' readiness to operate in extreme conditions [32]. In another psychodiagnostic study by O.O. Kyrylova *et al.* [33], which included 88 healthcare professionals (41 doctors and 47 nurses) in Kharkiv, the critical situation in 2022 necessitated systemic psychological interventions, leading to the establishment of a dedicated support office that year. Its activities encompassed organisational and psychological interventions, psychoeducational self-help and burnout-prevention modules (weekly 30-minute mini-seminars), as well as individual and group consultations aimed at developing resilient stress-coping skills. A follow-up assessment of staff in 2023 revealed a statistically significant 23% reduction in distress levels, alongside a marked decrease in the prevalence of anxiety symptoms (from 78% to 54% among doctors and from 81% to 60% among nurses), fear, and depression. These findings indicated the effectiveness of the implemented psychological support strategies, demonstrating that regular counselling, psychoeducation, and organisational assistance can substantially reduce psychological strain and enhance the resilience of healthcare workers in conflict settings. In this context, practices implemented in Ukraine and Sudan were compared, as summarised in Table 1, since both countries faced protracted crises that severely restricted access to conventional healthcare resources. This comparison allows for an analysis of how training programmes and educational initiatives can support healthcare personnel across different socio-cultural and political contexts, ensuring the continuity and stability of healthcare systems during emergencies.

Table 1. Psychological support strategies for healthcare workers in conflict zones (Ukraine vs Sudan)

Parameter	Ukraine (ACTT, 2023)	Sudan (Emergency ECHO, 2023)
Target audience	238 unique participants: 42.5% nurses/NP/PA; 32.1% feldshers/paramedics; 13.1% doctors; 5.8% mental health staff; remainder – laboratory, educational, and administrative personnel.	>5,000 healthcare workers (doctors, nurses, students) in Clinical Management Response Team Telegram group; audience grew to >14,000 after programme launch.
Format	16 webinars (twice weekly, 9 June – 2 August 2023); live and asynchronous access via YouTube.	43 interactive online sessions (Telegram+Zoom), April-October 2023; combination of basic emergency care materials and locally adapted content.
Language and lecturers	International instructors; English.	Sudanese experts and diaspora; Arabic (culturally relevant content).
Number of visits	758 (repeat visits).	2,697 (average attendance 65.8 per session).
Knowledge assessment	"Very competent" increased from 24.9% to 52.6%; "Extremely competent" from 5.4% to 11.3% (N = 708).	58.6% of participants reported acquiring new knowledge and readiness to apply it in practice (N = 986).
Confidence in applying knowledge	27.7% rated the training as "extremely relevant", 50.5% as "very relevant" (N = 708).	59.9% felt "extremely confident", 26.4% "moderately confident" in applying the material (N = 1,104).
Strengths	Involvement of the Ministry of Health, availability of recordings, minimisation of safety risks.	Utilisation of existing networks, low internet requirements, cultural relevance, rapid content adaptation.
Challenges	Low participant interaction (webinar format), recruitment difficulties during wartime.	Limited use of the consultation group, infrastructure and security challenges.

Note: NP/PA – Nurse Practitioner/Physician Assistant, healthcare professionals with extended clinical responsibilities; Emergency ECHO – educational initiative for Sudanese healthcare workers using the ECHO (Extension for Community Healthcare Outcomes) model for remote training and consultations
Source: compiled by the author based on S.M. D'Andrea *et al.* [32]

As shown in Table 1, the comparative analysis of psychological support strategies for healthcare workers in conflict zones in Ukraine and Sudan highlights the scale and complexity of the psychological challenges faced by medical personnel during armed conflict. It also demonstrated the critical importance of a systematic approach to mental health management as a foundation for preserving the healthcare workforce. Following training in Ukraine, among 238 participants, the proportion rating their knowledge as "very competent" increased from 24.93% to 52.60%, more than doubling, which indicates a systemic lack of preparedness among healthcare workers

to manage traumatic cases and their own psychological responses under sustained stress. The "extremely competent" category also doubled from 5.4% to 11.3%, showing significant progress in the development of specialised skills. The diversity of the target audience (42.5% nurses/NP/PAs, 32.1% feldshers and paramedics, 13.1% doctors) emphasises that psychological challenges affect all categories of healthcare personnel, not only those in direct patient contact. High programme engagement (758 repeat visits in Ukraine; 2,697 in Sudan) and substantial audience growth (from >5,000 to >14,000 participants in Sudan) underscore the critical need for psychological support and strategies for preventing burnout and PTSD. Overall, the training results indicate that systematic programmes effectively enhance the psychological competence of healthcare workers and strengthen their readiness to operate under crisis conditions.

The effectiveness of these programmes is reflected in participants' confidence in applying acquired knowledge: 78.2% of Ukrainian participants rated the training as very or extremely relevant, while 86.3% of Sudanese participants reported feeling moderately or extremely confident in the practical application of the skills gained [32]. At the same time, identified challenges, such as low participant interaction due to security constraints and difficulties in recruiting personnel during active hostilities, highlight the need for adaptive approaches to psychological support that account for the specific conditions of conflict. These findings substantiate psychological support as a critical component of workforce management in conflict zones, since without systematic prevention of burnout and PTSD, healthcare systems risk losing a significant proportion of qualified personnel – not through physical casualties, but through psychological exhaustion and professional impairment.

Such challenges were compounded by the lack of academic programmes training healthcare workers to cope with PTSD and burnout [34]. While international organisations such as Médecins Sans Frontières and the International Committee of the Red Cross (ICRC) develop safety protocols and train staff in negotiation with armed actors, formal academic preparation remains insufficient. Nurses are particularly vulnerable, constituting up to 70% of humanitarian personnel and often being the first to receive the flow of casualties, thereby increasing their risk of burnout and PTSD. Threats, abductions, and attacks remain widespread: in the first six months of 2017 alone, attacks on healthcare workers were recorded in 23 countries. Specialised programmes such as HELP and international humanitarian medicine training play a crucial role in mitigating these risks, emphasising triage, psychoprevention, and the ethical dimensions of working under conditions of violence [34]. In A.Y. Sydorenko's *et al.* [35] study, an analysis of the psychological state and needs of 1,442 healthcare workers in Ukraine found that 70% reported a negative impact of the war on their work, while 13.1% experienced bullying from colleagues or management. Regarding support strategies, 79.3% of participants highlighted the need for improved financial conditions, moral and emotional support, increased staffing, and adequate rest. Despite these challenges, job satisfaction remained high, although awareness of personal stress is low, indicating the presence of resilience mechanisms alongside a need for targeted psychological support. This underscored the importance of systematic training, including stress-management education and prevention of emotional exhaustion, as only a comprehensive approach to protection and resilience development can preserve professional effectiveness and reduce the risk of long-term psychological consequences.

A descriptive analysis of an online support programme for Israeli nurses facing the emotional and psychological challenges of war by V. Segev *et al.* [36] explored the difficulties encountered by healthcare workers. In December 2023, 30 volunteer nurses trained in cognitive-behavioural therapy offered up to three anonymous 30-minute online sessions. The findings revealed that nurses experience stress both in everyday professional duties and in the context of armed conflict. Among the key findings was a reduction in psychological isolation through access to anonymous support, an increase in confidence in the ability to manage professional challenges, and the development of a sense of support and solidarity within the team. These results highlighted the importance of flexible and accessible formats of psychological support for maintaining professional resilience among healthcare personnel in contexts of armed conflict.

A literature review by K. Goniewicz *et al.* [37] on the impact of the war on Ukraine's healthcare system revealed that, in response to rising demands, part of the financial aid was allocated to establishing mobile clinics that provide psychological support directly in combat zones. Concurrently, additional specialists were trained to work with trauma and stress-related disorders. These measures targeted not only civilians and military personnel but also healthcare workers, who face high levels of psychological pressure daily and require specialised support to preserve professional resilience. According to an analytical commentary by A.S. Niven *et al.* [38], the influx of 6.9 million internally displaced persons created an immense demand for basic healthcare services. To alleviate this pressure and support Ukrainian healthcare workers, an international team was established to provide online training in trauma care, supply access to clinical guidelines, and create a private messenger group comprising 946 participants. In addition to professional support, this initiative also served a psychological function: healthcare workers were given the opportunity to share experiences, discuss personal challenges, and receive emotional support from colleagues and international experts. This proved to be a crucial factor in preserving their mental health under conditions of war. Existing data reveal substantial differences in the prevalence of psychological disorders among healthcare personnel working in crisis situations. For instance, A.A. Faraj *et al.* [28] found anxiety in 73% of surgeons and 54% of mortuary staff, whereas M.V. Krasnoselskyi *et al.* [29] reported depression rates among doctors ranging from 11% to 47%.

Unlike the comparatively moderate levels of depression, anxiety among surgeons reaches critical levels, emphasising the need for a differentiated approach to psychological support across staff categories.

Divergent approaches are also evident in strategies for psychological support. O.S. Chaban & O.O. Khaustova [28] demonstrated the effectiveness of game-based therapy (Tetris) for cognitive blockade of traumatic imagery, which reduces intrusive memories during the first hours after trauma. This indicated the potential of rapid interventions in the acute phase of traumatic stress. In contrast to short-term interventions, M.V. Krasnoselskyi *et al.* [29] highlighted the importance of prolonged professional therapy, including cognitive-behavioural therapy and EMDR, as effective tools for burnout prevention. They also emphasised the importance of regular monitoring of therapeutic outcomes to ensure a stable and sustained effect. In addition, A.A. Faraj *et al.* [28] emphasised the role of group supervision in the systematic monitoring of mental health. Regular supervision facilitates the early identification of professional burnout. Meanwhile, O.O. Kyrylova *et al.* [33] demonstrated the effectiveness of weekly mini-seminars, psychoeducational modules, and individual consultations in developing resilient coping skills. These interventions resulted in a 23% reduction in distress among participants, highlighting the practical value of regular psychoeducational activities for enhancing staff psychological resilience. Similarly, A.S. Niven *et al.* [38] showed that international online groups via messaging platforms provide access to clinical guidelines and support Ukrainian healthcare workers remotely. Such online communities also promote experience-sharing and rapid problem-solving in crisis conditions. Thus, regardless of the specific method – whether game-based therapy, cognitive-behavioural interventions, EMDR, group supervision, or online consultations – the presence of structured psychological support is critically important for healthcare personnel working in conflict zones. Any organised form of assistance contributes to stress reduction, burnout prevention, and increased resilience to psychological strain.

Data on the effectiveness of psychological support demonstrated positive outcomes when comprehensive programmes are implemented. For example, S.M. D'Andrea *et al. [*32] found that an online programme increased the proportion of healthcare workers rating their knowledge as "very competent" from 24.93% to 52.60%. This indicated that remote training interventions can effectively enhance healthcare personnel's self-assessment of professional competence. At the same time, O.O. Kyrylova *et al.* [33] recorded a 23% reduction in distress levels and a decrease in the prevalence of anxiety symptoms among physicians from 78% to 54% following the establishment of a psychological support unit in Kharkiv. Thus, in contrast to individual morbidity indicators, organised psychological support programmes can substantially improve the psycho-emotional well-being of staff, particularly when combining online tools with local consultations.

The reviewed evidence suggests that effective management of healthcare personnel's mental health requires a systematic, multi-level approach encompassing early intervention, regular monitoring, individual and group interventions, and the use of modern digital platforms for support and education. In particular, the implementation of comprehensive programmes combining various psychotherapy methods helps to reduce the level of professional burnout and improve the psychological well-being of healthcare workers. Such an approach not only reduces anxiety and depression but also strengthens psychological resilience, thereby enhancing overall medical service effectiveness during crises.

Legal status and physical safety of healthcare personnel. The legal status of healthcare personnel and medical units in armed conflicts is defined by international humanitarian law, which guarantees their physical inviolability and neutrality. Legal protection for healthcare workers and medical facilities in international conflicts is further ensured by the provisions of the Geneva Conventions [39]. Protection extends not only to doctors and nurses but also to support staff and transport used exclusively for medical purposes. Mandatory requirements include the display of identifying symbols and the possession of proper credentials, as well as adherence to the principles of humanity and medical ethics. Such legal regulation provides the foundation for carrying out humanitarian missions and delivering effective aid to those affected in conflict zones [39]. The provisions guarantee the inviolability of personnel, prohibit attacks on hospitals and medical transport, and require the neutrality of humanitarian organisations such as the ICRC. Practical implementation involves the use of Red Cross and Red Crescent symbols, the establishment of protected zones, and the training of personnel in conduct under combat conditions. However, political factors – particularly the veto power within the UN Security Council – significantly complicate accountability for attacks on medical facilities [40]. This highlights that while international humanitarian law ensures the inviolability and neutrality of medical personnel, the effectiveness of this protection largely depends on political will and compliance by all parties to the conflict.

Analysis of the physical security of healthcare personnel and facilities by E.J. Breeze [41] indicated that international humanitarian law recognises their inviolability and obliges conflict parties to facilitate the evacuation of the wounded and access to humanitarian assistance. In practice, adherence to these norms is hindered by the intensity of hostilities, the actions of non-state armed groups, and the use of unconventional weapons. This necessitates careful evacuation planning, clear marking of medical facilities, and personnel training in safety procedures. At the same time, the enforcement of these protections is heavily dependent on the goodwill of the parties involved, emphasising the need for effective monitoring and legal enforcement.

M.A. Mamun [42] in a critical analysis highlighted those gaps in international humanitarian law allow perpetrators to evade accountability for systematic attacks on

medical personnel and facilities. Cases from Syria and Sudan illustrate extensive violations resulting in significant human losses: in Syria, government forces carried out 92% of attacks leading to fatalities among healthcare workers, while in Sudan, rebel groups targeted 52 medical facilities. The lack of precise definitions of "attack" and the concept of "dual-use" creates legal loopholes for aggressors. Proposed measures to strengthen protection include the decolonisation of international humanitarian law, training military personnel in legal norms, and the more decisive application of sanctions and diplomatic pressure on violators. Despite the existence of legal protections, attacks on medical infrastructure in conflict zones continue to rise [43]. In 2022, the number of documented incidents increased by 45% compared with the previous year, and in 2023, they exceeded 2,500. In Ukraine, between 24 February and 31 December 2022, 707 attacks on the healthcare system were recorded, including the bombing of a maternity hospital in Mariupol. Analysis indicated that international law alone is insufficient to ensure the safety of medical personnel, highlighting the need for professional associations and individual physicians to actively engage in public advocacy and demand protection from governments.

Additional evidence highlighted multiple forms of threats, including assault, arrest, intimidation, obstruction of facility operations, and armed attacks [44]. In some cases, medical facilities were physically destroyed, as occurred in eastern Ukraine in 2014, while demands from opposing parties for differentiated or complete denial of care placed personnel in direct conflict with the principles of international humanitarian law. Beyond physical risks, a significant threat arises from the development of post-traumatic stress disorder: up to 25% of nurses in some samples reported symptoms years after experiencing explosions, and rates of chronic PTSD among military medical personnel are comparable to those of combat troops.

In Ukraine, during the first year of the full-scale war, 106 civilian medical personnel were killed, 33 of them at their workplaces and the most affected regions were Kherson and Donetsk, where over 50% of medical facilities were damaged [45]. An analysis focusing on the protection of the rights and physical safety of medical workers in Ukraine during hostilities by O.G. Strelchenko *et al.* [46] showed that by 2024, Russian troops had damaged 1,468 medical facilities and completely destroyed 193, as well as destroying or capturing 481 ambulances. In addition, the main factors affecting the work of these facilities were staff shortages (51%), security issues (31%) and damage to buildings. Despite these challenges, the number of healthcare workers increased from 288,000 on 1 January 2022 to 345,000 on 1 January 2024, while the number of medical facilities grew from 7,393 to 8,444, demonstrating the healthcare system's capacity to adapt and recover even under conditions of armed conflict. These figures underscore the high vulnerability of medical personnel and infrastructure, highlighting the need for rigorous planning of safe evacuation routes, proper marking and protection of medical facilities, and strategic coordination with state and humanitarian organisations to minimise risks to the health and lives of both medical staff and patients. The distribution of the operational status of medical facilities in the most affected regions is illustrated in Figure 2.

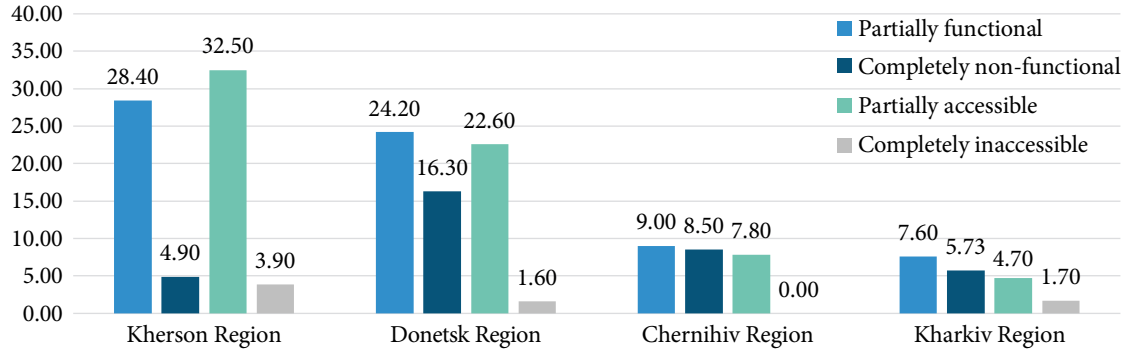

Figure 2. Percentage of medical facilities and their structural units experiencing partial or complete loss of functionality and accessibility in the most affected regions of Ukraine
Note: comparative analysis of reports from January 2023 and January 2024
Source: compiled by the author based on R.P. Brukhno *et al.* [45]

As shown in Figure 2, the distribution of operational capacity across medical facilities in the four most affected regions highlights the critical challenges in ensuring physical security and the continuity of healthcare services during armed conflict, emphasising the need for comprehensive strategies to protect medical infrastructure. Kherson Region presents the most dramatic picture of the destruction of the healthcare system, with only 28.40% of facilities partially operational, reflecting extensive infrastructure damage from direct attacks and the need to evacuate a substantial proportion of medical personnel from the zone of active hostilities. Donetsk Region shows similarly critical figures, with 24.20% of facilities partially operational and a high proportion of completely nonfunctional institutions (16.90%), indicating the chronic impact of the conflict on the healthcare system and the need for continuous

adaptation of operational modes under heightened risk conditions. In contrast, Chernihiv and Kharkiv regions display significantly better outcomes, with 9.00% and 7.60% of facilities partially operational, respectively. This may reflect the effectiveness of recovery and protection measures, the importance of geographical location, and the efficacy of air defence systems in preserving medical infrastructure. The sharp contrast between regions underscores that proximity to the frontline and the intensity of hostilities are decisive factors in maintaining the functionality of medical facilities. These data highlighted the critical need for differentiated strategies for the physical protection of healthcare institutions, including the establishment of underground medical complexes, emergency evacuation plans for patients and staff, and flexible protocols for transitioning between full and partial operational modes depending on the level of threat. The loss of operational capacity in medical facilities directly affects the provision of healthcare to the population and places additional strain on facilities that remain functional in less affected regions. To ensure the physical safety of medical personnel and reduce the risk of trauma, a range of strategies is employed. Analysis by R. Mugavero & M. Alkuhali [47] identified prevention of attacks, evacuation, and the organisation of operations in high-risk zones as key priorities. Practical measures included protocols for behaviour in dangerous areas during shelling, airstrikes, and explosions; safe movement by vehicle and on foot; procedures for encountering suspicious objects; and handling of bodies within a 50 m radius. Data indicated that up to 80% of injuries in buildings during explosions are caused by glass fragments, emphasising the critical importance of adhering to safety procedures.

Strict protocols – such as Confirm, Clear, Call, Cordon, Control (5 C) – combined with ongoing training, incident documentation, GPS navigation, and clear route marking, enhance operational effectiveness and coordination with humanitarian and engineering teams. Coordinates of bodies or unexploded ordnance are recorded in decimal degrees for subsequent work by bomb disposal specialists, safe zones and transit routes are marked with tape or indicators, and hazardous areas are delineated with warning signs and cordons. Compliance with protective measures and awaiting specialised assistance minimises the risk of injury and contamination: personnel use personal protective equipment including gloves, boots, waterproof suits, masks, and goggles, avoid contact with biological fluids, and follow the 5 C procedure when encountering hazardous items, prioritising team safety while awaiting explosive ordnance disposal or specialist intervention. International humanitarian law recognises medical personnel and healthcare facilities as protected entities, making adherence to safety procedures critically important in conflict zones [48]. This underscores that comprehensive compliance with physical safety protocols, strict operational procedures, and international norms is essential to minimise the risk of injury to medical staff. The effectiveness of these measures, however, depends heavily on adequate training, coordinated action, and timely access to support in extreme conditions. The performance of medical teams is also contingent on their level of preparedness. Surgical teams, including those deployed by the ICRC and Médecins Sans Frontières, conduct emergency operations amid unpredictable combat intensity, combining patient care with the training of local specialists. Specialised courses, such as Definitive Surgical Trauma Care and Emergency War Surgery, equip surgeons with the skills necessary for managing military trauma [48]. To anticipate resource requirements and plan safe operations for medical teams, the Red Cross Wound Score [49] has been developed. This scoring system is independent of vital signs and is particularly useful in resource-limited settings.

Despite the high risks, healthcare systems have demonstrated a remarkable capacity to adapt and maintain continuity of services. In Ukraine, even amid extensive destruction and ongoing hostilities, the number of medical personnel and healthcare facilities increased between 2022 and 2024, reflecting the system's flexibility and intrinsic resilience [46]. However, according to World Health Organization [9] analysis focused on the functioning of primary healthcare in Ukraine under conditions of armed conflict, a survey of 500 medical facilities revealed that 10% were forced to temporarily suspend operations, while 43% altered their service schedules for the population, highlighting the need for adaptive planning. Staff shortages remain a serious concern: 74% of facilities experienced personnel attrition or absence, with 41% of cases resulting from forced relocation of employees. Direct threats to staff were also significant, with 22 workers injured and one fatality reported due to hostilities. To enhance preparedness and reduce risks, 73% of facilities organised additional staff training, encompassing psychological support, emergency response for combat-related injuries, and procedures for radiation threats. Despite limited funding – only 16% of facilities received supplementary resources – 97% of employees continued to receive their salaries on time, and 47% received additional compensation for overtime work, demonstrating the system's ability to maintain basic economic stability under crisis conditions [9]. These findings underscored that the Ukrainian healthcare system continues to function and deliver services despite the destructive impacts of armed conflict. Nevertheless, the high levels of personnel loss and limited resources indicate a pressing need to strengthen training and support strategies to ensure the stability and effectiveness of medical care. Similar challenges in protecting medical personnel are observed in Syria, where the ongoing conflict since 2011 has precipitated a catastrophic collapse of the healthcare system. Despite the existence of international norms, such as the Geneva Conventions [50] and Resolution 2286 [51], healthcare workers remain highly vulnerable to attacks, and most hospitals and ambulances are unable to operate fully [52]. This underscores the urgent need for strategic measures to ensure the physical safety of medical personnel, healthcare facilities, and transport in order to maintain basic health services and protect civilian lives.

In the context of the conflict in Sudan, the legal status and physical safety of medical personnel remain critically compromised. Over the course of a conflict lasting more than eight months, the healthcare system has come under severe strain due to mass displacement, disease outbreaks, combatrelated injuries, and inadequate medical care for pregnant women and children. According to World Health Organization [10] data, 60 attacks on healthcare facilities were recorded, resulting in 34 deaths and 38 injuries among medical personnel, while approximately 70% of healthcare facilities in conflict zones were either partially operational or completely non-functional. A significant proportion of medical personnel have not received salaries for up to eight months, and access to medicines and medical supplies – including treatments for chronic diseases, laboratory reagents, and dialysis equipment – is severely limited. In response to these challenges, the WHO coordinates the national healthcare system, conducts staff training, deploys mobile clinics, and implements electronic early warning and response systems for disease outbreaks, thereby enhancing the safety and effectiveness of healthcare delivery in conflict settings.

Available data reveal substantial differences in the scale of attacks on medical facilities and personnel across countries and conflicts. For example, I. Bilgrami *et al.* [43] reported 707 attacks in Ukraine between February and December 2022, while O.G. Strelchenko *et al.* [46] recorded that, from the onset of the full-scale war until 2024, 1,468 healthcare facilities in Ukraine were damaged and 193 completely destroyed. By comparison, in Sudan, rebel groups carried out 52 attacks, according to M.A. Mamun [42], and A. Martin *et al.* [52] documented numerous attacks on healthcare facilities and personnel in Syria. These incidented severely impede the provision of medical care to civilians and place healthcare workers' lives at risk. This evidence indicates that, although threats to medical infrastructure represent a global challenge, their scale and intensity vary considerably depending on the region and the nature of the conflict. At the same time, data on injuries reveal variations according to weapon type and environmental context. M. Muhrbeck [49] reported that in urban areas, 42% of injuries were caused by firearms, whereas in rural settings, 70% of injuries resulted from explosions. R. Mugavero & M. Alkuhali [47] further clarified that 80% of injuries sustained in explosions within buildings were caused by glass shards, highlighting the context-specific nature of trauma in conflict zones.

Organisational and adaptive measures have demonstrated diverse approaches to ensuring the safety of medical personnel and enhancing the effectiveness of healthcare delivery. R. Mugavero & M. Alkuhali [47] emphasised the use of personal protective equipment and strict safety protocols, such as the 5 C system, whereas L. Yustitianingtyas & L.R. Habibah [40] highlighted the importance of specialised identification signs and credentials to protect personnel and vehicles used exclusively for medical purposes. Meanwhile, C. Ferreira & M. Correia [48] underscored the necessity of specialised training for surgeons and medical staff, including courses such as Definitive Surgical Trauma Care and Emergency War Surgery, to adapt standard surgical protocols to the conditions of war and the specific nature of combat-related injuries.

Thus, unlike isolated assessments of the number of attacks or the characteristics of injuries, the integration of organisational measures, personnel training, and adherence to international safety standards enables effective risk reduction for medical facilities and staff, ensures the provision of timely medical care, and increases the survival of the injured across diverse conflict environments. In addition, systematic monitoring and evaluation of the effectiveness of measures taken allow for prompt adjustments to strategies and procedures, ensuring continuous improvement in the safety and quality of medical services. Thanks to this comprehensive approach to the organisation of medical facilities, significant improvements have been achieved in the efficiency and accuracy of care provision in high-risk situations.

Conclusions

This review systematically compiled and analysed contemporary scientific evidence on the mobilisation, management, and psychological support of medical personnel in armed conflict settings. The study's objectives were achieved through an examination of international experience, organisational models, and legal frameworks. The analysis revealed that, in wartime conditions, medical personnel face resource shortages, elevated risks, and significant psychological strain, underscoring the critical importance of the timely implementation of integrated strategies. The use of HECP ensures clear operational coordination, mobility, and readiness of healthcare systems for crisis scenarios. The 10-1-2 doctrine demonstrated a marked ability to reduce mortality among battlefield casualties. Flexible staff rotation models also enable the continued functionality of medical facilities in active conflict zones. Regarding psychological support, cognitive-behavioural interventions, EMDR, group therapy, regular psychoeducational modules, and international online platforms proved effective in reducing stress levels and fostering adaptive coping strategies. Nevertheless, the legal and physical protection of medical personnel remains a complex challenge. Although international humanitarian law guarantees the inviolability of healthcare staff and facilities, numerous violations indicate the need for strengthened monitoring and enforcement of these norms.

The findings confirmed that maintaining the functionality of healthcare systems during armed conflict is achievable only through the integration of three key components: effective workforce mobilisation, systematic psychological support, and enhanced legal and physical protection. Only the comprehensive coordination of these strategies can minimise losses and ensure the continuity of medical care, even under the most extreme conditions. For workforce management, the implementation of flexible staff rotation

systems, the establishment of mobile medical teams, and training programmes for reservists were advisable. In the sphere of psychological support, systematic monitoring of mental health, the introduction of comprehensive PTSD prevention programmes, and access to online support platforms were recommended. For physical safety, protocols for marking facilities and transport should be improved, staff trained in emergency procedures, and the infrastructure of protected medical zones developed. Future directions include the development of unified protocols for medical personnel mobilisation, evaluation of the effectiveness of digital platforms for psychological support, study of the long-term impacts of conflict on the mental health of medical staff, and exploration of ways to enhance mechanisms of international legal protection. An important area of focus is also the analysis of adaptive strategies employed by healthcare facilities during the post-conflict period.

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Управління персоналом охорони здоров'я в умовах воєнного стану

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Анотація. Мобілізація та управління медичним персоналом у зоні бойових дій є одним із ключових викликів сучасних збройних конфліктів, що напряму впливає на безперервність надання медичної допомоги та збереження життя пацієнтів. Метою дослідження було узагальнити наявні наукові та практичні відомості щодо організації роботи медичного персоналу, методів психологічного супроводу та забезпечення безпеки під час воєного стану. Методологія включала пошук літератури у міжнародних базах даних з охопленням публікацій за період 2014-2025 років. У фокусі аналізу перебували приклади України, Сирії, Ізраїлю та Судану. Отримані результати показали, що впровадження Hospital Emergency and Contingency Planning сприяє підвищенню стійкості системи охорони здоров'я через формування мобільних груп, чітку координацію та кадрові резерви, тоді як доктрина 10-1-2 дозволила знизити смертність на полі бою з 13 % до 3 %. Використання математичних моделей ротації персоналу продемонструвало ефективність у збереженні безперервності медичних послуг. У сфері психологічної підтримки найбільш результативними виявилися когнітивно-поведінкові та Eye Movement Desensitisation and Reprocessing терапія, групові та індивідуальні програми, що знизили рівень дистресу в середньому на 23 %. Онлайн-платформи та міжнародні ініціативи створили додаткові можливості для дистанційної допомоги та обміну досвідом. У сфері фізичної безпеки ключове значення мають протоколи маркування медичних об'єктів, створення захищених зон і навчання персоналу тактичній медицині. Аналіз підтвердив, що інтеграція стратегій мобілізації кадрів, психологічної підтримки та правового захисту є необхідною умовою для збереження функціональності медичної системи у війні. Практична значимість результатів полягає у можливості застосування їх органами державного управління, медичними закладами та гуманітарними організаціями для оптимізації кадрової політики, впровадження програм підтримки та підвищення безпеки персоналу

Ключові слова: адаптивні кадрові стратегії; психологічна резильєнтність медиків; міжнародні протоколи безпеки; ротаційні моделі праці; травматичний стрес у зоні конфлікту; гуманітарна координація ресурсів

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Analysis of secondary deformities of the lip and nose in children after primary unilateral cheiloplasty

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Abstract. The aim of the study was a comprehensive investigation of the morphological and functional features of secondary deformities of the lip and nose in children after primary unilateral cheiloplasty and the identification of the key structural manifestations. The study was carried out in the Department of Plastic and Reconstructive Microsurgery of the National Children's Specialised Hospital "Okhmatdyt" in Kyiv from 2023 to 2025, within which 57 children with unilateral clefts were examined, with assessment of the parameters of three anatomical zones (upper lip, nose and oral vestibule) in the long-term period after primary cheiloplasty performed in other medical institutions. As a result of the study, it was established that secondary postoperative changes in the nasolabial region were observed in all children (100%), among whom aesthetic deformities were detected in 57.9% and anatomical defects in 42.1%. Moderate forms of severity predominated (47.4%), whereas mild forms accounted for 31.6% and severe forms for 21.0%. The most frequent morphological changes of the upper lip were asymmetry of Cupid's bow (80.7%), cicatricial changes (73.7%) and reduction of philtral height (66.7%), which were combined with impaired mobility in 50.9% of children. In the structure of the nose, asymmetry of the alae (77.2%), deformities of the columella (68.4%) and hypoplasia of the alar cartilage (63.1%) predominated. In the oral vestibule, common findings were reduction of depth (61.4%), scarring of the mucosa (70.2%) and oronasal communications (29.8%). A correlation was found between the severity of cicatricial changes and the reduction in vestibular depth ($r = 0.62$; $p = 0.008$), indicating the systemic nature of the secondary disturbances. The results obtained confirmed that secondary deformities of the nasolabial region after primary cheiloplasty are systemic in nature, with a predominance of aesthetic disturbances of moderate severity. The data can be used by surgeons, orthodontists, and speech therapists for planning reconstructive and rehabilitation interventions in children

Keywords: cleft; cartilage aplasia; palate; oral vestibule; reconstructive interventions; primary operation; asymmetry

Introduction

Congenital clefts of the lip and palate are among the most common developmental defects of the maxillofacial region in children, causing not only cosmetic but also functional disorders. Primary cheilorhinoplasty is the standard treatment for such patients and is aimed at restoring the anatomical integrity of the lip and nose, improving breathing, speech and facial aesthetics. However, even with technically correctly performed intervention, the results of the operation do not always remain stable during facial growth. As the child develops, secondary tissue displacements, asymmetry of the muscles and cartilaginous structures occur, which leads to disturbance of the shape of the nose and of the height and contours of the upper lip. The lack of timely diagnosis and correction of these changes results in a number of problems – persistent deformity of the nasolabial region, difficulty in nasal breathing, articulation disorders, formation of malocclusion and pronounced psychological discomfort. Such consequences significantly reduce the quality of life of patients and require repeat reconstructive operations, which are often more complex and less predictable than the primary intervention.

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The morphological prerequisites for the development of secondary deformities after primary unilateral cheiloplasty are determined by a combination of anatomical, functional and cicatricial factors that disrupt the harmonious development of the nasolabial region during facial growth. In the course of analysing the data of M.I. Dmytrenko *et al.* [1], it was established that persistent morphological changes in the musculo-cartilaginous complex of the upper lip were manifested several years after the operation as a result of incomplete restoration of the orbicularis oris muscle. The authors emphasised that these deformities were exacerbated by cicatricial contractures, which limited tissue mobility and reduced the elasticity. According to the observations of V. Filonenko *et al.* [2], secondary changes formed as a result of imbalance between the soft-tissue segments and uncoordinated regeneration after primary suturing. The researchers stressed that uneven distribution of tissue tension caused volume deficiency and depression in the central part of the lip. The results obtained by R.J. Alimdjanovich & T.N.K. Ugli [3] showed that asymmetric forms of deformity with hypoplasia of the lateral nasal wing and residual cicatricial changes predominated, reflecting the combined influence of the operative technique and individual features of healing. In addition, as demonstrated by N. Uchino *et al.* [4], primary postoperative symmetry was gradually disrupted with age due to uneven development of bony and soft tissues, and the severity of secondary deformities had a clear correlation with the age of the patients, which confirms the need for long-term morphological follow-up.

Diagnosis and morphometric characteristics of secondary deformities of the nasolabial region are crucial for determining the degree of disturbance, predicting the results of reconstructive interventions and choosing the optimal treatment strategy. As noted by M.Y. Oliinyk *et al.* [5], orthopaedic monitoring and systematic anthropometric control after primary repair made it possible to identify the initial signs of deformities at early stages of growth. The authors proved that regular registration of anthropometric parameters facilitated timely adjustment of orthopaedic appliances and prevented the formation of pronounced asymmetries. In the study by S.T. Shokirov *et al.* [6], an analysis of facial anthropometric indices in children after the main stages of surgical treatment was carried out, which made it possible to trace the dynamics of changes in the structure of the lip and nose. The researchers established a pattern of asymmetry development that increased with age due to uneven tissue growth. The use of modern technologies in the study by M.C. Neves [7] enabled quantitative assessment of lip and nasal parameters using stereophotogrammetry. The scientist demonstrated that this method provided high accuracy in determining deformities and allowed objective criteria for the classification to be formulated. In turn, S. Schmutz *et al.* [8] used 3D analysis for a comprehensive assessment of the shape of the nasolabial complex, which made it possible to compare individual variations of deformities with an average three-dimensional template. It was shown that this technique provides a standardised assessment of the results of reconstructive interventions and can be used to plan secondary corrections.

Surgical and multidisciplinary correction of secondary deformities of the lip and nose is aimed at the simultaneous restoration of anatomical integrity, functional capacity and aesthetic harmony of the nasolabial complex, which requires a combination of microsurgical, orthodontic, speech-therapy and psychosocial approaches. According to the results of the study by Y. Kovach *et al.* [9], experience was presented in the use of microvascular free tissue flaps in children with secondary defects after clefts, which made it possible to restore the volume and projection of the midface. The researchers demonstrated that this strategy provided not only closure of complex defects but also improvement of soft palate function and conditions for further rehabilitation. In the study by Y. Chen *et al.* [10], a new algorithm for revision correction of unilateral secondary deformities of the lip and nose was developed, combining reconstruction of soft tissues and structural rhinoplasty. The authors proved that comprehensive restoration of the supporting elements of the nose and redistribution of tissue tension in the upper lip ensured improved profile symmetry and nasal breathing and reduced the risk of further recurrence of deformities.

Previous studies mainly focused on the description of individual clinical cases and technical aspects of surgical correction; however, the structural mechanisms of formation of secondary deformities and the morphometric characteristics in children after primary unilateral cheiloplasty remained insufficiently studied. The aim of this work was to carry out a comprehensive morphometric and clinical analysis of secondary morphological changes in the nasolabial region in children after primary unilateral cheiloplasty in order to determine the frequency, structure and functional significance. To achieve this aim, it was planned to perform the following tasks: to determine the frequency, type, and degree of severity of secondary postoperative deformities; to establish the relationships between morphological changes of the lip, nose and oral vestibule and functional disorders.

Materials and Methods

The study had a retrospective-prospective clinical design and was carried out at the Department of Plastic and Reconstructive Microsurgery and the Consultative and Diagnostic Outpatient Clinic of the State Non-Commercial Enterprise "National Children's Specialised Hospital "Okhmatdyt" of the Ministry of Health of Ukraine" (Kyiv) [11]. In the period from from 2023 to 2025, children with secondary deformities of the upper lip and nose after surgical treatment of congenital clefts presented to the institution. This time interval was chosen because it provided a sufficient number of observations for a representative analysis and covered clinical cases that were homogeneous in terms of treatment techniques. During this period, a clinical analysis of the cases was carried out in

order to assess the morphological features and severity of postoperative changes. The patients underwent planned examination and treatment at the Department of Dentistry within the state programme of medical care for children with congenital facial clefts, which ensured a comprehensive multidisciplinary approach to rehabilitation. The study complied with the ethical principles laid down in the WMA Declaration of Helsinki [12]. Before the start of the study, written informed consent was obtained from the parents or legal representatives of all participants. Personal data were fully anonymised, and the work had an observational character and did not involve any additional medical intervention. The study protocol was approved by the Ethics Committee of the clinic, as confirmed by decision No. EK-24/117 of 31.08.2023. Generative artificial intelligence tools were not used at any stage of this study, including study design, data collection, data analysis, interpretation of results, or manuscript preparation.

In the study, 57 children with a diagnosis of unilateral complete cleft of the upper lip, alveolar process, and hard and soft palate were examined. Among these children were 23 boys and 34 girls aged from 4 to 6 years, which corresponded to the pre-school period, when the formation of functional and psycho-emotional reactions related to the aesthetic appearance of the face intensified. All patients had previously undergone primary surgical treatment: lip repair was performed at the age of 4 months to 1 year, and hard and soft palate repair at the age of 1-1.5 years. The mean postoperative period at the time of examination was 3.5 years after the primary cheiloplasty. From the medical records it was established that the primary operations had been performed using the classical Millard and Tennison-Randall techniques. In 9 children, both techniques were applied sequentially at different stages of treatment. These cases were included in the overall descriptive analysis of secondary deformities but were excluded from direct intergroup comparisons between single-technique cohorts. The Millard technique involved rotation of the medial segment of the lip with simultaneous advancement of the lateral flap to form a natural Cupid's bow. The Tennison-Randall technique was based on excision of a triangular flap, which provided precise approximation of the cleft margins and preservation of symmetry of the upper lip. The sample included children with complete unilateral clefts of the upper lip and palate, with existing morphological manifestations of secondary deformities of the lip or nose after primary cheiloplasty, in a stable somatic condition, and with complete clinical and photographic documentation required for morphometric analysis. Children with bilateral, atypical or incomplete cleft forms; with congenital syndromes accompanied by craniofacial anomalies; with severe somatic or neurological disorders that made correct examination impossible; with incomplete documentation; as well as those who had undergone non-standardised or out-of-protocol reconstructive procedures that could distort the morphological assessment of secondary deformities were not included.

Assessment of the nasolabial complex was carried out taking into account three anatomical components: nasal structures, the upper lip and the oral vestibule. The examination was performed by a maxillofacial surgeon together with an orthodontist under standard clinic conditions in daylight. Each patient was examined in a sitting position, with the head fixed in order to ensure symmetry of photographic documentation. Photography was performed according to a standardised protocol: with a digital camera at a focal length of 50 mm and a distance of approximately 1.0-1.2 m from the patient's face, under frontal lighting without shadows. Images were obtained in three main projections – frontal, right, and left lateral – as well as in an additional three-quarter projection to assess profile asymmetries. To assess the internal nasal structures and identify partial aplasia of the lateral crus, flattening of the alar cartilage, deformities of the nasal floor and residual oronasal communications, anterior rhinoscopy was additionally performed using a Killian nasal speculum and a cold frontal reflector. Clinical assessment was carried out according to morphometric criteria (Table 1).

Table 1. Criteria for clinical assessment of secondary nasolabial deformities

Assessment group	Assessment parameter	Description of the criterion	Score (0-3)	Deformity severity stratification
Upper lip	Symmetry of Cupid's bow	Preservation or disturbance of symmetry between the unaffected and operated sides	0 – symmetrical 1 – slight asymmetry 2 – moderate asymmetry 3 – pronounced disturbance	0 – normal 1-2 – mild/moderate 3 – severe
	Philtral columns	Equality of the height and direction of the philtral columns	0 – normal 1 – slight difference 2 – moderate 3 – significant asymmetry	0 – normal 1-2 – moderate 3 – severe
	Volume of the vermilion border	Hypo- or hypertrophy of the vermilion border	0 – symmetrical 1 – moderate difference 2 – pronounced 3 – deformity >50%	0 – normal 1-2 – mild/moderate 3 – severe
	Lip mobility	Amplitude of movements, participation in mouth closure	0 – full 1 – slight limitation 2 – moderate 3 – absence of mobility	0 – normal 1-2 – functional impairment 3 – severe

Continued Table 1

Assessment group	Assessment parameter	Description of the criterion	Score (0-3)	Deformity severity stratification
Upper lip	Cicatricial changes	Presence of retractions, contractures, excessive scars	0 – absent 1 – moderate 2 – pronounced 3 – marked with contracture	0 – normal 1-2 – moderate 3 – severe
Nose	Symmetry of the nasal wings	Equality of height, shape, and volume of the wings	0 – symmetrical 1 – moderate difference 2 – asymmetry up to 3 mm 3 – marked deformity	0 – normal 1-2 – moderate 3 – severe
	Position of the columella	Position, height, deviation from the midline	0 – centred 1 – deviation up to 1 mm 2 – 1-3 mm 3 – >3 mm	0 – normal 1-2 – mild/moderate 3 – severe
	Alar cartilage	Deformation or aplasia of the cartilaginous structure	0 – preserved 1 – slight deformity 2 – partial aplasia 3 – marked distortion	0 – normal 1-2 – structural disorders 3 – severe
	Nasal floor	Widening or narrowing, retraction of the nasal floor	0 – normal 1 – slight deviation 2 – pronounced 3 – communication with the oral cavity	0 – normal 1-2 – moderate 3 – severe
Vestibule of the oral cavity	Depth of the vestibule	Reduction or absence of the anterior vestibule	0 – normal 1 – reduction up to 2 mm 2 – 2-4 mm 3 – absence	0 – normal 1-2 – moderate 3 – severe
	Presence of oronasal connections	Fistulas between the nose and mouth	0 – absent 1 – microscopic 2 – single 3 – multiple	0 – normal 1-2 – moderate 3 – severe
	Condition of the mucous membrane	Scarring, hypertrophy, atrophy	0 – normal mucosa 1 – slight changes 2 – atrophy/hypertrophy 3 – coarse scarring	0 – normal 1-2 – moderate 3 – severe

Source: compiled by the author

The total scores were used to determine the severity of secondary deformities within the three anatomical zones – the upper lip, nose and oral vestibule. The maximum value of the integral index was 36 points. Severity stratification of deformities was carried out according to the accepted three-level scale: 0-12 points – mild deformities, characterised by minimal aesthetic or functional changes; 13-24 points – moderate deformities, which combined aesthetic disturbances with partial structural changes; 25-36 points – severe deformities, accompanied by pronounced asymmetry, structural defects or functional limitations of the nasolabial complex.

For systematisation of the clinical manifestations, the secondary deformities were divided into two main groups. Aesthetic deformities were characterised by disturbance of anthropometric proportions without loss of anatomical structures. These included reduction in the height of the philtral column on the cleft side, atrophic or hypertrophic tissue changes, retraction or excessive stretching of the skin, which led to distortion of the lip contours, while the muscle layer and mucosa remained restored. Anatomical defects, in contrast, included disruption of the integrity or absence of individual elements of the nasolabial region – the upper oral vestibule, fixation of the orbicularis oris muscle, as well as the formation of oronasal fistulae.

Statistical analysis was performed using SPSS Statistics version 26.0 (IBM Corp., USA). Descriptive statistics included absolute and relative frequencies (n, %) for categorical variables and mean values with standard deviations (mean ± SD) for quantitative variables. The normality of continuous data distributions was assessed using the Shapiro-Wilk test. As severity scores of secondary deformities did not follow a normal distribution ($p < 0.05$), non-parametric statistical methods were applied (Spearman's rank correlation coefficient for assessment of associations between severity-related variables). Associations between categorical variables, including type of deformity, severity category, sex, cleft laterality, and primary surgical technique, were analysed using Fisher's exact test. Statistical significance was defined as $p < 0.05$.

Results

Frequency and distribution of secondary deformities. In the course of the analysis, it was established that secondary postoperative changes in the nasolabial region were observed in 100% of cases (n = 57), although the severity

and character varied considerably. According to the results of a comprehensive assessment of secondary deformities based on morphological criteria, it was found that aesthetic disturbances (asymmetry, cicatricial changes, distortion of the lip contour or nasal shape) were detected in 33 children (57.9%), whereas anatomical defects (absence or deformation of structures, oronasal communications, cartilage aplasia, vestibular defects) were present in 24 children (42.1%). These results indicated a predominance of aesthetic changes, which mostly formed due to slight displacement of tissues and scarring after the primary surgical intervention. To assess the structure of secondary changes and the relationship with biological factors (sex, side of lesion), stratification of cases by type of deformity and severity was carried out. The obtained data were summarised in Table 2.

Table 2. Distribution of secondary deformities by type, sex, and side of lesion

Indicator	Total number (n=57)	Boys (n = 23)	Girls (n = 34)	Right-sided cleft (n = 31)	Left-sided cleft (n = 26)
Aesthetic deformities	33(57.9%)	11(47.8%)	22(64.7%)	18(58.1%)	15(57.7%)
Anatomical defects	24(42.1%)	12(52.2%)	12(35.3%)	13(41.9%)	11(42.3%)
Mild form (0-12 points)	18(31.6%)	8(34.8%)	10(29.4%)	9(29.0%)	9(34.6%)
Moderate form (13-24 points)	27(47.4%)	10(43.5%)	17(50.0%)	15(48.4%)	12(46.2%)
Severe form (25-36 points)	12(21.0%)	5(21.7%)	7(20.6%)	7(22.6%)	5(19.2%)

Note: comparisons between groups according to sex and cleft laterality were performed using Fisher's exact test. No statistically significant differences were found in the distribution of deformity types or severity categories between groups ($p > 0.05$)
Source: compiled by the author

The quantitative indicators obtained showed that aesthetic changes accounted for a greater proportion of secondary deformities compared with anatomical defects. However, comparative analysis did not reveal statistically significant differences in the distribution of deformity types or severity categories according to sex or cleft laterality ($p > 0.05$). The proportion of aesthetic deformities (57.9%) indicates that in most cases surgical reconstruction ensured anatomical integrity of the tissues but did not allow achievement of complete morphological symmetry. This situation is typical for the postoperative period in preschool children, when asymmetric growth of soft tissues and elements of cicatricial deformation begin to appear. In the group of anatomical defects (42.1%), combined disturbances were descriptively more frequent, including defects of the oral vestibule, partial absence of the nasal floor, or residual fistulae. These findings characterised the clinical spectrum of secondary postoperative deformities observed in the study population and emphasised the heterogeneity of their morphological presentation. Clinically, such children often required additional functional rehabilitation, including speech therapy and orthodontic treatment. Comparison by sex revealed numerical tendencies: anatomical defects were numerically more frequent in boys (52.2%), whereas aesthetic deformities were more commonly observed in girls (64.7%); these differences remained within the limits of random variation. Analysis by side of lesion demonstrated a comparable distribution of secondary deformities between right-sided (58.1%) and left-sided (57.7%) clefts, indicating no apparent influence of laterality on the frequency of postoperative changes.

In terms of severity, deformities of moderate degree accounted for the largest proportion of cases (47.4%), while mild forms represented 31.6% and severe forms 21.0%. This distribution reflects a descriptive pattern rather than a statistically tested difference between severity categories. In most cases, the primary operation ensured satisfactory anatomical restoration but did not guarantee complete symmetry or functional coherence of the nasolabial complex. The predominance of a moderate level of severity is clinically favourable, as such changes are generally amenable to correction through secondary reconstructive interventions or orthodontic treatment. Among children with severe deformities (n = 12), combined defects of the lip and nose with total scores above 25 were more frequently observed descriptively, often accompanied by pronounced cicatricial contractures and retractions. These observations characterised the clinical features of the severe subgroup and were not subjected to separate statistical comparison. Such cases represented a risk group for the formation of persistent cosmetic defects affecting appearance, articulation and nasal breathing. Thus, a qualitative analysis of the obtained data confirmed that the structure of secondary postoperative deformities was of a mixed nature, with aesthetic forms constituting a larger proportion and a tendency towards a moderate level of severity. These findings described overall patterns within the study sample rather than statistically significant group differences. This indicated that primary cheiloplasty generally provided anatomical restoration of the lip and nose but required further rehabilitation and, in some cases, revision surgery to eliminate residual asymmetry and cicatricial changes. To clarify the distribution of deformity severity within individual anatomical zones, a comparative analysis of average severity scores was performed, the results of which are shown in Figure 1.

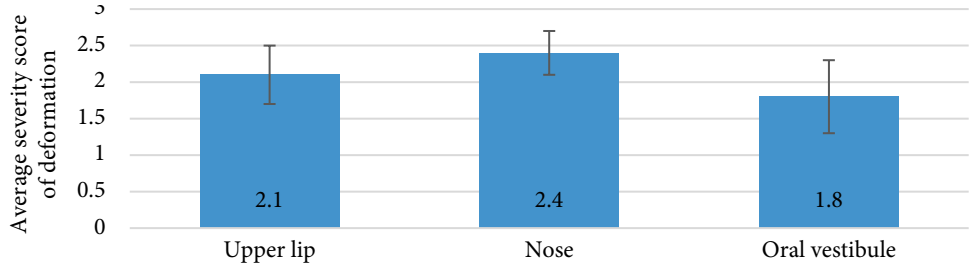

Figure 1. Mean severity scores of secondary deformities by anatomical region
Source: compiled by the author

Analysis of the total scores for the three anatomical regions (upper lip, nose, oral vestibule) showed that the highest mean severity score was observed in the nasal structures (2.4 ± 0.3), followed by the upper lip (2.1 ± 0.4) and the oral vestibule (1.8 ± 0.5). These values indicate a numerical gradient of severity across anatomical regions; however, no formal statistical comparison between regions was performed. This distribution can be explained by the complexity of three-dimensional nasal reconstruction during primary cheilorhinoplasty and the limited predictability of growth-related changes in cartilaginous structures. In most children with severe deformities, combined changes of the lip and nose were observed, and in cases of pronounced scarring these were accompanied by functional disorders such as incomplete lip closure, impaired nasal breathing and reduced mimetic activity.

Thus, the results of the comprehensive analysis showed that secondary deformities after primary unilateral cheilorhinoplasty were predominantly of moderate severity, with aesthetic changes prevailing over anatomical ones. This reflected the effectiveness of the primary restoration of tissue integrity but incomplete compensation of nasolabial symmetry. The most vulnerable areas remained the alar cartilages of the nose and the central part of the upper lip, where residual asymmetries and scar retractions formed, which could subsequently cause functional disorders. The results obtained underlined the need for early morphometric monitoring and planning of staged reconstructive interventions to ensure a stable functional and aesthetic outcome.

Morphological characteristics of secondary changes in the upper lip. The analysis showed that, after primary unilateral cheilorhinoplasty, most children retained secondary morphological changes of varying severity, caused both by technical features of the primary intervention and by asymmetric tissue growth during development. The assessment was performed according to five key morphological criteria: symmetry of the Cupid's bow, philtral columns, volume of the vermilion border, lip mobility and scar changes. The summarised results were presented in Table 3.

Table 3. Morphological changes of the upper lip after cheilorhinoplasty

Assessment parameter	Detection frequency, n (%)	Mean score ± SD	Nature of the predominant changes
Symmetry of the Cupid's bow	46(80.7%)	2.3 ± 0.4	Moderate arch asymmetry on the cleft side.
Philtral columns	38(66.7%)	2.1 ± 0.3	Reduced height or displacement of the column on the operated side.
Vermilion border volume	31(54.4%)	1.9 ± 0.4	Hypo- or hypertrophy, more often flattening.
Lip mobility	29(50.9%)	2.0 ± 0.5	Partial restriction on mouth closure.
Scar changes	42(73.7%)	2.2 ± 0.5	Retraction or hypertrophy in the postoperative scar zone.

Note: SD – standard deviation, characterising the variation in severity scores of morphological changes within the sample
Source: compiled by the author

Analysis of the results showed that in most cases the morphological changes of the upper lip were of moderate severity, combining aesthetic disproportions with minimal functional limitations. The most frequent manifestation was asymmetry of the Cupid's bow – in 80.7% of children. It appeared as a shift of the mid-point of the bow towards the former cleft, creating a visual shortening of the lip and disruption of its contour line. This defect was interpreted as a typical postoperative feature associated with asymmetric tissue tension and slight displacement of the cutaneous-muscular segments during primary repair. Disturbances in philtral column height were observed in 66.7% of cases and were characterised by a decrease in height or a change in the direction of the column on the operated side. This type of deformity affected the visual proportionality of the mid-face, and its formation was descriptively associated with the technique of triangular flap formation according to Tennison-Randall, which is characterised by increased local tissue tension. Scar changes represented the second most frequent group (73.7%) and often combined with impaired lip mobility. The mean score of 2.2 ± 0.5 indicated that most scars were moderately expressed, with retraction of the cutaneous-mucosal junction or formation of linear contractures. Such changes not only reduced aesthetic

attractiveness, but in some patients (about 15%) also caused functional complications – incomplete mouth closure and difficulties during articulation.

Changes in the volume of the vermilion border were observed in more than half of the children (54.4%) and were predominantly mild. Hypoplasia of the lip on the cleft side was more common than hypertrophy, which was explained by uneven filling of the musculo-cutaneous layer and residual scar retraction. Despite moderate manifestations, this defect created an impression of asymmetry of the lower third of the face, especially when smiling. Decreased lip mobility was observed in 50.9% of patients. The mean score of 2.0 ± 0.5 confirmed that, in most cases, this was partial limitation associated with scar changes in the region of the orbicularis oris muscle. Such disorders had not only an aesthetic but also a functional character, as the disorders affected articulation, swallowing and facial expression.

Comparison by type of primary operation revealed descriptive differences in the morphological patterns of secondary upper-lip changes. Residual asymmetries of the Cupid's bow were numerically more frequent in children treated using the Millard technique (59.1%), whereas hypertrophic scar changes were more commonly observed after the Tennison-Randall procedure (55.2%). These differences reflect clinical distribution patterns and were not subjected to formal statistical testing; therefore, they should not be interpreted as statistically significant. The overall cumulative index of morphological changes of the upper lip was 10.5 ± 1.8 points, corresponding to a moderate severity level. Most children had a combination of two or three types of defect, mainly asymmetry of the Cupid's bow, scar changes and reduced philtral height. These parameters had the greatest impact on the aesthetic perception of the face.

Thus, the study results indicated that, after primary cheilorhinoplasty in children, moderate aesthetic deformities predominated, caused by morphological disproportions rather than structural defects. The most vulnerable areas remained the central part of the upper lip and the Cupid's bow region, where residual asymmetries and scar retractions formed, which could subsequently cause functional limitations. This underlined the need for early morphometric monitoring, the use of gentle techniques for revision cheiloplasty and an interdisciplinary approach to restoring symmetry and mobility of the upper lip.

Characteristics of secondary nasal deformities. After primary cheilorhinoplasty, the nasal region is the most vulnerable part of the nasolabial complex, as it contains cartilaginous, muscular and cutaneous structures that change actively during a child's growth. Even with technically correct primary surgery, maintaining complete nasal symmetry is a challenging task, which explains the high frequency of secondary defects. To quantify the frequency and severity of secondary morphological changes in the nasal structures after primary cheilorhinoplasty, a detailed assessment was carried out according to four key parameters – symmetry of the nasal alae, position of the columella, condition of the alar cartilage and shape of the nasal floor. The summarised results were presented in Table 4.

Table 4. Morphological characteristics of secondary nasal changes in children after cheilorhinoplasty

Assessment parameter	Detection frequency, n (%)	Mean score ± SD	Nature of the predominant changes
Symmetry of the nasal alae	44(77.2%)	2.4 ± 0.3	Moderate or pronounced asymmetry of the alae, drooping of the operated side.
Position of the columella	39(68.4%)	2.2 ± 0.4	Deviation from the midline, shortening, or rotation.
Alar cartilage	36(63.1%)	2.5 ± 0.3	Partial aplasia or deformity of the lateral crus.
Nasal floor	28(49.1%)	1.9 ± 0.5	Narrowing or retraction of the nasal floor, sometimes fistulas.

Source: compiled by the author

The results obtained showed that 77.2% of children after primary cheilorhinoplasty retained nasal deformities of varying severity, most often asymmetry of the alae. In most cases, this manifested as drooping of the ala on the cleft side, creating a visual widening of the nostril. This deformity was mainly aesthetic, but in some patients, it led to partial narrowing of the nasal passage. The mean score of 2.4 ± 0.3 indicated a predominance of moderate severity, which is usually amenable to correction during secondary reconstruction. Deformities of the columella were recorded in 68.4% of cases. Deviation from the midline and shortening of the structure produced a visual effect of nasal asymmetry, particularly in frontal projection. These features were interpreted as postoperative morphological changes associated with uneven tissue adaptation and healing processes rather than as isolated structural defects. More pronounced columellar deviation was typically accompanied by deformation of the nasal ala and rotation of the nasal tip, which contributed to an increase in profile asymmetry.

Particular attention should be paid to the condition of the alar cartilages, which were altered in 63.1% of children, with a mean score of 2.5 ± 0.3 – the highest among all parameters. In most cases, partial aplasia or flattening of the lateral crus was observed, leading to nostril asymmetry. This observation reflects the relative vulnerability of the cartilaginous framework during early reconstructive stages. Deformities of the alar cartilage were often accompanied by inadequate projection of the nasal tip, which complicated breathing by reducing the lumen of the nasal passages. The nasal floor was altered in 49.1% of patients, mainly in the form of retraction of the nasal base or narrowing. Such changes limited aeration of the nasal

cavity and were accompanied by recurrent episodes of difficult breathing. In some children, residual fistulas were observed, which formed as a result of incomplete closure of the mucosal-muscular layers during the primary intervention. Comparative analysis showed that in most patients the deformities had a combined character: in 41.7% of cases a combination of alar asymmetry and columellar deviation was identified, while in 22.8% complex defects included disturbances in all four parameters. This tendency pointed to the morphological interdependence of the nasal structures, where even slight deviation of one element led to cascade changes in adjacent zones.

A statistically significant correlation was found between the severity of upper-lip scar changes and nasal deformities ($r = 0.68$; $p = 0.003$), indicating a shared morphogenetic mechanism related to scar tissue contraction. This process influenced the position of the columella and the configuration of the nasal alae, suggesting that the condition of lip scars may serve as a clinical indicator of secondary nasal changes. When nasal deformities were analysed according to the type of primary operation, children who underwent Millard repair numerically lower mean nasal deformity scores (2.1 ± 0.4) compared with those treated using the Tennison-Randall technique (2.4 ± 0.3). This comparison was descriptive and was not subjected to formal statistical testing, reflecting differences related to technical characteristics of the procedures, including greater mobilisation of the nasal alae in the Millard technique and more limited correction of nasal structures in the Tennison-Randall approach.

Thus, the study results showed that the nasal region remained the most problematic area after primary cheilorhinoplasty. The most frequent findings were alar asymmetry, columellar deformities and hypoplasia of the alar cartilage, which caused both aesthetic and functional disturbances. The combination of nasal defects with lip scarring indicated a single morphogenetic mechanism that required a comprehensive treatment approach. To improve reconstructive outcomes, it is necessary to take into account the biomechanics of cartilage growth and ensure symmetrical formation of the nasal supporting structures. The optimal option is to use combined techniques that integrate the principles of Millard repair with local columellar correction and reinforcement of the alar cartilage using autografts. Such an approach will minimise the risk of secondary deformities and improve both aesthetic and functional outcomes in the long term.

Disturbances of the anatomy of the oral vestibule and the clinical significance. The results of the analysis of morphological changes in the oral vestibule showed that this area often remained a source of residual anatomical and functional disorders after primary cheilorhinoplasty. To assess the degree of such changes, a detailed study of three parameters was carried out – vestibular depth, presence of oronasal communications and condition of the mucosa, the results of which were presented in Table 5.

Table 5. Morphological changes of the oral vestibule in children after primary cheilorhinoplasty

Assessment parameter	Detection frequency, n (%)	Mean score ± SD	Nature of the predominant changes
Vestibular depth	35(61.4%)	2.1 ± 0.4	Reduced depth of the anterior vestibule, restriction of lip mobility.
Oronasal communications	17(29.8%)	2.3 ± 0.5	Presence of residual fistulas in the anterior part of the alveolar process.
Condition of the mucosa	40(70.2%)	2.0 ± 0.4	Scarring, atrophic areas, hypertrophy in the postoperative suture zone.

Source: compiled by the author

The results obtained showed that the most frequent disturbance was insufficient depth of the oral vestibule – in 61.4% of cases. This defect was found both on the cleft side and partly on the opposite side due to asymmetry of attachment of the orbicularis oris muscle. Reduced depth led to restriction of upper-lip mobility, especially when smiling or articulating sounds requiring lip elevation. Children with this type of defect often complained of muscle fatigue when speaking and mild hypersalivation. In some cases, retraction of the mucosa in the postoperative scar area was observed, which indicated insufficient tissue mobilisation during the primary operation or excessive tension during wound closure. Oronasal communications were identified in almost one-third of patients (29.8%). These communications were most often localised in the anterior part of the hard palate and alveolar process and clinically reflected incomplete fusion of the mucosal-muscular layer. The presence of such communications had important functional significance – it disrupted the separation between the oral and nasal cavities, complicating sound formation and normal nasal breathing. During clinical examination, these children often showed air leakage through the nose when articulating or swallowing, indicating incomplete sealing of the oral cavity. In addition, these defects may be a chronic source of infection, promoting the development of rhinitis or inflammatory processes of the mucosa.

With regard to the condition of the mucosa, pathological changes were recorded in 70.2% of patients. The most common were scar changes (hypertrophic or retracted scars), which impaired mucosal elasticity, limited tissue mobility and led to formation of local depressions. In some cases, atrophic areas of mucosa in the postoperative scar zone were observed, formed due to disturbed microcirculation in the postoperative period. Such changes complicated repeated reconstructive interventions, as the changes reduced the possibility of adequate tissue mobilisation

without the risk of new scar formation. Comprehensive analysis showed that vestibular depth and mucosal status were functionally linked: correlation analysis revealed a moderate direct relationship between reduced vestibular depth and the severity score for mucosal changes ($r = 0.62$; $p = 0.008$). In children with pronounced reduction in vestibular depth (scores 2-3), scar-related mucosal contractures were more common, supporting the role of musculo-fascial tension in the development of secondary deformities. In such cases, the upper lip demonstrated a reduced range of motion, and tension during smiling promoted scar thinning and retraction of the vermilion border, contributing to secondary aesthetic asymmetry.

A descriptive co-occurrence was noted between oral-vestibular defects, particularly residual oronasal communications, and nasal asymmetry (columellar deviation and alar asymmetry), indicating morphological interdependence of nasolabial structures. From a clinical perspective, anatomical defects of the oral vestibule had pronounced functional significance. Insufficient vestibular depth or scar shortening of the anterior vestibule resulted in restricted upper-lip mobility and impaired lip closure, creating prerequisites for articulation disorders, particularly during the production of labial consonants (p, b, m), as well as for disturbed nasal breathing. In cases with more severe vestibular involvement (vestibular depth scores ≥3), incomplete lip closure at rest was observed, accompanied by compensatory chin elevation and increased activity of the lower-lip muscles. Prolonged muscular imbalance of this type contributed to secondary deformities of the lower facial region and disruption of profile harmony.

The results obtained showed that even minor morphological defects of the oral vestibule could have a substantial functional impact and therefore required early correction. Restoration of normal vestibular depth and mucosal elasticity should be regarded as a key stage in the comprehensive rehabilitation of children after cheilorhinoplasty. The optimal direction of correction is considered to be a combination of soft-tissue plasties with subsequent speech therapy and orthodontic support, which allows not only elimination of local anatomical defects but also restoration of the functional balance of the nasolabial complex. In summary, disturbances of the anatomy of the oral vestibule have a systemic character and are closely related to the severity of secondary changes in adjacent structures – the lip and nose. Timely diagnosis makes it possible not only to assess the extent of postoperative complications, but also to anticipate the potential need for revision interventions and prolonged rehabilitation.

Discussion

The study results showed that secondary postoperative deformities were observed in all children after primary unilateral cheilorhinoplasty, were predominantly of moderate severity and had a combined character. The most frequent changes were asymmetry of the nasal alae, displacement of the Cupid's bow, reduction in philtrum height and scar retractions in the central zone of the lip. The highest severity scores were recorded in the nasal structures, especially in the region of the alar cartilages. Disturbances of the anatomy of the oral vestibule were combined with restricted lip mobility and functional disorders of articulation and nasal breathing.

Secondary changes in the nasolabial region were identified in all children; in 57.9% aesthetic deformities prevailed, and in 42.1% anatomical defects with combined involvement of the lip, nose, and vestibule were present. In terms of severity, moderate forms dominated (47.4%), whereas severe deformities were recorded in 21.0% of cases, which indicated preservation of structural integrity with incomplete compensation of symmetry. A similar pattern was demonstrated in the study by C.A. Yao & J.B. Mulliken [13], where it was established that most patients required revision procedures during the first twenty years after primary correction. The authors linked this trend to the progressive influence of facial skeletal growth on reconstructed tissues, which explained the occurrence of residual deformities even after a technically sound operation. According to the classification presented by U.S. Hamdan *et al.* [14], secondary defects were regarded as a predictable stage of morphological adaptation after cheilorhinoplasty. In that work, it was emphasised that even with complete anatomical reconstruction, functional and aesthetic symmetry could be disrupted over time due to natural growth processes.

The dependence of the frequency of postoperative deformities on variants of the surgical protocol was shown in the work of P. Corthouts *et al.* [15], where the influence of different techniques on the growth of the midface was analysed. It was proved that the position of the flaps and the degree of tissue tension were key factors in the formation of residual asymmetries, which was consistent with the patterns identified. In the study by I. Roohani *et al.* [16] it was noted that early cheiloplasty improved the overall perception of appearance but was accompanied by an increase in the incidence of minor aesthetic defects. This effect was explained by the peculiarities of tissue healing in infants and incomplete prediction of growth processes, which corresponded to the obtained indicators of moderate forms of deformity. The importance of even slight morphological deviations was confirmed in the work of G. Bohneberger & N.M. Ernica [17], where the significant impact on the quality of life of children was established. The authors stressed that asymmetry in the lip and nose region affected not only appearance but also patients' social confidence. According to the meta-analysis by E.K. Branson *et al.* [18], the presence of facial asymmetry after cheilorhinoplasty was associated with an increased risk of psychological and social difficulties in childhood. The study showed that even moderate residual defects generated long-term emotional discomfort, which confirmed the clinical significance of the identified changes as a complex medico-psychosocial problem.

In the sample, the most frequent disorders of the upper lip were asymmetry of the Cupid's bow (80.7%), scar changes (73.7%) and reduction in philtrum height (66.7%), predominantly of moderate severity. The cumulative index of

morphological changes corresponded to a moderate severity level; most children had a combination of two or three defects. Persistence of asymmetry after reconstruction was confirmed in the study by Z.A. Khan *et al.* [19], where, even with the modified Delaire technique, significant differences between the operated and intact sides remained. It was established that the asymmetry was caused not only by surgical factors but also by asynchronous growth of the soft tissues in the philtral region. Difficulties in restoring stable upper-lip projection were described by M. Schwaiger *et al.* [20]. The researchers noted that complete symmetry after revision procedures was an exceptional phenomenon. The presence of residual asymmetries was regarded as an inevitable result of postoperative tissue remodelling. The influence of cheiloplasty technique on the formation of residual defects was shown by K.S. Abdullateef *et al.* [21], where the Millard method was more often accompanied by asymmetries, whereas the Tennison-Randall technique produced pronounced scar changes. Differences in flap tension and incision direction determined the morphological structure of the postoperative scar. A synthesis of techniques carried out by M. Zaidov [22] confirmed that excessive tissue tension in the suture zone increased the risk of forming retracted or hypertrophic scars. This pattern reflected the pathogenetic basis of the high frequency of scar contractures in cohorts after primary repair. The importance of the soft-tissue framework for lip stability and mobility was highlighted by A.I. Shaikh *et al.* [23], where it was noted that even anatomically correct reconstruction without an adequate muscular layer led to loss of functional range of motion. It was determined that the quality of restoration of the orbicularis oris muscle was the key factor in preventing postoperative deformities.

Within the structure of secondary changes, the nasal region proved to be the most problematic: asymmetry of the nasal alae was observed in 77.2% of children, deformities of the columella in 68.4%, and disturbances of the alar cartilages in 63.1%, with the cartilaginous framework showing the highest severity scores. The combination of these defects formed a typical picture of a "low" asymmetrical nose with functional limitation of nasal breathing. The need for structural reinforcement of the supporting framework in secondary reconstruction was substantiated by H. Yue *et al.* [24], who proved that the use of cartilage grafts ensured stability and long-lasting correction outcomes. It was demonstrated that deficiency of supporting elements was the main cause of recurrent deformities after primary cheilorhinoplasty. The effectiveness of open rhinoplasty with autologous cartilage was confirmed by B.C. Cho *et al.* [25], who showed the possibility of simultaneous restoration of nasal form and function. In the authors' study, it was emphasised that reconstruction of the cartilaginous structure directly influenced the patency of the nasal passages. The advisability of using septal cartilage to restore the columella and nostril symmetry was confirmed by H.L. Nguyen *et al.* [26]. The study demonstrated a reduction in residual columellar deviation and improvement in nasal aeration. The use of costal cartilage to correct asymmetry was described by Z. Zhang *et al.* [27], where increased reconstruction stability and a reduced risk of recurrence of deformity were noted. Both approaches reflected patterns similar to those observed in the present study, particularly the high frequency of hypoplasia of the alar cartilages. The functional effect of secondary rhinoplasty was summarised in the systematic review by J. Yuan & Y. An [28], which showed that elimination of morphological defects was accompanied by a significant improvement in nasal breathing. It was emphasised that reconstruction should be considered not only as an aesthetic, but also as a functional procedure.

In the present study, insufficient depth of the oral vestibule was found in 61.4% of children, pathological mucosal changes in 70.2%, and oronasal communications in 29.8%, which were accompanied by restricted lip mobility and impaired articulation. A moderate direct association between reduction in vestibular depth and severity of scar changes was established ($r = 0.62$; $p = 0.008$), as well as an association of oronasal fistulas with nasal deformities, indicating the systemic nature of involvement of the nasolabial complex. In the scoping review by M.S. Yusof & H.M. Ibrahim [29] it was demonstrated that residual oronasal and oropharyngeal disorders were associated with a marked reduction in quality of life in younger children. It was emphasised that even minimal functional defects of the mucosa or vestibule resulted in difficulties with speech, swallowing and social adaptation. The study by N.H. Nguyen *et al.* [30] showed that successful psychosocial rehabilitation of patients with clefts required a comprehensive multidisciplinary approach including speech therapy, orthodontic and psychological support. It was indicated that precisely such comprehensive rehabilitation minimised the consequences of vestibular scar contractures.

According to the data of S.M. Sarrami *et al.* [31], timely revision surgery for secondary deformities had to be based on an assessment of the functional potential of the mucosal-muscular layer, since its inadequate restoration determined the persistence of fistulas. The authors emphasised the importance of using soft-tissue plasties to prevent recurrent disruption of vestibular sealing. Similarly, V.L. Gille *et al.* [32] drew attention to the role of postoperative tissue stabilisation and the use of retainers in maintaining the restored anatomy after primary and revision procedures, which was consistent with the need to prevent repeated scarring of the mucosa. The dependence of local deformities on age-related anthropometric factors was shown by M.F. Arpacı *et al.* [33], where it was established that asymmetric development of facial structures intensified morphofunctional disorders of the vestibule. The study proved that even minor displacements of tissues in early childhood led to persistent anatomical defects, which fully corresponded to the identified tendency towards the formation of scar contractures in younger children.

Summarising the results, it was established that secondary morphological changes after primary unilateral cheilorhinoplasty involved all components of the nasolabial

complex and had a systemic character. The data obtained were consistent with the results of other studies confirming the interrelation between deformities of the lip, nose and vestibule, as well as the influence of surgical technique on long-term facial symmetry. The consistency of the results indicated common pathogenetic mechanisms and the need for a comprehensive approach to correction and rehabilitation of patients after cheilorhinoplasty.

Conclusions

Secondary postoperative changes in the nasolabial region were identified in 100% of children, which confirmed the regularity after primary unilateral cheilorhinoplasty. Aesthetic deformities accounted for 57.9%, anatomical defects for 42.1%, and the predominance of moderate forms (47.4%) indicated overall effective restoration of anatomical integrity while retaining residual asymmetry. Severe deformities with combined involvement of the lip and nose were recorded in 21.0% of children, which was explained by insufficient tissue mobilisation and disturbed functional integration of the orbicularis oris muscle. The absence of significant differences between right-sided (58.1%) and left-sided (57.7%) clefts indicated that the decisive factor in the formation of secondary defects was not the side of involvement but the quality of the primary reconstruction.

Within the structure of morphological changes of the upper lip, asymmetry of the Cupid's bow (80.7%), scar changes (73.7%) and reduction in philtrum height (66.7%) were most frequently detected, whereas the mean total severity score amounted to 10.5 ± 1.8 points, corresponding to a moderate level. Changes in the volume of the vermilion border (54.4%) and reduced lip mobility (50.9%) indicated a combination of aesthetic and functional components of the deformity. The highest mean severity scores were recorded for scar changes (2.2 ± 0.5) and asymmetry of the Cupid's bow (2.3 ± 0.4), which was explained by tissue tension in the suture zone and uneven reconstruction of the muscular layer. The identified dependence between operative technique and the nature of defects (Millard – asymmetry, Tennison-Randall – hypertrophic scars) highlighted the importance of choosing the surgical approach for the prevention of postoperative contractures.

The most frequent disorders were asymmetry of the nasal alae (77.2%), deviation of the columella (68.4%) and deformity of the alar cartilages (63.1%), which were accompanied by a mean severity score of 2.4 ± 0.3. The high frequency of combined defects (41.7%) indicated morphological interdependence of the nasal structures. The established correlation between the severity of upper-lip scarring and nasal deformities ($r = 0.68$; $p = 0.003$) confirmed a common mechanism of scar-induced tissue contraction. The highest severity scores were recorded for the alar cartilage (2.5 ± 0.3), which explained the instability of the cartilaginous framework under growth conditions. The obtained results indicated the need for structural reinforcement of the supporting nasal elements during primary and revision reconstructions to prevent secondary asymmetries.

Insufficient depth of the oral vestibule was found in 61.4% of children, scar changes of the mucosa in 70.2%, and oronasal communications in 29.8%, which pointed to a frequent combination of local anatomical defects with functional disorders. The moderate direct correlation between vestibular depth and severity of scarring ($r = 0.62$; $p = 0.008$) confirmed the role of musculo-fascial tension in the formation of secondary contractures. In children with pronounced reduction in depth (>2.5 points), difficulties with full lip closure and nasal breathing were observed, which indicated the functional nature of the deformities. It was revealed that vestibular defects had systemic significance, affecting articulation, breathing and aesthetics, and therefore should be a mandatory target of comprehensive rehabilitation after cheilorhinoplasty. The data obtained emphasised the need for a multidisciplinary approach to follow-up of such patients, involving a surgeon, orthodontist, speech therapist and otolaryngologist in order to achieve a complete functional and aesthetic outcome. Recommendations for further research included expanding the age range of the sample and introducing dynamic monitoring of long-term outcomes to clarify the influence of growth and surgical factors on the formation of secondary deformities.

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Conflict of Interest

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Аналіз вторинних деформацій губи та носа у дітей після первинної однобічної хейлоринопластики

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Анотація. Метою дослідження було комплексне вивчення морфологічних і функціональних особливостей вторинних деформацій губи й носа у дітей після первинної однобічної хейлоринопластики та визначення їхніх ключових структурних проявів. Дослідження проведено у відділенні пластично-реконструктивної мікрохірургії Національної дитячої спеціалізованої лікарні «Охматдит» у Києві з 2023 по 2025 рік, у межах якого обстежено 57 дітей з однобічними розщілинами із оцінкою параметрів трьох анатомічних зон (верхньої губи, носа та присінка порожнини рота) у віддаленому періоді після первинної хейлоринопластики, проведеної в інших медичних закладах. У результаті дослідження встановлено, що вторинні післяопераційні зміни назо-лабіальної ділянки спостерігалися у всіх дітей (100 %), серед яких естетичні деформації виявлено у 57,9 %, а анатомічні дефекти – у 42,1 %. Переважали помірні форми тяжкості (47,4 %), тоді як легкі становили 31,6 %, а тяжкі – 21,0 %. Найчастішими морфологічними змінами верхньої губи були асиметрія дуги Купідона (80,7 %), рубцеві зміни (73,7 %) та зниження висоти фільтрума (66,7 %), що поєднувалися з порушенням рухливості у 50,9 % дітей. У структурі носа переважали асиметрія крил (77,2 %), деформації колумели (68,4 %) і гіпоплазія крилоподібного хряща (63,1 %). У присінку порожнини рота частими були зменшення глибини (61,4 %), рубцювання слизової оболонки (70,2 %) та ороназальні сполучення (29,8 %). Виявлено кореляцію між вираженістю рубцевих змін і зменшенням глибини присінка (r=0,62; p=0,008), що вказує на системний характер вторинних порушень. Отримані результати підтвердили, що вторинні деформації назо-лабіальної ділянки після первинної хейлоринопластики мають системний характер із переважанням естетичних порушень помірного ступеня. Дані можуть бути використані хірургами, ортодонтами й логопедами для планування реконструктивних та реабілітаційних втручань у дітей

Ключові слова: розщілина; аплазія хряща; піднебіння; присінок порожнини рота; реконструктивні втручання; первинна операція; асиметрія

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Pharmacoeconomic evaluation of pharmacotherapy of patients with diabetic foot syndrome secondary to type 2 diabetes mellitus

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Abstract. The increasing prevalence of type 2 diabetes mellitus complicated by diabetic foot syndrome and the prescription of high-cost medicines, which are not included in treatment protocols, significantly raise healthcare expenditures and worsen patient outcomes, making the optimisation of pharmacotherapy both a clinical and economic priority. The purpose of this study was to evaluate the rationality of pharmacotherapy for patients with diabetic foot syndrome using an integrated frequency/ABC/VEN analysis. The study applied frequency analysis to assess prescription patterns ATC-classification, ABC-analysis to classify medicines by expenditure levels, and VEN-analysis to determine their therapeutic importance based on national and international treatment guidelines recommendations for type 2 diabetes mellitus. The results demonstrated that etiological and pathogenetic therapies approaches predominated in clinical practice. Antidiabetic agents accounted for the largest share of prescriptions (23%), while antibiotics were essential

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for managing infectious complications. The integrated frequency/ABC/VEN-analyses revealed that "essential" medicines (44.19%) predominated over "vital" (23.26%) and "non-essential" categories (32.56%), indicating the insufficiently rational prescribing practices in accordance with treatment recommendations. However, a high share of healthcare expenditures was concentrated in a group of high-cost medicines (34.89%), suggesting suboptimal allocation of healthcare resources. The results also highlighted the importance of evidence-based antibiotic selection due to the growing risk of antimicrobial resistance. The study provided a foundation for improving the economic efficiency of pharmacotherapy in patients with diabetic foot syndrome by supporting rational prescribing, optimisation of healthcare expenditures, and the promotion of cost-effective Ukrainian medicines alternatives

Keywords: integrated frequency/ABC/VEN analysis; medicinal prescriptions; anti-bacterial agents; antimicrobial resistance; pharmaceutical provision optimisation

Introduction

Type 2 diabetes mellitus (T2DM) is a significant global health challenge due to its increasing prevalence and severe complications, including diabetic foot syndrome (DFS). DFS, characterised by diabetic foot ulcers, infections, and gangrene, substantially exacerbates morbidity, mortality, and healthcare costs in patients with T2DM. Despite advances in pharmacotherapy, the management of DFS remains complex and resource-intensive. Pharmacoeconomic evaluation is essential in optimising pharmacotherapy for patients with DFS secondary to T2DM, given the significant clinical and economic burden of this complication. Careful assessment of treatment costs and outcomes can inform better resource allocation and improve patient outcomes in this complex disease area.

Numerous studies by both foreign and Ukrainian healthcare experts have been devoted to the issue of in-depth investigation about this pathology, its impact on the countries' budget, and the development of ways to optimise expenses for providing high-quality, effective, and safe pharmacotherapy DFS secondary to T2DM. The study by D.G. Armstrong *et al.* [1] provided a comprehensive review of diabetic foot ulcers, emphasising the high morbidity, including risk of amputation and death associated with these ulcers, and the critical role of multidisciplinary care involving surgical debridement, offloading, and infection management. This systemic approach impacts both clinical outcomes and healthcare expenditures profoundly. In turn, M.J. Carter *et al.* [2] highlighted the rising prevalence of chronic wounds among Medicare beneficiaries in the USA, elucidating increasing treatment costs over time and underscoring the urgent need for cost-effective therapeutic options to manage this growing patient population. Additionally, the findings of Y. Zhang *et al.* [3] quantified the global disability burden posed by diabetes-related lower-extremity complications, including diabetic neuropathy and foot ulcers. Moreover, M.A. Del Core *et al.* [4] provided an updated overview of diabetic foot ulcer evaluation and treatment, underscoring the multifactorial etiology involving neuropathy, vasculopathy, and immunopathy, and advocating for comprehensive multidisciplinary management and further randomised clinical trials to refine therapeutic and preventive protocols.

Furthermore, B.J. Petersen *et al.* [5] documented higher mortality rates and increased healthcare resource utilisation during episodes of care for diabetic foot ulceration, highlighting the profound economic and clinical consequences of this complication. National diabetes statistics report [6] further underscored the continued rise in diabetes prevalence and related complications, reinforcing the public health imperative to optimise pharmacotherapeutic management of DFS. The study by K. McDermott *et al.* [7] analysed disparities in diabetic foot ulcer incidence and outcomes, drawing attention to the socio-economic and racial factors contributing to suboptimal care and increased costs, thus illustrating the complexity of delivering equitable and cost-effective diabetes care.

N.W. Cortes-Penfield *et al.* [8] focused on diabetes-related foot infections, a major DFS complication, highlighting the substantial morbidity and financial costs associated with infection management, including antimicrobial therapy and potential surgery. This study stressed the necessity of multidisciplinary teams and individualised treatment strategies to improve outcomes and reduce costs. In addition, A. Jodheea-Jutton *et al.* [9] reviewed recent health economic trends in diabetic foot ulcer treatment, advocating for accelerated therapeutic approaches and enhanced care models aimed at cost containment and efficacy improvement. Ultimately, the study by E. Sutrisno *et al.* [10], through descriptive and statistical pharmacoeconomic analysis, compared the economic burden of conservative versus amputation treatment modalities for diabetic foot ulcers, emphasising the importance of strategic therapeutic decision-making to optimise healthcare spending without compromising patient outcomes.

In the context of DFS, a serious and costly complication of type 2 diabetes prevalent in Ukraine, rational pharmacotherapy is essential. T.H. Bakaliuk *et al.* [11] showed that rehabilitation methods, when combined with standard protocols, significantly improve ulcer healing rates and patient quality of life in diabetic foot syndrome, implying potential cost savings through enhanced clinical outcomes. Altogether, these studies affirm that complex ABC/VEN analysis is a valuable tool in pharmacoeconomics, enabling healthcare providers in Ukraine to efficiently allocate limited resources, prioritise essential medicines, and optimise treatment outcomes in challenging settings such as DFS. Case in point, the study by I.A. Kostiuk & K.L. Kosiachenko [12] demonstrated the application of integrated

ABC/VEN analysis to evaluate medicinal prescribing in pediatric bronchial asthma pharmacotherapy, highlighting the capacity of this approach to optimise resource allocation and ensure prioritisation of vital and essential medicines.

Therefore, the purpose of this study was to implement methodological approaches of frequency/ABC/VEN analyses for the assessment of the rationality of medicinal prescriptions in the pharmacotherapy of patients with diabetic foot syndrome resulting from T2DM.

Materials and Methods

The integrated frequency/ABC/VEN analyses was conducted using medical prescriptions of 80 inpatients with T2DM complicated by DFS admitted to the Surgical Department of the Municipal Non-Profit Enterprise "Ternopil City Municipal Hospital" during 2023, February-October. The inclusion criteria: adult patients of both sexes (≥18 years) verified diagnosed with T2DM complicated by DFS, who provided informed consent to participate. The exclusion criteria: the presence of chronic diseases in the acute or decompensated phase, ongoing glucocorticosteroid therapy, pregnancy, mental disorders, malignancies or suspected cancer, and refusal to participate. The methodology of pharmacoeconomic research involved the combined analysis of the frequency of medical prescriptions of inpatients, ranking of expenses for pharmacotherapy (ABC analysis), and ranking of prescribed drugs by degree of importance (VEN analysis). A frequency analysis is a type of quantitative assessment that reflects how often specific medicines or pharmacological groups are prescribed, and their proportion within the total number of prescriptions, arranged from highest to lowest frequent. The trade names (TN) of medicines and their Anatomical Therapeutic Chemical (ATC) classification system ATC/DDD Index 2025 [13] were used the frequency analysis of prescriptions to evaluate the main trends in pharmacotherapy of patients with T2DM complicated by DFS.

According to an expert approach of VEN analysis [14], medicines were divided into three categories: vital (V), essential (E), and non-essential (N), taking into consideration the compliance of patients' pharmacotherapy with current standards and clinical protocols: Order of the Ministry of Health of Ukraine No. 356 [15], Resolution of the Cabinet of Ministers of Ukraine No. 333 [16], Order of the Ministry of Health of Ukraine No. 971 [17], WHO Model List of Essential Medicines [18], the National Institute for Health and Care Excellence (NICE) guideline [19], Order of the Ministry of Health of Ukraine No. 1513 [20], AWaRe classification [21]. ABC analysis is the categorisation of medicines into three groups (high-cost, medium-cost, and low-cost) depending on the share of expenses for their use in the total amount of medicines costs over a certain period of time. According to the methodology group "A" should be formed by the most expensive drugs (80% of total costs), group "B" – medium-cost (15%) and group "C" – low-cost (5%). ABC analysis is based on the Pareto principle: 20% of the total number of prescribed medicines allows covering 80% of costs, while 80-85% of prescribed medicines require 20% of the funds raised. Indicators such as prescription frequency (PF), average retail price (ARP) per package, percentage of total costs, and cumulative cost percentage were used for ABC classification [14]. Statistical processing of the numerical data was performed using Excel software suite (Microsoft, USA), tabulating the data and expressing them in percents. The research was conducted following the principles set out in the Declaration of Helsinki [22] and Ethics and Data Protection [23]. Ethical approval for the publication of this case report was obtained from the Ethics Committee of Ivan Horbachevsky Ternopil National Medical University (protocol No. 83 dated November, 2025).

Thus, an integrated pharmacoeconomic evaluation combining frequency, ABC, and VEN analyses was applied to assess the rationality and economic structure of pharmacotherapy in inpatients with T2DM complicated by DFS. This approach helped to identify the most frequently prescribed medicines, determine their contribution to total treatment costs, and assess their clinical significance in accordance with treatment priorities. In addition, it provided a clear basis for optimising therapeutic strategies, ensuring both clinical and economic efficiency in the treatment of patients with T2DM complicated by diabetic foot syndrome.

Results and Discussion

Analysis of patients (n = 80) demographics by age and sex revealed that males accounted for 66.25% of the cohort, while females represented 33.75%. The mean age was 61-70 years and above 70 years, respectively. Gender distribution indicated that men with T2DM were more prone to developing DFS compared to women. Pharmacoeconomic frequency analysis of prescriptions identified 43 TNs of medicines, which were stratified into three groups based on PF: Group 1 – PF ≥ 40%, Group 2 – PF 20-40%, and Group 3 – PF < 20%. The analysis showed that only one medicine (Ceftriaxone, powder for solution for injection 1.0, vial) had a PF > 40% (Table 1). Group 2 included five drugs, three of which belonged to the pharmacological group J 01 (antimicrobials for systemic use). The largest number of medicines (37 drugs, mean PF 3.20%) was assigned to Group 3.

Table 1. TOP-10 medicines by PF in pharmacotherapy T2DM complicated with DFS

No.	INN	ATC-index	Trade name / Dosage form	PF, %
1.	Ceftriaxone	J01D D04	Ceftriaxone / powder for solution for injection, 1.0 g No. 1 vial	46.70%
2.	Cefazolin	J01D B04	Cefazolin-BChPhF / powder for solution for injection, 1.0 g No. 1 vial	18.60%
3.	Piperacillin and β-lactamase inhibitor	J01C R05	Tazpen / powder for solution for injection and infusion, 4.0/0.5 g No. 1 vial	16.30%
4.	Metformin	A10B A02	Metformin-Teva / film-coated tablets, 1.0 g No. 30 (15x2)	16.30%

Continued Table 1

No.	INN	ATC-index	Trade name / Dosage form	PF, %
5.	Metronidazole	J01X D01	Metronidazole / tablets, 250 mg No. 20 (10x2)	11.60%
6.	Metronidazole	D06B X01	Metrogyl Gel / gel for external use, 10 mg/g, 30 g tube	11.60%
7.	Insulin (human)	A10A B01	Actrapid NM / solution for injection, 100 IU/mL, 10 mL No. 1 vial	9.30%
8.	Arginine hydrochlorid	B05X B01	Tivortin / infusion solution, 42 mg/mL, 100 mL bottle	9.30%
9.	Electrolytes in combination	B05BB04	Reosorbilact / infusion solution, 200 mL vial	4.60%
10.	Insulin aspart	A10AB05	Novorapid Flexpen / solution for injection, 100 IU/mL, 3 mL No. 5, prefilled pen	4.60%

Note: INN – International Nonproprietary Names
Source: compiled by the authors

Regarding ATC/DDD Index 2025 [13], eight pharmacological groups were identified in prescriptions. As illustrated in Figure 1, the leading ATC groups were: A10 – Drugs used in diabetes (23%; leading drug – Metformin-Teva, film-coated tablets), J01 – Antibacterials for systemic use (21%; leading drug – Ceftriaxone, powder for injection solution), B01A – Antithrombotic agents (18.6%; leading drug – Tivortin, infusion solution).

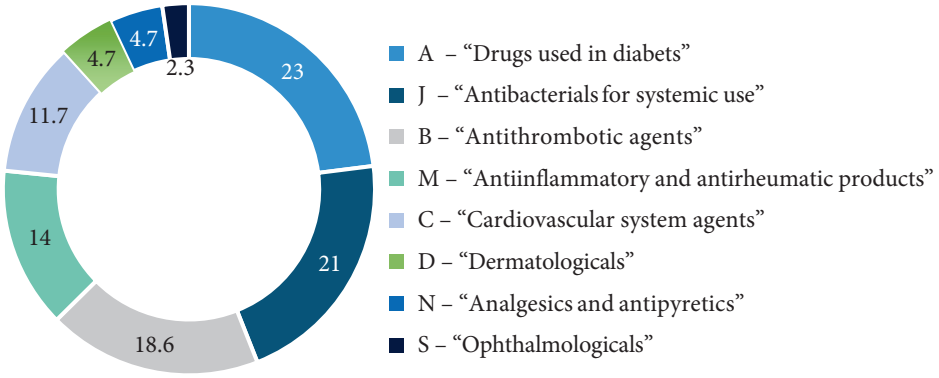

Figure 1. PF by ATC classification groups (%)
Source: compiled by the authors

Other pharmacological groups included agents for etiological therapy of DFS or pathogenetic therapy of comorbidities: M01 – Anti-inflammatory and antirheumatic products (14%), C – Cardiovascular system agents (11.7%), D – Dermatologicals (4.7%), and N02B – Analgesics and antipyretics (4.7%). Analysis of prescription forms revealed an equal distribution between oral (film-coated tablets) and parenteral formulations (injection and infusion solutions) at a 1:1 ratio. Oral administration was preferred for mild infections (occasionally with topical therapy), while moderate and severe infections typically required initial parenteral therapy with a subsequent switch to oral administration once clinical stability was achieved. Further analysis of antibiotics' prescription within the J01 group demonstrated that Ceftriaxone accounted for the highest PF (46.7%) and indicates compliance with the Ministry of Health of Ukraine's treatment guidelines for DFS [15], and simultaneous non-compliance with the requirements of rational pharmacotherapy [20] (Table 2).

Table 2. PF and expenses for antibacterial therapy T2DM complicated with DFS

No.	INN	ATC-index	Trade name / Dosage form	PF	AVR, UAH	Expenses, UAH
1.	Ceftriaxone	J01DD04	Ceftriaxone / powder for solution for injection, 1.0 g, No. 1 vial	20	44.00	880.00
2.	Cefazolin	J01DB04	Cefazolin-BHFZ / powder for solution for injection, 1.0 g, No. 1 vial	8	24.00	192.00
3.	Piperacillin and β-lactamase inhibitor	J01CR05	Tazpen / powder for solution for injection and infusion, 4.0/0.5 g, No. 1 vial	7	300.0	2,100.00
4.	Metronidazole	J01XD01	Metronidazole / tablets, 250 mg, No. 20 (10×2)	5	58.50	292.50
5.	Meropenem	J01DH02	Diapenem / powder for solution for injection and infusion, 1.0 g, No. 10 vials	1	3,600.00	3,600.00
6.			Meropenem / powder for solution for injection, 1.0 g, No. 1 vial	1	370.00	370.00

Continued Table 2

No.	INN	ATC-index	Trade name / Dosage form	PF	AVR, UAH	Expenses, UAH
7.	Levofloxacin	J01MA12	Supralev / infusion solution, 500 mg/100 mL, 100 mL vial	1	60.00	60.00
8.			Levofloxacin-Teva / film-coated tablets, 0.5 g, No. 10	1	228.00	228.00
9.	Ciprofloxacin	J01MA02	Ciprofloxacin-Astrafarm / film-coated tablets, 0.5 g, No. 10	1	97.00	97.00

Note: INN – International Nonproprietary Names; ARP – average retail price
Source: compiled by the authors

No evidence of polypharmacy was observed: the maximum number of prescribed drugs per patient was five, while the minimum was one, consistent with the therapeutic specificity of T2DM patients with DFS. Cost analysis revealed that expenditures on Tazpen (powder for injection and infusion solution) and Diapenem (powder for injection and infusion solution) significantly exceeded the median cost (UAH 303.00), potentially creating an additional financial burden under budget-reimbursed treatment schemes for DFS. The next stage of the study involved conducting a VEN-analysis of medicines prescribed to patients with DFS. To evaluate the rationality of anti-bacterial agents' prescriptions, information on the inclusion them in key national and international regulatory and clinical documents was systematised (Table 3).

Table 3. Analysis of the availability of anti-bacterial agents on the lists of rational pharmacotherapies of DFS

No.	INN	National List of Essential Medicines of Ukraine, 2023	State Formulary of Medicinal Products (edit), 2025	WHO Model List of Essential Medicines, 2025	NICE guideline, 2019	AWaRe classification, 2021
1.	Ceftriaxone	+	+	+	+	C
2.	Cefazolin	+	+	+	-	A
3.	Piperacillin and β-lactamase inhibitor	-	+	-	+	B
4.	Metronidazole	+	+	+	+	A
5.	Meropenem	-	+	+	-	B
6.	Levofloxacin	-	+	+	-	C
7.	Ciprofloxacin	+	+	+	+	B

Source: compiled by the authors

According to the obtained data, 100% of prescribed anti-bacterial agents were listed in the State Formulary of Medicinal Products [17]. Ceftriaxone, cefazolin, and metronidazole are consistently included in the National List of Essential Medicines [16], State Formulary of Medicinal Products [17], WHO Model List of Essential Medicines [18], reflecting their essential status and wide relevance in empiric and targeted antimicrobial therapy. However, according to AWaRe classification [21], ceftriaxone and levofloxacin are included in the "Reserve" category, so their use must be restricted and carefully monitored. Meropenem, although not included in all lists due to its classification as a broad-spectrum carbapenem, appears within the AWaRe "Watch" category, highlighting its importance for resistant infections but recommending restricted use. According to the NICE guideline [19], four of these agents (ceftriaxone, piperacillin-β-lactamase inhibitor, metronidazole, ciprofloxacin) are recommended for use in moderate to severe infections associated with DFS, confirming their clinical appropriateness. According to the WHO AWaRe classification [21], cefazolin and metronidazole are categorised in the "Access" group, due to their lower resistance risk. Three antibiotics (piperacillin-β-lactamase inhibitor, meropenem, and ciprofloxacin) are classified in the "Watch" group and reserved for specific indications and require stewardship oversight to prevent overuse and resistance development.

Based on the full (100%) inclusion of Ceftriaxone, Metronidazole, and Ciprofloxacin in all regulatory acts their prescriptions were classified as justified and assigned to the "V" (vital) category. However, the low PF of Ciprofloxacin (2.30%) may indicate either its empirically low clinical efficacy or high microbial resistance in wound infections in patients with T2DM complicated by DFS. Moreover, the high PF of Piperacillin and beta-lactamase inhibitors (16.30%) remains controversial, as these drugs are absent from the WHO Model List of Essential Medicines [18] and, accordingly, from the National List of Essential Medicines [16]. Similarly, prescriptions for Cefazolin, Meropenem, and Levofloxacin require further discussion, as they are not included in the NICE guideline [19].

According to the research, ten medicines were classified as "vital". Among them, three medicines (30%) were antibiotics, active against gram-positive wound pathogens, while six medicines (60%) belonged to the A10 pharmacological group (antidiabetic agents) aimed at pathogenetic correction in T2DM patients. One medicine, Reosorbilact (infusion solution) from the B05 group (blood substitutes

and perfusion solutions), was identified as essential for improving microcirculation, arterial and venous blood flow, and correcting metabolic acidosis. 19 medicines (44%) of the prescriptions were assigned to the "essential" category. Most of these were included in the National List of Essential Medicines [16] and the State Formulary of Medicinal Products [17], however were not always recommended in DFS treatment protocols [15]. These medicines largely contribute to maintaining the quality of life in patients with chronic comorbidities with high disease burden indices, such as arterial hypertension, diabetes mellitus, and venous thromboembolism. Antimicrobial agents predominated in this category (36.8%), followed by B01A antithrombotic drugs (26.3%), reflecting the cardiovascular comorbidity profile of T2DM patients. Ultimately, 32.6% of prescribed medicines were classified as "non-essential". These agents were absent in the National List of Essential Medicines [16] and primarily used for symptomatic management of conditions not directly affecting DFS progression. The majority of these medicines belonged to the M01A pharmacological group (nonsteroidal anti-inflammatory and antirheumatic drugs), particularly Dexketoprofen (35.8%).

The ABC analysis (Table 4) revealed that group "A" included 15 trade names (34.9%), dominated by antidiabetic agents (40%), blood and haematopoietic drugs (33.3%), and systemic antibacterials (20%). It reflects that the management of T2DM with complications, along with haematological support, is consuming a significant portion of healthcare resources. In contrast, group "B", representing only 15% of expenditures, includes medicines with a moderate cost impact, suggesting they are essential but not as financially demanding. It comprised 13 medicines (30.2%) with an equal share of antidiabetic and antimicrobial agents (23% each), reflecting etiological and pathogenetic treatment approaches. It suggests that these drugs support both disease management and infection control, but at a lower overall expense. Group "C" (5% of expenditures) consisted of 15 medicines (34.9%), mainly cardiovascular agents (26.7%) and anti-bacterial agents (20%). Their budgetary burden is minimal, indicating that either they are low-cost generics or have lower consumption volumes.

Table 4. Generalised results of integrated frequency/ABC/VEN analysis

ABC / VEN analysis	V			E			N		
	Number of medicines	Cost, UAH	Share, %	Number of medicines	Cost, UAH	Share, %	Number of medicines	Cost, UAH	Share, %
A	6	8,260.00	30.34	7	11,833.00	43.47	2	1,658.00	6.09
B	2	733.50	2.69	4	1,125.30	4.13	7	2,299.00	8.45
C	2	312.00	1.15	8	602.50	2.22	5	329.20	1.46
Total	10	9,305.50	34.18	19	13,560.80	49.82	14	4,355.20	16.00

ABC-analysis			VEN-analysis		
Class	Number of medicines	Share, %	Group	Number of medicines	Share, %
A	15	34.89	V	10	23.26
B	13	30.22	E	19	44.19
C	15	34.89	N	14	32.56
Total	43	100	Total	43	100

Source: compiled by the authors

The integration of antibacterial prescribing patterns with essential medicines lists and stewardship frameworks provides a deeper understanding of the rationality of antimicrobial therapy in patients with DFS. The results indicated a disproportionate cost burden for vital and essential medicines, emphasising the need for economic strategies to optimise resource allocation and improve access to cost-effective pharmacotherapy for DFS in patients with T2DM. The results of the frequency analysis indicated the predominance of etiological and pathogenetic therapy, consistent with the recommendations of regulatory documents [15, 19, 20]. The high concordance of frequently prescribed antibiotics (such as ceftriaxone, cefazolin, metronidazole, and piperacillin-tazobactam) with national and international essential medicines lists underscores the alignment of local prescribing practices with evidence-based standards. This suggests that the antimicrobial choices made in the study setting adhere to globally recognised therapeutic principles and emphasise the availability and affordability of core antibacterial agents.

The VEN analysis revealed that "non-essential" drugs were mainly prescribed for concomitant minor conditions. Their clinical efficacy is often poorly supported by evidence, and in some cases, these medicines are costly relative to their limited therapeutic benefit. Importantly, inclusion in the "N" category does not necessarily mean the drug is absent from the formulary system or essential medicines list; in many cases, drugs for minor illnesses appear on essential medicines lists but are considered lower priority for procurement compared to others. Overall, the VEN analysis helped to derive the following equation for drug distribution in prescriptions by clinical importance: E > V > N. The quantitative predominance of "essential" over "vital" medicines remains a subject of debate due to the

complexity of the pathology (patients were treated not only for the primary disease but also for chronic comorbidities) and the nature of DFS therapy. A positive finding was the relatively small share of "non-essential" drugs, indicating a preference for targeted, evidence-based pharmacotherapy (V + E > N by twofold) among healthcare professionals.

The ABC analysis revealed a shift toward a higher proportion of high-cost "A" medicines, indirectly suggesting a potentially irrational allocation of funds for DFS pharmacotherapy. In the study, the ABC-equation was A = C > B, indicating about the demand in the cost optimisation and a reduction in the share of expensive drugs through greater use of Ukrainian generic medicines. For practical implications for budget optimisation, it would be appropriate to prioritise negotiations for group "A" and "V"/"E" medicines to reduce procurement costs, especially high-cost antidiabetic and antimicrobial agents. Secondly, promote generics in the "E" group to maintain access while lowering expenses. And finally, rationalise or restrict "N" medicines, particularly those in group "A", to prevent wasteful spending healthcare costs.

Controversial results are also observed when comparing the principles of the WHO AWaRe classification with the medical prescriptions of the studied group of patients. Under this framework, "Access" antibiotics are intended to be broadly accessible, as they constitute first-line therapeutic options associated with a relatively low resistance potential. It is recommended that these agents comprise a minimum of 60% of all antibiotic use in inpatient settings and 95% in ambulatory practice [21]. In the case of the authors, ceftriaxone was the first-line antibiotic, with a prescription rate exceeding 40%, leading to the conclusion that healthcare costs are irrational. This is also confirmed by WHO data [21] and the study by K. Myroniuk-Konstantynovych *et al.* [24], where the share of antibiotics of "Access" category accounted for only 59.1% of total antibiotic consumption in outpatient settings and merely 39.8% in hospitals, in Ukraine in 2024, reflecting a substantial departure from the recommended global targets established by the WHO. This imbalance emphasises a concerning overuse of "Watch" and "Reserve" group antibiotics, which risks accelerating antimicrobial resistance and highlights the urgent need for improved antibiotic stewardship in the country. This observation aligns with global concerns regarding antibiotic overuse in DFS management, as highlighted by A. Jodheea-Jutton *et al.* [9] that antimicrobial therapy constitutes a substantial proportion of DFS-related costs and that irrational antibiotic use accelerates resistance while inflating healthcare expenditures. The results further confirmed their conclusion that optimising antimicrobial selection is central to improving both clinical outcomes and cost-effectiveness in DFS care.

Comparative analysis with the study by N. Rahayuningsih *et al.* [25] revealed similar trends in antibiotic-driven cost structures. Their cost-effectiveness analysis of antibiotic use in DFS patients in Indonesia demonstrated that broad-spectrum antibiotics significantly increased treatment costs without proportional improvements in outcomes when not guided by severity stratification and microbiological data. Likewise, the current study identified high-cost antibiotics (piperacillin – tazobactam, meropenem) as contributors to the "A" cost group, despite relatively low prescription frequencies. This reinforces the notion that even limited use of high-cost antimicrobials can disproportionately burden hospital budgets, particularly in resource-constrained health systems [26]. In contrast to studies focusing predominantly on pharmacotherapy costs, S. Russo *et al.* [27] evaluated the cost-effectiveness of platelet-rich plasma versus standard of care for DFS management in the United States. Their findings demonstrated that although platelet-rich plasma's therapy entails higher upfront costs, it may reduce long-term expenditures by accelerating wound healing and decreasing complications. While the present study did not assess advanced wound-healing technologies, the heavy reliance on prolonged systemic pharmacotherapy observed in the cohort may indirectly reflect limited access to such adjunctive interventions. This comparison suggests that investment in cost-effective non-pharmacological or biologic therapies could potentially reduce reliance on expensive antimicrobial regimens and improve overall economic efficiency in DFS management.

The systematic review by E. Sutrisno *et al.* [10] further contextualised the present findings by comparing conservative treatment strategies with amputation-related costs in gangrene associated with T2DM. Their analysis demonstrated that although conservative pharmacotherapy may appear costly in the short term, it is generally more cost-effective than surgical interventions, particularly amputations, when clinical outcomes are favourable. In this context, the high share of expenditures allocated to "vital" and "essential" medicines in the present study may be interpreted positively, as it reflects an emphasis on limb preservation strategies. However, the presence of a considerable proportion of "non-essential" medicines, including non-steroidal anti-inflammatory drugs, suggests the need for further rationalisation to ensure that resources are concentrated on interventions with direct impact on DFS outcomes. From a broader health-system perspective, the findings are consistent with those of A.M. Alshammari *et al.* [28], who reported that diabetes-related hospital costs are primarily driven by complications and inpatient pharmacotherapy. The hospital-centric cost analysis in Saudi Arabia identified medications and prolonged hospital stays as major cost drivers, emphasising the importance of formulary management and prescribing optimisation. Similarly, the present study's results of the ABC analysis demonstrated that approximately one-third of medicines accounted for the majority of expenditures, underscoring the relevance of targeted procurement strategies and price negotiations for high-cost, high-priority drugs, particularly those in the "A/V" and "A/E" categories.

The economic implications of alternative treatment modalities were further illustrated by K. Tochaiwat *et*

al. [29]. The paper reported favourable cost-effectiveness outcomes for high-power laser therapy compared to conventional treatment for diabetic foot ulcers in Thailand. Their results confirmed that innovative therapies may reduce total treatment costs by shortening healing time and decreasing medication use. In contrast, the pharmacotherapy-heavy approach observed in present study may contribute to prolonged treatment duration and sustained drug expenditures. This was further supported by L. Ge *et al.* [30], who found that specialised multidisciplinary management programmes significantly reduce costs and improve outcomes in diabetic foot ulcer treatment. This comparison highlighted the potential benefits of integrating pharmacoeconomic evidence into clinical decision-making when selecting DFS treatment strategies. Ultimately, A. Shankar [31] emphasised that effective diabetic foot care requires alignment between clinical guidelines, health policy, and economic evaluation. The present findings directly address this intersection by demonstrating partial alignment of prescribing practices with essential medicines lists and clinical guidelines, while simultaneously revealing deviations from antimicrobial stewardship principles, particularly under the WHO AWaRe framework. The overrepresentation of "Watch" and "Reserve" antibiotics observed in this study mirrors national trends reported for Ukraine and signals an urgent need for stewardship interventions to improve rational antibiotic use and long-term cost containment.

Overall, the findings of this study were consistent with international evidence demonstrating that DFS management imposes a substantial economic burden, largely driven by pharmacotherapy costs and antimicrobial use. Compared with global studies, the Ukrainian context is characterised by a high reliance on systemic antibiotics and a cost structure dominated by a limited number of high-cost medicines. The integrated frequency/ABC/VEN approach proved effective in identifying priority areas for optimisation, including promotion of "Access" antibiotics, increased use of cost-effective generics, and restriction of non-essential medicines. These measures, combined with broader adoption of evidence-based adjunctive therapies, may enhance the cost-effectiveness of DFS management and improve patient outcomes within constrained healthcare budgets.

Conclusions

The pharmacoeconomic research was conducted using 80 inpatient medical records of patients with T2DM complicated by DFS with an analysis of 43 TNs of prescribed medicines. The distribution of prescriptions according to the ATC classification revealed that the leading pharmacological groups were A10 – Drugs used in diabetes (23%), J01 – Antibacterials for systemic use (21%), and B01A – Antithrombotic agents (18.6%). Analysis of dosage form types demonstrated an equal distribution between oral formulations (film-coated tablets) and parenteral formulations (injection and infusion solutions) at a 1:1 ratio. The frequency analysis involved dividing the drugs into three groups, which revealed a significantly uneven distribution of prescriptions, characterised by the dominance of ceftriaxone in Group 1.

According to the VEN analysis identified 10 medicines in the "vital" category: 30% were antibiotics, 60% belonged to group A10 "Antidiabetic drugs", and 10% belonged to group B05 "Blood substitutes and perfusion solutions". The "essential" category comprised 19 medicines (44% of all agents prescribed to patients with T2DM and DFS), with antimicrobials accounting for 36.8%. Due to the high prevalence of comorbidities in T2DM, medicines from group B01A "Antithrombotic agents" constituted 26.3% of this category. The "non-essential" category accounted for 32.6% of all prescribed medicines and was predominantly represented by group M01A "Nonsteroidal anti-inflammatory and antirheumatic drugs". Thus, "essential" medicines dominated over "vital" categories (E > V > N), indicating rational prescribing practices with limited use of low-priority drugs. The results of the ABC analysis demonstrated that Group A (accounting for 80% of expenditures) comprised 15 TNs of medicines (34.90%), Group B (15% of expenditures) included 13 TNs (30.2%), and Group C (5% of expenditures) comprised 15 TNs (34.9%). The ABC correlation was A = C > B, indicating a shift toward an increased share of high-cost "A" category medicines, and therefore the need for economic optimisation and broader implementation of cost-effective domestic generics to improve affordability and reduce the financial burden on healthcare systems and patients.

Based on the results of the integrated analysis, the following correlations were formulated: 73% of expenditures – E/A > V/A indicates the high cost of "vital" and "essential" medicines; 27% of expenditures – N/B > N/A > E/B > V/B > E/C > N/C > V/C indicates an irrational approach to DFS therapy, as there is a predominance of medicines lacking sufficient evidence of effectiveness and associated with considerable economic burden. The study results emphasised the critical role in optimisation of antimicrobial therapy in DFS management while drawing attention to the growing risk of antimicrobial resistance, underlining the necessity of evidence-based selection of antibiotics. Future research should focus on developing pharmacoeconomic models for individualised therapy, assessing cost-utility parameters, and exploring innovative strategies to enhance treatment effectiveness while ensuring sustainable healthcare financing.

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Conflict of Interest

The authors declare no conflict of interest financial or otherwise.

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Фармакоекономічна оцінка фармакотерапії пацієнтів із цукровим діабетом 2 типу з синдромом діабетичної стопи

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Анотація. Зростання поширеності цукрового діабету 2 типу, ускладненого синдромом діабетичної стопи, та призначення дорогих ліків, які не включені до протоколів лікування, значно підвищують витрати на охорону здоров'я та погіршують результати лікування пацієнтів, що робить оптимізацію фармакотерапії як клінічним, так і економічним пріоритетом. Метою дослідження було оцінити раціональність фармакотерапії для пацієнтів із синдромом діабетичної стопи за допомогою інтегрованого частотного/ABC/VEN аналізу. У дослідженні застосовувався частотний аналіз для оцінки фармакотерапевтичних схем та частоти призначення ліків за АТС-класифікацією, ABC-аналіз для класифікації ліків за рівнем витрат та VEN-аналіз для визначення їх терапевтичної значущості на основі рекомендацій національних та міжнародних рекомендацій щодо лікування цукрового діабету 2 типу. Результати показали, що в клінічній практиці переважали етіологічні та патогенетичні підходи до терапії. Протидіабетичні засоби становили найбільшу частку призначень (23 %), тоді як антибіотики були необхідними для лікування інфекційних ускладнень. Інтегрований частотний/ABC/VEN-аналіз показав, що «необхідні» ліки (44,19 %) переважали над категоріями «життєво важливим» (23,26 %) та «другорядними» (32,56 %), що свідчило про недостатньо раціональну практику призначення відповідно до рекомендацій щодо лікування. Висока частка витрат на охорону здоров'я була зосереджена в групі високовартісних ліків (34,89 %), що свідчило про неоптимальний розподіл ресурсів охорони здоров'я. Результати також підкреслили важливість вибору антибіотиків на основі доказів через зростаючий ризик резистентності до антимікробних препаратів. Дослідження створює платформу для підвищення економічної ефективності фармакотерапії у пацієнтів із синдромом діабетичної стопи шляхом підтримки раціонального призначення ліків, оптимізації витрат на охорону здоров'я та просування економічно ефективних українських аналогів

Ключові слова: інтегрований частотний/ABC/VEN-аналіз; лікарські призначення; антимікробні засоби; антибіотикорезистентність; оптимізація фармацевтичного забезпечення

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