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Chemical structure and anti-inflammatory mechanisms of phenolic compounds from medicinal plants

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Abstract. The study was aimed at investigating the relationship between the chemical structure of phenolic compounds from medicinal plants *Curcuma longa*, *Hypericum perforatum* and *Salvia officinalis* and their anti-inflammatory activity. The research was conducted at the Educational and Scientific Institute of Applied Pharmacy, National University of Pharmacy (Kharkiv, Ukraine), between September and December 2024. Using high-performance liquid chromatography and mass spectrometry, 12 bioactive compounds were identified, including curcumin, rutin and rosmarinic acid. Experiments on macrophage cells demonstrated that curcumin reduced tumour necrosis factor-alpha levels by 72 ± 3% and interleukin-6 by 65 ± 2% (at a concentration of 50 μM) through inhibition of the transcription factor kappa-B (55 ± 4%) and p38 kinase (60 ± 5%). Rutin, despite its stability in blood plasma (half-life 4.2 hours), showed a lower permeability coefficient across cell membranes (2.1×10^{-6} cm/s). *Salvia officinalis* extract increased the level of the anti-inflammatory interleukin-10 by 20 ± 3%, while the correlation between the number of hydroxyl groups in the molecules and interleukin-6 inhibition was 0.89. Curcumin exhibited cytotoxicity at concentrations above 100 μM, reducing cell viability by 40%. The results confirmed that the anti-inflammatory effect depends on the presence of ortho-dihydroxyl groups and glycosylation, with the highest potential observed in curcumin. These findings highlighted the importance of structural analysis and comprehensive methods for developing plant-based preparations with targeted activity. The results may be applied by pharmaceutical companies and research laboratories in the creation of new anti-inflammatory agents of natural origin

Keywords: *Salvia officinalis*; curcumin; *Hypericum perforatum*; ortho-dihydroxyl groups; pharmacokinetic profile

INTRODUCTION

Phenolic compounds of medicinal plants remain the subject of intensive research due to their antioxidant, anti-inflammatory, and immunomodulatory properties. Between 2020 and 2025, interest in their application as alternatives to synthetic drugs has increased, particularly against the backdrop of the global prevalence of chronic inflammatory diseases such as arthritis, atherosclerosis, and autoimmune disorders. Despite significant progress in investigating the bioactivity of phenols, unresolved issues remain concerning the relationship between their chemical structure and mechanisms of action at the molecular level. In particular, the role of specific functional groups, such as ortho-dihydroxyls, in modulating inflammatory signalling pathways – including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) – is still insufficiently explored.

Research highlighted the potential of phenolic compounds as multitarget agents. For example, W. Liu *et al.* [1] systematised data on the anti-inflammatory properties of bioactive peptides of plant origin, emphasising their ability to modulate pro-inflammatory cytokine production and influence NF-κB and MAPK signalling pathways. The authors underscored the potential of these compounds in developing new nutritional or pharmaceutical products with anti-inflammatory action. Equally important are the findings of A.V. Lopez-Corona *et al.* [2], who studied phenolic components of raspberry (*Rubus idaeus*) and their impact on oxidative stress in model systems. Extracts rich in ellagic acid and quercetin were found to reduce levels of reactive

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oxygen species (ROS) and activate antioxidant enzymes, indirectly contributing to anti-inflammatory effects. A. Rakha *et al.* [3] made an important contribution to understanding structure–function relationships by summarising the effects of dietary flavonoids as natural anti-inflammatory and antiallergic agents. Their work focused on mechanisms such as inhibition of cyclooxygenase-2 (COX-2), suppression of histamine release, and regulation of T-cell-mediated responses, underlining the significance of flavonoids in nutraceutical prevention of inflammatory conditions. However, these conclusions require further *in vitro* empirical validation, particularly regarding transcription factor suppression.

Gaps in current knowledge become apparent when analysing studies devoted to specific medicinal plants. O.V. Soroka *et al.* [4] examined bioactive compounds of the genus Carlina, widespread in the flora of Ukraine, noting the presence of phenolic acids and flavonoids with potential antibacterial activity. However, data on their anti-inflammatory properties remain fragmentary, and no evaluation of their ability to modify the cytokine profile has been carried out. S. Jongrungraungchok *et al.* [5] analysed the antioxidant properties of mixtures of Thai plants and found that combined extracts demonstrated greater effectiveness than individual components. Phenolic compounds, in particular, were shown to reduce malondialdehyde (MDA) levels, indicating a decrease in lipid peroxidation, although the mechanisms of synergy remain unclear.

The mechanisms of action of phenols from species common in Europe, such as *Hypericum perforatum* (St John's Wort) or *Salvia officinalis* (sage), remain insufficiently explained. In particular, the effect of glycosylation on the bioavailability of flavonoids in these plants, and whether their activity results from synergism of different components, has not been thoroughly studied. I. Borysiuk *et al.* [6] demonstrated the promise of *in silico* modelling for predicting the biological activity of natural compounds, particularly in relation to enzymatic targets. However, the authors pointed out that empirical data on the efficiency of various extraction methods remain limited, which may affect the reliability of *in silico* conclusions.

The study of anti-inflammatory mechanisms is particularly relevant in the context of emerging medical challenges. For example, F. Shahzad *et al.* [7] showed that phenols can modulate immune responses during viral infections, including Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), by affecting the expression of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). This demonstrates the potential of phenolic extracts as adjuvant therapies in viral infections, although clinical confirmation remains limited. I. Mssillou *et al.* [8] identified the wound-healing potential of phenolic extracts through suppression of pro-inflammatory cytokine activity and stimulation of collagen production in dermal fibroblasts. Their study demonstrated that even local application of phenols can modulate inflammatory processes, although mechanisms of interaction with receptor structures require further research. L. Toma *et al.* [9] emphasised the epigenetic effects of phenols in cardiovascular diseases, particularly their impact on DNA methylation and gene expression associated with inflammatory reactions. However, their influence on NF-κB-dependent signalling cascades in the context of extracts such as St John's Wort or sage has yet to be sufficiently empirically substantiated.

A review of the literature indicated that previous studies have mainly focused on individual aspects of phenol bioactivity, whereas a comprehensive approach combining chemical analysis, *in vitro* experiments and structure-activity evaluation remains rare. For example, O.S. Nwozo *et al.* [10] summarised data on antioxidant activity, phytochemical composition and therapeutic potential across a wide spectrum of medicinal plants. The authors emphasised the roles of polyphenols, alkaloids and terpenes as key compounds mediating protective effects against oxidative stress, inflammation and metabolic disorders. However, they did not examine specific mechanisms of interaction between individual phenolic molecules and signalling pathways, leaving a significant niche for further research.

Thus, the necessity of this study stems from the need for a systematic approach to investigating phenolic compounds, combining advanced chemical-analytical methods with biological models to predict their therapeutic effectiveness. The aim of this study was to conduct an empirical analysis of the chemical structure of phenolic compounds from three medicinal plants (*Hypericum perforatum*, *Salvia officinalis*, *Curcuma longa*) and their influence on key inflammatory mechanisms *in vitro*. The hypothesis was based on the assumption that anti-inflammatory activity depends on three main factors: the presence of ortho-dihydroxyl groups in the phenolic ring, the degree of glycosylation, and the ability to inhibit NF-κB-dependent cytokine transcription.

MATERIALS AND METHODS

The study was conducted between September and December 2024 at the Educational and Scientific Institute of Applied Pharmacy, National University of Pharmacy (Kharkiv, Ukraine). The experimental work comprised three stages. The first stage – preparation of plant samples – lasted from May to July 2024: sage (*Salvia officinalis*) was collected in May, the aerial part of St John's Wort (*Hypericum perforatum*) in June, and standardised rhizomes of turmeric (*Curcuma longa*) were imported at the beginning of July. The second stage, dedicated to chemical analysis of phenolic compounds, was carried out in July-August 2024. The third stage (August-September 2024) involved evaluation of the anti-inflammatory activity of the obtained extracts using *in vitro* methods. The plant materials represented three species: *Hypericum perforatum* (collected in May 2024 in an ecologically clean area of Poltava region), *Salvia officinalis* (cultivated under controlled conditions in the botanical garden of the National University of Pharmacy), and *Curcuma longa* (rhizomes imported from India, certified under DSTU ISO 22000:2019 [11]). The selection of these species was justified by their long history of use in both traditional and modern medicine as sources of bioactive phenolic compounds, as well as by the reproducibility of results and the availability of standardised methods for component analysis.

Extracts were obtained using maceration, which ensures stable extraction of thermolabile phenolic compounds. The crushed raw material (particle size 0.5-1 mm) was immersed in a hydroethanolic solution (70% ethanol, 30% deionised water) in a ratio of 1:10 (w/v). The choice of 70% ethanol as the solvent was justified by its ability to

effectively extract both hydrophilic and lipophilic components. The mixture was incubated at 40°C in a thermostatic chamber with periodic stirring (100 rpm) for 48 hours. These conditions minimised degradation of thermolabile compounds (e.g., curcuminoids) and ensured complete saturation of the solvent with target components. After incubation, the suspension was filtered through nitrocellulose membrane filters (pore size 0.45 µm) under vacuum (500 mbar) to remove plant matrix residues. The filtrate was concentrated on a Heidolph vacuum evaporator (Germany) at 200 mbar and 50°C to 20% of the initial volume. Final solvent removal was performed by lyophilisation using a FreeZone 2.5 (Labconco, USA) at -50°C and 0.01 mbar for 24 hours. The resulting dry extracts were stored in hermetically sealed containers with silica gel at 4°C until further analysis.

Phenolic profiles were determined using high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity II system (USA). A Zorbax Eclipse XDB-C18 column (4.6×150 mm, 5 µm) was employed with a mobile phase consisting of acetonitrile (A) and 0.1% aqueous orthophosphoric acid (B). Gradient elution was performed at a flow rate of 1 ml/min: the proportion of acetonitrile increased from 10% to 50% over 1-25 minutes. Detection was carried out on a UV detector at 280 nm. To confirm compound structures, liquid chromatography–mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-9030 (Japan) with electrospray ionisation (ESI+) in the 100-2,000 m/z range. Identification of components was based on comparison with spectra from public databases (PubMed) and commercial standards (quercetin – Q4951-5MG, gallic acid – G7384-25G, curcumin – 34589-100MG). For each identified phenolic compound, structural analysis considered the number of hydroxyl groups, particularly ortho-dihydroxyl configurations, and the presence of glycosidic residues. Structures were verified against LC-MS spectra using PubChem (USA) and the Human Metabolome Database (HMDB) (USA). Functional groups were manually quantified using ChemDraw (PerkinElmer, USA).

Anti-inflammatory activity was studied in RAW 264.7 macrophages obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% foetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin mixture at 37°C and 5% CO_2. To induce inflammation, cells were treated with lipopolysaccharide (LPS, Sigma-Aldrich, USA) at a concentration of 1 µg/ml for 24 hours. After induction, cells were treated with extracts (10-100 µm) or isolated compounds (curcumin, rutin) for 12 hours. Levels of pro-inflammatory cytokines TNF-α and IL-6 were measured using commercial ELISA kits (R&D Systems, USA) following the manufacturer's protocol; optical density was determined on a Multiskan Sky microplate reader (USA) at 450 nm. For analysis of NF-κB p65 and phosphorylated p38 MAPK protein expression, western blotting was performed: cell lysates were separated on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes, and incubated with primary antibodies (Cell Signaling Technology, USA) at 1:1,000 dilution. Signal detection was performed using Clarity Max chemiluminescent substrate (Bio-Rad, USA) and visualised with a ChemiDoc MP system (Bio-Rad, USA). Data analysis was carried out using SPSS 26.0 (USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for group comparisons. Correlation between the number of hydroxyl groups in phenol molecules and cytokine inhibition was assessed using Pearson's coefficient. Statistical significance was considered at $p < 0.05$. Each experiment was repeated five times ($n = 5$) to ensure reproducibility. The experimental protocol was developed in accordance with the recommendations of the European Pharmacopoeia [12].

RESULTS

Chemical composition and antioxidant activity. The chemical profile of medicinal plants is a key factor determining their biological activity, particularly antioxidant and anti-inflammatory properties. Within the present study, it was established that antioxidant activity directly correlates with both the quantitative content and structural features of phenolic compounds. There is a direct relationship between the chemical structure of phenols – primarily the presence of ortho-dihydroxyl groups, the degree of glycosylation, and the ability to influence molecular targets such as the NF-κB pathway – and the intensity of the anti-inflammatory effect. Using HPLC and LC-MS methods, 12 phenolic compounds were identified in the extracts of the studied plants (Table 1). These compounds belong to various classes of polyphenols, including flavonoids, phenolic carboxylic acids, depsides and diterpene derivatives. The highest polyphenol content was recorded in the extract of *Curcuma longa* – 218 ± 5 mg/g – which can be attributed to the presence of curcuminoids. These demonstrated strong antioxidant properties due to the presence of two ortho-dihydroxyl groups in the phenyl ring. Such groups promote chelation of transition metal ions that catalyse the formation of reactive oxygen species (ROS), as well as efficient neutralisation of free radicals through electron transfer.

Table 1. Phenolic compounds identified in extracts of the studied plants

No.	Compound	Source plant	Structural features	Molecular formula	Molecular mass (g/mol)	Concentration (mg/g)
1	Curcumin	*Curcuma longa*	Two ortho-dihydroxyl groups, β-diketone	$C_{21}H_{20}O_6$	368.38	120 ± 4
2	Demethoxycurcumin	*Curcuma longa*	One ortho-dihydroxyl group	$C_{20}H_{18}O_5$	338.36	65 ± 3
3	Bisdemethoxycurcumin	*Curcuma longa*	No methoxyl groups	$C_{19}H_{16}O_4$	308.33	33 ± 2
4	Rutin	*Hypericum perforatum*	Quercetin glycoside (rhamnose + glucose)	$C_{27}H_{30}O_{16}$	610.52	85 ± 2
5	Hypericin	*Hypericum perforatum*	Naphthodianthrone, conjugated system	$C_{30}H_{16}O_8$	504.44	40 ± 1

Table 1. Continued

No.	Compound	Source plant	Structural features	Molecular formula	Molecular mass (g/mol)	Concentration (mg/g)
6	Quercetin	*Hypericum perforatum*	Rutin aglycone, 3 OH-groups	$C_{15}H_{10}O_7$	302.24	20 ± 1
7	Rosmarinic acid	*Salvia officinalis*	Depside (caffeic + 3,4-dihydroxyphenyllactic acid)	$C_{18}H_{16}O_8$	360.32	45 ± 2
8	Carnosol	*Salvia officinalis*	Diterpene phenol, ortho-quinone structure	$C_{20}H_{26}O_4$	330.42	25 ± 1
9	Carnosic acid	*Salvia officinalis*	Triterpene phenol, carboxyl group	$C_{20}H_{28}O_4$	332.43	15 ± 1
10	Luteolin	*Salvia officinalis*	Flavone, 4 OH-groups	$C_{15}H_{10}O_6$	286.24	8 ± 0.5
11	Chlorogenic acid	*Hypericum perforatum*	Ester of caffeic and quinic acids	$C_{16}H_{18}O_9$	354.31	12 ± 0.8
12	Caffeic acid	*Hypericum perforatum*	Hydroxycinnamic acid, 2 OH-groups	$C_9H_8O_4$	180.16	10 ± 0.6

Source: compiled by the author

Table 1 summarised the data on phenolic compounds, including their sources, structural features, molecular mass and concentration. For example, curcumin ($C_{21}H_{20}O_6$), the principal polyphenol of *Curcuma longa*, is characterised by a β-diketone structure and the presence of two ortho-dihydroxyl groups, which underpin its strong ability to inhibit inflammatory cascades. Its structural analogues – demethoxycurcumin and bisdemethoxycurcumin – demonstrate similar, though somewhat reduced, activity due to the absence of methoxyl or hydroxyl groups. In *Hypericum perforatum*, flavonoids predominate, including rutin (a glycoside of quercetin), hypericin (a naphthodianthrone derivative) and the aglycone quercetin. Glycosylation of phenols, as in the case of rutin, enhances hydrophilicity, bioavailability and affinity for cell membranes, positively influencing antioxidant activity. Hypericin, with its extended conjugated system, shows photosensitising capacity and inhibition of pro-inflammatory enzymes.

Compounds from *Salvia officinalis* are also of particular importance. Rosmarinic acid, a depside, exhibits dual antioxidant activity by both inhibiting ROS formation and stabilising free radicals. Carnosol and carnosic acid – diterpene phenols – exhibit not only antioxidant but also anticancer activity, associated with their quinone-like structure and their ability to influence gene expression in inflammation. Luteolin, a flavone, also acts as an effective antioxidant capable of inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the primary source of superoxide anion in immune cells. Thus, the data demonstrate that variability in chemical structures of phenolic compounds determines the spectrum of their biological activity. Ortho-dihydroxyl groups and glycosidic fragments play particularly critical roles, shaping both physicochemical properties and molecular targets of bioactivity. These results support the hypothesis that the antioxidant effect is multifactorial, dependent not only on redox potential but also on the ability to regulate cellular signalling pathways such as NF-κB, MAPK and nuclear factor erythroid 2-related factor 2 (Nrf2).

Anti-inflammatory activity and mechanisms of action. To assess the anti-inflammatory activity of phenolic extracts from the studied medicinal plants, a model of LPS-induced inflammation was applied using RAW 264.7 murine macrophages at a concentration of 1 μg/ml. Extracts were tested across a wide concentration range (10-100 μm), allowing assessment of dose dependence and effective thresholds. A 12-hour incubation was selected as the optimal interval for activation of inflammatory mediators without cytotoxic consequences. Curcumin, the principal bioactive component of *Curcuma longa*, demonstrated the strongest anti-inflammatory activity. At 50 μm, it significantly reduced production of key pro-inflammatory cytokines: tumour necrosis factor-alpha decreased by 72 ± 3%, and IL-6 by 65 ± 2%. These results indicated inhibition of both early and late phases of the inflammatory response. This effect is characteristic of compounds with strong redox activity and the ability to directly modify signalling proteins regulating cytokine transcription.

Rutin, isolated from *Hypericum perforatum*, exhibited moderate anti-inflammatory effects, reducing TNF-α by 60 ± 2% at the same concentration (50 μm). This confirmed a capacity to modulate inflammatory responses, likely by stabilising cell membranes and reducing endothelial permeability to inflammatory mediators. Of the compounds tested, rosmarinic acid (*Salvia officinalis*) showed the weakest activity, reducing TNF-α by only 45 ± 4%. Despite its strong antioxidant potential, its anti-inflammatory effects were less pronounced, possibly due to weaker interactions with transcription factors of inflammation.

Western blot analysis of key signalling proteins confirmed these findings. Curcumin significantly inhibited NF-κB activation (55 ± 4% reduction relative to LPS control), blocking nuclear translocation and suppressing transcription of pro-inflammatory cytokine and enzyme genes (e.g., iNOS, COX-2). Rutin reduced NF-κB activation by 40 ± 3%. Furthermore, curcumin strongly suppressed phosphorylation of p38 MAPK – a critical mediator that activates transcription via ATF-2 and Elk-1 – by 60 ± 5%, compared to 35 ± 3% for rutin. These data indicate that curcumin targets multiple signalling cascades, including both NF-κB- and MAPK-dependent pathways, consistent with its broad anti-inflammatory spectrum.

The obtained results confirmed that inhibition of NF-κB p65 activity closely correlates with the reduction in TNF-α levels (correlation coefficient $r = 0.92$, $p < 0.001$), indicating a causal relationship between the transcriptional activity of this factor and cytokine secretion. A high correlation was also established between the number of hydroxyl groups in the structure of phenolic compounds and the degree of IL-6 inhibition ($r = 0.89$, $p < 0.01$), highlighting

the important role of polar functional groups in ensuring the anti-inflammatory effect through antioxidant and signalling modulation. Thus, the study demonstratев a clear dose-dependent anti-inflammatory activity of phenolic extracts, with curcumin showing superiority due to its multi-component action on key cellular signalling pathways. These findings confirmed the hypothesis that the effectiveness of natural compounds in anti-inflammatory therapy is determined not only by their ability to neutralise free radicals, but also by their direct influence on transcriptional and post-transcriptional regulation of inflammatory processes.

Structure-function relationships of the phenolic compounds. An in-depth comparative analysis of the structural organisation of the phenolic compounds examined in this experiment revealed several patterns that determine their biological activity, particularly anti-inflammatory potential. The focus of the study was on functional groups, bond types, and the overall chemical architecture of the molecules, which are crucial for interaction with cellular molecular targets, membrane permeability, stability in biological fluids, and the ability to initiate cellular responses.

One of the most important factors defining antioxidant and anti-inflammatory activity is the presence of ortho-dihydroxyl groups in the aromatic ring. Curcumin, which contains two ortho-positioned OH groups on each phenolic fragment, exhibited 30% higher bioactivity compared to rosmarinic acid, which has only one such group. This structural feature greatly enhances the molecule's ability to chelate divalent metal ions (Fe^{2+}, Cu^{2+}), which in turn blocks the activity of metalloenzymes that catalyse ROS formation. Hence, ortho-hydroxyl groups not only enable direct neutralisation of free radicals but also modulate secondary signalling pathways associated with stress-induced transcription factor activation.

Another significant aspect is the degree of glycosylation, which determines the pharmacokinetic profile of the molecule. Rutin, a glycoside of quercetin, proved more stable in plasma, showing a prolonged half-life ($t_{1/2}$ = 4.2 h), more than double that of its aglycone form – quercetin ($t_{1/2}$ = 1.8 h). This stability is explained by the hydrophilic nature of the glycosidic moiety, which shields the reactive centres of the flavonoid structure from rapid metabolism. However, glycosylation markedly reduces membrane permeability, limiting diffusion across lipid bilayers of cellular membranes. This is evidenced by permeability coefficient (Papp) values: 2.1×10^{-6} cm/s for rutin compared with 8.7×10^{-6} cm/s for quercetin. Thus, there is a trade-off between metabolic stability and cellular availability, which is critical for optimising the structure of potential therapeutic agents.

Particular attention should also be drawn to the presence of conjugated π-systems in the structure of curcumin. Its system of conjugated double bonds between two phenolic rings facilitates delocalisation of π-electron density along the entire molecule. This not only enhances antioxidant potential but also promotes activation of apoptotic cascades in inflamed cells, since such delocalisation facilitates interactions with key cytoplasmic and nuclear proteins. Moreover, similar conjugated systems are capable of DNA intercalation and epigenetic modulation, which may also be relevant to chronic inflammatory processes. To illustrate these structure-function relationships, Table 2 compared inhibition of key pro-inflammatory cytokines and IC_{50} values for each of the three studied plants.

Table 2. Comparative effectiveness of plants

Plant	TNF-α inhibition (%)	IL-6 inhibition (%)	IC_{50} (μM)
Curcuma longa	72 ± 3	65 ± 2	18.4
Hypericum perforatum	60 ± 2	55 ± 3	32.7
Salvia officinalis	45 ± 4	38 ± 2	49.1

Source: compiled by the author

According to these data, *Curcuma longa* showed the lowest IC_{50}, reflecting high efficacy even at low concentrations – a direct result of its optimal structural configuration, particularly the combination of dihydroxyl groups, a conjugated bond system, and amphiphilic properties. By contrast, *Salvia officinalis*, despite possessing some active fragments, demonstrated the highest IC_{50}, indicating that higher concentrations are required to achieve a marked biological effect, and thus a lower therapeutic potential. Hence, structural features of phenolic compounds directly influence their bioactivity, and these relationships must be taken into account when designing natural anti-inflammatory agents with predictable pharmacodynamic and pharmacokinetic properties.

Hypothesis validation and mechanistic insights. The experimental results confirmed the initial hypothesis that the presence of ortho-dihydroxyl groups in phenolic structures is a key structural determinant of anti-inflammatory potential. Molecules with two adjacent hydroxyl groups on the benzene ring, such as curcumin, displayed significantly higher bioactivity due to their ability to stabilise phenoxyl radicals and efficiently chelate transition metal ions. These properties indirectly reduce ROS generation – a key promoter of the inflammatory response at the cellular level. In addition, experimental data confirmed the role of glycosylation as a determinant of the pharmacokinetic profile. Rutin, the glycosylated form of quercetin, demonstrated increased stability, resulting in a longer-lasting effect. At the same time, reduced membrane permeability of the glycosylated form suggests that glycosylation represents a balance factor between bioavailability and metabolic stability, requiring further optimisation to maximise therapeutic efficacy.

The study also confirmed that suppression of NF-κB is the principal molecular mechanism of anti-inflammatory action of the extracts, particularly curcumin and rutin. Decreased expression of NF-κB p65 correlated with reduced secretion of pro-inflammatory cytokines such as TNF-α and IL-6, consistent with the existing literature. However, inhibition of the MAPK pathway – particularly phosphorylated p38 – also proved significant, especially for curcumin, which exhibited a dual influence at both transcriptional

and signalling levels. This points to the multi-component nature of curcumin's action, making it a promising candidate for further investigation as a multifunctional modulator of inflammation.

Unplanned or unexpected observations, though not part of the initial hypothesis, are also of considerable importance for interpreting the results and generating new research questions. Notably, the extract of *Salvia officinalis*, despite showing the lowest activity in inhibiting pro-inflammatory cytokines, promoted an increase in the anti-inflammatory cytokine IL-10 by 20 ± 3%. This effect may indicate potential immunomodulatory properties of the extract, possibly via activation of regulatory T cells or modulation of alternative signalling pathways associated with TGF-β or IL-10-linked transcriptional profiles. This aspect merits further *in vivo* investigation, since IL-10 elevation can have both therapeutic and immunosuppressive effects depending on the inflammatory context.

Another important finding concerned the cytotoxic properties of curcumin at high concentrations. Specifically, at doses exceeding 100 µm, curcumin reduced the viability of RAW 264.7 cells by 40% (assessed by MTT assay: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). This indicated a narrow therapeutic window that must be considered when designing dosage regimens. The cytotoxic effect is likely associated with induction of the mitochondrial apoptotic pathway, which could be advantageous in anticancer therapy but poses potential risks for prolonged use in anti-inflammatory strategies. Thus, the experimental part of the study not only confirmed the pre-formulated hypotheses but also revealed new phenomena, indicating the complex, multi-component nature of plant phenol action.

DISCUSSION

The findings confirm a direct relationship between the chemical structure of phenolic compounds from *Curcuma longa*, *Hypericum perforatum* and *Salvia officinalis* and their anti-inflammatory activity. Specifically, the presence of ortho-dihydroxyl groups in the phenolic ring and the degree of glycosylation determine the effectiveness of inhibiting key inflammatory markers such as TNF-α and IL-6. These conclusions are consistent with the results of B.M. Razavi *et al.* [13], who showed that curcumin suppresses the NF-κB pathway, reducing pro-inflammatory cytokine levels. However, the present study extended understanding of curcumin's mechanism of action by identifying its ability to stabilise radicals through its conjugated π-system. This effect is supported by the findings of S.N.H. Jamil *et al.* [14], where structural analogues lacking ortho-dihydroxyl groups demonstrated significantly lower activity.

An important aspect is the cytotoxicity of curcumin at concentrations above 100 µm, confirming the observations of M. Cozmin *et al.* [15], who pointed to the narrow therapeutic window of this compound – the limited range between effective and toxic doses. Their study showed that curcumin, despite high bioactivity, can reduce cell viability at concentrations above 80-100 µm, particularly by inducing apoptosis through caspase-3 activation and mitochondrial membrane depolarisation. This creates potential risks in therapeutic use without prior standardisation of dosing. A similar effect was observed in the present study, where at 100 µm cell viability decreased by about 40%, underscoring the need for strict dose control during pharmaceutical development. Unlike T.S. Vo *et al.* [16], who focused on curcumin's antibacterial properties (notably against Staphylococcus aureus and Escherichia coli), this study examined its immunomodulatory potential in greater detail. It was found that curcumin at 50 µm significantly increased expression of IL-10, a key anti-inflammatory cytokine. This suggests that one mechanism of action may involve activation of regulatory T cells (Tregs), which play a role in dampening excessive inflammatory responses and establishing tolerance. However, the mechanism of IL-10 induction remains insufficiently studied and requires further exploration using specific signalling pathway inhibitors.

For *Hypericum perforatum*, a key finding was the high stability of rutin in plasma, consistent with data from Y. Tumbarski *et al.* [17], who reported a half-life of 4-5 hours depending on experimental conditions. This high stability is attributed to the glycosidic structure, which protects the compound against metabolic degradation. At the same time, the present study revealed markedly reduced permeability of rutin's glycosylated form across cell membranes ($Papp = 2.1 \times 10^{-6}$ cm/s), potentially limiting its bioavailability in target tissues. This phenomenon is linked to the high hydrophilicity of glycosidic forms, which poorly traverse lipid bilayers, hindering efficient intracellular delivery. Consequently, there is a need for nano-carriers or liposomal formulations to enhance rutin delivery in biological systems. This is particularly relevant in the context of gastrointestinal disorders, where pharmacological correction of peristalsis remains widespread, as evidenced by outpatient drug consumption data from Ukraine and neighbouring countries [18].

These observations are consistent with the review by E. Błońska-Sikora *et al.* [19], who emphasised the importance of optimising flavonoid pharmacokinetics using nanotechnological platforms such as polymeric nanoparticles, solid lipid nanoparticles, and microemulsions. They demonstrated that encapsulation of flavonoids in such systems can overcome cellular transport barriers, improve stability in biological environments, and enhance targeted delivery to inflamed tissues.

With regard to *Salvia officinalis*, the observed 20 ± 3% increase in IL-10 levels in macrophage cultures indicates an immunomodulatory effect not widely reported previously for this species in the context of anti-inflammatory activity. This finding contrasts with the results of M.Y. Boufadi *et al.* [20], where the main focus was antioxidant activity assessed via DPPH analysis, without in-depth exploration of cytokine profiles. Such differences may arise not only from variations in experimental models (*in vitro* vs *in vivo*) but also from methodological approaches to bioactive compound extraction. In the present study, unlike S. Đurović *et al.* [21], who applied hydrodistillation, maceration at 25°C for 48 hours was used, enabling better preservation of thermolabile components such as carnosol and carnosic acid.

Carnosol, as reported by M. Brindisi *et al.* [22], exhibits dual bioactivity – both as an antioxidant and as an antitumour agent, primarily due to its ability to inhibit phosphorylation of p38 MAPK, which is responsible for the transcriptional activation of pro-inflammatory genes. In

the experimental model of the present study, a significant reduction in phosphorylated p38 levels by 18 ± 2% was observed, indicating a potential contribution of this component to the mechanism of action of S. officinalis extract. Compared with the study of R. Mokhtari *et al.* [23], which investigated the antimicrobial properties of sage against pathogenic strains of *Candida* spp. and *Staphylococcus aureus*, the results obtained here broaden the spectrum of sage's bioactivity, highlighting its ability to influence both pro- and anti-inflammatory pathways of the immune response. In particular, the increase in IL-10 may result from activation of the transcription factor STAT3, or be a secondary effect of NF-κB inhibition, which is consistent with hypotheses about the synergistic action of phenolic acids and terpenes within the plant extract. This approach is corroborated by G. Margetts *et al.* [24], who found that a combination of phenolic compounds (rosmarinic acid, caffeic acid) and monoterpenes (eucalyptol, thujone) in *Salvia officinalis* significantly enhances inhibition of cyclooxygenase-2 (COX-2) activity, which plays a key role in prostaglandin synthesis during inflammation.

In the present study, a similar decrease in COX-2 expression by 25 ± 4% was recorded, along with suppression of NF-κB activity, confirming the presence of a multi-target mechanism of action of the extract. Moreover, rosmarinic acid, the dominant component of sage extract, is known for its ability to inhibit nuclear translocation of the NF-κB p65 subunit, thereby blocking transcription of pro-inflammatory mediator genes (TNF-α, IL-1β). This ensures a balanced immune effect that does not cause excessive immunosuppression but promotes the development of a regulatory anti-inflammatory response, in particular via IL-10. Overall, the results confirmed that the effect of *Salvia officinalis* cannot be reduced to a single molecule or function – its pharmacological activity is the outcome of a complex interplay of multiple bioactive components exerting both antioxidant and modulatory influences on cellular inflammatory signalling pathways. This underscored the importance of a phytocomplex approach in the study of medicinal plants and the need for research targeting cellular markers and transcriptional cascades, particularly MAPK/NF-κB/STAT3.

Despite the considerable progress achieved, there are limitations linked to the experimental conditions. Firstly, the study was conducted *in vitro*, which does not account for the influence of *in vivo* metabolic processes on bioavailability, as noted in the works of T.S. Vo *et al.* [16] and F. Righi *et al.* [25]. Secondly, the concentrations of extracts used (10-100 μm) may not reflect physiological conditions, thereby limiting clinical interpretation. This issue is also relevant to the research of Y. Sharma *et al.* [26], where high doses induced cytotoxicity. In the wider research context, the results obtained here complement the findings of E. Poulios *et al.* [27] on the antioxidant potential of sage and highlight the need for integration of structural analysis into phytopharmacology. At the same time, they reveal discrepancies with some previous studies, particularly regarding the role of glycosylation, which requires further clarification. For example, the results of M. Peić Tukuljac *et al.* [28] indicated reduced bioavailability of glycosides, whereas the present study established that glycosylation protects the molecule from rapid metabolism, thereby prolonging its half-life.

M. Khatun *et al.* [29] found that different turmeric species from Bangladesh vary in their curcuminoid content, which directly correlates with their ability to neutralise free radicals and suppress inflammation. The present study also confirmed that curcumin is the key component of *Curcuma longa* responsible for its effectiveness, particularly through NF-κB inhibition. However, unlike the study of M. Khatun *et al.*, which focused on antibacterial activity, the main emphasis here was on immune modulation via increased IL-10 levels, expanding understanding of turmeric's therapeutic potential. For *Hypericum perforatum*, the findings on the high stability of rutin and its role in suppressing pro-inflammatory cytokines are consistent with the work of M. Novelli *et al.* [30], who demonstrated that hypericin and hyperforin from St John's wort inhibit inflammatory signalling pathways associated with diabetes. However, in the present study it was observed that flavonoid glycosylation, such as in rutin, not only enhances stability but also limits membrane permeability, potentially necessitating auxiliary methods to improve bioavailability.

These findings complement the work of P. Rychlewski *et al.* [31], who compared commercial and wild samples of St John's wort: although both contained high levels of bioactive compounds, the present study emphasises the need for extract standardisation to ensure consistent pharmacological effects. Regarding *Salvia officinalis*, the data obtained on its ability to modulate IL-10 levels and inhibit p38 MAPK phosphorylation align with the research of E. Napoli *et al.* [32], who analysed hydrodistillation by-products of *Lamiaceae* plants. They found that phenolic components in these by-products retain antioxidant activity, underscoring the importance of rational utilisation of plant raw material. In contrast to E. Napoli *et al.*, the present work employed maceration to extract thermolabile compounds such as rosmarinic acid, which preserved their bioactivity and demonstrated a comprehensive effect on inflammatory pathways. The results of the study showed that the anti-inflammatory activity of the investigated plants is determined by their structural characteristics, in particular the presence of ortho-dihydroxyl groups and glycosylation

The results of the study confirmed that the anti-inflammatory activity of *Curcuma longa*, *Hypericum perforatum* and *Salvia officinalis* extracts is associated with the structural features of phenolic compounds, in particular the presence of ortho-dihydroxyl groups and glycosylated forms. It has been established that these factors affect the levels of cytokines TNF-α, IL-6 and IL-10, as well as the activity of key signalling pathways, in particular NF-κB and p38 MAPK. The data obtained confirm that the pharmacological effect of the studied plants cannot be reduced to individual molecules, as it is the result of the complex action of several bioactive components.

CONCLUSIONS

The study confirmed that the anti-inflammatory and antioxidant activities of phenolic compounds from medicinal plants directly depend on their chemical structure. Twelve bioactive compounds were identified, among which curcumin from *Curcuma longa* demonstrated the highest efficacy. Due to the presence of two ortho-dihydroxyl groups and a conjugated π-system, it inhibited TNF-α by 72 ± 3%

and IL-6 by 65 ± 2% (at 50 μm), primarily via inhibition of NF-κB (55 ± 4%) and p38 MAPK (60 ± 5%). These results highlighted the role of structural features such as hydroxyl group positioning and glycosylation in determining biological activity. For instance, rutin, a quercetin glycoside, proved more stable in plasma (half-life 4.2 h) but less permeable across cell membranes compared with its aglycone.

The practical significance of the study lied in substantiating the use of *Curcuma longa* as a basis for phytopharmaceutical development. The high activity of curcumin, its ability to influence several signalling pathways simultaneously, and its low IC_{50} (18.4 μm) make it a promising candidate for chronic inflammation therapy. Special attention should be paid to the unexpected effect of *Salvia officinalis* extract, which increased the level of the anti-inflammatory cytokine IL-10 by 20 ± 3%, suggesting immunomodulatory properties. To further develop these findings, preclinical trials on animal models are recommended to assess the *in vivo* efficacy and safety of curcumin.

Nevertheless, the study has limitations. Firstly, experiments were conducted *in vitro*, which does not reflect the influence of *in vivo* metabolism on compound bioavailability. Secondly, curcumin exhibited cytotoxicity at concentrations above 100 μm, reducing cell viability by 40%, indicating a narrow therapeutic window. Thirdly, synergistic effects between extract components, which may enhance or diminish their activity, were not investigated. A promising direction would be the development of methods to improve bioavailability, such as lipid nano-carriers or micellisation. Furthermore, interactions between phenolic compounds within complex extracts should be studied, as their synergism may open up new therapeutic opportunities. Special attention should also be given to exploring immunomodulatory potential, particularly mechanisms underlying the increase in IL-10 observed in *Salvia officinalis*.

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REFERENCES
[1] Liu W, Chen X, Li H, Zhang J, An J, Liu X. Anti-inflammatory function of plant-derived bioactive peptides: A review. Foods. 2022;11(15):2361. DOI: 10.3390/foods11152361
[2] Lopez-Corona AV, Valencia-Espinosa I, González-Sánchez FA, Sánchez-López AL, Garcia-Amezquita LE, Garcia-Varela R. Antioxidant, anti-inflammatory and cytotoxic activity of phenolic compound family extracted from raspberries (*Rubus idaeus*): A general review. Antioxidants. 2022;11(6):1192. DOI: 10.3390/antiox11061192
[3] Rakha A, Umar N, Rabail R, Butt MS, Lieliszek M, Hassoun A, et al. Anti-inflammatory and anti-allergic potential of dietary flavonoids: A review. Biomed Pharmacother. 2022;156:113945. DOI: 10.1016/j.biopha.2022.113945
[4] Soroka OV, Prokopiak MZ, Hrytsak LR, Drobyk NM. Biologically active compounds in species of *Carlina l.* genus of flora of Ukraine. Sci Iss Ternopil Volodymyr Hnatiuk Nat Ped Univ Series Biol. 2024;84(3-4):89–99. DOI: 10.25128/2078-2357.24.3-4.10
[5] Jongrungraungchok S, Madaka F, Wunnakup T, Sudsai T, Pongphaew C, Songsak T, et al. *In vitro* antioxidant, anti-inflammatory, and anticancer activities of mixture Thai medicinal plants. BMC Complement Med Ther. 2023;23:43. DOI: 10.1186/s12906-023-03862-8
[6] Borysiuk I, Valivodz I, Akisheva A, Molodan Y, Markova I, Saprunova V. Prediction the biological activity of substances from medicinal plant raw materials by the *in silico* method with evaluation of extraction efficiency of various types of extractions. SWorldJ. 2022;3(11-03):76–83. DOI: 10.30888/2663-5712.2022-11-03-094
[7] Shahzad F, Anderson D, Najafzadeh M. The antiviral, anti-inflammatory effects of natural medicinal herbs and mushrooms and SARS-CoV-2 infection. Nutrients. 2020;12(9):2573. DOI: 10.3390/nu12092573
[8] Mssillou I, Bakour M, Slighoua M, Laaroussi H, Saghrouchni H, Ez-Zahra Amrati F, et al. Investigation on wound healing effect of Mediterranean medicinal plants and some related phenolic compounds: A review. J Ethnopharmacol. 2022;298:115663. DOI: 10.1016/j.jep.2022.115663
[9] Toma L, Sanda GM, Niculescu LS, Deleanu M, Sima AV, Stancu CS. Phenolic compounds exerting lipid-regulatory, anti-inflammatory and epigenetic effects as complementary treatments in cardiovascular diseases. Biomolecules. 2020;10(4):641. DOI: 10.3390/biom10040641
[10] Nwozo OS, Effiong EM, Aja PM, Awuchi CG. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. Int J Food Prop. 2023;26(1):359–88. DOI: 10.1080/10942912.2022.2157425
[11] DSTU ISO 22000:2019. Food safety management systems. Requirements for any organization in the food chain (ISO 22000:2018, IDT) [Internet]. 2019 October 31 [cited 2025 March 13]. Available from: https://surl.li/fsemnt
[12] European Pharmacopoeia [Internet]. 2022 December [cited 2025 March 13]. Available from: https://surl.li/rahzrm
[13] Razavi BM, Ghasemzadeh Rahbardar M, Hosseinzadeh H. A review of therapeutic potentials of turmeric (*Curcuma longa*) and its active constituent, curcumin, on inflammatory disorders, pain, and their related patents. Phytother Res. 2021;35(12):6489–513. DOI: 10.1002/ptr.7224
[14] Jamil SNH, Ali AH, Feroz SR, Lam SD, Agustar HK, Mohd Abd Razak MR, et al. Curcumin and its derivatives as potential antimalarial and anti-inflammatory agents: A review on structure-activity relationship and mechanism of action. Pharmaceuticals. 2023;16(4):609. DOI: 10.3390/ph16040609
[15] Cozmin M, Lungu II, Gutu C, Stefanache A, Duceac LD, Şoltuzu BD, et al. Turmeric: From spice to cure. A review of the anti-cancer, radioprotective and anti-inflammatory effects of turmeric sourced compounds. Front Nutr. 2024;11:1399888. DOI: 10.3389/fnut.2024.1399888

[16] Vo TS, Vo TTBC, Vo TTTN, Lai TNH. Turmeric (*Curcuma longa* L.): Chemical components and their effective clinical applications. J Turk Chem Soc Sect A Chem. 2021;8(3):883–98. DOI: 10.18596/jotcsa.913136
[17] Tumbarski Y, Ivanov I, Todorova M, Gerasimova A, Dincheva I, Makedonski L, et al. Chemical composition and biological activities of St John's Wort (*Hypericum perforatum* L.) essential oil from Bulgaria. Appl Sci. 2024;14(24):11754. DOI: 10.3390/app142411754
[18] Gerasymova O, Iakovlieva L, Tkachova O. Analysis of outpatient consumption of propulsives in Ukraine compared with Norway and the Baltic states. Ukr J Med Biol Sport. 2025;10(1):8–15. DOI: 10.63341/ujmbs/1.2025.08
[19] Błońska-Sikora E, Zielińska A, Dobros N, Paradowska K, Michalak M. Polyphenol and flavonoid content and antioxidant activity of *Hypericum perforatum* L. (St. John's Wort) extracts for potential pharmaceutical and cosmetic applications. Appl Sci. 2025;15(5):2590. DOI: 10.3390/app15052590
[20] Boufadi MY, Keddari S, Moulaihacene F, Chaa S. Chemical composition, antioxidant and anti-inflammatory properties of *Salvia officinalis* extract from Algeria. Pharmacog J. 2021;13(2):506–15. DOI: 10.5530/pj.2021.13.64
[21] Đurović S, Micić D, Pezo L, Radić D, Bazarnova JG, Smyatskaya YA, et al. The effect of various extraction techniques on the quality of sage (*Salvia officinalis* L.) essential oil, expressed by chemical composition, thermal properties and biological activity. Food Chem X. 2022;13:100213. DOI: 10.1016/j.fochx.2022.100213
[22] Brindisi M, Bouzidi C, Frattaruolo L, Loizzo MR, Cappello MS, Dugay A, et al. New insights into the antioxidant and anti-inflammatory effects of Italian *Salvia officinalis* leaf and flower extracts in lipopolysaccharide and tumor-mediated inflammation models. Antioxidants. 2021;10(2):311. DOI: 10.3390/antiox10020311
[23] Mokhtari R, Fard MK, Rezaei M, Moftakharzadeh SA, Mohseni A. Antioxidant, antimicrobial activities, and characterization of phenolic compounds of thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and thyme-sage mixture extracts. J Food Qual. 2023;2023(1):2602454. DOI: 10.1155/2023/2602454
[24] Margetts G, Kleidonas S, Zaibi NS, Zaibi MS, Edwards KD. Evidence for anti-inflammatory effects and modulation of neurotransmitter metabolism by *Salvia officinalis* L. BMC Complement Med Ther. 2022;22:131. DOI: 10.1186/s12906-022-03605-1
[25] Righi N, Boumerfeg S, Deghima A, Fernandes PAR, Coelho E, Baali F, et al. Phenolic profile, safety assessment, and anti-inflammatory activity of *Salvia verbenaca* L. J Ethnopharmacol. 2021;272:113940. DOI: 10.1016/j.jep.2021.113940
[26] Sharma Y, Velamuri R, Fagan J, Schaefer J, Streicher C, Stimson J. Identification and characterization of polyphenols and volatile terpenoid compounds in different extracts of garden sage (*Salvia officinalis* L.). Pharmacognosy Res. 2020;12(2):149–57. DOI: 10.4103/pr.pr_92_19
[27] Poulios E, Giaginis C, Vasios GK. Current state of the art on the antioxidant activity of sage (*Salvia* spp.) and its bioactive components. Planta Med. 2020;86(4):224–38. DOI: 10.1055/a-1087-8276
[28] Peić Tukuljac M, Prvulović D, Gvozdenac S. The influence of extraction solvents on the antioxidant potential of St. John's wort (*Hypericum perforatum* L.). In: Proceedings of the 10th international symposium on agricultural sciences "AgroReS 2021". Banja Luka: University of Banja Luka; 2021. P. 69–77.
[29] Khatun M, Nur MA, Biswas S, Khan M, Amin MZ. Assessment of the anti-oxidant, anti-inflammatory and anti-bacterial activities of different types of turmeric (*Curcuma longa*) powder in Bangladesh. J Agricult Food Res. 2021;6:100201. DOI: 10.1016/j.jafr.2021.100201
[30] Novelli M, Masiello P, Beffy P, Menegazzi M. Protective role of St. John's Wort and its components hyperforin and hypericin against diabetes through inhibition of inflammatory signaling: Evidence from *in vitro* and *in vivo* studies. Int J Mol Sci. 2020;21(21):8108. DOI: 10.3390/ijms21218108
[31] Rychlewski P, Kamgar E, Mildner-Szkudlarz S, Kowalczewski PŁ, Zembrzuska J. Determination of the contents of bioactive compounds in St. John's wort (*Hypericum perforatum*): Comparison of commercial and wild samples. Open Chem. 2023;21(1):20220347. DOI: 10.1515/chem-2022-0347
[32] Napoli E, Ruberto G, Carrubba A, Sarno M, Muscarà C, Speciale A, et al. Phenolic profiles, antioxidant and anti-inflammatory activities of hydrodistillation wastewaters from five lamiaceae species. Molecules. 2022;27(21):7427. DOI: 10.3390/molecules27217427

Хімічна структура та протизапальні механізми дії фенольних сполук лікарських рослин

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Анотація. Дослідження було спрямоване на вивчення зв'язку між хімічною структурою фенольних сполук лікарських рослин *Curcuma longa*, *Hypericum perforatum* та *Salvia officinalis* та їхньою протизапальною активністю. Робота виконана на базі Навчально-наукового інституту прикладної фармації Національного фармацевтичного університету (м. Харків, Україна), протягом вересня-грудня 2024 року. За допомогою високоефективної рідинної хроматографії та мас-спектрометрії визначено 12 біоактивних сполук, включаючи куркумін, рутин і розмаринову кислоту. Експерименти на клітинах макрофагів показали, що куркумін знижує рівень фактора некрозу пухлин-альфа на 72 ± 3 % та інтерлейкіну-6 на 65 ± 2 % (при концентрації 50 мкМ) через пригнічення транскрипційного фактора каппа-В (55 ± 4 %) та кінази p38 (60 ± 5 %). Рутин, незважаючи на стабільність у плазмі крові (період напіврозпаду 4,2 години), мав нижчий коефіцієнт проникності через клітинні мембрани ($2{,}1\times10^{-6}$ см/с). Екстракт шавлії лікарської підвищив рівень антизапального інтерлейкіну-10 на 20 ± 3 %, а кореляція між кількістю гідроксильних груп у молекулах та інгібуванням інтерлейкіну-6 склала 0,89. Виявлено, що куркумін проявляє цитотоксичність при концентраціях понад 100 мкМ, знижуючи життєздатність клітин на 40 %. Результати підтвердили, що протизапальний ефект залежить від наявності орто-дигідроксильних груп та глікозидації, причому найвищий потенціал виявлено у куркуми. Отримані дані підкреслили важливість структурного аналізу та комплексних методів для розробки рослинних препаратів із цілеспрямованою дією. Результати дослідження можуть бути використані фармацевтичними компаніями та науково-дослідними лабораторіями при створенні нових протизапальних засобів природного походження

Ключові слова: шавлія лікарська; куркумін; звіробій звичайний; орто-дигідроксильні групи; фармакокінетичний профіль

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Peculiarities of degenerative changes in the proximal tibia according to knee joint morphotype

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Abstract. This study aimed to identify the characteristics of degenerative changes in the proximal tibia, taking into account the morphological variant of the knee joint. The analysis was based on radiographic examinations of 100 cases of gonarthrosis, predominantly affecting the medial compartment. The mean age of the patients was 63.56 ± 8.10 years. Among the radiographic criteria assessed were the anatomical medial proximal tibial angle (MPTA) and the femorotibial angle (FTA). In addition, morphometric parameters of the proximal tibia were evaluated relative to an axis drawn from the intercondylar eminence to the tibial condyles (LTCA, LTPA, MTPA, MTCA), as well as relative to an axis tangential to the condyles (LTCA2, LTPA2, MTPA2, MTCA2). The morphotypes of the knee joints were determined according to a proprietary cluster-based classification system. Statistical analysis was conducted using Statistica 13 software. Statistical significance was set at $p \leqslant 0.05$. Changes in LTCA and LTCA2 observed in patients with morphotypes I, II, and III indicated a medial deviation of the tibial axis. In patients with valgus deviation of the lower limb axis and knee joint morphotype IV, the decrease in LTCA and LTCA2 values indicated further destruction of the bone and cartilage tissues of the tibial condyle within the load-bearing area of the joint. It also reflected a shift of the axis tangential to the tibial condyles medially and downward, along with an upward deviation of the axis drawn from the intercondylar eminence to the lateral condyle. Changes in the LTPA, LTPA2, MTPA, and MTPA2 angles suggested a medial deviation of the tibia. An analysis of the MTCA and MTCA2 angles in patients with morphotypes II, III, and IV confirmed a medial shift of the tibia. Conversely, a decrease in MTCA and MTCA2 values in patients with morphotype I indicated further destruction of the bone and cartilage tissues of the medial condyle, as well as a downward and medial displacement of both the axis drawn from the intercondylar eminence to the medial condyle and the axis tangential to the condyles. These findings demonstrated a variation in the morphometric parameters of the proximal tibia depending on the identified knee joint morphotype in the context of degenerative-dystrophic conditions. The results may support the refinement of personalised approaches to orthopaedic interventions in degenerative dystrophic diseases of the knee joint

Keywords: osteoarthritis; degenerative-dystrophic diseases; morphology; radiographic parameters; gonarthrosis

INTRODUCTION

The study of anatomical variability in the proximal tibia, both under normal conditions and in the presence of degenerative-dystrophic diseases, is of significant relevance to orthopaedic practice. Assessing the shape of the tibia and the spatial relationships within the knee joint is essential for understanding the pathogenesis of degenerative-dystrophic diseases, identifying factors contributing to their progression, and selecting the most appropriate treatment method. An analysis of the morphological features of degenerative-dystrophic changes in the proximal tibia will support the development of individualised implants and enhance the personalised planning of surgical interventions such as proximal tibial osteotomy and total knee arthroplasty.

Degenerative-dystrophic joint diseases represent one of the most pressing challenges in modern orthopaedics due to their high prevalence and progressive nature. They are considered among the leading causes of chronic pain,

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functional limitations, and disability in the working-age population. Among all joints of the human musculoskeletal system, the knee is the most frequently affected by degenerativedystrophic changes [1-3]. Gonarthrosis is a comprehensive joint pathology characterised by structural alterations in the articular cartilage, subchondral bone, Hoffa's fat pad, synovial membrane, ligaments, and muscles [4, 5]. Therefore, the study of morphological changes in the knee joint under degenerative-dystrophic conditions has become a subject of considerable scientific interest.

In most cases, tissue degeneration begins in the medial compartment of the knee joint and, as the pathological process progresses, leads to disruption of the lower limb axis [6]. In the pathogenesis of gonarthrosis, particular attention should be paid to the condition of the proximal tibia, as its morphological changes determine the nature and severity of the resulting deformity. Understanding the anatomical variability and mechanisms of disease progression is key to improving existing treatment methods and developing more effective approaches for the management and prevention of gonarthrosis. The considerable variability of joint structures has been confirmed by the findings of A. Mullaji *et al*. [7], who, based on the analysis of 2,129 orthoradiographs, identified four phenotypic variations of knee osteoarthritis associated with varus alignment of the lower limb. The research group also demonstrated morphological variability in knee joints with valgus deviation.

In a separate study, A. Mullaji *et al*. [8] analysed orthoradiographs of 233 knee joints exhibiting valgus alignment and identified nine phenotypic variants of valgus knee osteoarthritis. Similarly, in a study by D. Yang *et al*. [9], five subtypes of valgus knee deformities were established based on the analysis of radiographs from 105 cases with valgus lower limb alignment. According to the researchers, the key factors contributing to the formation of distinct valgus knee morphologies include the pattern of local bone loss and deformation of the lateral tibial plateau, with or without valgus angulation at the level of the metaphysis.

Degenerative-dystrophic diseases of the knee joint affect not only the articular surface of the tibia but also cause significant deformation of the metaepiphyseal region. In a study by J. Tomczyk *et al*. [1], based on the analysis of computed tomography data from 23 patients with knee osteoarthritis and varus deformity, high variability was observed in the structural changes of the proximal epiphysis and metaepiphysis of the tibia. Using mathematical modelling, the researchers demonstrated significant differences in the three-dimensional dimensions of the tibial plateau, its inclination angle, and the medial and lateral tibial condyles, depending on body side and sex in patients with knee osteoarthritis and varus deformity. The study also confirmed a higher risk of varus osteoarthritis in the presence of deformation of the proximal tibial metaepiphysis.

Similar findings were reported by T. Ishibashi *et al*. [10], who used a three-dimensional (3D) model to demonstrate variations in the shape of the proximal tibia based on data from 31 patients with symptomatic knee osteoarthritis. The researchers found lower variability in the medial proximal cortical layer of the tibial metaphyseal region compared to the values for the tibial tuberosity area ($p = 0.004$) and the lateral cortex ($p = 0.020$). This was attributed to the greater load-bearing role of the medial compartment of the knee joint in cases of varus deviation of the lower limb axis. In contrast, the authors reported greater variability in the shape of the medial tibial plateau (1.46 mm) compared to the lateral plateau (1.16 mm) ($p = 0.044$), and noted less damage to the lateral compartment of the knee joint in patients with degenerative-dystrophic diseases. The researchers found that, as osteoarthritis progresses, cartilage destruction and bone erosion typically begin in the anteromedial area of the tibial plateau, with the pathological process subsequently extending posteriorly and laterally. However, it remains unclear whether changes in the proximal tibial metaepiphysis are a cause or a consequence of osteoarthritis.

This issue was addressed in a study by S. Kuriyama *et al*. [11], who used mathematical modelling of open wedge osteotomy with programmed values of the medial proximal tibial angle (MPTA) ranging from 90° to 97° in 1° increments. In all modelled scenarios, higher peak contact forces during walking were recorded in the lateral compartment of the knee joint compared to the medial one. The researchers demonstrated that an increase in MPTA is associated with excessive elevation of contact forces in the medial compartment and increased tension in the medial collateral ligament during knee flexion. The authors also observed non-physiological anterior rolling of the lateral femoral condyle during squatting, and the absence of the "screw-home" mechanism during knee extension in models with elevated MPTA values.

Morphological differences in the proximal tibia have been confirmed by the findings of J. Itou *et al*. [12]. Based on radiographic analysis of 109 knee joints, the authors identified three main types of tibial plateau shapes, according to the inclination of the medial and lateral articular surfaces. The majority of cases – 52 knees (47.71%) – exhibited a flat plateau (inclination up to 3°); a depressed shape was observed in 38 cases (34.86%), while a convex or "pagoda-shaped" form was identified in 19 knees (17.43%). The researchers highlighted the clinical relevance of analysing the morphology of the proximal tibia to improve outcomes of orthopaedic interventions for degenerative-dystrophic diseases of the knee joint.

Thus, most researchers share the view that a pronounced inclination of the tibial articular surface, as determined in the frontal plane, is associated with increased loading of the medial compartment of the knee joint, while the lateral compartment is subject to less stress. However, despite the available evidence, the influence of proximal tibial joint surface inclination on knee joint biomechanics remains insufficiently explored. This study aimed to characterise the morphological features of the proximal tibia in patients with degenerative-dystrophic diseases of the knee joint, according to knee joint morphotypes determined using a proprietary cluster-based classification system.

MATERIALS AND METHODS

This observational cross-sectional study analysed the radiographic findings of 100 cases of degenerativedystrophic knee joint disease, predominantly affecting the medial compartment, in 70 patients who received inpatient treatment at the Municipal non-commercial enterprise Vinnytsia City Clinical Emergency Hospital between 2017 and 2025. The mean age was 63.56 ± 8.10 years. The study group

included 26 men (37.14%) and 44 women (62.86%). Unilateral degenerative-dystrophic involvement of the knee joint was identified in 40 patients (57.14%), while a bilateral pattern was recorded in 30 cases (42.86%).

Inclusion criteria for the study were as follows: primary knee osteoarthritis (osteoarthritis), Kellgren-Lawrence grade II-III, with predominant medial compartment involvement; secondary knee osteoarthritis with medial compartment predominance, including cases following aseptic necrosis of the medial femoral condyle; satisfactory condition of the lateral compartment of the knee joint (intact meniscus and full-thickness articular cartilage); integrity of the knee ligamentous apparatus; ability to fully extend the knee joint or presence of a flexion contracture of less than 10°. Exclusion criteria for the study included: total involvement of all compartments of the knee joint by the degenerative-dystrophic process; secondary post-traumatic osteoarthritis following a tibial plateau fracture; previous surgical interventions involving the proximal tibia (excluding arthroscopic meniscectomy); instability of the knee ligamentous apparatus; presence of a flexion contracture greater than 10°; secondary osteoarthritis caused by dysplastic bone changes, metabolic or other disorders (e.g. ochronosis, Gaucher's disease, Paget's disease, osteopetrosis); active infectious processes or latent infection.

The assessment of degenerative changes in the proximal tibia was carried out using standard (short) radiographs of the knee joints, taken in the anteroposterior projection under weight-bearing conditions. Among the radiographic criteria, the MPTA was evaluated. It was defined as the angle between a line tangential to the proximal articular surface of the tibia and the anatomical axis of the tibia, measured from the medial side [13]. The angle between the anatomical axes of the femur and tibia was defined as the femorotibial angle (FTA). In addition to standard parameters, morphometric measurements of the proximal tibia were taken concerning the axis drawn from the intercondylar eminence to the tibial condyles (Fig. 1), including:

- lateral tibial condylar angle (LTCA) – the angle between the line drawn from the intercondylar eminence to the lateral tibial condyle and the line tangential to the outer surface of the lateral tibial condyle;
- lateral tibial plateau angle (LTPA) – the angle between the line drawn from the intercondylar eminence to the lateral tibial condyle and a line parallel to the longitudinal axis of the tibia that intersects the horizontal axis at its central point;
- medial tibial plateau angle (MTPA) – the angle between the line drawn from the intercondylar eminence to the medial tibial condyle and a line parallel to the longitudinal axis of the tibia that intersects the horizontal axis at its central point;
- medial tibial condylar angle (MTCA) – the angle between the line drawn from the intercondylar eminence to the medial tibial condyle and the line tangential to the inner surface of the medial tibial condyle.

Morphometric parameters of the proximal tibia were also evaluated in relation to the axis tangential to the tibial condyles (Fig. 2):

- LTCA2 – the angle between the line drawn along the proximal articular surface of the tibia and the line tangential to the outer surface of the lateral tibial condyle;
- LTPA2 – the angle between the line drawn along the proximal articular surface of the tibia and a line parallel to the longitudinal axis of the tibia that intersects the horizontal axis at the midpoint between the intercondylar eminence and the outermost point of the lateral tibial condyle;
- MTPA2 – the angle between the line drawn along the proximal articular surface of the tibia and a line parallel to the longitudinal axis of the tibia that intersects the horizontal axis at the midpoint between the intercondylar eminence and the medial edge of the tibial condyle;
- MTCA2 – the angle between the line drawn along the proximal articular surface of the tibia and the line tangential to the inner surface of the medial tibial condyle.

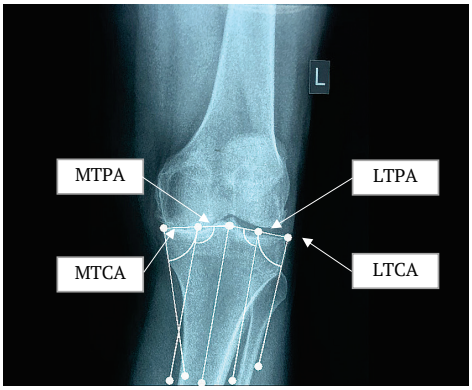


Figure 1. Anteroposterior radiograph of the left knee joint of patient M., aged 49
Note: diagnosis – primary osteoarthritis of the left knee, grade III, predominantly affecting the medial compartment. Knee joint morphotype – type II. LTCA – 88.6°; LTPA – 91.9°; MTPA – 102.6°; MTCA – 83.9°
Source: author's photo

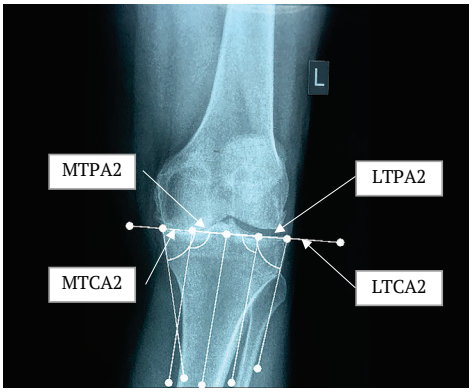


Figure 2. Anteroposterior radiograph of the left knee joint of patient M., aged 49
Note: diagnosis – primary osteoarthritis of the left knee, grade III, predominantly affecting the medial compartment. Knee joint morphotype – type II. LTCA2 – 81.6°; LTPA2 – 83.6°; MTPA2 – 93.0°; MTCA2 – 76.6°
Source: author's photo

The morphotypes of knee joints affected by the degenerative-dystrophic process were determined using a proprietary cluster classification system [14]. The study

was conducted in accordance with the ethical principles of the Declaration of Helsinki by the World Medical Association [15], the Convention on Human Rights and Biomedicine [16], as well as the applicable national ethical standards of Ukraine [17]. All participants were informed about their involvement in the study and provided written informed consent. To ensure confidentiality, all personal data of the patients examined were anonymised.

Statistical analysis of the numerical data was performed using the software package Statistica 13. Descriptive statistical methods were used to evaluate and analyse quantitative data. Continuous variables are presented as mean ± standard deviation (M ± SD), while categorical variables are expressed as absolute numbers of observations (n) and corresponding percentages (%). For comparisons between independent groups, the non-parametric Kruskal-Wallis test was applied. The functional relationship between variables was assessed using Kendall's tau (τ) rank correlation coefficient. The predictive value of the analysed parameters for determining the morphotype of the knee joint affected by degenerativedystrophic changes was evaluated using a binary logistic regression model, with odds ratios (OR) and 95% confidence intervals (CI) calculated. Statistical significance for predictive accuracy was set at $p \leqslant 0.05$.

RESULTS AND DISCUSSION

According to the proprietary cluster classification system [14], four groups were identified among the examined patients, corresponding to four morphological variants of knee joint structure in the context of degenerative-dystrophic disease. Morphotype I was identified in 21 patients (21.00%), morphotype II in 38 (38.00%), morphotype III in 29 (29.00%), and morphotype IV in 12 (12.00%). The mean FTA among all patients was 177.09° ± 5.57°. In patients with morphotype I, the mean FTA was 169.76° ± 1.48°, indicating varus deviation of the lower limb axis. Varus deviation of the limb axis was also observed in patients with morphotype II, whose mean FTA was 175.58° ± 1.32°. In patients with morphotype III, the mean FTA was 180.14° ± 1.49°, corresponding to a neutral lower limb alignment. Among individuals with morphotype IV, the mean value of the measured angle was 187.33° ± 3.75°, indicating valgus deviation of the axis. A comparison of the mean FTA values across the groups revealed a statistically significant difference ($p < 0.0001$).

Assessment of the morphometric parameters of the proximal tibia relative to the axis extending from the intercondylar eminence to the tibial condyles showed that the mean LTCA among all participants was 89.31° ± 7.09°. The lowest angle values were recorded in individuals with morphotype I – 86.12° ± 7.11°; in patients with morphotype II, the mean LTCA was 88.87° ± 6.09°; in those with morphotype IV – 88.17° ± 7.08°; and the highest values were observed in individuals with morphotype III – 92.67° ± 7.26°. The differences between these values were statistically significant ($p = 0.02$) (Table 1). No significant correlation was found between LTCA and FTA values ($\tau = +0.13$, $p = 0.05$).

Table 1. Characteristics of morphometric parameters of the proximal tibia relative to the axis drawn from the intercondylar eminence to the tibial condyles

	Variants of knee joint morphotypes				p
	I (n = 21)	II (n = 38)	III (n = 29)	IV (n = 12)	
LTCA	86.12 ± 7.11°	88.87 ± 6.09°	92.67 ± 7.26°	88.17 ± 7.08°	0.02*
LTPA	89.57 ± 5.47°	93.50 ± 3.79°	95.52 ± 2.97°	99.83 ± 4.97°	<0.00001*
MTPA	105.67 ± 6.69°	101.79 ± 10.07°	99.90 ± 7.22°	98.83 ± 4.59°	<0.00001*
MTCA	77.74 ± 6.14°	83.32 ± 6.33°	80.31 ± 6.67°	79.17 ± 4.43°	0.02*

Note: * – statistically significant difference at $p \leqslant 0.05$
Source: developed by the author

Analysis of the LTPA values demonstrated a statistically significant difference depending on the morphological characteristics of the knee joint structure in degenerative-dystrophic conditions ($p < 0.00001$). The highest LTPA values were observed in individuals with morphotype IV – 99.83° ± 4.97°, followed by patients with morphotype III – 95.52° ± 2.97°, morphotype II – 93.50° ± 3.79°, and morphotype I – 89.57° ± 5.47°. The overall mean LTPA among patients was 94.02° ± 5.07°. Moreover, higher LTPA values were significantly associated with higher FTA values, as confirmed by a direct correlation between the indicators ($\tau = +0.36$, $p < 0.0000001$).

The mean value of the MTPA among the participants was 101.7° ± 8.33°. The highest MTPA values were observed in patients with morphotype I – 105.67° ± 6.69°, followed by morphotype II – 101.79° ± 10.07°, morphotype III – 99.90° ± 7.22°, and morphotype IV – 98.83° ± 4.59°. Comparison of MTPA values based on the morphological structure of the knee joint confirmed a statistically significant difference ($p < 0.00001$). It was established that higher MTPA values are associated with varus alignment of the lower limb axis and correspondingly lower FTA values ($\tau = -0.37$, $p < 0.0000001$). The mean value of the MTCA was 80.78° ± 6.49°. Among individuals with morphotype I, the mean value of the MTCA was 77.74° ± 6.14°, in patients with morphotype II – 83.32° ± 6.33°, in those with morphotype III – 80.31° ± 6.67°, and in morphotype IV – 79.17° ± 4.43°. These differences were statistically significant ($p = 0.02$). No significant correlation was found between MTCA and FTA values ($\tau = -0.008$, $p = 0.91$).

Analysis of the morphometric parameters of the proximal tibia relative to the axis tangential to the femoral condyles revealed that the mean value of the LTCA2 was 80.78° ± 6.69°. The lowest values were observed in patients with morphotype I – 77.60° ± 6.59°, while in morphotype II the mean was 80.17° ± 7.61°, in morphotype III – 83.03° ± 4.04°, and morphotype IV – 82.83° ± 7.04°. The differences were statistically significant ($p = 0.008$) (Table 2). Furthermore,

higher LTCA2 values were found in patients with valgus alignment of the lower limb axis and correspondingly higher FTA values, which were confirmed by a direct correlation between the two indicators ($\tau = +0.23$, $p = 0.0009$).

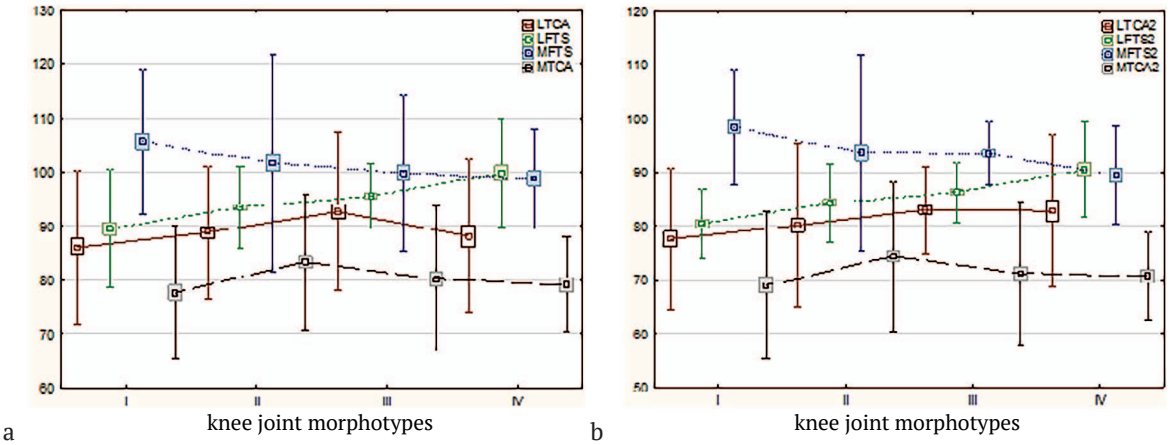
Table 2. Characteristics of morphometric parameters of the proximal tibia relative to the axis tangential to the femoral condyles

	Variants of knee joint morphotypes				p
	I (n = 21)	II (n = 38)	III (n = 29)	IV (n = 12)	
LTCA2	77.60 ± 6.59°	80.17 ± 7.61°	83.03 ± 4.04°	82.83 ± 7.04°	0.008*
LTPA2	80.43 ± 3.23°	84.37 ± 3.65°	86.29 ± 2.78°	90.46 ± 4.43°	< 0.00001*
MTPA2	98.36 ± 5.34°	93.68 ± 9.08°	93.53 ± 2.89°	89.5 ± 4.59°	< 0.00001*
MTCA2	69.05 ± 6.82°	74.39 ± 6.96°	71.14 ± 6.64°	70.67 ± 4.06°	0.03*

Note: * – statistically significant difference at $p \leq 0.05$
Source: developed by the author

A highly significant difference in LTPA2 values across the groups was confirmed ($p < 0.00001$). The overall mean LTPA2 was 84.83° ± 4.49°. The highest values were observed in individuals with valgus deviation of the lower limb axis – 90.46° ± 4.43°, followed by patients with morphotype III – 86.29° ± 2.78°, morphotype II – 84.37° ± 3.65°, and the lowest in morphotype I – 80.43° ± 3.23°. A moderate direct correlation was identified between the LTPA2 and FTA values ($\tau = +0.53$, $p < 0.0000001$). The mean value of the MTPA2 among the participants was 94.12° ± 6.92°. The highest MTPA2 values were recorded in individuals with morphotype I – 98.36° ± 5.34°, followed by morphotype II – 93.68° ± 9.08°, morphotype III – 93.53° ± 2.89°, and morphotype IV – 89.5° ± 4.59°. These differences were statistically significant ($p < 0.00001$). It was demonstrated that higher MTPA2 values are associated with lower FTA values, confirmed by a negative correlation between the two indicators ($\tau = -0.45$, $p < 0.0000001$).

Analysis of MTCA2 values across the groups confirmed a statistically significant difference depending on the morphological characteristics of the knee joint structure ($p = 0.03$). The mean MTCA2 value in patients with morphotype I was 69.05° ± 6.82°, in morphotype II – 74.39° ± 6.96°, in morphotype III – 71.14° ± 6.64°, and in morphotype IV – 70.67° ± 4.06°. The overall mean value of this angle was 71.88° ± 6.80°. No statistically significant correlation was found between MTCA2 and FTA values ($\tau = -0.01$, $p = 0.87$). The differences in morphometric parameters of the proximal tibia, as measured relative to the axis drawn from the intercondylar eminence to the condyles, and relative to the tangent to the tibial condyles, are illustrated in Figure 3.



a b

Figure 3. Box plot of morphometric parameters of the proximal tibia
Note: a – relative to the axis drawn from the intercondylar eminence to the condyles; b – relative to the tangent to the tibial condyles
Source: developed by the author

Based on the obtained data, it was established that changes in the morphological configuration of the medial condyle of the tibia played a key role in the development of lower limb axis deviation. Significant destruction of the medial tibial compartment in cases of pronounced varus deformity was reported in the study by E.B. Demir *et al*. [18]. The researchers found that patients with HKA angle values greater than 20.6° had approximately a sixfold increased risk of requiring medial augmentation during total knee arthroplasty. In the study by H. Nilsson *et al*. [19], a higher risk of developing medial compartment osteoarthritis of the knee was observed in patients with more severe varus deformity and correspondingly lower HKA angle values. Deformation of the medial femoral condyle in the presence of varus alignment of the lower limb axis was also confirmed by the findings of Z. Zhang *et al*. [20]. The authors noted that correction of varus deviation through osteotomy and restoration of optimal mechanical MPTA and

FTA values leads to a reduction in cartilage loading and a shift in stress from the medial to the lateral compartment of the joint. This, in turn, promotes redistribution of the centre of gravity in the frontal plane.

Further support for this conclusion is provided by the study of H. Zhang *et al*. [21], who used computer modelling to develop three-dimensional simulation models of knee osteoarthritis with varying degrees of varus deformity (0°, 3°, 6°, 9°, 12°, 15°, 18°). They analysed the distribution of Von-Mises stress and peak stress values for the cartilage of the femoral condyles and the medial and lateral tibial plateaus. The researchers observed a marked increase in axial load on the lower limb in the medial direction, as well as elevated peak stress values in the medial compartment of the knee joint, corresponding with the degree of varus deviation of the lower limb axis. They identified higher levels of loading on the medial cartilage and a greater frequency of damage in areas of peak stress when the varus deviation exceeded 6°.

In an analysis of 1,158 computed tomography scans of knee joints from 193 patients with osteoarthritis and 965 healthy individuals, A. Siddiqi *et al*. [22] found significant differences in several morphometric parameters. These included the posterior condylar axis (0.3° ± 1.5° versus 1.2° ± 1.9°, respectively), the medial-to-lateral posterior condylar offset ratio (1.01 ± 0.06 versus 1.04 ± 0.07, respectively), the medial-to-lateral condylar radius ratio (0.98 ± 0.07 versus 1.03 ± 0.07, respectively), and the tibial slope in both sagittal and frontal planes. Specifically, medial posterior plateau slope was 8.4° ± 4.0° versus 9.2° ± 4.0°, lateral posterior plateau slope was 9.2° ± 3.6° versus 7.2° ± 3.3°, and medial frontal tilt was 82.1° ± 4.3° versus 83.9° ± 3.3°, respectively.

Distinct findings were reported in the study by S.H. Alruwaili *et al*. [23], who assessed differences in the orientation of the joint lines of the medial and lateral tibial plateaus about the horizontal line of the mechanical axis of the tibia (TPD, tibial plateau difference) in 181 women with degenerative-dystrophic knee joint conditions (181 knee joints). The researchers did not identify any statistically significant differences in TPD values ($p = 0.662$), the levels of the medial and lateral femoral condyles ($p = 0.54$), MPTA ($p = 0.169$), or the posterior slope of the tibial plateau ($p = 0.466$) between groups stratified by the severity of osteoarthritis. However, the authors noted a statistically significant correlation between increased TPD and greater mechanical FTA ($p < 0.01$).

The morphological characteristics of the lateral compartment of the knee joint in gonarthrosis, particularly in relation to the alignment of the lower limb axis, remain insufficiently studied. In the study by W. Ma *et al*. [24], the authors observed lateral displacement of the tibial plateau, curvature of the proximal fibula, and elevation of the fibular head in cases of knee osteoarthritis accompanied by varus deviation of the lower limb axis. In a retrospective study by Z. Wang *et al*. [25], based on analysis of radiographs from 414 patients (789 knee joints), the researchers concluded that varus deformity of the proximal tibia was linked to the morphological features of the epiphyseal and metaepiphyseal regions. Among women, progressive and asymmetrical subsidence of the tibial epiphysis was observed with age, which, according to the authors, contributed to the development of dynamic varus deformity of the proximal tibia. In a separate study, M. Kulynych & Yu. Mochalov [26] found that the degree of midface deformity in children with congenital defects of the alveolar process of the maxilla was directly proportional to the size of the defect. The anthropometric parameters of the nasolabial complex prior to surgery are critically important for selecting the appropriate surgical approach and type of bone graft.

The findings of the present study are broadly consistent with existing literature and confirm the morphological variability of degenerative-dystrophic changes in the proximal tibia across different knee joint morphotypes. Moreover, the results highlight the complex and multifactorial relationship between the morphometric characteristics of the proximal tibia and the alignment of the lower limb axis. The identified morphometric differences support the rationale for differentiating knee joint morphotypes in degenerative-dystrophic conditions and underscore the need to individualise orthopaedic treatment planning. This is particularly relevant for corrective osteotomy, unicondylar, and total knee arthroplasty.

CONCLUSIONS

The study established differences in degenerative changes in the proximal tibia depending on the morphotype of the knee joint, as classified using a proprietary clustering system. In groups categorised according to the morphological variant of the knee joint, statistically significant differences were identified in the structural and spatial characteristics of the proximal tibia. These included angular values measured relative to an axis drawn from the intercondylar eminence to the tibial condyles – namely, LTCA ($p = 0.02$), LTPA ($p < 0.00001$), MTPA ($p < 0.00001$), and MTCA ($p = 0.02$) – as well as parameters measured relative to an axis tangential to the condyles – LTCA2 ($p = 0.008$), LTPA2 ($p < 0.00001$), MTPA2 ($p < 0.00001$), and MTCA2 ($p = 0.03$). Changes in the morphology of the proximal tibia depending on the alignment of the lower limb axis were confirmed by significant correlations between FTA values and LTPA ($\tau = +0.36$, $p < 0.0000001$), MTPA ($\tau = -0.37$, $p < 0.0000001$), LTCA2 ($\tau = +0.23$, $p = 0.0009$), LTPA2 ($\tau = +0.53$, $p < 0.0000001$), and MTPA2 ($\tau = -0.45$, $p < 0.0000001$). The absence of significant correlations between FTA and LTCA ($\tau = +0.13$, $p = 0.05$), MTCA ($\tau = -0.008$, $p = 0.91$), and MTCA2 ($\tau = -0.01$, $p = 0.87$) indicates the complexity and multifactorial nature of the spatial relationships among the components of the deformity. It has been demonstrated that degenerative-dystrophic changes in the proximal tibia in individuals with knee joint morphotypes II and III are primarily associated with medial deviation of the longitudinal tibial axis. In contrast, in morphotypes I and IV, such changes are largely attributable to additional destruction of the cartilage and bone tissues of the medial femoral condyle. These findings confirm the appropriateness of a personalised approach when planning orthopaedic interventions for patients with degenerative-dystrophic knee joint disorders. Taking into account the specific morphological variant of the knee joint allows for more accurate prediction of pathological progression mechanisms and provides a rationale for selecting the most suitable surgical strategy. A promising direction for further research is the analysis of morphological features of the distal femur in degenerative-dystrophic knee conditions, with consideration of the identified morphological variant. Equally important is the assessment of structural changes within the knee joint occurring in the sagittal and

axial planes to enable a comprehensive understanding of joint biomechanics in three-dimensional space.

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REFERENCES
[1] Tomczyk J, Taczała Warga J, Lesman J, Sawicki J, Domżalski M. Assessment of the deformation model of the proximal tibia in the course of degenerative disease: Analysis of the 3-dimensional mathematical model. Quant Imaging Med Surg. 2022;12(8):4202–12. DOI: 10.21037/qims-21-1210
[2] Nelson AE, Keefe TH, Schwartz TA, Callahan LF, Loeser RF, Golightly YM, et al. Biclustering reveals potential knee OA phenotypes in exploratory analyses: Data from the Osteoarthritis Initiative. PLoS One. 2022;17(5):e0266964. DOI: 10.1371/journal.pone.0266964
[3] Langworthy M, Dasa V, Spitzer AI. Knee osteoarthritis: Disease burden, available treatments, and emerging options. Ther Adv Musculoskelet Dis. 2024;16. DOI: 10.1177/1759720X241273009
[4] Primorac D, Molnar V, Rod E, Jeleč Ž, Čukelj F, Matišić V, et al. Knee osteoarthritis: A review of pathogenesis and state-of-the-art non-operative therapeutic considerations. Genes. 2020;11(8):854. DOI: 10.3390/genes11080854
[5] He Y, Li Z, Alexander PG, Ocasio-Nieves BD, Yocum L, Lin H, et al. Pathogenesis of osteoarthritis: Risk factors, regulatory pathways in chondrocytes, and experimental models. Biology. 2020;9(8):194. DOI: 10.3390/biology9080194
[6] Van Oevelen A, Van den Borre I, Duquesne K, Pizurica A, Victor J, Nauwelaers N, et al. Wear patterns in knee OA correlate with native limb geometry. Front Bioeng Biotechnol. 2022;10:1042441. DOI: 10.3389/fbioe.2022.1042441
[7] Mullaji A, Shah R, Bhoskar R, Singh A, Haidermota M, Thakur H. Seven phenotypes of varus osteoarthritic knees can be identified in the coronal plane. Knee Surg Sports Traumatol Arthrosc. 2022;30(8):2793–805. DOI: 10.1007/s00167-021-06676-8
[8] Mullaji A, Bhoskar R, Singh A, Haidermota M. Valgus arthritic knees can be classified into nine phenotypes. Knee Surg Sports Traumatol Arthrosc. 2022;30(9):2895–904. DOI: 10.1007/s00167-021-06796-1
[9] Yang D, Zhou Y, Shao H, Deng W. Different deformity origins and morphological features in subtypes of valgus knees: A radiological classification system. Orthop Surg. 2022;14(1):96–103. DOI: 10.1111/os.13178
[10] Ishibashi T, Konda S, Tamaki M, Okada S, Tomita T. Tibial morphology of symptomatic osteoarthritic knees varies according to location: A retrospective observational study in Japanese patients. Sci Rep. 2024;14(1):3250. DOI: 10.1038/s41598-024-53222-w
[11] Kuriyama S, Watanabe M, Nakamura S, Nishitani K, Tanaka Y, Sekiguchi K, et al. Large medial proximal tibial angles cause excessively medial tibiofemoral contact forces and abnormal knee kinematics following open-wedge high tibial osteotomy. Clin Biomech. 2020;80:105190. DOI: 10.1016/j.clinbiomech.2020.105190
[12] Itou J, Kuwashima U, Itoh M, Okazaki K. Effect of bone morphology of the tibia plateau on joint line convergence angle in medial open wedge high tibial osteotomy. BMC Musculoskelet Disord. 2022;23(1):568. DOI: 10.1186/s12891-022-05526-z
[13] Cassar-Pullicino VN, Davies AM, editors. Measurements in musculoskeletal radiology. Berlin: Springer; 2020. 860 P. DOI: 10.1007/978-3-540-68897-6
[14] Kylymniuk LO, Matsipura MM, Iaremyn SIu. Clustering of knee joint types affected by the degenerative-dystrophic process. Ukr Med J. 2025;2(168):1–6. DOI: 10.32471/umj.1680-3051.264438
[15] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2025 March 29]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[16] Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine [Internet]. 1997 April 4 [cited 2025 March 29]. Available from: https://zakon.rada.gov.ua/laws/show/994_334#Text
[17] Order of the Ministry of Health of Ukraine No. 690. On Approval of the Procedure for Conducting Clinical Trials of Medicinal Products and Examination of Clinical Rrial Materials and Model Regulation on Ethics Committees [Internet]. 2009 September 23 [cited 2025 March 29]. Available from: https://zakon.rada.gov.ua/laws/show/z1010-09#Text
[18] Demir EB, Barça F, Dinçer A, Atilla HA, Akdoğan M, Ateş Y. Predicting the need for medial augmentation for primary total knee arthroplasty with varus deformity. Jt Dis Relat Surg. 2025;36(1):129–36. DOI: 10.52312/jdrs.2025.1973
[19] Nilsson H, Englund M, Frobell R, Lohmander LS, Struglics A, Swärd P. Varus alignment of the hip and knee 2 years after anterior cruciate ligament injury is associated with medial tibiofemoral osteoarthritis 3 years later. J Exp Orthop. 2025;12(1):e70143. DOI: 10.1002/jeo2.70143
[20] Zhang Z, Tao H, Zhao Y, Xiang W, Cao H, Tao F. High tibial osteotomy improves balance control in patients with knee osteoarthritis and a varus deformity. J Orthop Surg Res. 2023;18(1):538. DOI: 10.1186/s13018-023-04041-8
[21] Zhang H, Ma J, Tian A, Lu B, Bai H, Dai J, et al. Analysis of cartilage loading and injury correlation in knee varus deformity. Medicine. 2024;103(19):e38065. DOI: 10.1097/MD.0000000000038065
[22] Siddiqi A, Anis H, Borukhov I, Piuzzi NS. Osseous morphological differences in knee osteoarthritis. J Bone Joint Surg Am. 2022;104(9):805–12. DOI: 10.2106/JBJS.21.00892

[23] Alruwaili SH, Park KK, Yang IH, Lee WS, Cho BW, Kwon HM. Difference between medial and lateral tibia plateau in the coronal plane: Importance of preoperative evaluation for medial unicompartmental knee arthroplasty. BMC Musculoskelet Disord. 2022;23(1):342. DOI: 10.1186/s12891-022-05298-6
[24] Ma W, Wang F, Sun S, Ding L, Wang L, Yu T, et al. Novel ideas for the comprehensive evaluation of varus knee osteoarthritis: Radiological measurements of the morphology of the lateral knee joint. J Orthop Surg Res. 2023;18(1):196. DOI: 10.1186/s13018-023-03684-x
[25] Wang Z, Zheng Y, Meng D, Li H, Ji C, Wang J. Anatomical imaging study on uneven settlement of the proximal tibia. Orthop Surg. 2023;15(1):239–46. DOI: 10.1111/os.13632
[26] Kulynych M, Mochalov Yu. Anthropometric characteristics of the face in patients with congenital defects of alveolar process on maxilla before surgical intervention. Ukr J Med Biol Sport. 2023;8(1):165–70. DOI: 10.26693/jmbs08.01.165

Особливості дегенеративних змін проксимального відділу великогомілкової кістки залежно від морфотипу колінного суглоба

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Анотація. Мета роботи полягала у визначенні особливостей дегенеративного ураження проксимального відділу великогомілкової кістки, враховуючи морфологічний варіант колінного суглоба. Проаналізовано результати рентгенографічного обстеження 100 випадків гонартрозу з переважним ураженням медіального відділу. Середній вік – 63,56 ± 8,10 років. Серед рентгенологічних критеріїв оцінювались анатомічний медіальний проксимальний великогомілковий кут (MPTA) та стегново-гомілковий кут (FTA). Додатково оцінювались морфометричні показники проксимального відділу великогомілкової кістки відносно осі, проведеної від міжвиросткового підвищення до виростків великогомілкової кістки (LTCA, LTPA, MTPA, MTCA) та відносно осі, дотичної до виростків (LTCA2, LTPA2, MTPA2, MTCA2). Морфотип колінних суглобів було встановлено відповідно до власної кластерної системи. Статистичний аналіз було виконано з використанням програмного засобу Statistica 13. Рівень статистичної значущості визначено при $p \leqslant 0,05$. Встановлені зміни показників LTCA та LTCA2 у пацієнтів з морфотипом I, II, III свідчили про відхилення осі великогомілкової кістки досередини. У пацієнтів з вальгусним відхиленням осі нижньої кінцівки та морфотипом колінного суглоба IV зниження показників кутів LTCA та LTCA2 вказувало на додаткове руйнування кісткової та хрящової тканин виростка великогомілкової кістки опорної зони суглоба та зміщення осі, дотичної до виростків великогомілкової кістки, досередини і донизу та відхилення осі, проведеної від міжвиросткового підвищення до латерального виростка, догори. Зміни кутів LTPA, LTPA2, MTPA та MTPA2 свідчили про відхилення великогомілкової кістки досередини. Аналізуючи значення кутів MTCA та MTCA2 у пацієнтів з морфотипом II, III та IV, встановлено відхилення великогомілкової кістки досередини. Натомість, зменшення значення кутів MTCA та MTCA2 у пацієнтів з морфотипом I свідчило про додаткове руйнування кісткової та хрящової тканин медіального виростка та зміщення як осі, проведеної від міжвиросткового підвищення до медіального виростка, так і осі, дотичної до виростків, донизу та досередини. Таким чином, доведено відмінність морфометричних показників проксимального відділу великогомілкової кістки залежно від встановленого морфотипу колінного суглоба при дегенеративно-дистрофічних захворюваннях. Отримані дані дозволять удосконалити персоніфікований підхід до ортопедичного втручання при дегенеративно-дистрофічних захворюваннях колінного суглоба

Ключові слова: остеоартроз; дегенеративно-дистрофічні захворювання; морфологія; рентгенологічні параметри; гонартроз

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Molecular and immune predictors of survival in lung squamous cell carcinoma: A TCGA-based analysis

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Abstract. Lung squamous cell carcinoma remains one of the most aggressive forms of non-small cell lung cancer with limited options for personalised therapy, highlighting the need for new prognostic biomarkers. The purpose of this study was to analyse the impact of somatic mutations and their co-mutations in key oncogenes (TP53, TTN, PIK3CA, and KEAP1/NFE2L2) on the survival of patients with lung squamous cell carcinoma, considering the tumour immune subtype. Data from open-access oncology repositories (TCGA, UCSC Xena, cBioPortal) were used for 419 patients with complete clinical, immune, and mutational profiles. Patients were stratified by tumour immune activity (high/intermediate vs low subtype), and the mutational analysis included both individual genes and co-mutations. Survival was assessed using the Kaplan-Meier method with log-rank testing. It was found that TP53 mutations were significantly associated with improved survival in both the high/intermediate immune subtype group (median 57.9 vs 27.8 months, $p = 0.0141$) and the low immune subtype group ($p = 0.0361$). TTN mutations showed a positive trend in the high/intermediate group ($p = 0.0582$) and a statistically significant association with survival in the low immune activity group ($p = 0.0123$). The strongest effect was observed for the TP53+TTN co-mutation, which significantly improved survival in both immune subtypes (high/intermediate: $p = 0.0065$; low: $p = 0.0006$). In contrast, PIK3CA and KEAP1/NFE2L2 mutations and their combinations did not show a statistically significant impact on survival. Cluster analysis of the mutational profile revealed two primary patterns, with TP53 and TTN mutations tending to cluster, though no clear visual association with survival status was observed. Thus, the mutational status of TP53 and TTN, particularly their co-mutation, has substantial prognostic value in patients with lung squamous cell carcinoma, especially when considered alongside immune microenvironment characteristics. Isolated PIK3CA and KEAP1/NFE2L2 mutations showed no significant effect. These results emphasised the importance of integrating mutational and immune profiling to guide personalised treatment strategies

Keywords: TP53; TTN; PIK3CA; KEAP1/NFE2L2; lung cancer; co-mutations

INTRODUCTION

Lung squamous cell carcinoma (LUSC) is an aggressive histological subtype of non-small cell lung cancer, associated with high mortality rates and limited options for targeted treatment. Although immune checkpoint inhibitors have significantly transformed the therapeutic landscape, clinical outcomes in LUSC remain highly variable. This variability is partly explained by the complex interplay between the tumour's molecular background and its immune microenvironment. Consequently, identifying robust prognostic biomarkers is essential for improving patient stratification and developing personalised therapeutic approaches, especially in the context of immunotherapy resistance.

Increasing scientific attention has been devoted to investigating the role of somatic mutations in shaping the

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tumour microenvironment and determining survival outcomes in patients with LUSC. J. Zhao *et al.* [1] described the immune infiltration landscape in LUSC and emphasised the impact of key oncogenic mutations on either enhancing or suppressing anti-tumour immune responses. Their findings underscored the close relationship between genetic alterations and the immunological behaviour of tumours. In a comparative mutational analysis conducted by C. Pop-Bica *et al.* [2], TP53 (tumour protein p53) and TTN (titin) mutations were identified as dominant events in LUSC, suggesting their relevance as molecular drivers. These mutations were among the most frequent and may play a central role in the initiation and progression of the disease. Y. Xu *et al.* [3] examined the prognostic significance of genomic variation, tumour mutational burden (TMB), and programmed death-ligand 1 (PD-L1) expression in LUSC patients treated with immunotherapy. Their study showed a positive association between tumour protein p53 (TP53) mutations and favourable treatment response. P.K. Paik *et al.* [4] explored Kelch-like ECH-associated protein 1/nuclear factor erythroid 2-related factor 2 (KEAP1/NFE2L2) mutations as potential therapeutic targets. Their trial involving the target of rapamycin complex 1/2 (TORC1/2) inhibitor TAK-228 revealed only limited clinical activity, but highlighted the need for combination treatment strategies in these molecular subsets. Meanwhile, D. Wang *et al.* [5] developed a prognostic signature based on TMB, which correlated with survival and immunotherapy efficacy in LUSC, particularly in patients with TTN mutations.

X. Deng *et al.* [6] investigated pyroptosis-related genes and demonstrated their influence on the immune composition of the tumour microenvironment. Their study provided evidence that specific genomic alterations can shape distinct immune phenotypes in LUSC. J.A. Hellyer *et al.* [7] similarly confirmed that KEAP1/NFE2L2 mutations are associated with "immunologically silent" microenvironments and reduced response to PD-1 blockade. These data highlighted the immunosuppressive potential of NRF2-driven signalling pathways. C. Su *et al.* [8] identified circulating TTN mutations in blood as potential markers of immunotherapy response. The researchers showed that TTN mutations increase tumour antigenicity and promote immunogenic microenvironments, thereby enhancing the likelihood of immunotherapeutic benefit. Overall, findings from international studies emphasise the prognostic importance of TP53, TTN, KEAP1/NFE2L2, and PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) mutations in LUSC and their influence on treatment response. However, most available data rely either on mutational analysis alone or on isolated assessments of immune phenotype, without integrating both dimensions. This limits the clinical applicability of current evidence in real-world settings.

Despite the limited number of studies on this topic in Ukraine, a research group has already been formed in the scientific community that is actively working in this area. The Ukrainian cohort study by D. Kozakov *et al.* [9] investigated the relationship between genetic alterations and the immune contexture of NSCLC, laying the groundwork for exploring the link between genomic profiles and immune activation in lung cancer. Their findings provided an important national perspective and contribute to the global understanding of tumour-immune interactions. Additionally, in a related review by O. Sulaieva *et al.* [10], the role of molecular biomarkers in guiding personalised management of NSCLC was discussed, emphasising the relevance of integrated molecular and immune profiling. These studies mark an important step toward establishing a national framework for translational research in lung cancer.

The purpose of this study was to analyse the association between somatic mutations and co-mutations in TP53, TTN, PIK3CA, and KEAP1/NFE2L2 and overall survival in patients with lung squamous cell carcinoma, taking into account the tumour's immune subtype.

MATERIALS AND METHODS

Patient selection criteria and procedure. This retrospective cohort study was conducted between January and March 2025 at the Educational and Scientific Medical Institute of Sumy State University (Sumy, Ukraine). The analysis was based on de-identified data obtained from publicly accessible oncological repositories: The Cancer Genome Atlas (TCGA) [11], the UCSC Xena Browser [12], and cBioPortal [13]. These platforms provided comprehensive clinical, immunological, and genomic information on patients with various malignancies, including LUSC. The initial dataset comprised of 504 patients diagnosed with histologically confirmed LUSC and enrolled in the TCGA-LUSC project. To ensure the completeness and reliability of the data, specific inclusion and exclusion criteria were applied. Eligible patients were aged 18 years or older, had complete clinical information (including age, sex, tumour stage, overall survival time, and status), a defined tumour immune subtype according to the Pan-Cancer immune classification by V. Thorsson *et al.* [14], and sequencing data for four key genes: TP53, TTN, PIK3CA, and KEAP1/NFE2L2. Patients were excluded if they lacked valid entries for age, survival status, or follow-up time (n = 44), if immune subtype data were unavailable (n = 33), or if mutation data were missing (n = 8). The final cohort included 419 patients who fulfilled all eligibility criteria and had a complete set of clinical, immunological, and genomic variables.

The mean age of the study cohort was 67.0 ± 8.53 years (range: 39-84), and 74.7% of patients were male. Most patients were diagnosed at early stages: 43.2% were stage 1 and 29.6% were stage 2. Distribution by immune subtype revealed that 41.3% of patients (n = 173) belonged to the high or intermediate immune subtype group (C2, C3, C6), while 58.7% (n = 246) were classified as low immune subtype (C1, C4). Regarding mutational status, TP53 mutations were detected in 90.0% of patients (n = 377), TTN mutations in 85.0% (n = 356), and PIK3CA mutations in 13.4% (n = 56). KEAP1 and NFE2L2 mutations were combined into a single variable due to their functional overlap and mutual exclusivity, and were present in 27.9% of cases (n = 117). The most common co-mutation was TTN+TP53, observed in 76.1% of patients (n = 319), while TP53 + PIK3CA was found in 11.5% (n = 48). These frequencies did not significantly differ between immune subtypes, except for KEAP1/NFE2L2 mutations, which were more prevalent in the low immune subtype group (p = 0.012).

All datasets were fully anonymised and complied with ethical standards for secondary data analysis. Thus, individual informed consent was not required. Nevertheless, the study adhered to the principles of the Declaration of Helsinki [15], ensuring the protection of human dignity,

privacy, and data integrity. The research protocol was reviewed and approved by the Bioethics Committee for Experimental and Clinical Studies at the Educational and Scientific Medical Institute of Sumy State University (protocol No. 3/12, approved on December 17, 2024). Limitations of the study included its retrospective design and reliance on public datasets, which may contain incomplete or heterogeneous information. The lack of treatment data also limited interpretability, particularly regarding immunotherapy effects. Additionally, the small number of cases with high immune activity reduced the statistical power of subgroup comparisons. External validation using independent cohorts with detailed clinical data was necessary to confirm these results.

Methods of statistical data analysis. All statistical analyses were performed using Stata software suite, version 19.5 (StataCorp LLC, College Station, TX, USA). Prior to analysis, all datasets were examined for completeness, inconsistencies, and outliers. Continuous variables were assessed for normality using visual inspection of histograms and the Shapiro-Wilk test. Given the non-parametric distribution of age, results are presented as median and interquartile range (IQR), and comparisons between age groups were conducted using the Mann-Whitney U-test. Categorical variables, including sex, tumour stage, mutational status, and immune subtype, were expressed as absolute counts and percentages. Their distribution across groups was compared using Pearson's chi-square test (χ^2), with p-values <0.05 considered statistically significant.

Overall survival was the primary endpoint and was defined as the time from diagnosis to death or last follow-up. Survival curves were estimated using the Kaplan-Meier method, and differences between groups were evaluated using the log-rank test. Separate survival analyses were performed for each gene (TP53, TTN, PIK3CA, and KEAP1/NFE2L2) and for co-mutations (TP53+TTN and TP53 + PIK-3CA) within both high/intermediate and low immune subtypes to explore potential interaction effects between mutational status and immune phenotype. To visualise mutational patterns and potential patient clusters, a binary matrix was created to reflect the presence or absence of mutations in each gene. This matrix was used to generate a clustered heatmap using Ward's method for hierarchical clustering. Patients were colour-coded by survival status to explore possible associations between mutation clusters and outcomes. Although this was an exploratory, descriptive component, it provided additional insights into the molecular heterogeneity of LUSC. No multiple comparison correction was applied, given the hypothesis-driven nature of the analysis. Nonetheless, all interpretations were contextualised with caution.

RESULTS AND DISCUSSION

Comparative analysis revealed no statistically significant differences in the frequencies of TP53 (p = 0.910), TTN (p = 0.779), or PIK3CA (p = 0.584) mutations between high/medium and low immune subtypes. However, KEAP1/NFE2L2 mutations were significantly more frequent in the low immune subtype group (21.4% vs 32.5%, p = 0.012), suggesting a potential link with an immunosuppressive tumour environment. Co-mutations of TP53 + TTN and TP53+PIK3CA were similarly distributed across subtypes (p = 0.594 and p = 0.713, respectively), indicating that their occurrence is independent of immune phenotype. These findings highlighted that among all examined mutations, only KEAP1/NFE2L2 status may partially correlate with immunological features of the tumour (Table 1).

Table 1. Associations between clinical, molecular-genetic features and immune subtypes in patients with lung squamous cell carcinoma

Variables	Total, n = 419	High / medium immune subtype, n = 173	Low immune subtype, n = 246	p-value
Median age (years, range)	67.0±8.53 (39-84)	66.3±8.68 (40-84)	67.4±8.40 (39-84)	0.2497
Sex:				
Female	106 (25.3)	49 (28.3)	57 (23.2)	0.232
Male	313 (74.7)	124 (71.7)	189 (76.8)	
Stage:				
I	181 (43.2)	74 (42.8)	107 (43.5)	0.977
II	124 (29.6)	53 (30.6)	71 (28.9)	
III	58 (13.8)	22 (12.7)	36 (14.6)	
IV	5 (1.2)	2 (1.2)	3 (1.2)	
Unknown	51 (12.2)	22 (12.7)	29 (11.8)	
Mutation TTN:				
Present	356 (85.0)	148 (85.5)	208 (84.6)	0.779
Absent	63 (15.0)	25 (14.5)	38 (15.4)	
Mutation TP53:				
Present	377 (90.0)	156 (90.2)	221 (89.8)	0.910
Absent	42 (10.0)	17 (9.8)	25 (10.2)	
Mutation PIK3CA:				
Present	56 (13.4)	25 (14.5)	31 (12.6)	0.584
Absent	363 (86.6)	148 (85.5)	215 (87.4)	
Mutation KEAP1/NFE2L2:				0.012
Present	117 (27.9)	37 (21.4)	80 (32.5)	
Absent	302 (72.1)	136 (78.6)	166 (67.5)	

Table 1. Continued

Variables	Total, n = 419	High / medium immune subtype, n = 173	Low immune subtype, n = 246	p-value
Co-mutation TTN+TP53:				0.594
Present	319 (76.1)	134 (77.5)	185 (75.2)	
Absent	100 (23.9)	39 (22.5)	61 (24.8)	
Co-mutation TP53+PIK3CA:				0.713
Present	48 (11.5)	21 (12.1)	27 (11.0)	
Absent	371 (88.5)	152 (87.9)	219 (89.0)	

Source: The Cancer Genome Atlas (TCGA) [11], UCSC Xena [12], and cBioPortal [13]

In summary, comparative analysis showed that the majority of gene mutations and co-mutations occurred independently of immune subtype classification. The only statistically significant difference was observed for KEAP1/NFE2L2 alterations, which were more common in tumours with low immune activity. These findings suggest a potential association between oxidative stress-related mutations and an immunosuppressive tumour microenvironment, warranting further investigation into their biological role in immune escape mechanisms. A comparison of patient survival based on immune subtypes revealed that the median overall survival was 57.1 months in the high/medium immune activity group, compared to 44.9 months in the low immune activity group. Despite a trend toward better survival in patients with higher immune activity, the difference between the groups was not statistically significant (Log-rank test: $\chi^2(1) = 1.14$, $p = 0.2854$). These results indicate that immune subtype did not have a significant impact on overall survival in this cohort of patients with lung squamous cell carcinoma (Fig. 1).

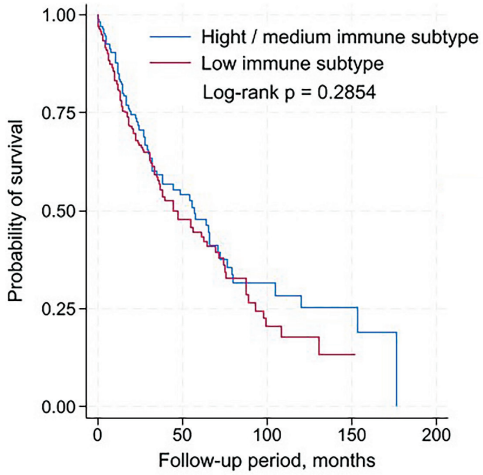

Figure 1. Kaplan-Meier survival curves for patients with lung squamous cell carcinoma according to tumour immune subtype
Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

Among patients with lung squamous cell carcinoma classified as having high/medium immune subtypes, stratification was performed based on the presence of mutations in TP53, TTN, PIK3CA, KEAP1/NFE2L2, and their combinations. Kaplan-Meier analysis with log-rank testing revealed no statistically significant differences in survival associated with PIK3CA mutations ($p = 0.2913$), KEAP1/NFE2L2 mutations ($p = 0.3796$), or the TP53+PIK3CA co-mutation ($p = 0.8296$). In contrast, patients harbouring TP53 mutations demonstrated significantly better overall survival compared to those without such mutations (median survival 57.9 vs 27.8 months, $p = 0.0141$). Similarly, TTN mutations were associated with a trend toward improved survival (median 57.9 vs 27.8 months), though this difference did not reach statistical significance ($p = 0.0582$). The most pronounced effect was observed for the TTN+TP53 co-mutation, which was significantly associated with better survival compared to patients lacking this co-mutation (median survival 63.7 vs 27.8 months, $p = 0.0065$) (Fig. 2).

Among patients with lung squamous cell carcinoma classified as having a low immune subtype, no statistically significant differences in survival were observed across groups with mutations in KEAP1/NFE2L2 ($p = 0.8091$), PIK3CA ($p = 0.5478$), or the TP53+PIK3CA co-mutation ($p = 0.7477$), indicating a lack of prognostic impact for these alterations in this cohort. In contrast, the presence of TP53 mutations was significantly associated with improved overall survival ($p = 0.0361$), as was the presence of TTN mutations ($p = 0.0123$). The strongest prognostic effect was observed in patients with co-mutations in TTN and TP53, who demonstrated the highest survival rates ($p = 0.0006$). Overall, these findings suggest that mutations in TP53 and

TTN – particularly in combination – may serve as favourable prognostic markers in patients with lung squamous cell carcinoma, in contrast to isolated alterations in PIK3CA or KEAP1/NFE2L2 (Fig. 3).

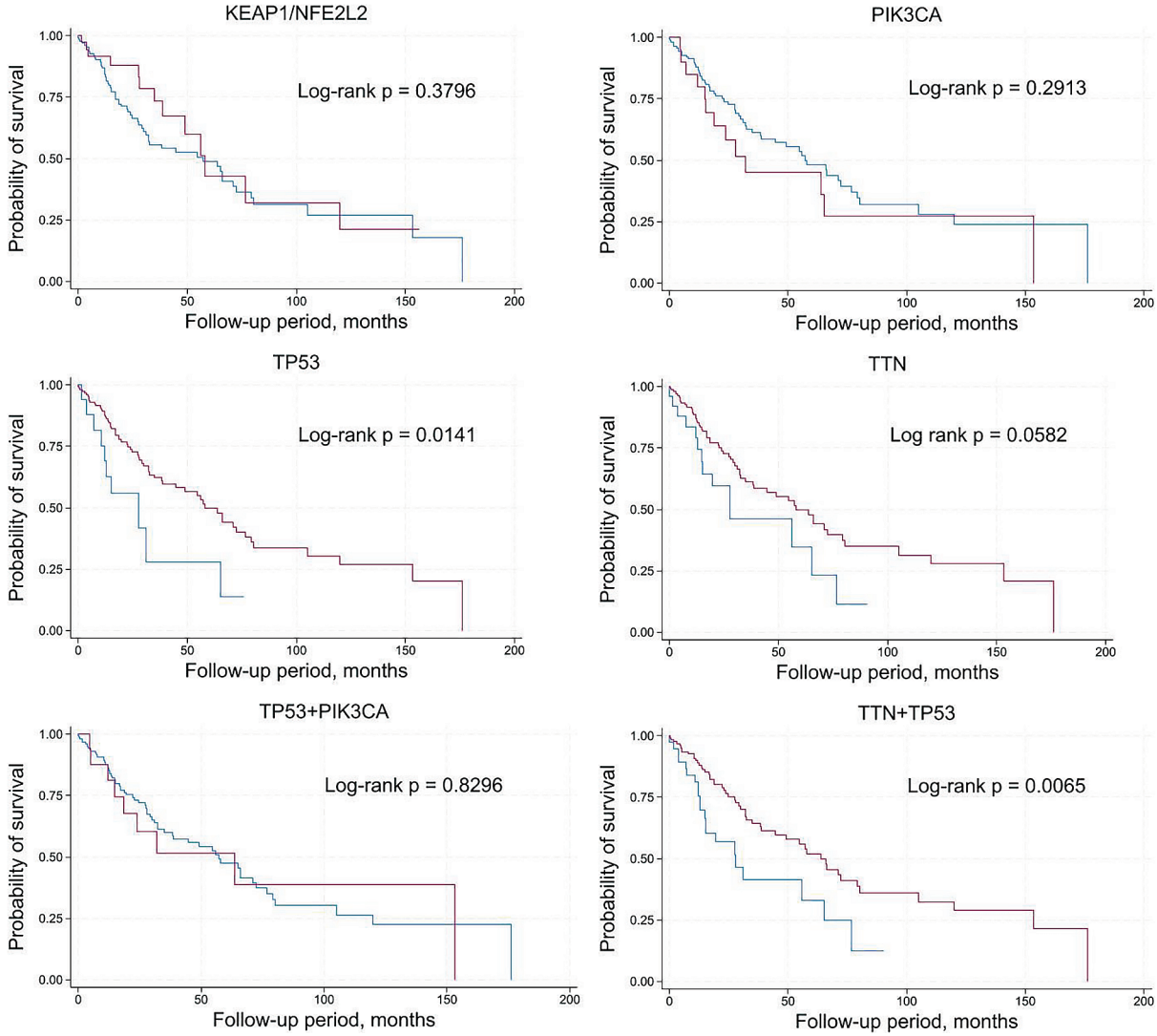

Figure 2. Kaplan-Meier survival curves according to mutation status in patients with lung squamous cell carcinoma and high/medium immune subtype

Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

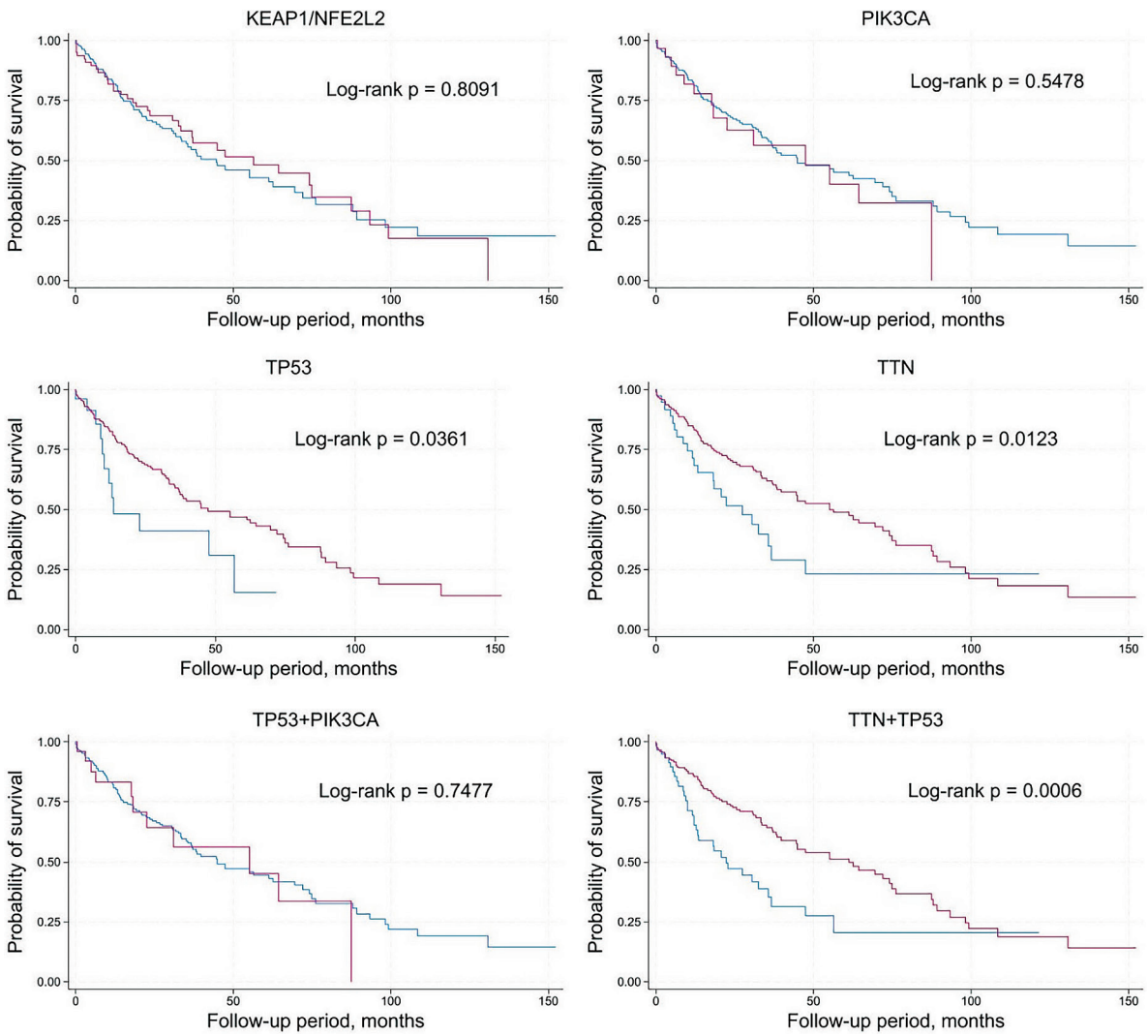

Figure 3. Kaplan-Meier survival curves according to mutation status in patients with lung squamous cell carcinoma and low immune subtype

Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

In conclusion, survival analysis revealed that individual mutations such as KEAP1/NFE2L2 and TTN, and specific co-mutation profiles, have prognostic relevance in LUSC. The effect of these mutations on overall survival was further modulated by immune subtype, emphasising the importance of integrating molecular and immunological features when assessing patient outcomes. To investigate the relationships between somatic mutations in key genes among patients with lung squamous cell carcinoma, a clustered heatmap was generated based on mutations in TTN, TP53, PIK3CA, and KEAP1/NFE2L2 (Fig. 4).

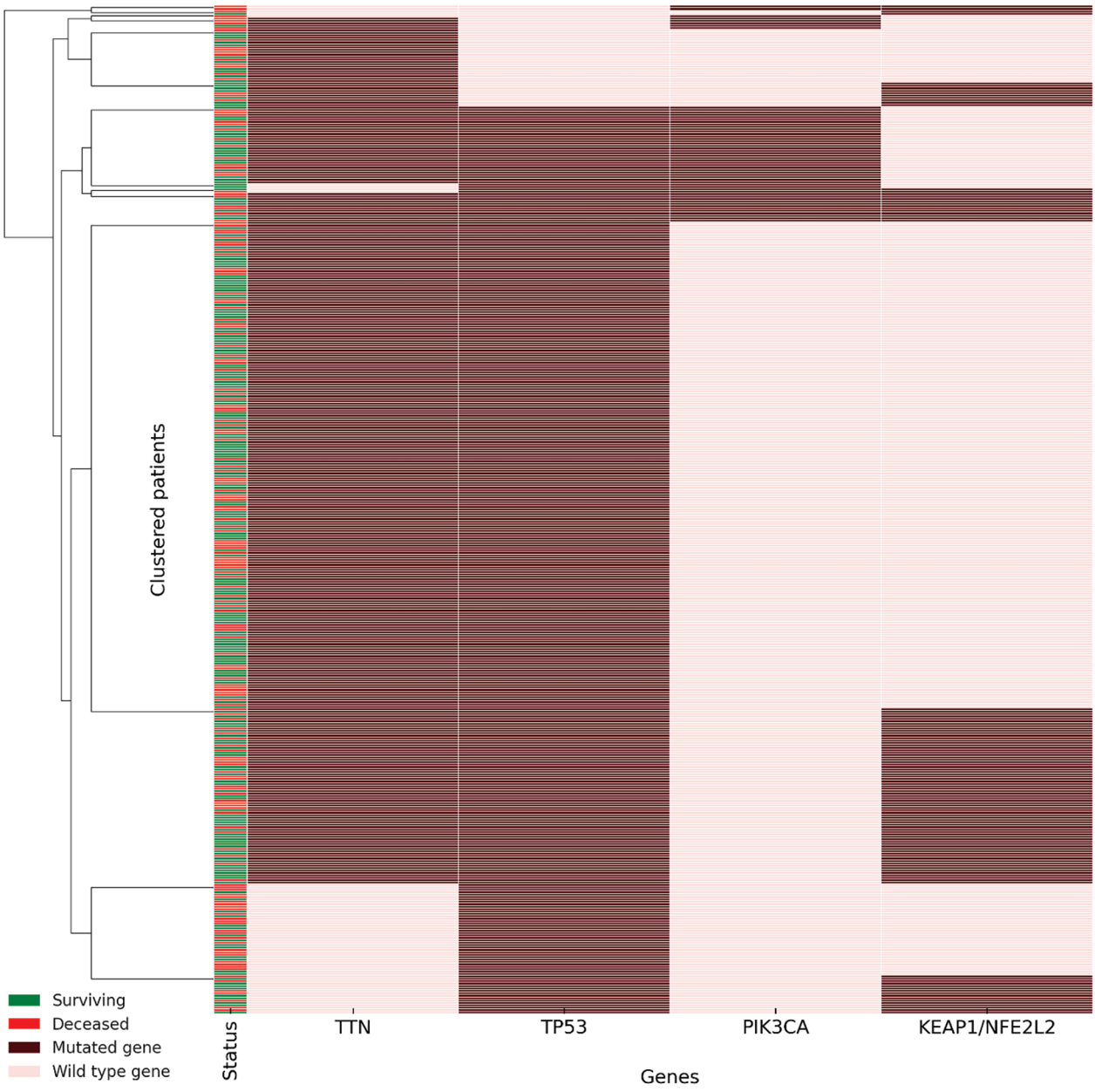

Figure 4. Clustered heatmap of mutations in patients with lung squamous cell carcinoma
Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

During the analysis, notable heterogeneity in the mutational landscape of LUSC was observed, allowing for the tentative identification of two major patient clusters based on shared mutational features. Mutations in TTN and TP53 were the most prevalent, occurring in over 76% of cases, and frequently co-occurred. This co-occurrence appears to define a substantial subgroup of patients with potentially common molecular drivers. In contrast, PIK3CA and KEAP1/NFE2L2 mutations showed a more scattered distribution, appearing independently across patients without consistent co-localisation. To explore possible prognostic implications, patients were colour-coded based on survival status (green for surviving, red for deceased). However, no distinct spatial clustering of deceased individuals was evident on the heatmap. Patients with poor outcomes were distributed throughout both clusters, suggesting that mutational status alone does not fully account for prognosis. The lack of visually dominant patterns linking mutational combinations to survival highlights the multifactorial nature of disease progression in LUSC. These findings emphasised the structural complexity and individual variability of mutational alterations in LUSC. While TTN and TP53 mutations may represent shared early events in tumorigenesis, mutations in KEAP1/NFE2L2 – which are known to affect oxidative stress pathways – may contribute to an immunosuppressive microenvironment. This underscores the necessity for integrated molecular-immunological approaches and further stratified analyses to better characterise prognostic subgroups and guide personalised treatment strategies.

The analysis of the mutational landscape in LUSC revealed intricate interactions between genetic alterations

and the tumour immune microenvironment. A prominent finding was the association between TP53 and TTN co-mutations and improved overall survival, particularly in patients with high or intermediate levels of immune infiltration. This observation was consistent with findings by K. Ying *et al.* [16], who demonstrated that TP53 + TTN co-mutations are predictive of enhanced immunotherapy responses in LUSC, likely due to increased tumour mutational burden and elevated neoantigen load. S. Zou *et al.* [17] further confirmed the prognostic value of TTN mutations, showing their association with prolonged survival and potential to stratify patients for immune checkpoint blockade. These findings underlined the relevance of TTN as a candidate biomarker for immunotherapy response prediction. X. Xie *et al.* [18] attributed this association to the ability of TTN mutations to drive immune activation, including the upregulation of cytotoxic T-cell markers and pro-inflammatory chemokines. Such immunogenic changes may explain the enhanced sensitivity of TTN-mutant tumours to immune checkpoint inhibition observed in clinical settings. Consistent with these studies, authors' analysis demonstrated that TTN mutations – particularly in combination with TP53 – were associated with significantly better overall survival, reinforcing their potential role in patient stratification. These immunostimulatory effects may contribute to reshaping the tumour microenvironment, thereby increasing the probability of durable response to immune checkpoint inhibitors in patients harbouring such alterations.

TP53, a canonical tumour suppressor gene, plays a central role in maintaining genomic integrity. Z. Fan *et al.* [19] reported that TP53-mutant tumours display distinct transcriptional programs associated with immune activation, including interferon signalling. These molecular features may explain why patients harbouring TP53 mutations tend to exhibit better immunotherapy outcomes. Moreover, J. Yu *et al*. [20] emphasised the importance of TP53 co-status with LRP1B, indicating that the absence of mutations in both genes correlates with favourable prognosis in LUSC patients treated with PD-L1 inhibitors. Collectively, these findings support the notion that the mutational background of tumours directly influences the efficacy of immunotherapies. These insights also imply that biomarker-driven patient stratification should account for not only single-gene alterations but also specific mutation combinations.

Importantly, the observed benefit of TP53 + TTN co-mutations extended, albeit to a lesser extent, to patients within the low immune subtype group. This suggests that even in immunologically "cold" tumours, the underlying genomic architecture may confer some degree of immunogenicity. L. Yang *et al.* [21] underscored the relevance of integrating immune cell infiltration profiles with molecular subtyping to better predict therapy responses, reinforcing the concept that both immune and genetic features must be jointly considered. A similar view was presented by J. Wang *et al*. [22], who proposed that specific autophagy-related gene signatures modulate immune infiltration and may underlie differences in survival among molecular subtypes of LUSC. Such findings collectively point to the necessity of combining mutational and immunologic parameters when developing predictive models for immunotherapy outcomes.

In contrast, mutations in the KEAP1/NFE2L2 pathway were predominantly observed in tumours with low immune activity. P.K. Paik *et al.* [4] demonstrated that alterations in this axis contribute to resistance against TORC1/2 inhibitors and promote tumour progression by suppressing oxidative stress responses. These findings highlighted the complexity of redox signalling in NSCLC and the challenges in targeting this pathway. J.A. Hellyer *et al.* [7] explored the clinical relevance of KEAP1-NFE2L2 mutations and linked them to immune silencing and reduced efficacy of immune checkpoint inhibitors. This further emphasised the immunosuppressive role of the KEAP1/NRF2 pathway. Supporting this, M. Sánchez-Ortega *et al.* [23] showed that wild-type NRF2/KEAP1 tumours exhibit higher oxidative stress susceptibility, suggesting a potential therapeutic window that is absent in mutant tumours. This finding underscored the therapeutic relevance of redox imbalance and highlighted the importance of molecular subtyping when designing targeted interventions. In the current cohort, the higher prevalence of these mutations among low immune subtype patients aligns with a model of immune evasion mediated by aberrant antioxidant regulation. These insights further confirm that KEAP1/NFE2L2 mutations not only affect tumour metabolism but also alter immune escape dynamics.

Mutations in PIK3CA, although not statistically significant in this analysis, have been the subject of extensive investigation. Q. Huang *et al.* [24] characterised the mutational landscape of PIK3CA in Chinese patients and reported substantial heterogeneity in mutation co-occurrence patterns. S. Cokpinar *et al.* [25] also highlighted the variable prognostic role of PIK3CA, emphasising that its clinical implications may depend on the presence of co-mutations such as TP53. In the present study, PIK3CA mutations exhibited a dispersed distribution across immune subtypes and were not linked to survival outcomes. However, their potential role as modifiers in polygenic contexts warrants further exploration. The interaction between PIK3CA and immune signalling pathways remains an open area of research, with implications for therapy resistance and immune modulation.

Cluster analysis of the mutational heatmap provided additional insights. Two main clusters of patients emerged, reflecting distinct mutation combinations and partially correlating with survival status. C. Su *et al*. [8] demonstrated that circulating TTN mutations can serve as dynamic markers for predicting responses to immunotherapy in advanced NSCLC. Similarly, D. Kozakov *et al.* [9] examined Ukrainian patients with NSCLC and concluded that specific mutation patterns shape the immune milieu of the tumour, impacting clinical trajectories. These findings reinforce the concept that the mutational composition of tumours shapes immune interactions and may serve as a basis for patient stratification. Moreover, they support the incorporation of genomic clustering analyses in the interpretation of immune-related phenotypes.

From a broader perspective, the interplay between immune contexture and genomic alterations in LUSC reflects a multifactorial and dynamic landscape. In their seminal study, V. Thorsson *et al.* [14] proposed six pan-cancer immune subtypes based on transcriptomic features, highlighting differences in leukocyte infiltration, antigen

presentation, and expression of immune checkpoints. The current results were congruent with this framework, particularly the observed heterogeneity among tumours with similar mutational loads but divergent immune infiltration patterns. Integrating such immune classification systems into genomic profiling may enhance the precision of personalised therapies. This approach may be especially relevant in tumours that lack conventional biomarkers but exhibit atypical immune behaviour [26].

Several additional studies included in the reference list provide complementary perspectives. For instance, X. Deng *et al.* [6] utilised pyroptosis-related genes to model immunotherapy efficacy, demonstrating that cell death pathways intersect with immune signalling in LUSC. Their model highlighted the relevance of immune cell infiltration patterns in shaping treatment response. D. Wang *et al.* [5] employed a multi-omics approach to build a prognostic signature incorporating TMB and gene expression, further supporting the integration of multiple data layers. This approach underscored the importance of combining genomic and transcriptomic variables to improve prognostic accuracy. W. Tao *et al.* [27] reviewed the predictive value of omics-derived markers in immunotherapy, advocating for combinatorial models over single biomarkers. They emphasised that such models offer greater robustness in real-world clinical settings. The researchers' conclusions were consistent with this integrative perspective, as it has been demonstrated that combined consideration of mutation and immune profiles improves the identification of prognostically significant subgroups in LUSC. Together, these studies reinforce the current findings and advocate for broader, systems-level approaches to immuno-oncology in LUSC.

In terms of clinical translation, these insights suggest that patient selection for immunotherapy in LUSC should move beyond PD-L1 status and encompass genomic features such as TTN and TP53 mutations, KEAP1/NFE2L2 status, and multi-parameter immune profiling. C. Pop-Bica *et al.* [2] noted the importance of comprehensive genomic analysis via NGS in both NSCLC and SCLC, stressing that divergent mutation profiles require tailored approaches. Their findings also highlighted the potential of genomic profiling to inform histology-specific therapeutic decisions. Y. Xu *et al.* [3] and A. Burlaka [28] provided evidence that PD-L1 expression alone does not capture the complexity of immune responsiveness, reinforcing the need for integrative biomarkers. Moreover, their study showed that a combination of high TMB and TP53 mutation was associated with improved clinical outcomes in patients receiving immunotherapy. These observations were consistent with authors' findings, which indicated that TP53 co-mutations – particularly with TTN – may enhance survival specifically in immune-inflamed tumour phenotypes. Overall, expanding the biomarker panel beyond PD-L1 may improve therapeutic outcomes by identifying subgroups most likely to benefit from immunotherapy.

Despite considerable progress in elucidating the genomic and immunological features of LUSC, few studies have integrated these parameters in a comprehensive survival-based analysis. Unlike previous research, which primarily focused either on mutational frequency or immune classification, the current study examined the prognostic impact of both individual and concomitant mutations in relation to immune tumour subtypes. This integrated approach offers a broader understanding of how specific genetic alterations interact with the immune contexture to influence patient outcomes. By stratifying survival analyses based on immune subtype, the research provided new insights into the heterogeneity of LUSC and highlighted potential avenues for improving risk stratification and developing personalised therapy.

CONCLUSIONS

In this study, a comprehensive analysis of the mutational profile, immune subtypes, and overall survival was conducted in 419 patients with LUSC using data from TCGA. Particular attention was given to four frequently mutated genes – TP53, TTN, PIK3CA, and KEAP1/NFE2L2 – which have been implicated in oncogenesis and immune regulation. Mutations in TP53 and TTN, both individually and in combination, demonstrated significant prognostic impact, especially among patients with high or intermediate immune activity in the tumour microenvironment. The highest overall survival was observed in patients with TP53+TTN co-mutations, with a median survival of 63.7 months compared to 27.8 months in patients without such alterations ($p = 0.0065$). A similar trend was observed in the subgroup with low immune activity, where patients harbouring TP53+TTN co-mutations also demonstrated the most favourable survival outcomes ($p = 0.0006$). In contrast, individual mutations in PIK3CA and KEAP1/NFE2L2 showed no statistically significant association with prognosis, suggesting limited prognostic relevance when considered in isolation from the broader genomic context. KEAP1/NFE2L2 was the only mutation significantly associated with immune subtype. It was more frequently observed in tumours with low immune activity (32.5% vs 21.4%, $p = 0.012$), indicating a potential role in shaping an immunosuppressive tumour microenvironment through dysregulation of oxidative stress pathways. Cluster analysis further confirmed the high frequency of TP53 and TTN co-mutations but did not reveal any distinct visual patterns related to survival outcomes. These findings highlighted the molecular heterogeneity of LUSC and underscore the necessity for a multifactorial approach to risk stratification and the development of integrated, personalised therapeutic strategies based on both genomic and immunological characteristics, which may be considered in the future.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Zhao J, Bao W, Cai W. Immune infiltration landscape in lung squamous cell carcinoma implications. Biomed Res Int. 2020;2020:5981870. DOI: 10.1155/2020/5981870

[2] Pop-Bica C, Ciocan CA, Braicu C, Haranguș A, Simon M, Nutu A, et al. Next-generation sequencing in lung cancer patients: A comparative approach in NSCLC and SCLC mutational landscapes. J Pers Med. 2022;12(3):453. DOI: 10.3390/jpm12030453
[3] Xu Y, Li H, Huang Z, Chen K, Yu X, Sheng J, et al. Predictive values of genomic variation, tumor mutational burden, and PD-L1 expression in advanced lung squamous cell carcinoma treated with immunotherapy. Transl Lung Cancer Res. 2020;9(6):2367–79. DOI: 10.21037/tlcr-20-1130
[4] Paik PK, Fan PD, Qeriqi B, Namakydoust A, Daly B, Ahn L, et al. Targeting NFE2L2/KEAP1 mutations in advanced NSCLC with the TORC1/2 inhibitor TAK-228. J Thorac Oncol. 2023;18(4):516–26. DOI: 10.1016/j.jtho.2022.09.225
[5] Wang D, Wang Y, Peng Y, Peng L. Utilizing multi-omics analysis, a new signature has been identified and validated for predicting prognosis and response to immunotherapy in lung squamous cell carcinoma, which is based on tumor mutation burden. Discov Oncol. 2025;16(1):539. DOI: 10.1007/s12672-025-02166-2
[6] Deng X, Wang Z, Luo Y, Li Z, Chen L. Prediction of lung squamous cell carcinoma immune microenvironment and immunotherapy efficiency with pyroptosis-derived genes. Medicine (Baltimore). 2022;101(37):e30304. DOI: 10.1097/MD.0000000000030304
[7] Hellyer JA, Padda SK, Diehn M, Wakelee HA. Clinical implications of KEAP1-NFE2L2 mutations in NSCLC. J Thorac Oncol. 2021;16(3):395–403. DOI: 10.1016/j.jtho.2020.11.015
[8] Su C, Wang X, Zhou J, Zhao J, Zhou F, Zhao G, et al. Titin mutation in circulatory tumor DNA is associated with efficacy to immune checkpoint blockade in advanced non-small cell lung cancer. Transl Lung Cancer Res. 2021;10(3): 1256–65. DOI: 10.21037/tlcr-20-1118
[9] Kozakov D, Kobyliak N, Livshun S, Seleznov O, Koshyk O, Matvieieva A, et al. Genetic alterations affect immune contexture of non small cell lung cancer: Ukrainian study. Front Med. 2025;12:1558016. DOI: 10.3389/fmed.2025.1558016
[10] Sulaieva O, Pototska O, Kozakov D, Livshun S, Panko M, Vynnychenko O, et al. Molecular biomarkers in the management of patients with non-small cell lung cancer. Pract Oncol. 2024;7(1):27–35. DOI: 10.22141/2663-3272.7.1.2024.97
[11] High-quality datasets. From foundational cancer genomic studies [Internet]. [cited 2025 April 4]. Available from: https://portal.gdc.cancer.gov
[12] UCSC Xena [Internet]. [cited 2025 April 4]. Available from: https://xenabrowser.net
[13] CBioPortal for cancer genomics [Internet]. [cited 2025 April 4]. Available from: https://www.cbioportal.org
[14] Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, et al. The immune landscape of cancer. Immunity. 2018;48(4):812–30. DOI: 10.1016/j.immuni.2018.03.023
[15] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2025 April 4]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[16] Ying K, Zou L, Wang D, Wang R, Qian J. Co-mutation of TP53 and TTN is correlated with the efficacy of immunotherapy in lung squamous cell carcinoma. Comb Chem High Throughput Screen. 2024;27(18):2699–711. DOI: 10.2174/0113862073246841230922052004
[17] Zou S, Ye J, Hu S, Wei Y, Xu J. Mutations in the TTN gene are a prognostic factor for patients with lung squamous cell carcinomas. Int J Gen Med. 2022;15:19–31. DOI: 10.2147/IJGM.S343259
[18] Xie X, Tang Y, Sheng J, Shu P, Zhu X, Cai X, et al. Titin mutation is associated with tumor mutation burden and promotes antitumor immunity in lung squamous cell carcinoma. Front Cell Dev Biol. 2021;9:761758. DOI: 10.3389/fcell.2021.761758
[19] Fan Z, Zhang Q, Feng L, Wang L, Zhou X, Han J, et al. Genomic landscape and prognosis of patients with TP53-mutated non-small cell lung cancer. Ann Transl Med. 2022;10(4):188. DOI: 10.21037/atm-22-412
[20] Yu J, Fan Z, Zhou Z, Zhang P, Bai J, Li X, et al. TP53 and LRP1B co-wild predicts improved survival for patients with LUSC receiving anti-PD-L1 immunotherapy. Cancers (Basel). 2022;14(14):3382. DOI: 10.3390/cancers14143382
[21] Yang L, Wei S, Zhang J, Hu Q, Hu W, Cao M, et al. Construction of a predictive model for immunotherapy efficacy in lung squamous cell carcinoma based on the degree of tumor-infiltrating immune cells and molecular typing. J Transl Med. 2022;20(1):364. DOI: 10.1186/s12967-022-03565-7
[22] Wang J, Zhu J, Tang Y, Zhang A, Zhou T, Zhou Y, et al. Characteristic of molecular subtypes in lung squamous cell carcinoma based on autophagy-related genes and tumor microenvironment infiltration. J Oncol. 2022;2022:3528142. DOI: 10.1155/2022/3528142
[23] Sánchez-Ortega M, Garrido A, Cirauqui C, Sanz-Gonzalez L, Hernández MC, González-García A, et al. A potential therapeutic strategy based on acute oxidative stress induction for wild-type NRF2/KEAP1 lung squamous cell carcinoma. Redox Biol. 2024;75:103305. DOI: 10.1016/j.redox.2024.103305
[24] Huang Q, Zhou Y, Wang B, Zhao Y, Zhang F, Ding B. Mutational landscape of pan-cancer patients with PIK3CA alterations in Chinese population. BMC Med Genomics. 2022;15(1):146. DOI: 10.1186/s12920-022-01297-7
[25] Cokpinar S, Erdogdu IH, Orenay-Boyacioglu S, Boyacioglu O, Kahraman-Cetin N, Meteoglu I. PIK3CA mutations and co-mutations in operated non-small cell lung carcinoma. J Clin Med. 2024;13(23):7472. DOI: 10.3390/jcm13237472
[26] Rocha P, Bach R, Masfarré L, Hernandez S, Navarro-Gorro N, Rossell A, et al. Molecular and immunological features associated with long-term benefits in metastatic NSCLC patients undergoing immune checkpoint blockade. Oncoimmunology. 2025;14(1):2469377. DOI: 10.1080/2162402X.2025.2469377

[27] Tao W, Sun Q, Xu B, Wang R. Towards the prediction of responses to cancer immunotherapy: A multi-omics review. Life. 2025;15(2):283. DOI: 10.3390/life15020283
[28] Burlaka A. Adipose tissue and its role in microenvironment of the colorectal adenocarcinoma cancer cell. Int J Med Med Res. 2019;5(1):26–32. DOI: 10.11603/ijmmr.2413-6077.2019.1.9819

Молекулярні та імунні предиктори виживаності при плоскоклітинній карциномі легень: аналіз даних TCGA

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Анотація. Плоскоклітинна карцинома легень залишається однією з найбільш агресивних форм недрібноклітинного раку з обмеженими можливостями для персоналізованого лікування, що зумовлює потребу у нових прогностичних біомаркерах. Мета дослідження – проаналізувати вплив соматичних мутацій та їх ко-мутацій у ключових онкогенах (TP53, TTN, PIK3CA та KEAP1/NFE2L2) на виживаність хворих на плоскоклітинну карциному легень з урахуванням імунного підтипу пухлини. Було використано дані відкритих онкологічних репозиторіїв (TCGA, UCSC Xena, cBioPortal) для 419 пацієнтів з повним клінічним, імунним та мутаційним профілем. Пацієнтів стратифікували за рівнем імунної активності пухлини (високий/середній або низький підтип), а аналіз мутацій охоплював окремі гени та ко-мутації. Виживаність оцінювали за методом Каплана-Майєра з лог-ранговим тестом. Було встановлено, що мутація TP53 асоціюється з достовірно кращою виживаністю як у групі з високою/середньою імунною активністю (медіана 57,9 проти 27,8 міс., p = 0,0141), так і в групі з низькою імунною активністю (p = 0,0361). Мутація TTN продемонструвала позитивну тенденцію у групі з високою/середньою активністю (p = 0,0582) та достовірний зв'язок з виживаністю у групі з низькою активністю (p = 0,0123). Найсильніший ефект виявлено для ко-мутації TP53 + TTN, яка значуще покращувала виживаність у пацієнтів з обома імунними підтипами (високий/середній: p = 0,0065; низький: p = 0,0006). Водночас мутації PIK3CA, KEAP1/NFE2L2 та їх комбінації не продемонстрували статистично значущого впливу на виживаність. Кластерний аналіз мутаційного профілю виявив два основні патерни, де TP53 і TTN мутації мали тенденцію до кластеризації. Водночас не було виявлено чіткої візуальної асоціації між мутаційними підтипами та статусом виживаності. Таким чином, мутаційний статус генів TP53 і TTN, а також їх ко-мутація TP53+TTN мають суттєве прогностичне значення у пацієнтів з плоскоклітинною карциномою легень, особливо у поєднанні з характеристиками імунного мікрооточення. Натомість ізольовані мутації в PIK3CA та KEAP1/NFE2L2 не мали достовірного впливу на виживаність. Отримані результати підкреслили важливість інтеграції мутаційного та імунного профілю для формування персоналізованих стратегій лікування

Ключові слова: TP53; TTN; PIK3CA; KEAP1/NFE2L2; рак легень; ко-мутації

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Modern experimental systems for studying various functions of the gastrointestinal tract

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Abstract. The integrity and functioning of intestine are crucial for the digestion and absorption of nutrients, immunological homeostasis and the prevention of the ingestion of pathogens. Currently, the improvement and development of new experimental systems for studying various functions of the intestine is a pressing task in biology and medicine. The aim of this review was to describe modern experimental systems for studying various functions of the intestine, focusing on their application, advantages and limitations. To achieve this goal, publications from 2019-2025 selected from PubMed and Google Scholar were analysed and summarised. Based on the analysis of literature data and author's experimental studies, it was established that methods such as "everted intestine" and "Ussing chamber" are effective for studying the mechanisms of kinetics and absorption of drugs in the small and large intestines. The "InTESTine™" system is a tool for assessing intestinal permeability and predicting intestinal absorption of food and drugs. Systems such as "intestinal organoids", "enteroid systems" and "gut-on-a-chip" used to study dynamic processes, secretion, and absorption in the intestine have been analysed. It has been shown that transformed and cultured "epithelial cell lines" (Caco-2, HT-29 and T84) have certain disadvantages in use and cancerous origin of cells. An "epithelial cell suspension" was investigated, which is a simple and promising *ex vivo* system for studying the direct and immediate effects of chemicals on the intestinal epithelium. The practical value of the work lies in comparing experimental methods for studying the intestine, which can contribute to the effectiveness of preclinical research, the improvement of medical care diagnostics, and the development of personalised medicine

Keywords: intestine; *in vitro* model; *ex vivo* mode; enterocytes; intestinal epithelial cell suspension

INTRODUCTION

Research into experimental systems for studying gastrointestinal (GI) functions is an important area in modern physiology, gastroenterology and biomedicine. Improvements in methods allow for a deeper understanding of the mechanisms of digestion, nutrient absorption, microbiota regulation, and the pathogenesis of various gastrointestinal diseases (in particular, inflammatory bowel disease, irritable bowel syndrome and cancer). One of the main reasons for the relevance of this research is the high prevalence of gastrointestinal diseases. Using experimental methods (*in vitro*, *in vivo*, *ex vivo* and *in silico*), researchers model pathological conditions, test drugs, and analyse the impact of various factors on intestinal function. Thus, the development and improvement of experimental methods for studying intestinal functions remains a relevant task that contributes to the progress of both fundamental science and clinical practice.

Models for studying the human and animal digestive systems have undergone significant improvements with the aim of enhancing their predictive ability regarding the bioavailability of various compounds, studying absorption mechanisms, and investigating the immune response of the intestine. In their work, R. Moerkens *et al*. [1] developed a "small intestine-on-a-chip" model, which is characterised by its ability to self-organise into a villous structure and includes mesenchymal and nerve cells. This platform provided apical/basolateral access and demonstrated mature barrier and metabolic gene programmes. This approach promotes the development of personalised *in vitro* modelling of intestinal physiology and drug response.

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In a study by S.M. Parigi *et al.* [2], spatial transcriptomics was developed for the entire mouse colon. They revealed previously underappreciated molecular regionalisation and identified spatially organised healing programmes (including p53-associated zones), providing a rich reference atlas for intestinal regeneration studies. Comparing colon tissues at rest and after mucosal healing (in treated mice), spatial transcriptional processes of tissue repair, immune cell activation/recruitment, pro-regenerative pathways, and tissue remodelling were identified.

In a Ukrainian study by S. Soloviov *et al.* [3], the authors created an *in vitro* model to assess how metabolites derived from *Lactobacillus* modulate the course of enterovirus infection in intestinal cells. This practical experimental approach made it possible to analyse the interactions between the host, the microbe and the virus, which is important for studying the barrier function of the intestine. In a research study conducted by M.N. Zhayvoronok & V.M. Zalesky [4], *ex vivo* samples of human intestinal tissue were used to investigate fibrogenesis in inflammatory bowel disease. The authors demonstrated that the accuracy of the obtained results strongly depended on the quality of resected specimens, highlighting the potential of such models for translational research. In a paper by E.M. Holloway *et al*. [5], a vascular component was formed in intestinal organoids, including endothelial cells. This significantly increased their physiological plausibility and potential for modelling tissue invasion or inflammation. In the work of J. Kassis & Z. Wan [6] presented the integration of organoids and microfluidic intestine-on-a-chip platforms for testing drug permeability – in particular, a Caco-2 chip directly connected to mass spectrometry, which allows for high-throughput parallel testing of drug efficacy. M.R. Kuhn *et al*. [7] used hydrogels with a specific natural protein composition (collagen, fibrin, hyaluron) that proved suitable for culturing human intestinal organoids. These matrices promoted long-term growth, were capable of differentiation, and maintained cellular heterogeneity.

Generally, the studies presented demonstrated a wide range of approaches, from microfluidic platforms to organoid models with vascular and immune components. At the same time, a number of problems remained: lack of standardisation of methods, limited reproducibility of experiments, and the complexity of modelling the full interaction between the microbiota, immune and nervous systems. Therefore, the aim of this work was to describe, analyse and systematise current experimental models used to study the functions of the small and large intestines.

To achieve these goals, a detailed literature review was conducted using the PubMed, Google Scholar and Scopus databases only sources published within the last six years were considered. Initially, about 120 sources (2019-2025) were selected, of which 45 articles were retained for detailed consideration after critical analysis. The selection criteria included the originality of the data, the availability of clinical or experimental results, and the novelty and relevance of the research area. Experimental publications describing methods for studying the gastrointestinal tract, namely the processes of absorption, transport and absorption of nutrients and drugs, as well as review articles, were analysed. A systematic search strategy was applied using relevant keywords and search terms. The information was systematised by model type (*ex vivo*, *in vivo*, *in vitro*, etc.), which made it possible to identify the main trends and determine the existing gaps in methods for studying intestinal functions.

TYPES OF EXPERIMENTAL APPROACHES TO INTESTINAL RESEARCH

There are many models of the GIT that allow the study of transport mechanisms and predict the pharmacokinetics of drugs. Establishing the *in vitro* – *in vivo* relationship is extremely important for patient-centered drug development from a product quality perspective. This review summarised and structured information about modern experimental systems for studying various intestinal functions, focusing on their application, advantages and limitations (Table 1).

Table 1. Modern experimental models for intestinal research

Model	Description	Examples of use
In vivo	Animal systems preserving systemic circulation and neural pathways.	Mice, rats, pigs, dogs, monkeys.
Ex vivo	Intestinal tissue sections isolated from animals, incubated under controlled conditions.	Everted gut sac, Ussing chamber, InTESTine™, intestinal suspension.
In vitro	Intestinal epithelial cell lines cultivated on flat surfaces.	Caco-2, HT-29, T84.
3D cell culture	Scaffold-based cultures mimicking the intestinal microenvironment and cell architecture.	Hydrogel-based systems, ECM scaffolds.
Organoids	Mini-gut structures derived from stem cells.	Human intestinal organoids, enteroids.
Microfluidic devices	«Gut-on-chip» platforms simulating intestinal mechanics, flow, and epithelial interactions.	Intestine-on-a-chip, enteroid chip.
In silico	Computational simulations of absorption, metabolism, and barrier function based on biological data.	Physiologically based pharmacokinetic modelling, AI-driven GI-simulations.

Source: compiled by the author based on A.P. Lytvynenko [8] and F. Abbas *et al*. [9]

Modern 3D bioprinting models of the intestine allow the creation of complex three-dimensional structures with crypt villi and laminated layers of epithelium and stroma. In 2024, a tubular model of the intestine with bioinclusions has been developed that independently generates epithelial micromorphogenesis, which allows for *in virto* modelling of drug cytotoxicity [10]. Bioprinting technology made it possible to recreate a model of the human small intestine. The process involved forming tubular structures consisting of inner and outer layers formed by epithelial cells. This ensured the integration of vascular endothelial cells and smooth muscle cells necessary to reproduce the

characteristic structure of the intestinal wall. The proposed model is significantly closer to the natural architecture of the small intestine and exceeds the level of structural similarity achieved in previous *in vitro* systems. Thus, this method of coaxial bioprinting to construct 3D tubular structures somewhat overcomes the limitations associated with structural and functional similarity to native tissue. *In silico* models provide mechanistic simulation of drug absorption, effectively predicting pharmacokinetic parameters within 2-fold accuracy, which facilitates early bioavailability assessment [11]. Nonclinical models of absorption to study transport mechanisms, determine intestinal permeability, and predict plasma pharmacokinetic profiles are extremely important.

Thus, modern microfluidic systems facilitate drug testing by simulating intestinal absorption and barrier function, providing greater relevance for pharmacokinetic and toxicological studies. The development of new platforms allows for the co-cultivation of intestinal epithelium with immune cells, vascular endothelium, and microbial communities to model host-microbiome interactions and immunological interactions *in vitro*. The prospect of developing microfluidic devices requires their verification, testing, and comparison with living native systems.

MODERN *EX VIVO* INTESTINAL EXAMINATION METHODS

Ex vivo methods involve the use of functional tissues extracted from organisms and maintained under controlled laboratory conditions; however, the absence of blood circulation and neural regulation in such setups may influence the reliability of certain results. One of the earliest and still widely used *ex vivo* models is the everted intestinal sac, introduced by Wilson and Wiseman in the 1950s to investigate the kinetics and mechanisms of drug absorption across various segments of the gastrointestinal tract. This model remains relevant, especially for evaluating the absorption rates of bioactive compounds in experimental studies on rats [12]. The intestinal sac evisceration method involves isolating a specific segment of the intestine (duodenum, ileum, or colon) and then thoroughly washing it with saline solution. The intestine is then everted onto a glass plate and filled with Krebs buffer solution in an oxygenated incubation system at 37°C. The result depends on age, sex, species, segment origin, and experimental factors (pH and temperature). Data obtained by K. Miyazaki *et al*. [13] indicated that modifications such as removal of the serous and muscular layers can improve permeability assessment but lead to inaccuracy and biological variability.

Using an inverted intestinal sac model, it was found that lanthanum interacts with iron during absorption. This indicated pharmacokinetic barriers to the simultaneous administration of these elements [14]. *Ex vivo* methods, including isolated intestinal segments and tissue explants, allow for accurate assessment of the effect of nanoparticles on intestinal physiology without systemic *in vivo* exposure. For example, using *ex vivo* perfused rat intestinal explants, M. Qi *et al.* [15] assessed the effect of Ag nanoparticles on neurotransmitter release (NO, serotonin) and local motility in real time. It was noted that even short-term exposure to metal nanoparticles can alter intestinal neuroregulation, which is critical for understanding potential toxicological risks. Based on this, S. Guo *et al.* [16] conducted a detailed analysis of the absorption stages of model nanoparticles of different sizes, surface charges, and levels of hydrophobicity or hydrophilicity. It has been proven that chemical composition and physicochemical parameters influence the interaction of nanoparticles with the intestinal barrier. Subsequently, research by Y. Zheng *et al.* [17] confirmed that particle size and surface modifications play a decisive role in trans-epithelial transport. The results obtained point to potential strategies for improving the oral administration of nanoparticle-based drugs by adapting the surface properties of the particles. Additional evidence was also obtained in studies using the inverted intestinal sac method [18], which demonstrated that surface characteristics may be a key factor determining absorption efficiency. These findings support the view that *ex vivo* intestinal models, despite their limitations, remain highly relevant for the mechanistic assessment of nanomaterial safety. Nevertheless, the model remains an accessible, reproducible, and effective method for studying the kinetics of transport and absorption of drugs, including nanoparticles, in both the small and large intestines *ex vivo* [19].

The Ussinger chamber was developed in the 1950s by Hans Ussinger to measure GI-permeability using paracellular flow and electrical measurements and has been adapted for both mouse and human tissues [20]. The chamber consists of two compartments separated by monolayers of tissue or cells, isolating the apical and basolateral sides of the epithelium. The Ussinger chamber is used to measure permeability and ion transport through epithelial tissues and cells. This led to the discovery of the Na+/K+ATPase pump. Thus, it has been established that a decrease in mucosal integrity is closely associated with the pathogenesis of ulcerative colitis, Crohn's disease, and colorectal cancer [21, 22]. These disorders are accompanied by impaired barrier function, which can subsequently cause chronic inflammation. In this context, S.C. Pearce *et al.* [23] emphasised the value of the Ussing chamber as a versatile experimental tool. Unlike many *in vitro* cell models, this system allows researchers to measure permeability, tissue integrity, and transport processes in intact intestinal samples under controlled conditions. However, *ex vivo* tissue explants used in Ussinger chambers are viable for five hours. Thus, the Ussinger chamber remains a valuable tool for measuring intestinal integrity (for measuring the permeability of *ex vivo* colon tissue samples from mice and humans). This method preserves tissue physiology, allowing for accurate assessment of absorption, barrier function, and transporter activity. Low throughput, laborious setup, and the need for fresh tissue remain drawbacks of this system.

The InTESTine™ platform was developed to study the passage and absorption of biological, nutritional, and pharmacological substances through the intestinal barrier. This system also uses different segments of the intestine, such as the duodenum, jejunum, ileum, and colon. The segments are arranged in such a way as to maintain distinct apical and basal-lateral divisions [23]. However, unlike a traditional chamber, the InTESTine™ system is compatible with standard 6- and 24-well plates, providing higher throughput and simple horizontal positioning [24]. Due to its practicality and convenience, this platform is well suited for *ex vivo* analysis of various gastrointestinal functions.

Thus, viable intestinal tissue mounted in the InTESTine™ system can be used as a reliable tool for evaluating intestinal permeability. This is suitable for predicting intestinal absorption in humans of substances such as digested foods and drugs and for studying complex intestinal processes due to the presence of many cell types in the tissue *ex vivo*.

In study A. Fedi *et al*. [25] a suspension of small intestinal epithelial cells was obtained from the upper parts of villi. It has been shown that such enterocytes can be used as an effective alternative for comparative analysis of the characteristics of nutrient transport in the epithelium. Light and immunofluorescence microscopy confirmed that the isolated cells retain a differentiated phenotype, form intercellular contacts and restore polarity, with a clear division into apical and basolateral domains. All experiments were performed after 7-9 hours of incubation, when the cells remained viable. Y. Kimura *et al*. [26] and A.P. Lytvynenko *et al*. [27] described a method for obtaining functional epithelium using differentiated enterocytes collected from the upper part of the villi of the small intestine of adult mice. Three main types of differentiated intestinal epithelial cells can be identified in the area of differentiated villi: enterocytes, goblet cells and enteroendocrine cells. The method allows the isolation of morphologically identifiable intestinal epithelial cells: elongated columnar and spherical cells. Columnar cells usually originate from the tip of the villi, and spherical cells from the middle and lower parts of the villi. Thus, the effect of dextran-polyacrylamide polymers, charged and uncharged, as well as their carriers of gold and silver nanoparticles, on the regulated cell death of ileal enterocytes in mice was evaluated. Preparation of epithelial monolayers from freshly isolated mouse enterocytes allowed quantitative assessment of fluid movement in the apical part of villi and paracellular filtration. These processes depend on gap junctions and their integrity. Thus, there is a direct mechanical link between intestinal motility and intestinal fluid regulation. The isolation of enterocyte suspension is a simple method, although it has certain limitations, such as possible mechanical damage to the cells. Therefore, to avoid significant deviations, the enterocytes are stained with trypan blue and morphological assessment of cell viability is performed. This method is authorised for use in experimental research, testing and improvement.

2D and 3D cultures of GI-organoids are actively developing, so immunohistochemistry on *ex vivo* tissues is a relevant area for improving these systems. C.M. Wang *et al*. [28] have attempted to recreate the tissue environment, matrix, and receptors that influence cell signalling, changing basic cell phenotypes. S. Deyaert *et al*. [29] developed a dynamic, long-term model of the ileal microbiota using SHIME® technology. This system mimics a synthetic bacterial consortium of 12 species. This provides representative modelling of the ileal bacterial community, facilitating the study of microbiota dynamics and activity. This approach allows the interaction between the food matrix, the microbiome and the intestine to be modelled. However, the role of this specific microbial community in influencing the absorption of nutrients and drugs remains unclear. Table 2 summarised the described methods for *ex vivo* intestinal research.

Table 2. *Ex vivo* intestinal examination methods

Method	Advantages	Disadvantages
Ex vivo models using resected tissues	✦ high biological relevance; ✦ preserved tissue architecture.	✦ limited tissue viability; ✦ donor variability.
Ussing chambers	✦ classic method for epithelial ion transport; ✦ barrier function study.	✦ technically demanding; ✦ requires fresh samples.
Intestinal epithelial cell suspension	✦ simple method isolating enterocytes.	✦ some mechanical damage to the cells.
Immunohistochemistry on *ex vivo* tissues	✦ precise localisation of proteins and cytokines; ✦ preserved microenvironment.	✦ static snapshot, lacks dynamic context.

Source: compiled by the author

Thus, *ex vivo* systems remain effective for studying the mechanisms of kinetics and absorption of drugs in the small and large intestines. *Ex vivo* models provide high biological relevance, but their use is complicated by the short viability of tissues and the variability of donor material. Ussing chambers remain the gold standard for analysing barrier function and ion transport, but require high technical skills and access to fresh samples. The use of enterocyte suspensions is a simple approach, but is accompanied by mechanical damage to the cells. Immunohistochemistry on *ex vivo* samples allows for clear determination of the localisation of proteins and cytokines, although its static nature limits the ability to study dynamic processes.

✦ EPITHELIAL CELL LINES: CACO-2, HT-29 AND T84

Immortalised cell lines derived from the human intestine, such as Caco-2, HT-29 and T84, remain indispensable in permeability and transport studies. Caco-2, first described in 1977, consistently differentiated into polarised enterocyte-like monolayers containing microvilli and tight junctions. It continues to serve as the gold standard for assessing intestinal drug absorption, despite limitations such as overly tight barrier, low hydrophilic permeability, limited metabolism [30]. N. Panse & P.M. Gerk [31] have improved its physiological characteristics by co-culturing with mucin-secreting cells (HT29-MTX). Thus, although Caco-2 retains its status as the “gold standard”, its limitations point to the need for combined systems. Table 3 provided specific examples of co-cultivation. Combination with other cell lines gradually improves the system, reduces the artificiality of the model, and brings it closer to *in vivo* conditions. T84, obtained from human colorectal carcinoma in the 1980s, is characterised by the formation of dense cell layers with a large number of intercellular contacts. This cell line is actively used to study electrolyte transport and barrier properties of the epithelium [32]. T84 does not reproduce the biochemical phenotype of mature small intestine

enterocytes characteristic of Caco-2. Similar to Caco-2, T84 cells can form absorptive monolayers of epithelial cells and are capable of differentiating into crypt-like structures [33]. The T84 line is less suitable for modelling nutrient absorption, but it is valuable for studying electrolyte transport and the functioning of tight junctions. At the same time, its ability to form crypt-like structures opens up prospects for researching regenerative processes in the intestine.

Table 3. Co-culture of Caco-2 epithelial cells

	Caco-2	Caco-2 + HT29-MTX	Caco-2 + HT29 + T84
Application	Drug transport model.	Mucus barrier model for nutrient uptake and bacterial adhesion.	Complex intestinal physiology (barrier + mucus + Cl^- secretion).
Advantages	High reproducibility; widely accepted for permeability screening.	Closer to *in vivo* mucus interaction; modulates permeability via 90:10 cell ratio.	Simulates inflammation, leaky gut; includes secretory and immune-like activity.
Disadvantages	No mucus layer; limited immune function modelling.	Incomplete MUC2 expression; variable transporter levels.	Complex culture setup; low throughput; interpretation requires caution.

Source: compiled by the author based on M. Anjum *et al*. [34], S. Peddibhotla *et al*. [35], M. Belaid *et al*. [36], D. Marescotti *et al*. [37]

HT-29, a colorectal adenocarcinoma cell line established in 1964, can differentiate into heterogeneous epithelial populations, including goblet cells that secrete mucus. M.J. Haddad *et al*. [30] have focused on the effects of specific stimuli, such as methotrexate or sodium butyrate. Co-cultures HT-29 with Caco-2 contribute to the creation of more physiologically relevant epithelial models by combining the barrier properties of mucus with absorptive functions. Caco-2 and HT-29 cell lines have similar properties of enterocytes during differentiation, but they also have some differences. While Caco-2 cells differentiate to a monolayer of absorptive phenotype without mucus granules, HT-29 cells show high heterogeneity in the formation of absorptive and goblet cells and, therefore, are capable of producing mucus [31]. The unique ability of HT-29 cells to form goblet-like cells makes them an important tool for modelling the intestinal mucosa. Their use in co-cultures with Caco-2 cells allows for the reproduction of more complex barrier and secretory properties of the epithelium.

Transformed and cultured "epithelial cell lines" (Caco-2, HT-29 and T84) have certain disadvantages: cancer origin, lack of specific epithelial function, different expression, in particular, of glucose transporter protein, the absence of a mucosal layer, etc. Whereas, the 'epithelial cell suspension' of enterocytes collected from the upper part of the small intestinal villi provides an alternative approach for comparative studies and responses to various conditions and stimuli in the GIT. However, these cell systems continue to evolve through new approaches of advanced engineering and co-culture strategies. These systems are very valuable in predicting drug permeability, mimicking epithelial function and modelling intestinal barrier physiology.

3D CELL CULTURE, INTESTINAL ORGANOID AND MICROFLUIDIC DEVICES

In 2023, microfluidic technologies have been intensively developed to create organ-on-a-chip devices designed to reproduce the three-dimensional topography of organs. A new coaxial bioprinting of small intestine technique has enabled the fabrication of multicellular 3D constructs that show improved structure and gene expression compared to traditional 2D monolayers [38]. New models with an open apical surface of organoids facilitate the study of pathogen invasion and nutrient uptake. Advances in the field of scaffolds for biomaterials have enabled the transition from Matrigel to synthetic hydrogels based on polyethylene glycol and collagen. This improves reproducibility and allows for the influence on the morphology and differentiation of organoids [39, 40]. Such developments highlighted the rapid advancement of 3D intestinal culture technologies. These models combine microengineering, advanced biomaterials and automation to more accurately mimic the *in vivo* gut environment. In 2021, the development of three-dimensional culture systems known as organoids has enabled the cultivation of cryptic villi that mimic many aspects of intestinal physiology. Organoids are cellular aggregates cultured in three dimensions and have the same characteristics as the tissue of origin. Due to their ability to self-renew and proliferate, organoids can be maintained in culture for a long time. Organoids have become a key platform for studying the intestinal barrier, tight junction regulation, and epithelial renewal *in virto*. These systems are suitable for studying permeability and inflammation because they respond to external stimuli such as cytokines, bacterial components, etc. [21, 41].

Organoid culturing has also been used I.A. Parente *et al.* [42] to study GI-diseases, gut-microbe interactions, and colorectal cancer. Organoids have proven to be valuable in the reproduction of inflammatory and neoplastic intestinal disorders. It has been shown that the destruction of tight junction proteins, such as claudin-2, claudin-7 and claudin-15, in mouse organoids allows us to study the mechanisms and causes of barrier disruption associated with chronic intestinal inflammation. Organoids provide a platform for researching infectious diseases, hereditary pathologies and cancer using genetic engineering and stem cell techniques. Moreover, transplantation of organoids into animal models allows tissues to be reconstituted and their reactions to be monitored *in vivo* [43]. These living biobanks offer insight into inter-patient variability and facilitate the identification of personalised therapeutic options. Thus, significant progress has been made in the development of intestinal epithelial cell cultivation technologies. The intestinal organoid is a widely used model system for studying the dynamic processes occurring at

the host-microbe interface and the mutual interactions between intestinal epithelial cells and cells of local immune homeostasis. Despite the obvious advantages of using multicellular "intestinal organoids" compared to single-cell lines, organoids still lack certain parts of intestinal physiology, such as the stroma, vasculature, immune system and microbiome, which limits the representation of the *in vivo* situation.

Intestine-on-a-chip allows for dynamic analysis of epithelial barrier function, providing simultaneous access to the apical and basolateral sides. This platform maintains continuous fluid perfusion, which promotes the development of a mucus layer and the creation of an oxygen gradient, allowing the cultivation of intestinal cells simultaneously with commensal or pathogenic microorganisms [43]. Compared to 3D organoid cultures, the gut-on-a-chip model more accurately reflects the duodenal transcriptome *in vivo*. In addition, this system allows for real-time sampling of light flux, which allows for monitoring mucus production, nutrient absorption, and barrier integrity. Intestine-on-a-chip allows for the formation of a protective mucus layer and an oxygen gradient, which supports the co-cultivation of cells and microbes for a longer time [44]. This model is technically complex and expensive, but it allows for the integration of different cell types (epithelial, stromal and microbial components), making it ideal for multivariate studies of intestinal function and disease mechanisms. Enteroid or mini-gut is derived from $LGR5^+$ intestinal stem cells isolated from the small or large intestine. It is a physiological model for the study of intestinal transport and host-pathogen interactions, for the study of viral, bacterial and protozoan parasitic infections [45]. This model allows the study of secretion and absorption processes in specific areas of the intestine under the influence of various stimuli.

Organoids derived from the intestine serve as valuable systems for studying dynamic interactions, including host-microbe relationships and communication between epithelial and immune cells. However, these systems do not fully reproduce the physiology of the intestine, as they lack components such as stromal tissue, blood vessels, immune cells, and resident microbiota, which limits their ability to completely mimic *in vivo* conditions. The enteroid model of the intestine, in turn, allows the study of segmental secretory and absorptive functions. Whereas the "gut chip" system is a new approach to studying gastrointestinal functions by incorporating several cell types and/or the intestinal microbiome into the system. Table 4 summarised the advantages and disadvantages of the systems described.

Table 4. Advantages and disadvantages of 3D organoids, Enteroid, Intestine-on-a-chip

Model	3D Organoids	Enteroid	Intestine-on-a-chip
Key features	Three-dimensional cellular aggregates capable of self-renewal and proliferation; cultured from LGR5+ stem cells.	Models derived from small or large intestine segments; LGR5+ cells.	Microfluidic systems with continuous fluid flow, mucus layer formation, and oxygen gradient.
Advantages	Closely resemble the original tissue, long-term culture possible, suitable for genetic studies.	Segment-specific functional studies, early molecular events can be observed.	Dynamic modeling of intestinal physiology, integration of multiple cell types, realistic transcriptome.
Limitations	Lack stroma, vasculature, immune system, and microbiome.	Limited modelling of multicellular interactions.	High technical complexity and cost.

Source: compiled by the author

Thus, modern technologies allow reproducing three-dimensional intestinal architecture with varying levels of complexity. 3D organoids are a powerful tool for studying cellular processes, barrier function, and pathogenic influences, while enteroids provide segment-specific functional research. Innovative microfluidic platforms such as intestine-on-a-chip allow the integration of multiple cell types and microbiota, providing a more accurate modelling of intestinal physiology *in vitro*. At the same time, none of the models fully reproduces all aspects of *in vivo*, so their selection depends on the specific research goal, and the results require accurate interpretation and comparison with living systems.

CONCLUSIONS

It has been found that modern models of intestinal research allow for detailed study of intestinal physiology, substance transport, and its barrier function. The analysis showed that *ex vivo* tissues allow for accurate assessment of absorption kinetics and ion transport. Suspensions of enterocytes collected from the villi tip are a simple and effective tool for studying the absorption of nutrients, chemical reagents, and nanoparticles. Co-culture of epithelial cells, such as Caco-2 with HT29-MTX or T84, has been shown to improve physiological fidelity by combining absorption, secretory, and barrier functions. Studies have shown that 3D organoids and enteroids reproduce the architecture of the intestine and segment-specific functionality, allowing the study of interactions with the microbiota and early molecular events during infections. Microfluidic intestine-on-a-chip platforms further enhance modelling accuracy by integrating multiple cell types, forming oxygen gradients, and co-culturing with microbes over extended periods. It has been proven that no single model can fully reproduce the physiology of the intestine *in vivo*, which emphasises the need for combined approaches. In addition, the problem of standardising methods and reproducibility of results between laboratories remains relevant. Promising areas for improving gastrointestinal research methods include the use of enterocyte suspensions in preclinical studies of intestinal effects, personalised organoid models, and the use of multi-omics approaches. This will deepen

fundamental knowledge and expand the clinical application of experimental systems in the diagnosis and treatment of gastrointestinal diseases.

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REFERENCES
[1] Moerkens R, Mooiweer J, Ramírez-Sánchez AD, Barrett RJ, Jonkers IH, Withoff S, et al. An iPSCderived small intestineonchip with selforganizing epithelial, mesenchymal, and neural cells. Cell Rep. 2024;43(7):114247. DOI: 10.1016/j.celrep.2024.114247
[2] Parigi SM, Larsson L, Das S, Ramirez Flores RO, Frede A, KP Tripathi, et al. The spatial transcriptomic landscape of the healing mouse intestine following damage. Nat Commun. 2022;13:828. DOI: 10.1038/s41467-022-28497-0
[3] Soloviov S, Trokhimenko O, Polishchuk V, Pits V, Vasylenko V, Vasylenko Y, et al. *In vitro* modeling of the effect of *Lactobacillus* metabolites on the systemic response of the body in intestinal viral infection. Innov Biosyst Bioeng. 2024;8(2):38–52.
[4] Zhayvoronok MN, Zalesky VM. Intestinal fibrogenesis in inflammatory bowel diseases. Gastroenterology. 2022;56(4):258–65.
[5] Holloway EM, Capeling MM, Spence JR. Biologically inspired approaches to generate functional human gastrointestinal organoids. Development. 2019;146(8):dev166173. DOI: 10.1242/dev.166173
[6] Kassis J, Wan Z. Editorial: Latest advancements in organ-on-a-chip technology. Front Pharmacol. 2025;16:1631320. DOI: 10.3389/fphar.2025.1631320
[7] Kuhn MR, Wolcott EA, Langer EM. Developments in gastrointestinal organoid cultures to recapitulate tissue environments. Front Bioeng Biotechnol. 2025;13:1521044. DOI: 10.3389/fbioe.2025.1521044
[8] Lytvynenko AP. The effect of dextran-polyacrylamide polymers on regulated cell death of enterocytes in mice. In: The 7th international scientific conference current problems of biochemistry, cell biology and physiology. Dnipro: Oles Honchar Dnipro National University; 2024. P. 144–5.
[9] Abbas F, Beigh A, Khuroo MS, Farooq S, Khuroo NS, Tazeen S. Histopathological profile of gastrointestinal neuroendocrine tumors in a tertiary care hospital. Int J Med Med Res. 2021;7(2):83–90. DOI: 10.11603/ijmmr.2413-6077.2021.2.12595
[10] Song H, Hong Y, Lee H. Rapid automated production of tubular 3D intestine-on-a-chip with diverse cell types using coaxial bioprinting. Lab Chip. 2025;25:90–101. DOI: 10.1039/D4LC00731J
[11] Khamoushian S, Madrakian T, Afkhami A, Ghoorchian A, Ghavami S, Tari K, et al. Transdermal delivery of insulin using combination of iontophoresis and deep eutectic solvents as chemical penetration enhancers: *In vitro* and *in vivo* evaluations. J Pharm Sci. 2023;112(8):2249–59. DOI: 10.1016/j.xphs.2023.03.005
[12] Yaghoobian M, Haeri A, Bolourchian N, Shahhosseini S, Dadashzadeh S. An investigation into the role of Pglycoprotein in the intestinal absorption of repaglinide: Assessed by everted gut sac and Caco2 cell line. Iran J Pharm Res. 2019;18(1):102–10. DOI: 10.22037/ijpr.2019.2345
[13] Miyazaki K, Sasaki A, Mizuuchi H. Advances in the evaluation of gastrointestinal absorption considering the mucus layer. Pharmaceutics. 2023;15(12):2714. DOI: 10.3390/pharmaceutics15122714
[14] Noorman L, van der Hee B, Gilbert MS, de Vries S, van der Hoek S, Gerrits WJJ. Assessing seromuscular layer and serosa removal on intestinal permeability measurements in weaned piglet everted sac segments. J Anim Sci. 2024;102:skae148. DOI: 10.1093/jas/skae148
[15] Qi M, Wang X, Chen J, Liu Y, Liu Y, Jia J, et al. Transformation, absorption and toxicological mechanisms of silver nanoparticles in the gastrointestinal tract following oral exposure. *ACS Nano*. 2023;17(10):8851–65. DOI: 10.1021/acsnano.3c00024
[16] Guo S, Liang Y, Liu L, Yin M, Wang A, Sun K, et al. Research on the fate of polymeric nanoparticles in the process of the intestinal absorption based on model nanoparticles with various characteristics: Size, surface charge and pro-hydrophobics. J Nanobiotechnol. 2021;19:32. DOI: 10.1186/s12951-021-00770-2
[17] Zheng Y, Luo S, Xu M, He Q, Xie J, Wu J, et al. Transepithelial transport of nanoparticles in oral drug delivery: From the perspective of surface and holistic property modulation. Acta Pharm Sin B. 2024;14(9):3876–900. DOI: 10.1016/j.apsb.2024.06.015
[18] Khan H, Nazir S, Farooq RK, Khan IN, Javed A. Fabrication and assessment of diosgenin encapsulated stearic acid solid lipid nanoparticles for its anticancer and antidepressant effects using *in vitro* and *in vivo* models. Front Neurosci. 2022;15:806713. DOI: 10.3389/fnins.2021.806713
[19] Grieger KM, Schröder V, Dehmel S, Neuhaus V, Schaudien D, Fuchs M, et al. *Ex vivo* modeling and pharmacological modulation of tissue immune responses in inflammatory bowel disease using precision-cut intestinal slices. Eur J Immunol. 2025;55(7):e70013. DOI: 10.1002/eji.70013
[20] Joshi A, Soni A, Acharya S. *In vitro* models and *ex vivo* systems used in inflammatory bowel disease. In Vitro Model. 2022;1(3):213–27. DOI: 10.1007/s44164-022-00017-w
[21] Thomson A, Smart K, Somerville MS, Lauder SN, Appanna G, Horwood J, et al. The Ussing chamber system for measuring intestinal permeability in health and disease. BMC Gastroenterol. 2019;19:98. DOI: 10.1186/s12876-019-1002-4

[22] McCormick J, Hoffman K, Thompson H, Skinner D, Zhang S, Grayson J, et al. Differential chloride secretory capacity in transepithelial ion transport properties in chronic rhinosinusitis. Am J Rhinol Allergy. 2020;34(6):830–7. DOI: 10.1177/1945892420930975
[23] Pearce SC, Coia HG, Karl JP, Pantoja-Feliciano IG, Zachos NC, Racicot K. Intestinal *in vitro* and *ex vivo* models to study host-microbiome interactions and acute stressors. Front Physiol. 2018;9:1584. DOI: 10.3389/fphys.2018.01584
[24] Stevens LJ, van Lipzig MM, Erpelinck SL, Pronk A, van Gorp J, Wortelboer HM, et al. A higher throughput and physiologically relevant two-compartmental human *ex vivo* intestinal tissue system for studying gastrointestinal processes. Eur J Pharm Sci. 2019;137:104989. DOI: 10.1016/j.ejps.2019.104989
[25] Fedi A, Vitale C, Ponschin G, Ayehunie S, Fato M, Scaglione S. *In vitro* models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review. J Control Release. 2021;335:247–68. DOI: 10.1016/j.jconrel.2021.05.028
[26] Kimura Y, van der Merwe M, Bering SB, Penmatsa H, Conoley VG, Sangild PT, et al. Glucose transport by epithelia prepared from harvested enterocytes. Cytotechnology. 2013;67(1):39–49. DOI: 10.1007/s10616-013-9656-1
[27] Lytvynenko AP, Kaleynikova OM, Voznesenska TYu. Effect of gold and silver nanoparticles on the enterocytes cell death in mice. Biopolym Cell. 2024;40(3):223. DOI: 10.7124/bc.000ACC
[28] Wang CM, Oberoi HS, Law D, Li Y, Kassis T, Griffith LG, et al. Human mesofluidic intestinal model for studying transport of drug carriers and bacteria through a live mucosal barrier. Lab Chip. 2025;25(12):2990–3004. DOI: 10.1039/D4LC00774C
[29] Deyaert S, Moens F, Pirovano W, van den Bogert B, Klaassens ES, Marzorati M, et al. Development of a reproducible small intestinal microbiota model and its integration into the SHIME®-system, a dynamic *in vitro* gut model. Front Microbiol. 2023;13:1054061. DOI: 10.3389/fmicb.2022.1054061
[30] Haddad MJ, Sztupecki W, Delayre-Orthez C, Rhazi L, Barbezier N, Depeint F, et al. Complexification of *in vitro* models of intestinal barriers, a true challenge for a more accurate alternative approach. Int J Mol Sci. 2023;24(4):3595. DOI: 10.3390/ijms24043595
[31] Panse N, Gerk PM. The Caco-2 model: Modifications and enhancements to improve efficiency and predictive performance. Int J Pharm. 2022;624:122004. DOI: 10.1016/j.ijpharm.2022.122004
[32] Hoffmann P, Burmester M, Langeheine M, Brehm R, Empl MT, Seeger B, et al. Caco-2/HT29-MTX co-cultured cells as a model for studying physiological properties and toxin-induced effects on intestinal cells. PLoS One. 2021;16(10):e0257824. DOI: 10.1371/journal.pone.0257824
[33] Wang Y, Zuo Y, Deng S, Zuo F, Lui Q, Wang R, et al. Using caffeine and free amino acids to enhance the transepithelial transport of catechins in Caco-2 Cells. J Agric Food Chem. 2019;67(19):5477–85. DOI: 10.1021/acs.jafc.9b01701
[34] Anjum M, Laitila A, Ouwehand AC, Forssten SD. Current perspectives on gastrointestinal models to assess probiotic-pathogen interactions. Front Microbiol. 2022;13:831455. DOI: 10.3389/fmicb.2022.831455
[35] Peddibhotla S, Boone LA, Taylor EL, McQueen BE, Boazak EM. A scalable human gut-immune co-culture model for evaluating inflammatory bowel disease anti-inflammatory therapies. SLAS Discovery. 2025;35:100248. DOI: 10.1016/j.slasd.2025.100248
[36] Belaid M, Javorovic J, Pastorin G, Vllasaliu D. Development of an *in vitro* co-culture model using Caco-2 and J774A.1 cells to mimic intestinal inflammation. Eur J Pharm Biopharm. 2024;197:114243. DOI: 10.1016/j.ejpb.2024.114243
[37] Marescotti D, Lo Sasso G, Guerrera D, Renggli K, Ruiz Castro PA, Piault R, et al. Development of an advanced multicellular intestinal model for assessing immunomodulatory properties of anti-inflammatory compounds. Front Pharmacol. 2021;12:639716. DOI: 10.3389/fphar.2021.639716
[38] Valiei A, Aminian-Dehkordi J, Mofrad MRK. Gut-on-a-chip models for dissecting the gut-brain axis. APL Bioeng. 2023;7(1):011502. DOI: 10.1063/5.0110584
[39] Mohan TS, Datta P, Nesaei S, Ozbolat V, Ozbolat IT. 3D coaxial bioprinting: Process mechanisms, bioinks and applications. Prog Biomed Eng (Bristol). 2022;4(2):022003. DOI: 10.1088/2516-1091/ac631c
[40] Liu T, Li X, Li H, Qin J, Xu H, Wen J, et al. Intestinal organoid modeling: Bridging the gap from experimental model to clinical translation. Front Oncol. 2024;14:1334631. DOI: 10.3389/fonc.2024.1334631
[41] Li Y, Yang N, Chen J, Huang X, Zhang N, Yang S, et al. Next-generation porcine intestinal organoids: An apical-out organoid model for swine enteric virus infection and immune response investigations. J Virol. 2020;94:e01006-20. DOI: 10.1128/JVI.01006-20
[42] Parente IA, Bertoni S, Chiara L. Exploring the potential of human intestinal organoids: Applications, challenges, and future directions. Life Sci. 2024;352:122875. DOI: 10.1016/j.lfs.2024.122875
[43] Verduin M, Hoeben A, De Ruysscher D, Vooijs M. Patient-derived cancer organoids as predictors of treatment response. Front Oncol. 2021;11:641980. DOI: 10.3389/fonc.2021.641980
[44] Tian CM, Yang MF, Xu HM. Stem cell-derived intestinal organoids: A novel modality for IBD. Cell Death Discov. 2023;9:255. DOI: 10.1038/s41420-023-01556-1
[45] Saxena K, Blutt SE, Ettayebi K, Zeng XL, Broughman JR, Crawford SE, et al. Human intestinal enteroids: A new model to study human rotavirus infection, host restriction, and pathophysiology. J Virol. 2015;90(1):43–56. DOI: 10.1128/JVI.01930-15

Сучасні експериментальні системи для вивчення різних функцій шлунково-кишкового тракту

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Анотація. Цілісність та функціонування кишечника мають вирішальне значення для перетравлення та поглинання поживних речовин, імунологічного гомеостазу та запобіганню потрапляння шкідливих антигенів. Вдосконалення та розробка нових експериментальних систем для вивчення різноманітних функцій кишечника є актуальним завданням біології та медицини. Метою даної статті був опис сучасних експериментальних систем для вивчення різних функцій кишечника, зосереджуючись на їх застосуванні, перевагах та обмеженнях. Для досягнення мети проведено аналіз та узагальненння публікацій 2019-2025 рр., відібраних у PubMed та Google Scholar. На основі аналізу даних літератури та авторських експериментальних досліджень встановлено, що такі методи як «вивернута кишка» та «камера Усенга» є ефективними для вивчення, механізмів кінетики та всмоктування ліків у тонкій і в товстій кишці. Системи «InTESTine™» є інструментом для оцінки кишкової проникності й для прогнозування кишкового всмоктування їжі та ліків. Проаналізовано такі системи як «кишкові органоїди», «система ентероїду» та «кишечник на чіпі», що використовуються для дослідження динамічних процесів, секреції та всмоктування в кишечнику. Показано, що трансформовані та культивовані «лінії епітеліальних клітин» (Caco-2, HT-29 і T84) мають певні недоліки у використанні та ракове походження клітин. Було досліджено «суспензію епітеліальних клітин», яка є простою перспективною системою *ex vivo* для вивчення прямого та негайного впливу хімічних речовин на кишковий епітелій. Практична цінність роботи полягає в порівнянні експериментальних методів вивчення кишечника, що може спияти ефективності доклінічних досліджень, удосконалення діагностики медичної допомоги та розвитку персоналізованої медицини

Ключові слова: кишечник; моделі *in virto*; моделі *ex vivo*; ентероцити; суспензія епітеліальних клітин кишечника

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Biofilm formation and antibiotic resistance of clinical isolates from diabetic foot ulcers

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Abstract. Diabetic foot ulcers are among the most debilitating complications of type 2 diabetes mellitus, often leading to persistent infections and lower limb amputations. Microbial colonisation and biofilm formation contribute significantly to the chronicity and antibiotic resistance observed in these wounds. This study aimed to investigate the spectrum of microorganisms isolated from diabetic foot ulcers, assess their antibiotic susceptibility, and evaluate the biofilm-forming capacity of *Staphylococcus aureus* strains. Microbiological examination of wound discharge was performed for 68 patients with clinically diagnosed diabetic foot syndrome. A total of 78 microbial isolates were identified using morphological and biochemical methods. Most wound infections were monocultures (85%), with mixed infections identified in 10 cases. Antibiotic susceptibility was tested using the Kirby-Bauer disk diffusion method. Biofilm formation in *Staphylococcus aureus* isolates was assessed under static conditions using gentian violet staining and semi-quantitative scoring. Gram-positive bacteria predominated (73%), with *Staphylococcus aureus* and *Staphylococcus haemolyticus* being the most frequently isolated. Among Gram-negative organisms (27%), *Klebsiella* spp. and *Pseudomonas aeruginosa* were common. Antibiotic susceptibility testing revealed moderate methicillin sensitivity in *Staphylococcus aureus* (40%) and *Staphylococcus*

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haemolyticus (44%), while vancomycin and tigecycline showed the highest activity. Macrolides were largely ineffective, and *Corynebacterium* spp. demonstrated extensive resistance. Gram-negative isolates displayed higher resistance overall, with *Klebsiella* spp. resistant to most tested antibacterials. Biofilm formation analysis of 25 *Staphylococcus aureus* isolates revealed biofilm formation in 48%, including weak (58%), moderate (17%), and strong (25%) producers. Routine screening for biofilm-producing pathogens may improve clinical management and outcomes in diabetic foot infections

Keywords: antibiotic susceptibility; chronic wounds; multidrug resistance; *S. aureus*; wound microbiota

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most widespread endocrine disorders globally, characterised by chronic hyperglycemia resulting from impaired insulin action and relative insulin deficiency. The global prevalence of T2DM continues to rise at an alarming rate, driven largely by aging populations, sedentary lifestyles, and increasing rates of obesity. According to the International Diabetes Federation, cited by A. Kumar *et al.* [1], over 530 million people worldwide are currently (2024) living with diabetes, with T2DM accounting for the vast majority of cases, and this number is projected to exceed 643 million by 2030. By K. Nabrdalik *et al.* [2] this chronic condition poses a growing public health concern, significantly increasing the risk of cardiovascular disease, kidney failure, neuropathy, and other long-term complications. In addition to the medical burden, T2DM represents a substantial economic challenge for healthcare systems globally due to the high cost of ongoing treatment, complication management, and disability-related care.

B. Stancu *et al.* [3] stated that among the many complications associated with diabetes mellitus, diabetic foot ulcers (DFUs) represent one of the most serious and prevalent outcomes, significantly contributing to patient morbidity and healthcare burden. These lesions often result from a complex interplay of factors, including peripheral neuropathy, peripheral arterial disease, and impaired microcirculation, all of which compromise wound healing and tissue integrity. Reduced sensation in the feet leads to unnoticed trauma, while diminished blood supply hinders immune response and tissue regeneration. According to Z. Moore *et al.* [4] and K. Parveen *et al.* [5] if not properly managed, DFUs can progress rapidly to soft tissue and bone infections, gangrene, and ultimately, limb amputation. In severe cases, systemic infection and sepsis may occur, posing a direct threat to the patient's life.

Diabetic foot ulcers are particularly susceptible to microbial colonisation due to compromised tissue integrity, impaired immune responses, and poor vascularisation. According to I. Volch *et al.* [6], chronic diabetic foot ulcers are often colonised by a wide range of microorganisms, including both Gram-positive and Gram-negative bacteria. Among them, *Staphylococcus aureus* and *Klebsiella* spp. were found to predominate, but other frequently isolated pathogens included *Staphylococcus haemolyticus*, *Pseudomonas aeruginosa*, *Corynebacterium* spp., *Escherichia coli*, and *Proteus* spp., as well as opportunistic fungi such as *Candida* spp. Similarly, A.C. Afonso *et al.* [7] documented a polymicrobial nature of diabetic foot infections, noting that the pathogens involved may range from aerobic to anaerobic species and include both Gram-positive and Gram-negative bacteria. Reported biofilm-producing organisms included members of the *Enterobacteriaceae* family (*E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, Morganella morganii, Citrobacter* spp., and *Vibrio* spp.), as well as *P. aeruginosa, Acinetobacter baumannii*, and other *Acinetobacter* spp. Among Gram-positive isolates, *S. aureus* (including MRSA), coagulase-negative *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus faecalis*, and *Corynebacterium* spp. were highlighted. In a separate study, Y.V. Ivanova *et al.* [8] also reported a wide microbial spectrum in chronic diabetic foot ulcers, with frequent recovery of *S. aureus, A. baumannii, E. coli, K. pneumoniae,* and *Candida albicans*. Importantly, the authors emphasised that these clinical isolates exhibited markedly higher adhesive properties than reference strains. Once colonised, bacteria in diabetic foot ulcers may adopt survival strategies that include the development of antibiotic resistance. As highlighted by M. Piksa *et al.* [9], this complication is of particular concern because the diabetic foot provides a favorable environment for chronic infection, sometimes extending to the bone, and is frequently associated with pathogens resistant to conventional therapy. Another important mechanism described by T. Naaz *et al.* [10] is the ability to form biofilms, which are complex, structured communities of microorganisms surrounded by a self-produced extracellular matrix, composed of polysaccharides, DNA, and proteins. As stated by N.Y. Kravets *et al.* [11], within these biofilms, bacteria are protected from various environmental stresses, including host immune defenses and antimicrobial agents, making them significantly more difficult to eradicate than planktonic cells.

The biofilm mode of growth has been strongly associated with chronic, recurrent infections and increased antibiotic resistance, particularly in individuals with diabetes, whose compromised healing capacity further exacerbates the risk. According to C. Pouget *et al.* [12], biofilm-associated infections often do not respond to conventional antibiotic therapy, leading to prolonged treatment courses and frequent recurrence. Consequently, identifying and characterising biofilm-producing clinical isolates from diabetic foot ulcers is crucial for guiding more effective treatment strategies. Despite its clinical relevance, biofilm formation is not routinely assessed in microbiological diagnostics, potentially contributing to persistent infections in patients who repeatedly fail to respond to antibiotic therapy alone. The purpose of this study was to assess the antibiotic resistance and biofilm-forming ability of pathogens isolated from diabetic foot ulcers.

MATERIALS AND METHODS

This study builds upon authors' previous research on the microbiota of diabetic foot ulcers and its patterns of antibiotic resistance [6]. It included 68 patients diagnosed with type 2 diabetes mellitus and diabetic foot syndrome, who

were treated at the Municipal Non-Profit Enterprise "Ternopil City Emergency Hospital" in 2024-2025. The inclusion criteria were: patients of both sexes, age over 18 years, verified diagnosis of type 2 diabetes mellitus, presence of diabetic foot syndrome. The exclusion criteria were: presence of chronic diseases in the acute phase or in the phase of decompensation, current treatment with glucocorticosteroids, pregnancy, mental disorders, confirmed or suspected cancer. All included patients were diagnosed with complicated diabetic foot syndrome, characterised by purulent-necrotic lesions and ulcers, and were indicated for surgical intervention. As part of the preoperative assessment, microbiological testing of wound discharge was performed.

Sterile cotton swabs were used to collect samples from the wound discharge under aseptic conditions. The collected material was inoculated onto selective culture media and incubated at 37°C for 24-48 hours. Pure microbial cultures were identified based on their morphological characteristics and a series of biochemical tests. Gram-negative rods were identified using the following assays: fermentation in Kligler Iron Agar, Simmons' citrate agar (Farmaktiv, LLC, Kyiv, Ukraine), indole production, catalase activity, and motility testing. Gram-positive cocci were identified by evaluating catalase, lecithinase, and coagulase activity (Biolik Pharma LLC, Kharkiv, Ukraine). In addition, blood agar (Farmaktiv, LLC, Kyiv, Ukraine) was used to assess hemolytic activity. The antibiotic susceptibility of the isolated microbial strains was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following standard protocols. The tested antibiotics included ciprofloxacin, ceftazidime, chloramphenicol, amikacin, azithromycin, ceftriaxone, doxycycline, erythromycin, methicillin, tigecycline, vancomycin, and ertapenem. Zone diameters were measured after incubation and interpreted according to CLSI [13].

The ability of the isolated strains to form biofilms was studied under static conditions by growing the cultures on sterile glass coverslips. After incubation, the coverslips were stained with gentian violet and examined under a light microscope (TM MICROmed, Poltava, Ukraine), which was equipped with a Swift 5.0 Megapixel Digital Camera (Swiftmicroscopes, China). Biofilm formation was semi-quantitatively evaluated using a 0-3 point scale based on the density and morphology of adherent cells [14, 15]. *S. aureus* was selected as a representative Gram-positive pathogen for biofilm assessment due to its high prevalence and well-established role in diabetic foot infections. The collected data were organised and tabulated using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics were applied, and the results were presented as percentages and proportions.

This study was conducted in accordance with the principles outlined in the UNESCO Universal Declaration on Bioethics and Human Rights [16] and the Declaration of Helsinki "Ethical principles of medical research involving human subjects" [17]. The research was approved by the Ethics Committee of Ternopil National Medical University (Protocol No. 81, April 3, 2025), and all participants provided written informed consent prior to participation. This study focused on biofilm formation in *S. aureus* isolates only, despite the presence of other clinically relevant species such as *S. haemolyticus* and *P. aeruginosa*. The selection was based on the high prevalence and clinical importance of *S. aureus* in diabetic foot infections. Additionally, microbial identification was qualitative in nature, and therefore the presence of a microorganism does not necessarily imply a causative role in infection.

RESULTS AND DISCUSSION

Microbiological testing was performed for all 68 patients enrolled in the study, resulting in a total of 78 microbial isolates. The majority of patients (n = 58) had monoculture infections, while mixed infections involving two distinct pathogens were identified in 10 cases. Gram-positive bacteria predominated, accounting for 57 isolates (73.08%), while 21 isolates (26.92%) were Gram-negative. *Staphylococcus* spp. were the most prevalent Gram-positive organisms, with *S. aureus* and *S. haemolyticus* being the most commonly isolated species. Among the Gram-negative bacteria, *Klebsiella* spp. were the most frequently detected. The complete distribution of isolated pathogens is presented in Table 1.

Table 1. Distribution and frequency of pathogens isolated from diabetic foot ulcers

Pathogen	Number of isolates	Frequency of isolation, %
Staphylococcus aureus	25	36.76
Staphylococcus haemolyticus	25	36.76
Klebsiella spp.	9	13.24
Corynebacterium spp.	7	11.76
Pseudomonas aeruginosa	8	10.29
Escherichia coli	4	5.88

Source: compiled by the authors

This distribution of pathogens reflects the well-recognised predominance of Gram-positive cocci, particularly *Staphylococcus* spp., in diabetic foot infections, while the presence of Gram-negative organisms such as *Klebsiella* spp. highlights the role of encapsulated bacteria that complicate host immune clearance and contribute to the chronicity of these wounds. Antibiotic susceptibility testing revealed distinct resistance patterns among Gram-positive and Gram-negative pathogens isolated from diabetic foot ulcers (Table 2). Among the Gram-positive isolates, *S. aureus* and *S. haemolyticus* demonstrated moderate sensitivity to methicillin (40% and 44%, respectively), with tigecycline and vancomycin showing the highest activity. In contrast, macrolides such as erythromycin and azithromycin exhibited poor efficacy, with only 36% of strains being sensitive. *Corynebacterium* spp. exhibited resistance to the majority of tested antibiotics, showing full susceptibility only to tigecycline and amikacin. This finding of

extensive resistance in *Corynebacterium* spp. is noteworthy, as this organism is often underestimated in clinical practice yet may contribute substantially to treatment failures in chronic wounds.

Table 2. Antibiotic sensitivity of pathogens isolated from diabetic foot ulcers

Antibiotic	*S. aureus* (n = 25)	*S. haemolyticus* (n = 25)	*Klebsiella* spp. (n = 9)	*Corynebacterium* spp. (n = 7)	*P. aeruginosa* (n = 8)	*E. coli* (n = 4)
Methicillin	10 (40.0%)	11 (44.0%)	R	R	R	R
Tigecycline	25 (100%)	22 (88.0%)	4 (44.4%)	7 (100%)	R	4 (100%)
Vancomycin	22 (88.0%)	24 (96.0%)	1 (11.1%)	3 (42.9%)	8 (100%)	R
Ciprofloxacin	14 (56.0%)	14 (56.0%)	R	R	8 (100%)	4 (100%)
Ceftazidime	13 (52.0%)	12 (48.0%)	R	R	R	2 (50.0%)
Levomycetin	17 (68.0%)	20 (80.0%)	R	5 (71.4%)	R	4 (100%)
Amikacin	21 (84.0%)	19 (76.0%)	4 (44.4%)	7 (100%)	7 (87.5%)	3 (75.0%)
Azithromycin	9 (36.0%)	9 (36.0%)	R	R	R	4 (100%)
Ceftriaxone	17 (68.0%)	14 (56.0%)	R	R	R	R
Erythromycin	9 (36.0%)	9 (36.0%)	R	R	R	R

Note: R – all tested isolates were resistant (0% sensitivity)
Source: compiled by the authors

Gram-negative isolates showed a higher degree of resistance overall. *Klebsiella* spp. were resistant to most antibiotics tested, including methicillin, ciprofloxacin, and ceftriaxone. *P. aeruginosa* was fully sensitive to ciprofloxacin and vancomycin but resistant to several other antibiotics, including tigecycline and ceftazidime. *E. coli* strains demonstrated good susceptibility to multiple agents such as ciprofloxacin, levomycetin, azithromycin, and tigecycline, but were completely resistant to vancomycin and ceftriaxone. Out of the 25 *S. aureus* isolates obtained from patients with diabetic foot ulcers, 12 (48%) demonstrated the ability to form biofilms under static conditions. Among these biofilm-producing strains, 7 (58.3%) exhibited weak biofilm formation (1 point), 2 strains (16.7%) showed moderate formation (2 points), and 3 strains (25.0%) demonstrated strong biofilm-forming ability (3 points), as shown in the sectoral distribution (Fig. 1).

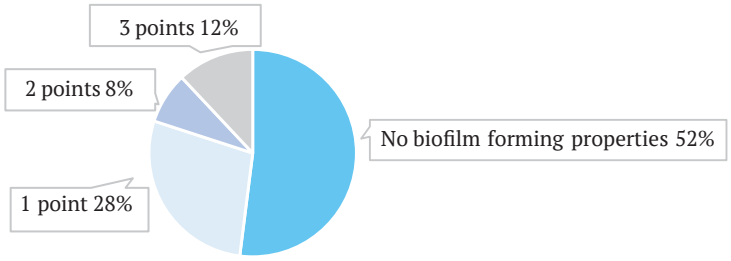

Figure 1. Distribution of biofilm-forming ability among *S. aureus* isolates from diabetic foot ulcers
Source: compiled by the authors

Although less than half of the *S. aureus* isolates demonstrated biofilm-forming ability and their overall resistance profile was moderate, the presence of strong biofilm producers within this group highlights that even a relatively small subset of strains may disproportionately drive persistence and treatment failure in diabetic foot ulcers. Representative microscopic images of each biofilm formation category are presented in Figure 2.

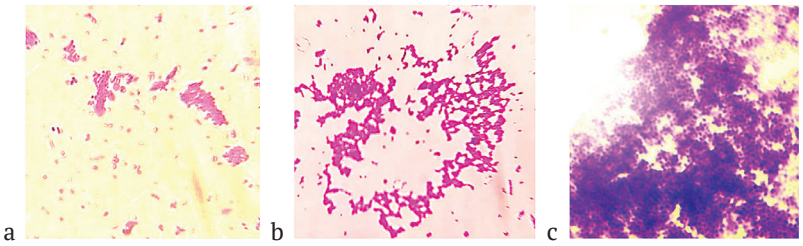

Figure 2. Biofilm formation in *Staphylococcus aureus* isolated from diabetic foot ulcer discharge in patients with type 2 diabetes mellitus. Gentian violet, ×1000
Note: a – weak biofilm formation (1 point); b – moderate biofilm formation (2 points); c – strong biofilm formation (3 points)
Source: compiled by the authors

The findings of this study demonstrated that diabetic foot ulcers harbor a diverse microbial community composed of both Gram-positive and Gram-negative organisms, with notable differences in antimicrobial susceptibility profiles. The high prevalence of multidrug resistance together with the biofilm-forming ability observed in nearly half of the *S. aureus* isolates, underscores the clinical challenges associated with effective treatment and wound healing. Other studies have shown that diabetic foot ulcers are colonised by a wide range of Gram-positive and Gram-negative microorganisms. For example, A. Banu *et al.* [18] reported that *S. aureus* was the predominant microorganism, followed by *P. aeruginosa*, among 100 samples collected from diabetic foot ulcer contents. These findings are consistent with the current study. The same authors also noted that biofilm formation occurred predominantly in *S. aureus* isolates (20%). In contrast, F. Du *et al.* [19] reported a higher prevalence of Gram-negative bacteria (52.4%) compared to Gram-positive bacteria (43.4%). In their study, the most frequently isolated pathogens included *S. aureus* (17.7%), *E. coli* (10.9%), *P. aeruginosa* (10.5%), *Klebsiella pneumoniae* (6.2%), *Staphylococcus epidermidis* (5.3%), *Enterococcus faecalis* (4.9%), and fungi (3.7%).

Corynebacterium spp. are generally regarded as skin commensals; however, under certain conditions, they may act as opportunistic pathogens and contribute to chronic wound colonisation. In this study, *Corynebacterium* spp. were identified in 7 cases, though the qualitative nature of the analysis does not allow for differentiation between colonisation and infection. Notably, these isolates exhibited resistance to 6 out of 10 tested antibiotics. In investigation of the diabetic foot ulcer microbiome, L. Soldevila-Boixader *et al.* [20] reported a higher abundance of *Corynebacterium* spp. in non-infected DFUs, suggesting a possible commensal or modulatory role. Their findings also indicated that *Corynebacterium* may influence the growth dynamics of *S. aureus*. In the present study, *P. aeruginosa* was isolated in 10.29% of cases. According to M. Garousi *et al.* [21], *P. aeruginosa* is one of the most common causes of diabetic foot infections globally. In their study, 16.6% of diabetic foot wound infections were attributed to *P. aeruginosa*. The clinical relevance of *P. aeruginosa* lies in its intrinsic resistance to many commonly used antibiotics, its ability to thrive in moist wound environments, and its capacity to form biofilms, which complicates eradication. Infections caused by *P. aeruginosa* are often associated with delayed wound healing, prolonged hospital stays, and increased risk of amputation [22].

The current study revealed diverse antibiotic resistance profiles among pathogens isolated from diabetic foot ulcers, with Gram-negative isolates generally exhibiting a higher degree of resistance. *S. aureus* and *S. haemolyticus* showed moderate sensitivity to methicillin, while *Corynebacterium* spp. were resistant to most antibiotics, remaining fully susceptible only to tigecycline and amikacin. Among Gram-negative isolates, *Klebsiella* spp. displayed the highest level of resistance, whereas *P. aeruginosa* retained sensitivity to ciprofloxacin and vancomycin. *E. coli* showed favorable susceptibility to multiple agents but demonstrated complete resistance to vancomycin and ceftriaxone. These findings are consistent with those reported in studies and meta-analyses, which have confirmed a high global prevalence of multidrug-resistant (MDR) pathogens in diabetic foot ulcers [23, 24]. According to S. Yang *et al.* [25], the most prevalent MDR Gram-positive bacterium was *S. aureus*, with a reported rate of 12.13%, while *E. coli* (6.93%) and *P. aeruginosa* (6.01%) were the leading MDR Gram-negative pathogens.

In addition to the widespread emergence of multidrug resistance, another major factor contributing to the persistence and poor healing of diabetic foot ulcers is the ability of certain pathogens to form biofilms. In the present study, 48% of *S. aureus* isolates demonstrated biofilm-forming ability, with varying degrees of biofilm density. This proportion is lower than what has been reported in other studies. For instance, H. Mamdoh *et al.* [26] found that all isolated *Staphylococcus* spp. from diabetic foot ulcers were capable of forming biofilms, albeit with differing intensities. Their dataset included *S. aureus* alongside *S. haemolyticus*, *S. epidermidis*, and other coagulase-negative staphylococci. While some studies have reported a high prevalence of biofilm formation among *S. aureus* isolates from diabetic foot ulcers, others have shown much lower rates in different clinical contexts. For example, a study on *S. aureus* strains isolated from the oropharynx of children with chronic tonsillitis found that only 28.6% demonstrated biofilm-forming ability [11].

The findings of this study highlighted the complex microbial ecology of diabetic foot ulcers, where both Gram-positive and Gram-negative organisms contribute to chronicity through antibiotic resistance and biofilm formation. The detection of multidrug-resistant strains such as *Klebsiella* spp., *P. aeruginosa*, and methicillin-sensitive but biofilm-producing *S. aureus* highlights the challenge of antimicrobial resistance and microbial persistence in wound environments. The identification of *Corynebacterium* spp. further illustrates the potential role of less frequently reported organisms in shaping wound microbiota and influencing pathogenic dynamics. This reinforced the need for integrated diagnostic and therapeutic approaches that address not only antimicrobial susceptibility but also microbial interactions and biofilm-related resilience in diabetic foot ulcers.

CONCLUSIONS

This study provided important microbiological insights into the management of diabetic foot ulcers, highlighting both the diversity of isolated pathogens and their complex antimicrobial resistance profiles. The predominance of Gram-positive bacteria (73%), particularly *S. aureus* and *S. haemolyticus*, along with notable Gram-negative isolates such as *Klebsiella* spp. and *P. aeruginosa*, reflects the polymicrobial nature of diabetic foot infections. Antibiotic susceptibility testing confirmed widespread resistance, especially among Gram-negative bacteria, which frequently exhibited multidrug resistance patterns. Importantly, *Corynebacterium* spp., often considered mere commensals, also demonstrated significant resistance, suggesting their potential underrecognised role in chronic infections. The assessment of biofilm formation in *S. aureus* added an important detail, showing that nearly half of the isolates (48%) were capable of forming biofilms of varying intensity. This finding emphasised the role of biofilms in persistent infections and reduced antibiotic

efficacy, and supported the consideration of biofilm assessment as part of advanced diagnostic protocols. Taken together, these results stressed the urgent need for integrated diagnostic strategies that combine pathogen identification, susceptibility testing, and biofilm evaluation. Such approaches can inform more targeted therapies, reduce treatment failures, and ultimately improve patient outcomes. In addition to conventional antibiotic-based regimens, future research should also explore the role of adjuvant therapies aimed at reducing microbial load and enhancing wound healing. Among these, ozonated water and oils have shown promising antimicrobial and antibiofilm activity in other clinical contexts, and their potential integration with standard care may represent a valuable complementary approach. Investigating such strategies, alongside novel antibiofilm agents, could expand the therapeutic arsenal available for managing chronic and resistant diabetic foot infections.

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REFERENCES

[1] Kumar A, Gangwar R, Ahmad Zargar A, Kumar R, Sharma A. Prevalence of diabetes in India: A review of IDF diabetes atlas 10th edition. Curr Diabetes Rev. 2024;20(1):e130423215752. DOI: 10.2174/1573399819666230413094200

[2] Nabrdalik K, Kwiendacz H, Moos J, Moos Ł, Kulpa J, Brzoza Z, et al. Diabetic peripheral neuropathy is associated with diabetic kidney disease and cardiovascular disease: The Silesia Diabetes-Heart Project. Curr Probl Cardiol. 2023;48(8):101726. DOI: 10.1016/j.cpcardiol.2023.101726

[3] Stancu B, Ilyés T, Farcas M, Coman HF, Chiș BA, Andercou OA. Diabetic foot complications: A retrospective cohort study. Int J Environ Res Public Health. 2023;20(1):187. DOI: 10.3390/ijerph20010187

[4] Moore Z, Avsar P, Wilson P, Mairghani M, O'Connor T, Nugent L, et al. Diabetic foot ulcers: Treatment overview and cost considerations. J Wound Care. 2021;30(10):786–91. DOI: 10.12968/jowc.2021.30.10.786

[5] Parveen K, Hussain MA, Anwar S, Elagib HM, Kausar MA. Comprehensive review on diabetic foot ulcers and neuropathy: Treatment, prevention and management. World J Diabetes. 2025;16(3):100329. DOI: 10.4239/wjd.v16.i3.100329

[6] Volch I, Mykhailyshyn H, Pokotylo O, Hetman U, Bukata V. Microbiological analysis of wound content in patients with type 2 diabetes mellitus with diabetic foot syndrome. Int J Med Med Res. 2024;10(1):6–14. DOI: 10.61751/ijmmr/1.2024.06

[7] Afonso AC, Oliveira D, Saavedra MJ, Borges A, Simões M. Biofilms in diabetic foot ulcers: Impact, risk factors and control strategies. Int J Mol Sci. 2021;22(15):8278. DOI: 10.3390/ijms22158278

[8] Ivanova YV, Gramatiuk SM, Kryvoruchko IA, Goloborodko MM, Miasoiedov KV, Knyhin MV, et al. Biofilm-forming mechanisms in diabetic foot syndrome pathogens: Adhesive properties and interaction in associations. Clin Prev Med. 2025;5:37–44. DOI: 10.31612/2616-4868.5.2025.05

[9] Piksa M, Fortuna W, Lian C, Gacka M, Samuel IDW, Matczyszyn K, et al. Treatment of antibiotic-resistant bacteria colonizing diabetic foot ulcers by OLED induced antimicrobial photodynamic therapy. Sci Rep. 2023;13:14087. DOI: 10.1038/s41598-023-39363-4

[10] Naaz T, Lahiri D, Pandit S, Nag M, Gupta PK, Al-Dayan N, et al. Antimicrobial peptides against microbial biofilms: Efficacy, challenges, and future prospect. Int J Pept Res Ther. 2023;29(3):48. DOI: 10.1007/s10989-023-10519-0

[11] Kravets NY, Klumnyk SI, Romanyuk LB, Borak VP. Biofilm-forming properties of pathogenic microorganisms in children with recurrent tonsillitis. World Med Biol. 2022;80(2):210–3. DOI: 10.26724/2079-8334-2022-2-80-210-213

[12] Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in diabetic foot ulcers: Significance and clinical relevance. Microorganisms. 2020;8(10):1580. DOI: 10.3390/microorganisms8101580

[13] Weinstein MP, Lewis JS. Performance standards for antimicrobial susceptibility testing 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2020. 332 P.

[14] Wilson C, Lukowicz R, Merchant S, Valquier-Flynn H, Caballero J, Sandoval J, et al. Quantitative and qualitative assessment methods for biofilm growth: A mini-review. Res Rev J Eng Technol. 2017;6(4):1–42.

[15] Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods. 2000;40(2):175–9. DOI: 10.1016/s0167-7012(00)00122-6

[16] UNESCO. Universal Declaration on Bioethics and Human Rights [Internet]. 2005 October 19 [cited 2025 April 25]. Available from: https://www.unesco.org/en/legal-affairs/universal-declaration-bioethics-and-human-rights?hub=66535

[17] The World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2025 April 25]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-princip.2024

[18] Banu A, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. Australas Med J. 2015;8(9):280–5. DOI: 10.4066/AMJ.2015.2422

[19] Du F, Ma J, Gong H, Bista R, Zha P, Ren Y, et al. Microbial infection and antibiotic susceptibility of diabetic foot ulcer in China: Literature review. Front Endocrinol (Lausanne). 2022;13:881659. DOI: 10.3389/fendo.2022.881659

[20] Soldevila-Boixader L, Carrera-Salinas A, Mur I, Morata L, Rivera A, Bosch J, et al. Exploring the microbiome of diabetic foot ulcers: A focus on cases with a clinical worse outcome. Antibiotics. 2025;14(7):724. DOI: 10.3390/antibiotics14070724
[21] Garousi M, Monazamitabar S, Mirazi H, Farrokhi Z, Khaledi A, Shakerimoghaddam A. Epidemiology of *Pseudomonas aeruginosa* in diabetic foot infections: A global systematic review and meta-analysis. Germs. 2023;13(4):362–72. DOI: 10.18683/germs.2023.1406
[22] Dörr S, Holland-Letz AK, Weisser G, Chatzitomaris A, Lobmann R. Bacterial diversity, antibiotic resistance, and the risk of lower limb amputation in younger and older individuals with diabetic foot infection. Int J Low Extrem Wounds. 2021;22(1):63–71. DOI: 10.1177/1534734621992290
[23] Anafo RB, Atiase Y, Dayie NTKD, Kotey FCN, Tetteh-Quarcoo PB, Duodu S, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection of diabetic foot ulcers at a tertiary care hospital in Accra, Ghana. Pathogens. 2021;10(8):937. DOI: 10.3390/pathogens10080937
[24] Matta-Gutiérrez G, García-Morales E, García-Álvarez Y, Álvaro-Afonso FJ, Molines-Barroso RJ, Lázaro-Martínez JL. The influence of multidrug-resistant bacteria on clinical outcomes of diabetic foot ulcers: A systematic review. J Clin Med. 2021;10(9):1948. DOI: 10.3390/jcm10091948
[25] Yang S, Hu L, Zhao Y, Meng G, Xu S, Han R. Prevalence of multidrug-resistant bacterial infections in diabetic foot ulcers: A meta-analysis. Int Wound J. 2024;21(4):e14864. DOI: 10.1111/iwj.14864
[26] Mamdoh H, Hassanein KM, Eltoony LF, Khalifa WA, Hamed E, Alshammari TO, et al. Clinical and bacteriological analyses of biofilm-forming staphylococci isolated from diabetic foot ulcers. Infect Drug Resist. 2023;16:1737–50. DOI: 10.2147/IDR.S393724

Біоплівкоутворення та антибіотикорезистентність клінічних ізолятів з виразок діабетичної стопи

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Анотація. Виразки діабетичної стопи є одними з найбільш виснажливих ускладнень цукрового діабету 2 типу, які часто призводять до стійких інфекцій та ампутації нижніх кінцівок. Мікробна колонізація та утворення біоплівок значно сприяють хронічності перебігу та резистентності до антибіотиків, що спостерігаються у цих ранах. Мета цього дослідження полягала у вивченні спектру мікроорганізмів, виділених з діабетичних виразок стопи, оцінці їх чутливості до антибіотиків та оцінці здатності штамів *Staphylococcus aureus* до утворення біоплівок. Мікробіологічне дослідження виділень з ран було проведено у 68 пацієнтів з клінічно діагностованим діабетичним синдромом стопи. За допомогою морфологічних та біохімічних методів було ідентифіковано 78 мікробних ізолятів. Більшість ранових інфекцій були представлені монокультурами (85 %), а у в 10 випадках було виявлене полімікробне інфікування. Чутливість до антибіотиків було перевірено за допомогою методу дифузії дисків Кірбі-Бауера. Утворення біоплівки в ізолятах *Staphylococcus aureus* було оцінено в статичних умовах за допомогою фарбування генціан-фіолетовим. Переважали грампозитивні бактерії (73 %), серед яких найчастіше виділяли *Staphylococcus aureus* та *Staphylococcus haemolyticus*. Серед грамнегативних організмів (27 %) поширеними були *Klebsiella* spp. та *Pseudomonas aeruginosa*. Оцінка чутливості до антибіотиків виявила помірну чутливість до метициліну у *Staphylococcus aureus* (40 %) та *Staphylococcus haemolyticus* (44 %), тоді як ванкоміцин та тигециклін показали найвищу активність. Макроліди були значною мірою неефективними, а *Corynebacterium* spp. продемонстрували значну резистентність. Грамнегативні ізоляти загалом продемонстрували вищу резистентність, при цьому *Klebsiella* spp. були стійкими до більшості протестованих антибактеріальних препаратів. Аналіз біоплівкоутворення 25 ізолятів *Staphylococcus aureus* виявив утворення біоплівки у 48 %, включаючи слабких (58 %), помірних (17 %) та сильних (25 %) продуцентів. Регулярний скринінг на наявність збудників, що утворюють біоплівки, може покращити клінічне лікування та наслідки у випадках інфекцій діабетичної стопи

Ключові слова: чутливість до антибіотиків; хронічні рани; множинна стійкість до антибіотиків; *S. aureus*; ранова мікробіота

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Clinical assessment of the risk of amputation in diabetic foot: A clinical case

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Abstract. The aim of the study was to develop a clinical algorithm for assessing the risk of amputation in patients with diabetic foot syndrome and to test it on a clinical case, with an emphasis on determining the leading role of a podiatrist within a multidisciplinary team. The study was conducted as a description of a clinical case using the author's 15-point risk stratification card, gentle local podiatric interventions, individual offloading, behavioural modification and multidisciplinary routing, followed by assessment of the dynamics according to a 7- and 14-day observation protocol. During 14 days of observation, the implementation of a structured clinical algorithm ensured a progressive improvement in the local condition of the foot: on the 7th day, there was a reduction in hyperemia, pastosity, and pain, and by the 14th day, a pronounced epithelialisation ridge had formed along the edge of the defect, indicating the activation of reparative processes. The total score on the assessment card increased from 7 to 11, reflecting the patient's transition from high risk of amputation to moderate risk. The section scores for local examination increased from 1 to 3, for behavioural section – from 1 to 2, and for load correction – from 1 to 2, confirming the effectiveness of gentle sanitation, antiseptics, offloading pads and interdigital orthosis. The positive dynamics were accompanied by an increase in patient compliance and the refusal of traumatic home manipulations. At the same time, the level of glycaemia remained elevated (9-11 mmol/L), which necessitates long-term endocrinological control and prevention of relapses. The implementation of a podiatric algorithm with early stratification, gentle local interventions, individual offloading and behavioural correction allowed a clinically significant reduction in the risk of amputation to be achieved within 14 days of treatment. The results obtained can be used by podiatrists, family doctors and endocrinologists in outpatient care for early risk stratification, optimisation of the scope of interventions and timely referral of patients with diabetic foot syndrome for the purpose of preventing amputations

Keywords: podiatrist; stratification; offloading; affected area; inflammation; antiseptic treatment; sanitation

INTRODUCTION

The rise in diabetes is putting a lot of pressure on healthcare systems worldwide: by 2024, about 589 million adults aged 20-79 will be living with the disease (that is one in nine adults) [1]. One of the most serious complications is diabetic foot ulcer, which is diagnosed in approximately 18.6 million people each year, with these lesions accounting for up to 80% of lower limb amputations among people with diabetes [2]. The formation of ulcers is accompanied by a high risk of hospitalisation, amputation and death, and also reduces the quality of life of patients. In the context of an ageing population, poor adherence to treatment and limited access to specialised care, there has been a steady increase in the number of patients with complications, leading to significant costs for the healthcare system. This necessitates the improvement of clinical risk stratification methods and the development of timely therapeutic and diagnostic interventions to prevent serious consequences, including amputations.

The pathogenesis of diabetic foot involves the formation of clinical factors that determine the unfavourable

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course of local lesions. A study by H. Gong *et al*. [3] emphasised that the combination of infection and peripheral arterial insufficiency significantly increases the risk of limb loss in hospitalised patients. It has been shown that such changes lead to an increase in the frequency of surgical interventions shortly after the initial visit. At the same time, clinical predictors of major and minor amputations were studied in a study by K.T. Okur *et al*. [4], which emphasised the need for stratification according to the location and depth of the lesion. The authors stressed that even with the same lesion size, the prognosis can vary significantly depending on the degree of tissue ischaemia. A systematic review by R. Forsythe *et al*. [5] analysed the prognostic value of inflammatory markers, leukocytes, C-reactive protein and albumin, which could potentially be used as laboratory indicators of the risk of amputation. However, the significant variability of these indicators between populations limits the possibility of standardising laboratory stratification. The promise of new treatment approaches in Ukrainian practice is demonstrated in the work of O. Petrenko *et al*. [6], where the use of topical plasminogen was accompanied by a decrease in the frequency of amputations. At the same time, the authors emphasised the need for standardised clinical pathways for the management of such patients.

Classification systems for foot lesions served as the basis for clinical stratification of amputation risk, but their predictive accuracy varied significantly. Within the Wagner scale, D.G. Armstrong *et al*. [2] established a relationship between stage progression and an increase in the frequency of surgical interventions, but noted that this classification did not take into account the severity of the infectious process. The integration of the Perfusion, Extent, Depth, Infection, Sensation (PEDIS) and Site, Ischemia, Neuropathy, Bacterial Infection, Area, Depth (SINBAD) systems, as shown by A.S. Kaka *et al*. [7], allowed for a more accurate prediction of the risk of complications through a combined assessment of ischaemia, infection and neuropathy. However, even these systems remained limited in their interpretation of the complex interaction of pathogenetic factors. A study conducted by A.S. Ivanova *et al*. [8] presented the experience of implementing combined classifications in specialised institutions in Ukraine, demonstrating their practical effectiveness in reducing the frequency of amputations. The authors emphasised the importance of adapting international scales to the clinical conditions of the national healthcare system.

Researchers have focused on creating models for predicting amputations using machine learning and artificial intelligence (AI) methods. In a comparative study by Z. Liu *et al*. [9], gradient boosting models showed higher accuracy in predicting major amputations among patients with diabetic foot ulcers than traditional logistic algorithms. The authors emphasised the importance of variables such as duration of diabetes, C-reactive protein level, and patient age as key predictors. The use of explainable AI in risk factor analysis was demonstrated by C.W. Oei *et al*. [10], who noted that the inclusion of the systemic immune inflammation index increased the clinical interpretability of the models. In a study by S.D. Shapoval *et al*. [11], clinical and analytical prediction algorithms were combined with multidisciplinary patient management, which contributed to improved organ preservation outcomes. The authors noted the need for further standardisation and adaptation of AI approaches to the conditions of specialised surgical centres.

Despite numerous studies, the issue of early prediction of amputations in diabetic foot ulcers remained understudied, especially in terms of combining clinical, laboratory, and instrumental indicators. The aim of the study was to test the developed algorithm for assessing the risk of amputation in patients with diabetic foot syndrome in a clinical case, followed by determining the role of a podiatrist in the team management of such patients.

MATERIALS AND METHODS

The clinical case was assessed using the author's step-by-step algorithm for clinical assessment of the risk of amputation, developed on the basis of international recommendations from the International Working Group on the Diabetic Foot [12], the American Diabetes Association [13] and the National Institute for Health and Care Excellence [14] (Ukrainian patent No. 137472, 2025). As part of the algorithm testing, the following clinical case was analysed. An 82-year-old female patient, a resident of Brovary (Kyiv region), with type 2 diabetes mellitus for more than 12 years and low adherence to therapy, complained of swelling, redness and increased local temperature in the anterior part of the right foot, which occurred after self-application of celandine juice to remove a callus. Medical history: arterial hypertension, peripheral sensorimotor neuropathy, no previous ulcers or amputations. Family history includes diabetes mellitus (mother had type 2 diabetes mellitus with the development of neuro-ischaemic diabetic foot syndrome), hypertension and cardiovascular events before the age of 60 (father had a myocardial infarction). At the time of admission, the patient was taking metformin (1,000 mg/day) and amlodipine (5 mg/day) irregularly, and her glycaemia was monitored sporadically (fasting glucose level – 11 mmol/L). During the examination, pastosity and hyperemia of the I-II toes and the dorsal surface of the right foot, hyperkeratosis, scales, and isolated skin cracks were detected. The nail plates were deformed and thickened, characteristic of chronic onychomycosis (Fig. 1).

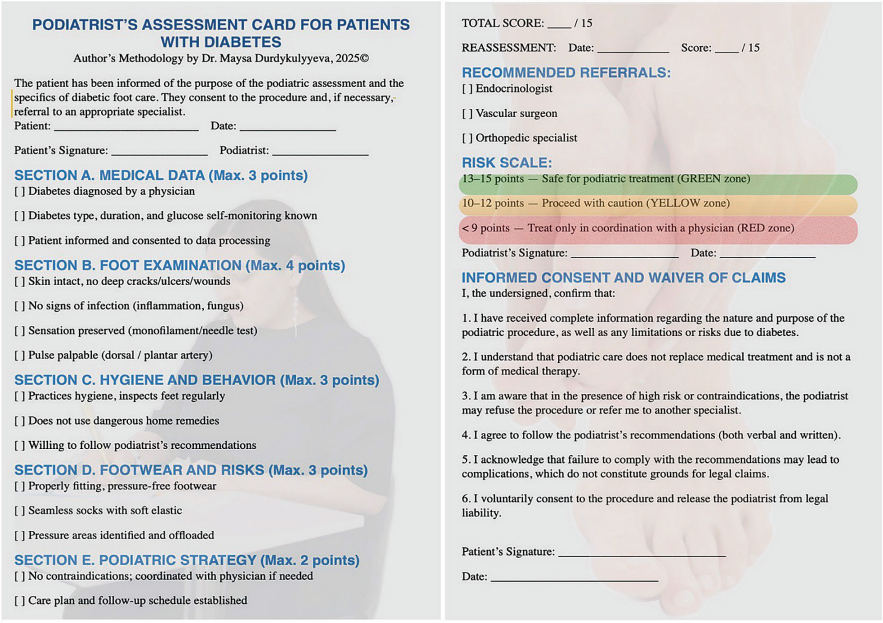
Figure 1. Initial condition of the anterior part of the right foot when the patient was admitted
Source: created by the author

There was no palpation pain, and temperature and pain sensitivity were reduced when tested with a 10-gram monofilament. The pulsation in the dorsal artery of the foot was determined to be sharply weakened, indicating a decrease in peripheral blood flow. The patient's shoes did not fit her feet, had stiff edges and created excessive pressure on the affected area, and foot hygiene and prevention rules were partially followed. Based on the podiatrist's 15-point assessment card, the patient was classified as being in the red zone of high risk of amputation (7 out of 15 points), which required urgent referral to an endocrinologist and vascular surgeon, as well as limiting podiatric interventions to non-traumatic manipulations. Repeat examinations were performed after 7 and 14 days in accordance with the standards for monitoring the treatment of diabetic foot syndrome [14]. The examination on the 7th day allowed to monitor the early clinical response to therapy, ensure that there was no deterioration, and evaluate the effectiveness of offloading. The examination on the 14th day coincided with the end of the treatment course, included a reassessment using the author's risk chart, and made it possible to determine the consistency of positive dynamics and changes in the total risk score. The study used a sterile anatomical scalpel, manual podiatric instruments (scissors, forceps, dissector), 0.05% aqueous chlorhexidine solution, soft offloading pads, interdigital fixator (I-II fingers) and sterile aseptic dressings.

The preparation and description of the clinical case were carried out in accordance with the ethical principles of the Declaration of Helsinki World Medical Association [15] and the requirements of Good Clinical Practice ICH GCP E6(R2) [16]. Before collecting data, the patient's written informed consent was obtained to include clinical information and photographs in a scientific publication, provided that personal data was completely anonymised. The patient was informed about the purpose of using the information, possible risks, benefits, confidentiality, and the right to refuse participation at any stage without prejudice to further medical care.

RESULTS AND DISCUSSION

The study resulted in the development of a step-by-step algorithm for clinical assessment of the risk of amputation, which is presented in the form of a 15-point podiatrist's card (Fig. 2). The card contained five sections: medical data (up to 3 points), foot examination (up to 4 points), hygiene and behaviour (up to 3 points), footwear and pressure factors (up to 3 points), and the podiatrist's action strategy (up to 2 points). the final score allowed the risk zone to be determined: green (low risk 13-15 points), yellow (moderate risk 10-12 points) or red (high risk <9 points). Further application of the algorithm demonstrated its effectiveness in clinical settings.



PODIATRIST'S ASSESSMENT CARD FOR PATIENTS WITH DIABETES

Author's Methodology by Dr. Maysa Durdykulyyeva, 2025©

The patient has been informed of the purpose of the podiatric assessment and the specifics of diabetic foot care. They consent to the procedure and, if necessary, referral to an appropriate specialist.

Patient: _____ Date: _____

Patient's Signature: _____ Podiatrist: _____

SECTION A. MEDICAL DATA (Max. 3 points)

[ ] Diabetes diagnosed by a physician

[ ] Diabetes type, duration, and glucose self-monitoring known

[ ] Patient informed and consented to data processing

SECTION B. FOOT EXAMINATION (Max. 4 points)

[ ] Skin intact, no deep cracks/ulcers/wounds

[ ] No signs of infection (inflammation, fungus)

[ ] Sensation preserved (monofilament/needle test)

[ ] Pulse palpable (dorsal / plantar artery)

SECTION C. HYGIENE AND BEHAVIOR (Max. 3 points)

[ ] Practices hygiene, inspects feet regularly

[ ] Does not use dangerous home remedies

[ ] Willing to follow podiatrist's recommendations

SECTION D. FOOTWEAR AND RISKS (Max. 3 points)

[ ] Properly fitting, pressure-free footwear

[ ] Seamless socks with soft elastic

[ ] Pressure areas identified and offloaded

SECTION E. PODIATRIC STRATEGY (Max. 2 points)

[ ] No contraindications; coordinated with physician if needed

[ ] Care plan and follow-up schedule established

TOTAL SCORE: ____ / 15

REASSESSMENT: Date: _____ Score: ____ / 15

RECOMMENDED REFERRALS:

[ ] Endocrinologist

[ ] Vascular surgeon

[ ] Orthopedic specialist

RISK SCALE:

13–15 points — Safe for podiatric treatment (GREEN zone)

10–12 points — Proceed with caution (YELLOW zone)

< 9 points — Treat only in coordination with a physician (RED zone)

Podiatrist's Signature: _____ Date: _____

INFORMED CONSENT AND WAIVER OF CLAIMS

I, the undersigned, confirm that:

1. I have received complete information regarding the nature and purpose of the podiatric procedure, as well as any limitations or risks due to diabetes.

2. I understand that podiatric care does not replace medical treatment and is not a form of medical therapy.

3. I am aware that in the presence of high risk or contraindications, the podiatrist may refuse the procedure or refer me to another specialist.

4. I agree to follow the podiatrist's recommendations (both verbal and written).

5. I acknowledge that failure to comply with the recommendations may lead to complications, which do not constitute grounds for legal claims.

6. I voluntarily consent to the procedure and release the podiatrist from legal liability.

Patient's Signature: _____

Date: _____

Figure 2. Podiatrist's assessment card for working with patients with diabetes
Note: patented (No. 137472, 2025)
Source: created by the author

During initial visit, the patient showed signs of inflammatory damage to the front part of right foot, which led to the start of intensive medical intervention according to the author's algorithm. Accordingly, a comprehensive podiatric approach was applied, including local gentle intervention, antiseptic therapy, offloading of the affected area, medical and health education of the patient, systemic antibacterial support, and referral to specialised specialists. At the first stage, gentle mechanical treatment of the wound surface was performed with a sterile anatomical scalpel and a manual podiatric instrument, removing superficial necrotic layers and hyperkeratosis in order to reduce peripheral inflammation and improve tissue oxygenation. Immediately after that, the affected area was treated with a 0.05% chlorhexidine solution, offloaded with soft pads, which allowed to eliminate local pressure on the front of the foot and fix the I-II toes, and a sterile aseptic bandage was applied (Fig. 3). The patient and her relatives were trained in the

technique of bandaging, after which they independently changed the dressings daily at home for the next 6 days, observing a gradual decrease in hyperemia and pastosity.

Figure 3. Offloading and initial bandaging of the affected area of the foot
Note: a – soft foam pads installed to offload the forefoot; b – aseptic bandage after mechanical and antiseptic treatment
Source: created by the author

After the swelling went down and the soft tissues stabilised (on day 7), a customised interdigital orthosis made of medical-grade silicone was made and fitted, which kept the contractured areas separated and reduced surface friction when walking (Fig. 4). An aseptic bandage continued to be applied daily for 3 days after the orthosis was installed, which prevented re-traumatisation of the damaged area and created conditions for the formation of a granulation ridge, confirming the activation of recovery processes.

Figure 4. Fitting an individual silicone interdigital orthosis
Source: created by the author

The next step was to teach the patient and her relatives how to care for her feet: daily inspection of the soles and between the toes using a mirror, washing with warm water and neutral pH soap without soaking, thorough drying of the interdigital spaces, application of emollients to dry areas of skin, but not between the toes; wearing socks made of natural fabrics, changing them twice a day, wearing comfortable shoes that fit well and have soft insoles. Emphasis was placed on the mandatory control of glycaemia, refusal of aggressive home methods of self-treatment of calluses (salt, celandine, peroxide, salicylic acid) and the need for immediate medical attention in case of cracks, blisters or skin discolouration. Educational work was aimed at increasing adherence to prevention and recurrence prevention.

Due to the high systemic risk, urgent referral was made on the day of the initial visit to an endocrinologist for correction of hypoglycaemic therapy and to a vascular surgeon to rule out critical ischaemia of the lower extremities and assess the need for further examination (Doppler ultrasound of the vessels, determination of the ankle-brachial index). This confirmed the absence of acute arterial obstruction and allowed the conservative strategy of local podiatric treatment to be continued. To prevent the spread of the infection, Augmentin 875/125 mg (1 tablet twice daily for 5 days) was prescribed. To prevent antibiotic-associated intestinal dysbiosis, the probiotic Neoflorum (1×10^9 colony-forming units/tablet) was recommended at a dose of 1 tablet once a day for 14 days. The patient tolerated the treatment well, with no side effects noted, which allowed for high adherence to the treatment strategy. During the first 7 days of therapy, the clinical condition was characterised by a progressive reduction in oedema and stabilisation of skin colour, with the colour tone changing from hyperemic pink to a pale pink more characteristic of epithelialisation, without perifocal areas of inflammation.

During the second week of observation, against the background of continued systemic antibacterial and probiotic therapy, local podiatric care and the use of an individual orthosis, a gradual increase in signs of regeneration was observed. The appearance of the affected area was characterised by the absence of hyperemia and oedema, clear delineation of the contours of the toes, a decrease in the density of hyperkeratotic layers, and the appearance of a

pale pink area of epithelialisation in the centre of the primary lesion (Fig. 5). The patient did not report pain when walking, and complaints of a feeling of fullness and local heat disappeared.

Figure 5. Condition of the right foot on the 14th day of treatment
Source: created by the author

On the 14th day of treatment, the foot condition was reassessed using the author's 15-point podiatrist scorecard. The total score increased from 7 to 11, which corresponded to a transition from the red (high risk of amputation) to the yellow (moderate risk) zone. This was the result of improved scores in section B "Foot examination"), where the score increased from 1 to 3 due to reduced inflammation and signs of epithelialisation, as well as in sections C ("Hygiene and behaviour") and D ("Footwear and pressure factors"), where, thanks to training and the selection of orthoses, the score rose to 2 instead of the initial 1. The maximum score of 2 remained stable in section E ("Podiatrist's treatment strategy") as the plan of therapeutic and diagnostic interventions was adhered to. Clinically, this was reflected in the stabilisation of foot tissue homeostasis, the absence of indications for amputation, and the formation of an epithelialisation ridge. At the same time, blood glucose levels remained elevated (9-11 mmol/L), necessitating increased endocrinological monitoring. The objective dynamics are summarised in Table 1, which showed a clear improvement in four of the five sections of the card and confirms the effectiveness of the comprehensive algorithm for clinical assessment of the risk of amputation.

Table 1. Dynamics of clinical assessment according to the author's 15-point podiatrist card against the background of treatment

Card section	Maximum points	Before treatment	After treatment	Comments on dynamics
A. Medical data	3	2	2	Therapy control has been partially improved.
B. Foot examination	4	1	3	Reduction of hyperemia, edema, epithelialisation.
C. Hygiene and behaviour	3	1	2	Refusal of self-help, compliance with recommendations.
D. Footwear and pressure factors	3	1	2	Offloading, use of orthosis.
E. Podiatrist's treatment strategy	2	2	2	The action plan has been followed.
Total score	15	7	11	Transition from the red to the yellow zone.

Source: created by the author

The results confirmed that the use of a structured algorithm for clinical assessment of the risk of amputation using the author's 15-point podiatrist card, gentle local interventions, offloading, educational work and multidisciplinary routing allows to quickly stabilise the condition of the diabetic foot and reduce the risk of amputation, emphasising the key role of the podiatrist as a primary care specialist capable of timely identifying critical changes and initiating preventive measures. The application of the author's algorithm with a step-by-step assessment of the clinical risk of amputation showed high efficiency during a two-week observation period. The patient's transition from the critically dangerous red zone to the moderate yellow zone was accompanied by a reduction in local inflammation, improvement in perfusion parameters, and the creation of conditions for wound healing. The greatest contribution to the positive dynamics was demonstrated by gentle local interventions and offloading technologies, while glycaemic control and behavioural modification remained the most difficult components for the patient, limiting the possibility of achieving low risk in the short term.

The reduction in the risk score from 7 to 11 within 14 days reflected not only an improvement in the local condition of the foot, but also a clear impact of behavioural modification and the implementation of a structured podiatric stratification algorithm. The patient experienced a reduction in inflammation, restoration of microcirculation, stabilisation of tissue homeostasis and formation of an epithelialisation ridge, which, combined with the abandonment of aggressive home treatment methods, allowed to move from the red to the yellow risk zone. Compared to the data of T. Korkmaz *et al*. [17], where even minimal behavioural modification reduced the risk of amputation from 35.4% to 21.7%, the results obtained confirmed the importance of the educational component of podiatric care. A study by C. Gazzaruso *et al*. [18] showed that comprehensive multidisciplinary interaction with a podiatrist, vascular surgeon, and endocrinologist forms the basis for successful conservative management, which is fully consistent with the routing applied in this case. At the same time, M.S. Aydın *et al*. [19] emphasised that a normalised systemic inflammatory background is necessary for a long-term reduced risk of amputation, which was not achieved in the patient under study, determining the need for further endocrinological correction. This observation is fully consistent with the conclusions of M. Klymenko [20], who

highlighted that persistent low-grade inflammation underlies multiple chronic metabolic and microvascular complications, including impaired tissue repair and recurrent lesions, thus complicating the conservative management of diabetic foot syndrome. Comparable conclusions are presented by F. Afshar *et al.* [21], where it was emphasised that optimisation of the ratio of neutrophils to lymphocytes in peripheral blood <3.8 after two weeks of treatment served as a predictor of stability of the result, which requires additional laboratory monitoring in further management.

The reduction in hyperemia, pastosity, and local signs of inflammation during the first two weeks of treatment demonstrated the important role of regular antiseptic treatment and gentle mechanical sanitation in stabilising early diabetic lesions. It was the removal of necrotic layers and hyperkeratosis that minimised the risk of bacterial colonisation and provided the tissues with access to oxygen, creating optimal conditions for epithelialisation. Scientists É. Senneville *et al.* [22] proved that systematic surface sanitation in combination with antiseptics reduced the risk of osteomyelitis formation within the diabetic foot by two times, confirming the feasibility of early local intervention. The work of J. Hüsers *et al*. [23] showed that the inclusion of the indicator of the frequency of mechanical treatments in prediction systems significantly improved the accuracy of amputation risk assessment tools, which emphasised the importance of podiatric sanitation as an important variable in mathematical models. A study by D. Demirkol *et al.* [24] demonstrated that the frequency and quality of local wound treatment determined the difference between conservative healing and progression to surgical intervention in more than 30% of cases, proving the critical role of biofilm removal. Similarly, R.G. Frykberg *et al*. [25] noted that the absence of mechanical sanitation and antiseptic prophylaxis significantly reduces the effectiveness of even surgical reconstructive care, emphasising the leading role of the podiatrist as the first specialist capable of preventing the penetration of infection into deep tissues and thereby reducing the need for amputation.

In this case, the improvement in the local condition of the foot was largely due to the correct selection of the method of offloading the affected area, which ensured a reduction in microtrauma to the tissues, stabilisation of microcirculation and the creation of conditions for physiological recovery. The use of soft silicone pads and an interdigital orthosis contributed to the reduction of pressure in the critical area of the I-II toes, which was accompanied by the formation of an epithelialisation ridge and a transition from the red to the yellow risk zone. A similar effect of biomechanical correction was described by Z. Huang *et al*. [26], who showed that the use of individual offloading devices was associated with a 38% reduction in the frequency of lesion progression, but noted that the maximum effect is achieved only with simultaneous modification of footwear. In the work of F.S. Yen *et al*. [27], it was proven that the use of orthopaedic offloading devices can prevent the formation of ulcers in 64% of patients with monoregional lesions, which is comparable to the dynamics obtained in this study, although the authors emphasised the limited result in the absence of daily compliance monitoring. A study by A. Azhar *et al*. [28] demonstrated that the combined use of antibacterial therapy and orthotics prevented amputation in 79.4% of cases, confirming the feasibility of combining orthopaedic and pharmacological approaches used in this case. On the other hand, S. Stefanopoulos *et al*. [29] emphasised that the long-term effectiveness of offloading depends on the individualisation of the approach and the ability to adapt to changes in the shape of the foot, which determined the prospect of further improvement of stratification algorithms for the needs of a specific patient.

The patient's adherence to hygiene recommendations, refusal of aggressive home procedures, and regular monitoring of the condition of the foot proved to be an important behavioural component that supported the positive local dynamics achieved by podiatric interventions. Daily visual inspection, thorough drying of the interdigital spaces, application of emollients, and wearing of adapted footwear minimised the risks of re-traumatisation and secondary infection and contributed to the formation of an epithelial ridge. In the work of B. Peng *et al*. [30], it was shown that systematic implementation of preventive measures led to a reduction in the frequency of amputations from 27.1% to 8.6%, which emphasised the critical role of adherence to recommendations even in patients with high baseline risk. W.W. Han *et al*. [31] emphasised that strict adherence to hygiene and routing recommendations reduces the risk of sepsis by almost half, which is consistent with the positive evolution of the process in the patient under study. According to A.D. Popa *et al*. [32], a combination of daily care and wearing appropriate footwear can prolong the period of remission in almost 70% of cases, confirming the importance of the behavioural component in the prevention of recurrent lesions. At the same time, S. Smith *et al*. [33] noted that without preventive measures, the risk of recurrence and increasing neurological complications may exceed 50%, which justifies long-term podiatric support even after clinical stabilisation.

This clinical case clearly demonstrated the leading role of podiatrists in the system of primary prevention of amputations in patients with diabetic foot syndrome, where rapid risk stratification and early intervention are crucial for the formation of organ-preserving tactics. The use of the author's 15-point card allowed not only to assess the degree of threat, but also to determine the limit of the permissible scope of podiatric manipulations, avoiding excessive actions that could provoke the progression of the lesion. The importance of rapid stratification is confirmed by the Australian recommendations of P.A. Lazzarini *et al*. [34], which state that a delay in risk assessment of more than 48 hours almost doubles the likelihood of amputation. In turn, the authors W. Jeffcoate *et al.* [35] emphasised that it is the podiatrist who should be the first point of contact, capable of early identification of prognostically unfavourable changes and initiating patient education. At the same time, F. Farine *et al*. [36] noted that adequate stratification allows both underestimation and oversaturation of interventions to be avoided, since it is errors in the choice of the scope of procedures that are associated with a 2-3-fold increase in the frequency of amputations. The study by C.K. Perng *et al.* [37] showed that patients who were referred to a vascular surgeon within the first ten days of the onset of the lesion had a lower rate of progression to critical ischaemia (15.8% vs 34.1%), reflecting the need for rapid referral. The study by P.O. Gerasymchuk *et al*. [38] proved

that the results of modern technologies (e.g., vacuum therapy) are most effective in early intervention, when the local process remains under the control of the podiatrist, which emphasised the importance of the author's algorithm as a tool for timely routing.

The use of a structured algorithm with the author's risk map ensured the timely detection of a high risk of amputation, the determination of the acceptable scope of local intervention, and the rapid referral of the patient to specialists, which is critically important in preventing the progression of the lesion. Thanks to a combination of gentle mechanical sanitation, offloading of the affected area, antiseptic therapy, and behavioural modification, it was possible to stabilise the condition of the foot tissues and reduce the clinical risk of amputation in less than two weeks. The results obtained are consistent with current international approaches, in which the podiatrist is considered the first point of contact, which is crucial in the primary prevention of diabetic foot complications through rapid stratification, tactical flexibility, and integration of the patient into a multidisciplinary care system. The use of such algorithms is particularly appropriate in high-risk groups – elderly patients with low treatment adherence and severe neuropathy, where even minimal local lesions are highly likely to progress rapidly to amputation.

CONCLUSIONS

The use of a structured algorithm with the author's 15-point podiatrist card in the management of a patient with diabetic foot syndrome allowed for timely stratification according to the risk of amputation and the formation of a personalised trajectory of therapeutic and preventive interventions. The initial risk score was 7 out of a possible 15, which corresponded to the red zone (high risk of amputation) and led to restrictions on invasive procedures and urgent referral to an endocrinologist and vascular surgeon. The introduction of a complex of local interventions (gentle sanitation, antiseptic treatment, aseptic dressing), individual offloading and behavioural modification, combined with educational work, contributed to the formation of conditions for the restoration of microcirculation on the 5th day and the appearance of an epithelialisation ridge by the 14th day of treatment.

The positive dynamics of the local condition was reflected in the increase in assessment scores in the sections of the "Foot Examination" card, "Hygiene and Behaviour" and "Footwear and Offloading" sections of the card from 1-1-1 to 3-2-2, respectively, which indicated the effectiveness of the sanitation and offloading approach and the patient's increasing adherence to the recommendations received. The total score increased from 7 to 11, i.e. by 57.1%, which corresponds to a transition from the red to the yellow risk zone. At the same time, the preservation of suboptimal control of systemic parameters (fasting glucose level 9-11 mmol/L) and incomplete adherence to the preventive regimen indicated the need for continued endocrinological and behavioural supervision to prevent recurrence or progression of the lesion.

The presented clinical case confirmed the leading role of the podiatrist not only in the local prevention of diabetic complications, but also in the formation of a multidisciplinary referral network that allows for the implementation of organ-preserving tactics in outpatient settings. The implementation of this algorithm contributedd to a reduction in the time from referral to clinical improvement (on average to 14 days), individualisation of the therapeutic strategy and increased objectivity of risk assessment, which makes it a promising tool for early intervention in podiatric care, especially among elderly patients with low treatment adherence and severe neuropathy. Further research should include multicentre prospective studies involving a larger sample of patients to verify the algorithm and refine the prognostic value of the author's card.

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CONFLICT OF INTEREST

The author is the developer and owner of the patent of Ukraine No. 137472 (2025) for the podiatrist's assessment card for diabetes mellitus, which could potentially be perceived as a factor that could affect the objectivity of the presentation of the results. There are no other commercial or financial conflicts of interest.

REFERENCES

[1] International Diabetes Federation. Diabetes facts & figures [Internet]. [cited 2025 February 25]. Available from: https://idf.org/about-diabetes/diabetes-facts-figures/
[2] Armstrong DG, Tan TW, Boulton AJ, Bus SA. Diabetic foot ulcers: A review. Jama. 2023;330(1):62–75. DOI: 10.1001/jama.2023.10578
[3] Gong H, Ren Y, Li Z, Zha P, Bista R, Li Y, et al. Clinical characteristics and risk factors of lower extremity amputation in the diabetic inpatients with foot ulcers. Front Endocrinol. 2023;14:1144806. DOI: 10.3389/fendo.2023.1144806
[4] Okur KT, Ozan F, Kahraman M, Melez M, Ünlü ÖC, Altun İ. Assessment of the risk factors determining the prognosis of major and minor limb amputations in patients with diabetic foot ulcers. Adv Clin Exp Med. 2024;33(12):21–30. DOI: 10.17219/acem/163240
[5] Forsythe RO, Apelqvist J, Boyko EJ, Fitridge R, Hong JP, Katsanos K, et al. Performance of prognostic markers in the prediction of wound healing or amputation among patients with foot ulcers in diabetes: A systematic review. Diabetes Metab Res Rev. 2020;36(1):e3278. DOI: 10.1002/dmrr.3278
[6] Petrenko O, Badziukh S, Korsa V, Kolosovych I, Tykhomyrov A. Topical application of autologous plasma-derived plasminogen accelerates healing of chronic foot ulcers in type 2 diabetes patients. Int J Low Extrem Wounds. 2024;15347346241256025. DOI: 10.1177/15347346241256025

[7] Kaka AS, Landsteiner A, Ensrud KE, Logan B, Sowerby C, Ullman K, et al. Risk prediction models for diabetic foot ulcer development or amputation: A review of reviews. J Foot Ankle Res. 2023;16(1):13. DOI: 10.1186/s13047-023-00610-6
[8] Ivanova AS, Melekhovets OK, Melekhovets IV. A comprehensive approach to diabetic foot ulcers treatment in patients with anisomelia. Int J Endocrinol. 2025;21(2):140–6. DOI: 10.22141/2224-0721.21.2.2025.1511
[9] Liu Z, Wei D, Wang J, Gao L. Predicting major amputation risk in diabetic foot ulcers using comparative machine learning models for enhanced clinical decision-making. Sci Rep. 2025;15:28103. DOI: 10.1038/s41598-025-13534-x
[10] Oei CW, Chan YM, Zhang X, Leo KH, Yong E, Chong RC, et al. Risk prediction of diabetic foot amputation using machine learning and explainable artificial intelligence. J Diabetes Sci Technol. 2024;19(4):1008–22. DOI: 10.1177/19322968241228606
[11] Shapoval SD, Tribushniy OV, Savon IL, Sophylkanych MM, Shidlovskyi VO, Vasilevska LA, et al. Complex treatment of patients with complicated syndrome of diabetic foot and sepsis. Rom J Diabetes Nutr Metab Dis. 2021;28(2):131–6. DOI: 10.46389/rjd-2021-1021
[12] International Working Group on the Diabetic Foot. Practical guidelines on the prevention and management of diabetesrelated foot disease [Internet]. 2023 [cited 2025 February 25]. Available from: https://iwgdfguidelines.org/wp-content/uploads/2023/07/IWGDF-Guidelines-2023.pdf
[13] American Diabetes Association Professional Practice Committee. Retinopathy, neuropathy, and foot care: Standards of care in diabetes – 2025. Diabetes Care. 2025;48(1):252–65. DOI: 10.2337/dc25-S012
[14] National Institute for Health and Care Excellence. Diabetic foot problems: Prevention and management [Internet]. 2015 August 26 [cited 2025 February 25]. Available from: https://www.nice.org.uk/guidance/ng19
[15] World Medical Association. Declaration of Helsinki: Ethical principles for medical research involving human subjects [Internet]. [cited 2025 February 25]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki/
[16] European Medicines Agency. Guideline for good clinical practice E6(R2) (EMA/CHMP/ICH/135/1995) [Internet]. [cited 2025 February 25]. Available from: https://www.ema.europa.eu/en/ich-e6-good-clinical-practice-scientific-guideline
[17] Korkmaz T, Afacan MY, Davulcu CD, Elibollar C, Değer GU, Şeker A. Depression as a prognostic factor in lower extremity amputation for diabetic foot: Insights from a prospective study on wound healing, infections, and early mortality. J Foot Ankle Surg. 2024;63(6):705–12. DOI: 10.1053/j.jfas.2024.07.005
[18] Gazzaruso C, Gallotti P, Pujia A, Montalcini T, Giustina A, Coppola A. Predictors of healing, ulcer recurrence and persistence, amputation and mortality in type 2 diabetic patients with diabetic foot: A 10-year retrospective cohort study. Endocrine. 2021;71:59–68. DOI: 10.1007/s12020-020-02431-0
[19] Aydın MS, Eren MA, Uyar N, Kankılıç N, Karaaslan H, Sabuncu T, et al. Relationship between systemic immune inflammation index and amputation in patients with diabetic foot ulcer. J Orthop Sci. 2024;29(4):1060–3. DOI: 10.1016/j.jos.2023.07.015
[20] Klymenko M. Progress and prospects in research on low-grade diffuse chronic inflammation: A literature review. Ukr J Med Biol Sport. 2025;10(1):16–29. DOI:10.63341/ujmbs/1.2025.16
[21] Afshar F, Daraie M, Mohammadi F, Seifouri K, Amin Afshari S, Heidari Some'eh S, et al. Neutrophil-lymphocyte ratio (NLR); an accurate inflammatory marker to predict diabetic foot ulcer amputation: A matched case-control study. BMC Endocr Disord. 2025;25:120. DOI: 10.1186/s12902-025-01941-0
[22] Senneville É, Albalawi Z, Van Asten SA, Abbas ZG, Allison G, Aragón-Sánchez J, et al. IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections (IWGDF/IDSA 2023). Diabetes Metab Res Rev. 2024;40(3):e3687. DOI: 10.1002/dmrr.3687
[23] Hüsers J, Hafer G, Heggemann J, Wiemeyer S, John SM, Hübner U. Predicting the amputation risk for patients with diabetic foot ulceration – a Bayesian decision support tool. BMC Med Inform Decis Mak. 2020;20:200. DOI: 10.1186/s12911-020-01195-x
[24] Demirkol D, Erol ÇS, Tannier X, Özcan T, Aktaş Ş. Prediction of amputation risk of patients with diabetic foot using classification algorithms: A clinical study from a tertiary center. Int Wound J. 2024;21(1):e14556. DOI: 10.1111/iwj.14556
[25] Frykberg RG, Attinger C, Smeets L, Koller A, Bal A, Kavarthapu V. Surgical strategies for prevention of amputation of the diabetic foot. J Clin Orthop Trauma. 2021;17:99–105. DOI: 10.1016/j.jcot.2021.02.019
[26] Huang Z, Zheng X, Liang H, Zhong SY, Meng J, Yao J. Association of systemic immune-inflammatory index with risk of foot ulcer amputation in patients with type 2 diabetes mellitus: Insights from a cross-sectional study. J Inflamm Res. 2025;18:8295–304. DOI: 10.2147/JIR.S517693
[27] Yen FS, Yen YH, Hung YM, Wei JCC, Tsai FJ, Hung YT, et al. Diabetic microvascular disease and risk of peripheral artery disease, foot ulcer, leg infection, and amputation. Thromb Haemost. DOI: 10.1055/a-2661-2537
[28] Azhar A, Basheer M, Abdelgawad MS, Roshdi H, Kamel MF. Prevalence of peripheral arterial disease in diabetic foot ulcer patients and its impact in limb salvage. Int J Low Extrem Wounds. 2023;22(3):518–23. DOI: 10.1177/15347346211027063
[29] Stefanopoulos S, Qiu Q, Ren G, Ahmed A, Osman M, Brunicardi FC, et al. A machine learning model for prediction of amputation in diabetics. J Diabetes Sci Technol. 2024;18(4):874–81. DOI: 10.1177/19322968221142899
[30] Peng B, Min R, Liao Y, Yu A. Development of predictive nomograms for clinical use to quantify the risk of amputation in patients with diabetic foot ulcer. J Diabetes Res. 2021;2021(1):6621035. DOI: 10.1155/2021/6621035
[31] Han WW, Fang JJ. Analysis of risk factors and predictive value of a nomogram model for sepsis in patients with diabetic foot. World J Diabetes. 2025;16(4):104088. DOI: 10.4239/wjd.v16.i4.104088

[32] Popa AD, Gavril RS, Popa IV, Mihalache L, Gherasim A, Niță G, et al. Survival prediction in diabetic foot ulcers: A machine learning approach. J Clin Med. 2023;12(18):5816. DOI: 10.3390/jcm12185816
[33] Smith S, Normahani P, Lane T, Hohenschurz-Schmidt D, Oliver N, Davies AH. Prevention and management strategies for diabetic neuropathy. Life. 2022;12(8):1185. DOI: 10.3390/life12081185
[34] Lazzarini PA, Raspovic A, Prentice J, Commons RJ, Fitridge RA, Charles J. Australian evidence-based guidelines for the prevention and management of diabetes-related foot disease: A guideline summary. Med J Aust. 2023;219(10):485–95. DOI: 10.5694/mja2.52136
[35] Jeffcoate W, Boyko EJ, Game F, Cowled P, Senneville E, Fitridge R. Causes, prevention, and management of diabetes-related foot ulcers. Lancet Diabetes Endocrinol. 2024;12(7):472–82. DOI: 10.1016/S2213-8587(24)00110-4
[36] Farine F, Rapisarda AM, Roani C, Giuli C, Comisi C, Mascio A, et al. Predictive factors of amputation in diabetic foot. Biomedicines. 2024;12(12):2775. DOI: 10.3390/biomedicines12122775
[37] Perng CK, Chou HY, Chiu YJ. Identifying major predictors of lower-extremity amputation in patients with diabetic foot ulcers. J Chin Med Assoc. 2021;84(3):285–9. DOI: 10.1097/JCMA.0000000000000473
[38] Gerasymchuk PO, Pavlyshyn AV, Fira DB, Volotovska NV. Study of the vacuum therapy influence on the wound process in patients with diabetic foot syndrome. Rom J Diabetes Nutr Metab Dis. 2024;31(4):428–32. DOI: 10.46389/rjd-2024-1741

Клінічна оцінка ризику ампутації при діабетичній стопі: клінічний випадок

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Анотація. Метою роботи було створення клінічного алгоритму оцінки ризику ампутації для пацієнтів із синдромом діабетичної стопи та його апробація на прикладі клінічного випадку, з акцентом на визначенні провідної ролі подолога в межах мультидисциплінарної команди. Дослідження виконано як опис клінічного випадку із застосуванням авторської 15-бальної карти стратифікації ризику, щадних локальних подологічних втручань, індивідуального розвантаження, поведінкової модифікації та мультидисциплінарної маршрутизації з подальшим оцінюванням динаміки за 7- та 14-денним протоколом спостереження. Упродовж 14 діб спостереження впровадження структурованого клінічного алгоритму забезпечило прогресивне покращення локального стану стопи: вже на 7-й день спостерігалося зменшення гіперемії, пастозності та болю, а до 14-го сформувався виражений епітелізаційний валик по краю дефекту, що свідчило про активацію репаративних процесів. Загальний бал за оцінковою картою підвищився з 7 до 11, відображаючи перехід пацієнтки з високого ризику ампутації до помірного. Секційні бали локального огляду зросли з 1 до 3, поведінкової секції – з 1 до 2, а корекції навантаження – з 1 до 2, що підтвердило ефективність щадної санації, антисептики, розвантажувальних прокладок та міжпальцевого ортеза. Позитивна динаміка супроводжувалась підвищенням комплаєнсу пацієнтки й відмовою від травмувальних домашніх маніпуляцій. Водночас рівень глікемії залишався підвищеним (9-11 ммоль/л), що зумовлює необхідність тривалого ендокринологічного контролю та профілактики рецидивів. Впровадження подологічного алгоритму з ранньою стратифікацією, щадними локальними втручаннями, індивідуальним розвантаженням та поведінковою корекцією дозволило досягти клінічно значущого зниження ризику ампутації вже протягом 14 днів лікування. Отримані результати можуть бути використані подологами, сімейними лікарями та ендокринологами у практиці амбулаторної допомоги для ранньої стратифікації ризику, оптимізації обсягу втручань і своєчасного направлення пацієнтів із синдромом діабетичної стопи з метою профілактики ампутацій

Ключові слова: подолог; стратифікація; розвантаження; уражена ділянка; запалення; антисептична обробка; санація

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Assessment of the effectiveness of teaching patients with heart failure in hospital settings

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Abstract. Heart failure is one of the leading causes of hospitalisation, disability and mortality among elderly people. Effective secondary prevention requires adequate patient education, and nurses play a key role in implementing educational measures at a professional level. The aim of the study was to comprehensively evaluate the effectiveness of an educational programme in improving the knowledge of patients with heart failure in hospital settings. The study involved 62 patients with stage IIA,B heart failure aged 33 to 82 (56.3 ± 11.7) years, 76% men and 24% women. All patients underwent a knowledge assessment at the beginning of hospitalisation and on the day of discharge after receiving training in a hospital setting. Information on the effectiveness of training was collected over a period of 6 months. The main results showed that patients who participated in the training programme improved their knowledge of heart failure (from 72.58% to 82.25%), symptom recognition (from 36.51% to 78.06%), behaviour (from 44.89% to 58.86%), nutrition (from 31.12% to 69.34%), and recommended treatment (from 35.24% to 58.51%). Most realised the importance of daily weighing (64.51%), limited their consumption of table salt (77.41%), fluids (74.19%) and alcohol (77.41%). A significant proportion gave up smoking during hospitalisation (91.93%) and partially maintained this trend after discharge (67.74%). Patients visited their cardiologist more often (61.29%), kept a symptom monitoring diary (96.77%), independently adjusted their intake of diuretics (72.58%) and potassium-containing drugs (59.67%), and did not miss any doses of medication (80.64%). It has been proven that higher patient knowledge was associated with the educational information received from a nurse during hospitalisation. The practical value of the results was to justify the need to master the skills of weighing, determining the degree of oedema, and keeping a diary of monitoring symptoms of heart failure in outpatient settings

Keywords: nurse; educational programme; awareness; effectiveness; diary; continuous care

INTRODUCTION

The leading cause of death worldwide is cardiovascular disease, and its final stage is considered to be heart failure (HF). It is one of the major health problems, accompanied by high rates of morbidity, hospitalisation and mortality. It is a clinical syndrome with symptoms and/or signs caused by structural or functional changes in the heart and objective manifestations of pulmonary or systemic congestion. The prevalence of heart failure is expected to continue to increase worldwide, both among people over 65 and in younger age groups [1]. This trend is due to demographic factors (ageing population), an increase in the prevalence of obesity and arterial hypertension, increased survival after myocardial infarction, and high comorbidity.

A study by M. Montalto *et al*. [2] noted that in people over 70 years of age, the prevalence of HF exceeds 10%; in the group under 55 years of age, it is about 1%. This confirms that heart failure is not exclusively a problem of older patients, but has a tendency to "rejuvenate". Epidemiological forecasts indicate a steady increase in the number of patients. For example, B. Chong *et al*. [3] reported that as of 2024, approximately 6.7 million Americans over the age of 20 have HF, and the prevalence is expected to increase to 8.7 million in 2030, 10.3 million in 2040, and 11.4 million by 2050. Similar trends have been observed in Europe: according to B. Bozkurt *et al*. [4], around 1.7% of the population are affected, with the proportion continuing to increase. In

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their review, H.O. Slabkiy & I.I. Koshelia [5] presented the dynamics of mortality from cardiovascular diseases among the adult population. In the Chernivtsi region, 1,038.5 deaths per 100,000 population were recorded in 2021, with ischaemic heart disease among the causes (about 958.4 cases per 100,000 population). This level exceeds the average in many European countries, which highlights the high local significance of the problem and the need for targeted preventive measures. This trend has emphasised the need for large-scale preventive and medical-social measures.

In such conditions, the need to involve patients in actively managing their condition becomes obvious. Educational nursing interventions proposed by T. Jaarsma *et al*. [6] improve the self-management and self-care skills of patients with heart failure, especially when using a personalised action plan and early post-discharge follow-up. At the same time, low patient motivation and limited resources remain barriers, requiring personalised and culturally adapted approaches. An analysis by S.R.G. Marques *et al*. [7] showed that post-discharge educational interventions by nurses reduced the risk of rehospitalisation by 36% and the risk of death by 35% compared to the control group, highlighting the clinical significance of such programmes. An important condition for the effectiveness of such measures is their continuity and the availability of feedback between the nurse and the patient, in particular through platforms or regular visits. At the same time, a study by S. Stahlman *et al*. [8] revealed gaps in patients' knowledge prior to discharge, including limited understanding of exacerbation symptoms, rules for adhering to therapeutic regimens, and dietary guidelines. This indicated a need for a more systematic approach to pre- and post-discharge education, which should be an integrated part of the patient's journey. In addition, psycho-emotional factors such as stress, anxiety, and low social support were associated with poorer treatment adherence, according to a review by A.V. Metts *et al*. [9]. Therefore, nursing interventions should also include basic psychological support skills, teaching patients stress management techniques, and, if necessary, referral to mental health specialists. In Ukraine, the educational programme for patients with arterial hypertension proposed by N. Palibroda *et al*. [10] for patients with arterial hypertension has demonstrated high effectiveness: it increased knowledge about the disease by 47.07%, risk factors by 50.88%, and possible complications by 58.82%, and also contributed to an increase in the proportion of patients with controlled blood pressure and pulse. This confirmed that educational interventions are an important complement to drug treatment and should be integrated into family medicine practice.

No systematic official programmes or initiatives aimed at educating patients with heart failure were identified as of 2025. Given this absence, the aim of the study was to conduct educational activities for patients in hospital settings with the active participation of nurses in order to increase their knowledge and further analyse the results obtained.

MATERIALS AND METHODS

An anonymous survey was conducted to collect data, ensuring complete confidentiality and protection of respondents' personal information. Participants were informed about the purpose of the study and voluntary participation, and had the opportunity to refuse to participate without any negative consequences. The training was conducted at the regional communal non-profit enterprise (RCNPE) "Chernivtsi Cardiology Centre", with the participation of the senior nurse of General Cardiology Ward No. 1. The study was carried out over six months, covering the entire data collection and analysis period. Patients were purposely selected according to established inclusion and exclusion criteria. Sixty-two patients (76% men, 24% women) aged 33 to 82 (56.3 ± 11.7) years with diagnosed HF of NYHA functional classes I-IV and II-A (79%), II-B (21%) stages according to the classification of M.D. Strazhesko and V.H. Vasilenko, which corresponds to stage C according to the Recommendations of the All-Ukrainian Association of Cardiologists of Ukraine on the diagnosis, treatment and prevention of chronic heart failure (CHF) [11]. During the development of the training programme, factors that could potentially influence the assimilation of information by patients were taken into account, such as education (32 people (52.51%) had higher education, 4 people (6.45%) had incomplete higher education, 23 (37.18%) had secondary education, and 3 people (4.83%) had incomplete secondary education); employment (36 patients (58%) were employed); marital status (47 (76%) were married and had a family, 13 (21%) were widowed); comorbidity (17 (27%)); left ventricular ejection fraction (LVEF) (average 50% (32;66)); duration of heart failure (7.2 ± 1.5 years); length of hospitalisation (7.3 ± 0.4 days); complaints during hospitalisation (33 patients (53%) had shortness of breath, 32 (52%) had discomfort, tightness behind the breastbone, palpitations, 32 (52%) had general weakness, dizziness, headache, 28 (45%) had increased blood pressure, 21 (34%) had peripheral oedema in the lower extremities, and pastiness of the lower legs. All patients (100%) had a history of ischaemic heart disease, 35 (56%) had atherosclerotic disease, 8 (13%) had a history of myocardial infarction, 17 (27%) had chronic atrial fibrillation, and 39 (63%) were receiving outpatient treatment with cardiac and diuretic drugs. The exclusion criteria were patients under 30 years of age, with stage I HF, hospitalisation for less than 5 days, those who were transferred to other departments, had functional limitations and cognitive impairments, insufficient digital literacy, and no access to the internet.

The training programme consisted of one 2-hour session covering theoretical material and practical skills. The theoretical part included a discussion with relevant patients about the causes of heart failure, symptoms, clinical manifestations, a low-salt diet, fluid control, weight control, behaviour in case of exacerbation of symptoms, physical activity, and drug treatment. The practical part included skills for determining the degree of oedema, weighing, determining sodium in food portions, and keeping a diary to monitor symptoms of heart failure in an outpatient setting. To reinforce the knowledge gained, patients were given paper handouts based on the colour-coded "traffic light" system with a QR code containing practical recommendations for self-care in an outpatient setting. Green indicated acceptable symptoms, "yellow" indicated symptoms that should raise concern and be reported to a nurse or family doctor, and "red" indicated symptoms that required urgent action: immediately call the special number "5103" "Emergency Cardiological Care" (a service provided by the RCNPE "Chernivtsi Regional Clinical Cardiology Centre") (Fig. 1)

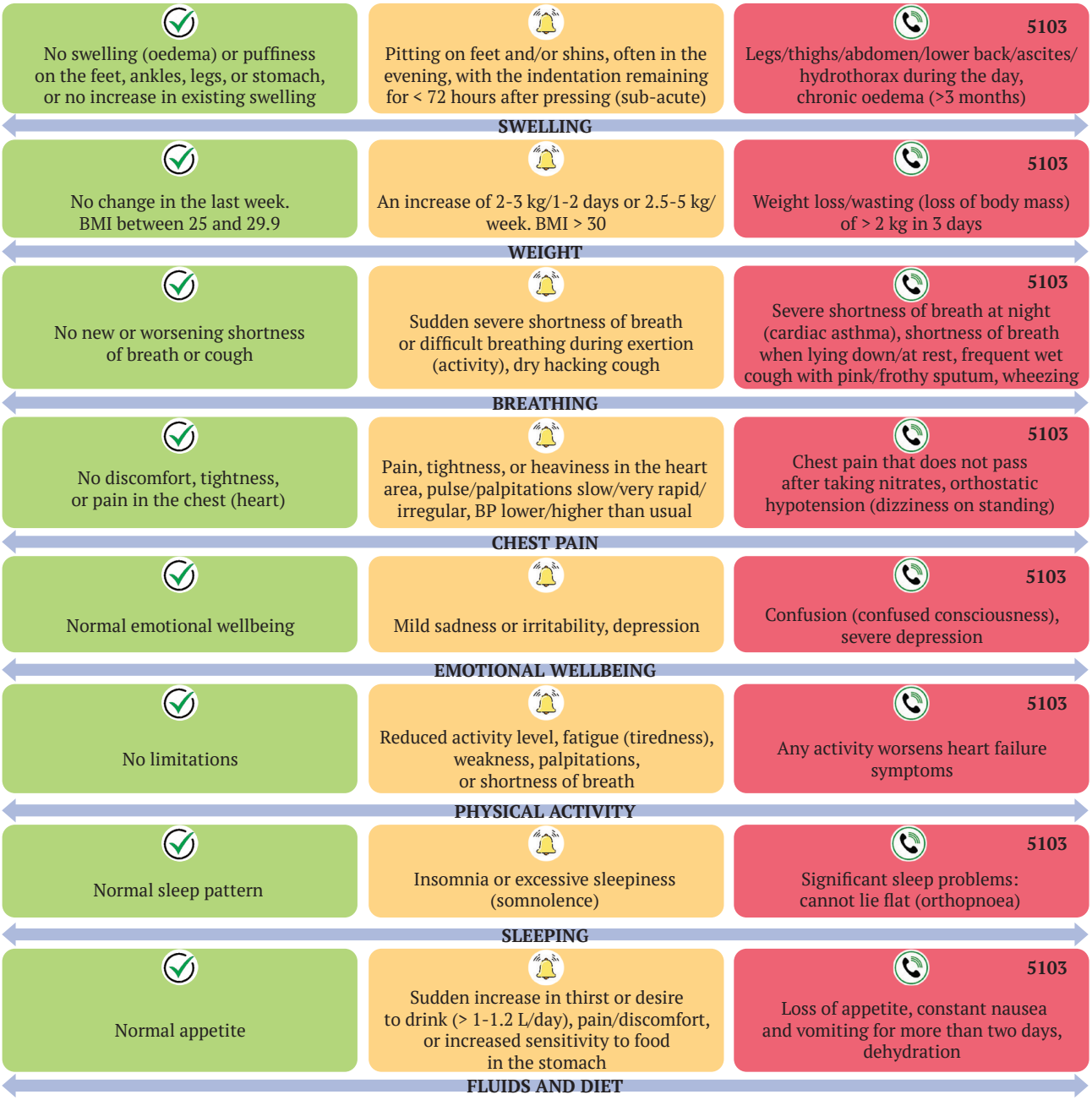

Figure 1. Memo for patients with heart failure
Note: BMI – body mass index. BP – blood pressure. 5103 – "Emergency cardiac care" (service provided by the RCNPE "Chernivtsi Regional Clinical Cardiology Centre")
Source: developed by the author

Patients' knowledge was assessed before and after training using the written version of the Atlantic Heart Failure Knowledge Test (AHFKT) version 3 (AHFKTv3), updated by B. Butts *et al*. [12]. The test consisted of 30 questions focusing on five areas of knowledge about self-care in HF: knowledge of the disease (2 questions), nutrition (10 questions), behaviour (6 questions), medication (7 questions) and symptoms (5 questions). Patients answered the questions by selecting one answer from the options provided. Individual answers were recorded and coded as correct (1 point) or incorrect (0 points). The total number of correct answers was counted and presented as a sum of points (from 0 to 30 possible correct answers) and a percentage of correct answers (from 0% to 100%).

Statistical analysis and graphical presentation of the results were performed using the Microsoft Excel computer program package (Microsoft 365 licence, subscription status – active). The indicators are presented as the mean value with a confidence interval and standard error. Primary and secondary results were compared using parametric data for dependent (paired) samples using the paired t-test and Pearson's χ^2 criterion for independent proportions. The mean values were given as $M \pm m$, where M is the arithmetic mean and m is the standard error of the mean.

Rank data in the diagrams are presented in the format of the median with lower and upper quartiles (M [Q1; Q3]). Categorical data are presented as the absolute number of cases (n) and the percentage (%) of the total number of the study group. Differences were considered statistically significant at $p \leqslant 0.05$ [13]. The study was approved by the Bioethics Committee of Bukovinian State Medical University (protocol No. 10, 2025) in accordance with the principles of bioethics set out in The World Medical Association [14] and the Universal Declaration on Bioethics and Human Rights [15]. All participants in this study provided informed written consent for the use of their medical records for scientific purposes, and personal identifiers were anonymised during data processing.

RESULTS AND DISCUSSION

When assessing the overall level of knowledge of patients based on 30 questions covering five areas of knowledge, significant unevenness in patients' preparedness for training was revealed. The most effective (in terms of the number of correct answers) was the area of knowledge about the disease in 72.58% (45/62), which indicates a basic understanding of the nature of heart failure. At the same time, the most vulnerable area was behaviour at 56.45% (35/62), indicating an insufficient understanding of the necessary changes in daily life to control the condition. A similar trend was observed in the area of symptom recognition (58.06% – 36/62) and medication adherence (65.51% – 40/62), highlighting the need for targeted training on early detection of exacerbations and self-monitoring of drug therapy. The level of knowledge about nutrition (66.12% – 41/62) showed average awareness, but leaves room for improvement, especially given the importance of dietary restrictions in the management of patients with heart failure. Thus, the results showed that prior to training, patients had basic knowledge about the disease but serious gaps in the practical aspects of self-management, which is of direct clinical importance for the prevention of exacerbations and rehospitalisations. The participation of a nurse in training activities conducted in a hospital setting significantly increased patient awareness in all areas of knowledge from an average of 45.16% (28/62) to 69.35% (43/62) ($p = 0.0024$), which demonstrated the effectiveness of structured training in improving patients' understanding of key aspects of their disease. The dynamics of changes in patients' knowledge levels before and after short-term training are shown in Figure 2.

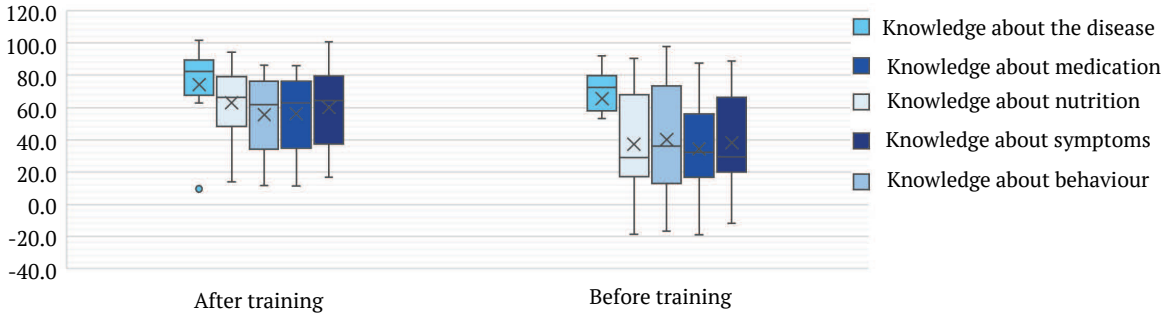

Figure 2. Dynamics of patients' knowledge levels based on survey results after and before training (%), Median [Q1; Q3]
Source: developed by the author

A detailed analysis of knowledge prior to training revealed that patients were most familiar with the basic concepts of heart failure, in particular its definition, causes and prognosis, and 62.90% (39/62) were aware that heart failure cannot be cured but must be controlled. The assessment of patients' knowledge of the disease after training did not reveal any statistically significant changes, as this section was already quite familiar. This indicates the presence of basic knowledge, but at the same time highlighted gaps in understanding the more complex aspects of self-care and symptom control, which was the subject of the educational programme. In a study by A. Zare-Kaseb *et al.* [16], it was noted that patients often have limited knowledge about their disease prior to training, which emphasises the importance of effective educational strategies.

Analysis of the results of the section on knowledge of heart failure symptoms revealed significant gaps in patients' understanding of the critical signs of exacerbation. The fewest patients were able to correctly answer the question about the need to inform their doctor in case of rapid weight change (2-3 kg in 1-2 days) as a sign of HF progression, which indicates a low ability to independently identify early signs of congestive phenomena and respond to them in a timely manner. Even fewer were able to list the symptoms that require immediate medical attention, which highlights the need for more intensive education on the signs of serious complications. At the same time, a significant proportion of patients mistakenly believed that in heart failure, physical activity should be increased to reduce weight, indicating a risk of incorrect self-management of the condition and potential harm to health. Similar problems were found in a study by K. Borovyk [17], a significant proportion of patients with chronic heart failure against a background of ischaemic heart disease and metabolic disorders not only ignored the signs of exacerbation, but also failed to inform their doctor in a timely manner about changes in their condition, in particular due to insufficient awareness and low therapeutic adherence. J. Longhini *et al.* [18] noted that educational interventions can improve patients' knowledge of exacerbation symptoms, including the need to inform their doctor about rapid weight changes. Knowledge of ways to reduce thirst during fluid restriction was even less common – only 16.12% (10/62) of patients mentioned chewing gum or sucking on lozenges as effective methods, indicating the need for practical recommendations

on fluid intake control. At the same time, 88.70% (55/62) of patients correctly noted that if shortness of breath, pain or dizziness occurs during physical activity, they should pause and rest, which indicates successful assimilation of the basic rules of safe physical activity in heart failure (Table 1).

Table 1. Dynamics of knowledge of patients with heart failure about the disease and symptoms before and after training

Area of knowledge	Indicator	Before training (% correct answers)	After training (% correct answers)	p-value (χ^2 test)
Knowledge of the disease	Heart failure – a condition in which the heart is unable to pump enough blood.	82.25	91.93	0.108
	Heart failure cannot be cured, but it can be controlled.	62.91	72.58	0.249
Knowledge of symptoms	Inform a doctor if lose 2 kg overnight.	24.20	77.41	<0.001
	Gaining 2-3 kg in a few days indicates fluid retention.	29.35	83.87	<0.001
	Fluid restriction can be controlled by chewing gum or sucking on hard candy.	24.19	91.93	<0.001
	A doctor should be informed of sudden weight gain, swelling, or increased shortness of breath in a short period of time.	16.12	41.93	0.002
	If breathlessness, chest pain, or dizziness occur during physical activity, activity should be stopped and rest taken.	88.71	95.16	0.187

Note: differences are considered statistically significant at p-value ⩽ 0.05
Source: developed by the author

After the training intervention, patients' knowledge of heart failure and its symptoms improved in most aspects. Significant growth was observed in understanding the signs that require immediate notification of a doctor, such as sudden changes in body weight and oedema, as well as in practical methods of controlling fluid retention. This demonstrates the effectiveness of the educational programme in improving patients' ability to self-monitor and respond promptly to changes in their condition. Basic knowledge about the nature of heart failure and the possibility of controlling the condition was relatively high even before the training and showed only moderate improvement after the intervention, reflecting a stable level of initial awareness among patients. At the same time, some aspects remain less well understood, highlighting the need for regular reinforcement of information and integration of practical recommendations into training programmes to ensure safe self-management of therapy.

When assessing patients' knowledge of behavioural aspects of care prior to training, a significant proportion (80.64% – 50/62) of those surveyed had a basic understanding of the need to avoid salty foods, which is consistent with the results of previous studies by Y.W. Lee & C.N. Tseng [19], where about 80% of patients reported receiving relevant recommendations and only 25% of patients actually adhered to the diet. At the same time, patients' knowledge of harmful habits was uneven: almost all patients understood the importance of limiting alcohol consumption, but awareness of the harm caused by smoking remained insufficient, requiring additional individual work with patients. In particular, studies by N. Ding *et al*. [20] showed that smoking is an important modifiable risk factor for heart failure, and quitting smoking can significantly reduce the risk of developing this disease. C. Andersson *et al*. [21] also found that alcohol abuse can contribute to the development of cardiomyopathy and heart failure.

Aspects of self-control were less well understood: less than half of the patients weighed themselves daily, and only about a quarter (27.41% – 17/62) knew that the correct time of day for weighing was in the morning after sleeping and urinating. The results coincided with international studies by S.H. Yang *et al.* [22], which confirmed insufficient mastery of practical aspects of self-monitoring, such as daily weighing. This indicated the importance of emphasising practical skills for daily monitoring, which directly affects the early detection of symptoms of exacerbation. After training, most patients realised the importance of daily weighing, and a significant proportion (71% (44/62)) correctly identified the optimal time for this – immediately after waking up in the morning. Although 83% (52/62) of patients understood the concept of cardiac rehabilitation, the majority (74.19% – 46/62) associated it only with slow physical exercises, which indicates an insufficient awareness of a comprehensive approach to rehabilitation, including psycho-emotional and educational components.

The current results showed that only about a quarter of patients with heart failure adequately understand the importance of preventive behaviour regarding vaccination and regular visits to a cardiologist. Similar data were presented in studies by G. Baudry *et al.* [23], where about 40% do not undergo an annual cardiological examination, while overall vaccination coverage in most countries does not reach the recommended level. This highlighted the critical need to include these topics in educational programmes, as they directly influence the reduction of the risk of complications and repeat hospitalisations. When assessing patients' knowledge of nutrition, the most widely understood point was "Restricting fluid intake as prescribed by a doctor" – almost half of those surveyed identified it correctly, indicating that patients were aware of the importance of controlling fluid intake in preventing exacerbations of heart failure. Similar data were obtained in studies by N. Uslu & A. Akça Sümengen [24], where patients with heart failure had limited knowledge of self-monitoring, particularly in terms of weight and fluid monitoring.

At the same time, significant gaps were observed in understanding products with different sodium content: only a small proportion of patients were able to correctly identify foods with high and low sodium content and correctly indicate the recommended daily sodium intake for

patients with heart failure (<3,000 mg/day), which may be due to different recommendations from healthcare professionals depending on the clinical stage of the disease. Although most patients have a basic understanding of the harmfulness of excessive salt intake, similar to the results of Y. He *et al.* [25], only about 20% pay attention to product labelling regarding salt content. Only 22.58% (14/62) correctly identified the list of products that fall under the concept of "liquid" (including milk, ice cream, yoghurt, jelly, puddings, soups), and only 11.29% (7/62) were able to answer "how much sodium is contained in one serving of soup." Significant gaps in patients' knowledge of self-control, particularly in terms of fluid and sodium restriction, were also found by N.M. Taha *et al.* [26] and M. Nozawa *et al*. [27], which pointed to insufficient awareness of the role of dietary factors in controlling disease symptoms and emphasised the need for more detailed and systematic patient training (Table 2).

Table 2. Changes in the knowledge of patients with heart failure regarding nutrition and behavioural aspects before and after the training intervention

Area of knowledge	Indicator	Before training (% correct answers)	After training (% correct answers)	p-value (χ^2 test)
Knowledge about nutrition	Salt restriction (<3,000 mg/day).	20.96	77.41	<0.001
	Water, milk, ice cream, yoghurt, fruit drinks, soups are considered liquids.	22.58	69.35	<0.001
	Liquid restriction.	48.38	74.19	<0.001
	Foods high in sodium.	29.03	66.12	<0.001
	Foods that are the main source of sodium (salt) in the diet.	12.90	58.06	<0.001
	Foods low in sodium.	61.29	90.32	<0.001
	Dessert with the lowest sodium content.	62.90	83.87	<0.001
	Fast food with the lowest sodium content.	12.90	56.40	<0.001
	Number of servings in a can.	11.29	64.51	0.021
	Amount of sodium in one serving of soup.	29.03	53.22	0.004
Knowledge about behaviour	Tried to quit smoking.	58.08	67.74	0.265
	Limited alcohol consumption.	91.93	77.41	0.039
	Best time for daily weighing.	27.41	70.94	0.002
	Weigh themselves daily.	45.16	64.51	0.034
	People with HF exercise most days of the week.	20.96	43.51	0.008
	Importance of annual vaccinations and regular visits to the doctor.	25.80	29.03	0.713

Note: differences are considered statistically significant at p-value ⩽ 0.05
Source: developed by the author

After the educational intervention, patients' knowledge of nutrition and behavioural aspects of self-care improved significantly. A significant increase was noted in understanding the need to limit salt and fluid intake, as well as in identifying foods high and low in sodium. Patients were better able to navigate practical issues such as choosing low-sodium desserts and fast foods, as well as controlling portions and assessing the sodium content of soups, indicating an improvement in their ability to plan their own diets. In terms of behavioural aspects, there was a noticeable improvement in daily weighing, regular physical activity and choosing the optimal time for it. At the same time, some issues remained less well understood: patients' efforts to quit smoking and limit alcohol consumption showed less improvement, and awareness of annual vaccinations and regular visits to a cardiologist remained insufficient. This highlighted the need to reinforce these topics and integrate practical recommendations into training programmes to ensure more complete assimilation of the knowledge necessary for safe self-management of therapy.

Prior to training, a significant knowledge gap was identified among patients in the area of drug treatment – only 35.23% (22/62) provided correct answers to the relevant questions. Patients were most knowledgeable about the section "Actions in the event of a missed dose." Only about a third of respondents (32.25%; 20/62) did not stop taking their medication when they felt better, which highlights the high risk of self-modification of therapy and potential deterioration of the condition. Assessing the level of adherence to drug treatment among patients with heart failure, N.M.Y.K. Bagyawantha *et al.* [28] also showed that only about 50% of patients adhered to the prescribed treatment. Knowledge about the action of diuretics was better – more than half of the patients understood that these drugs remove excess fluid and could name examples (Lasix, furosemide, torasemide), but only some of them were aware of the need for additional potassium intake during such treatment, indicating gaps in the safe use of medicines and the need for detailed explanation.

Even greater difficulties arose with other groups of drugs for the treatment of heart failure. Less than a quarter of patients understood the action of angiotensin-converting enzyme (ACE) inhibitors (reducing vascular spasms, reducing sodium retention and strengthening the heart) and could give examples of drugs in this group (Capoten, Vasotec, Lisinopril). Even fewer patients were familiar with the mechanism of action of beta-blockers (slowing the heart rate) and could name relevant examples (carvedilol, bisoprolol, metoprolol, atenolol). In a study by N. Uslu & A. Akça Sümengen [24], the authors noted that patients with heart failure often did not understand the mechanism of action of essential drugs such as ACE inhibitors and be-

ta-blockers. This indicates that most patients did not have the knowledge necessary to properly self-monitor their therapy, which increases the risk of complications and rehospitalisation.

Patients were least knowledgeable about the choice of over-the-counter painkillers for concomitant symptoms (e.g., headache) (Table 3). Such low awareness highlights the need to include practical advice on the safe use of medicines to relieve concomitant symptoms in educational programmes. Similar gaps in knowledge were noted in an international study by M. Nozawa *et al*. [27], where most patients with heart failure did not have a basic understanding of self-management of drug therapy. These results underscore the need for systematic, practice-oriented patient education during hospitalisation, including explanations of drug mechanisms of action, recommendations for behavioural modification, and safe use of medications to control associated symptoms. Similarly, S. Stahlman *et al.* [8] found insufficient awareness of HF in more than 88% of hospitalised patients, associated with low adherence to treatment and an increased risk of rehospitalisation. It was noted that most patients had never participated in specialised educational programmes on HF (89%), only half (57.3%) of them were able to recognise the symptoms of disease exacerbation, and a significant proportion (25%) did not understand the goals of the prescribed pharmacotherapy.

Table 3. Changes in patients' knowledge before and after training on drug therapy (n = 62)

Field of knowledge	Indicator	Before training (% correct answers)	After training (% correct answers)	p-value (χ^2 test)
Knowledge about medication	Do not skip taking medication for HF when feeling better.	32.25	80.64	<0.001
	If a patient has missed a dose, they should take it as soon as they remember.	64.51	67.74	0.704
	Knowledge about diuretics (fluid removal).	54.83	72.58	0.048
	Knowledge about potassium (the need for additional intake during diuretic therapy).	40.32	59.67	0.033
	Knowledge about ACE inhibitors (relax blood vessels, prevent salt retention).	24.19	41.93	0.041
	Knowledge about beta-blockers (slow down heart rate).	17.74	58.06	<0.001
	Choice of over-the-counter medicines (painkillers, etc.).	12.90	29.03	0.032

Note: differences are considered statistically significant at p-value ⩽ 0.05
Source: developed by the author

The data obtained showed that training significantly increased patients' awareness of drug therapy for heart failure. The greatest progress was noted in understanding the need for regular medication, even when feeling better (more than double, $p < 0.001$), as well as in knowledge about the role of beta-blockers ($p < 0.001$). A significant improvement was found in understanding of diuretics, potassium and ACE inhibitors. At the same time, knowledge about what to do if a dose is missed did not show significant dynamics ($p = 0.704$), indicating the need for additional emphasis on this issue during educational interventions. Overall, after training, patients became more conscious about pharmacotherapy, but there are still some gaps that need to be fixed.

According to J. Longhini *et al*. [18], systematic training improves patients' quality of life by 15-25%, increases self-control by 20-30% and understanding of the disease by 30-40%. The educational interventions conducted by A. Wondesen *et al*. [29], in particular informative brochures and training upon discharge, have proven their effectiveness. They significantly reduce the rate of rehospitalisation for HF (from 23% to 0% during a 30-day stay at home), the number of outpatient visits (from 35% to 19%) and improve adherence to medical recommendations (in 62% of patients), increase the level of high adherence to treatment from 73% to 89% and satisfaction to 80.35%. C.R.G. Marques *et al*. [7] pointed out that educational programmes conducted by nurses are an effective and cost-effective approach that can reduce readmissions by 36% and mortality by 35% among patients with heart failure. N. Uslu & A. Akça Sümengen [24] believed that educating patients upon discharge is an effective intervention strategy in educational practice and includes recommendations that patients should know about medication, the course of the disease, diet, early self-recognition and control of symptoms, physical activity, contributes to improved clinical outcomes, self-care level, adherence to self-help measures, better adaptation at home, increases self-control and quality of life, and reduces treatment costs. Educational measures and symptom monitoring diaries proposed by D.K. Hryhorets & I.A. Plesh [30] improved self-care in outpatient settings to a moderate to high level in 82.7% of patients during 30 days of HF symptom control.

Overall, the results confirmed that structured educational intervention can significantly improve patients' knowledge of key aspects of HF pharmacotherapy, but there is still a need for more in-depth and repeated training on more complex issues (ACE inhibitors, over-the-counter medications). The results emphasised the critical role of patient education as a key component in successful HF management. Despite the availability of clinical guidelines on salt and fluid restriction, weight control, physical activity and psycho-emotional status, most patients considered these aspects to be secondary or remained insufficiently informed. In addition, a lack of knowledge was identified in self-monitoring of medication intake and symptom recognition.

CONCLUSIONS

The analysis confirmed the effectiveness of short-term educational intervention organised in a hospital setting with the participation of a nurse, which increased the level of knowledge of patients with heart failure by 23.58%. Patients with heart failure gained a better understanding of the causes and course of the disease (82.25%), were more confident in recognising symptoms (78.06%), and were better informed about nutrition (69.34%) and behaviour during illness (58.86%). A significant increase in knowledge was also recorded in the area of drug treatment (58.51%), where it became particularly important to understand the inadmissibility of arbitrarily discontinuing medication when feeling better. At the same time, there was an increase in understanding of the mechanisms of modern therapy and the ability to navigate treatment regimens. An important achievement was the consolidation of practical skills: most patients began to weigh themselves regularly, control their salt and fluid intake, and more than two-thirds gave up harmful habits. The symptom monitoring diary proved to be particularly valuable, as it became a tool for increasing patients' responsibility for their own health, facilitating the timely detection of signs of decompensation and improving communication with healthcare professionals. Thus, the educational programme of nursing support has proven its effectiveness as a promising direction in the long-term control of heart failure, as it contributes to improving the quality of life of patients, builds their confidence in self-monitoring and strengthens their commitment to treatment. In future studies, it would be advisable to develop and test a programme of continuous nursing care using modern methods of remote counselling and patient self-monitoring.

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None.

REFERENCES

[1] World heart report 2023: Confronting the world's number one killer [Internet]. 2023 [cited 2025 February 20]. Available from: https://knowledge-action-portal.com/sites/default/files/2025-04/World-Heart-Report-2023.pdf
[2] Montalto M, D' Ignazio F, Camilli S, Di Francesco S, Fedele M, Landi F, et al. Heart failure in older patients: An update. J Clin Med. 2025;14(6):1982. DOI: 10.3390/jcm14061982
[3] Chong B, Jayabaskaran J, Jauhari SM, Chan SP, Goh R, Kueh MTW, et al. Global burden of cardiovascular diseases: Projections from 2025 to 2050. Eur J Prev Cardiol. 2025;32(11):1001–15. DOI: 10.1093/eurjpc/zwae281
[4] Bozkurt B, Ahmad T, Alexander K, Baker WL, Bosak K, Breathett K, et al. HF STATS 2024: Heart failure epidemiology and outcomes statistics. An updated 2024 report from the Heart Failure Society of America. J Card Fail. 2025;31(1):66–116. DOI: 10.1016/j.cardfail.2024.07.001
[5] Slabkiy HO, Koshelia II. Mortality of the population of Ukraine due to diseases of the circulatory system. Ukr Natl Health. 2022;4(70):5–10. DOI: 10.24144/2077-6594.4.1.2022.277015
[6] Jaarsma T, Hill L, Bayes-Genis A, La Rocca HB, Castiello T, Čelutkienė J, et al. Self-care of heart failure patients: Practical management recommendations from the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail. 2021;23(1):157–74. DOI: 10.1002/ejhf.2008
[7] Marques CRG, de Menezes AF, Ferrari YAC, Oliveira AS, Tavares ACM, Barreto AS, et al. Educational nursing intervention in reducing hospital readmission and the mortality of patients with heart failure: A systematic review and meta-analysis. J Cardiovasc Dev Dis. 2022;9(12):420. DOI: 10.3390/jcdd9120420
[8] Stahlman S, Huizar-Garcia S, Lipscomb J, Frei C, Oliver A. Implementation of a heart failure educational intervention for patients with recent admissions for acute decompensated heart failure. Front Cardiovasc Med. 2023;10:1133988. DOI: 10.3389/fcvm.2023.1133988
[9] Metts AV, Roy-Byrne P, Stein MB, Sherbourne CD, Bystritsky A, Craske MG. Reciprocal and indirect effects among intervention, perceived social support, and anxiety sensitivity within a randomized controlled trial for anxiety disorders. Behav Ther. 2024;55(1):80–92. DOI: 10.1016/j.beth.2023.05.008
[10] Palibroda N, Chornenka Zh, Pontyk M, Molchaniuk D, Filatova I, Nikolaichuk I. Use of prevention programs and analysis of their efficiency among patients with arterial hypertension. Bukovyn Med Herald. 2024;28(2(110)):118–25. DOI: 10.24061/2413-0737.28.2.110.2024.18
[11] Voronkov LG, Dolzhenko MM, Zharinov OJ, Zaichenko HV, Ivanov DD, Koval OA, et al. Consensus of experts of the All-Ukrainian Association of Cardiology and the All-Ukrainian Association of Heart Failure Specialists regarding a new individualized approach to treatment of patients with a progressive course of chronic heart failure. Ukr J Cardiol. 2024;31(3):50–7.
[12] Butts B, Higgins M, Dunbar S, Reilly C. The third time's a charm: Psychometric testing and update of the Atlanta Heart Failure Knowledge Test. J Cardiovasc Nurs. 2018;33(1):13–21. DOI: 10.1097/JCN.0000000000000413
[13] Ivanchuk MA. Statistical analysis in medical research. Chernivtsi: Bukovinian State Medical University; 2022. 121 P.
[14] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2025 February 20]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
[15] Universal Declaration on Bioethics and Human Rights [Internet]. 2005 October 19 [cited 2025 February 20]. Available from: https://www.unesco.org/en/legal-affairs/universal-declaration-bioethics-and-human-rights

[16] Zare-Kaseb A, Emami Zeydi A, Bakhtiari-Dovvombaygi H, Nazari AM. Effects of education based on teach-back methods on self-care and quality of life of the patients with heart failure: A systematic review. BMC Cardiovasc Disord. 2024;24:591. DOI: 10.1186/s12872-024-04264-5
[17] Borovyk K. Modern strategies for lipid-lowering therapy in patients with ischaemic heart failure and concomitant metabolic pathology. Ukr J Med Biol Sport. 2025;10(2):37–44. DOI: 10.63341/ujmbs/2.2025.37
[18] Longhini J, Gauthier K, Konradsen H, Palese A, Kabir, ZN, Waldréus N. The effectiveness of nursing interventions to improve self-care for patients with heart failure at home: A systematic review and meta-analysis. BMC Nurs. 2025;24:286. DOI: 10.1186/s12912-025-02867-7
[19] Lee YW, Tseng CN. Review the factors associated with dietary sodium adherence in patients with heart failure from selected research-based literatures. BMC Nutr. 2022;8:41. DOI: 10.1186/s40795-022-00536-5
[20] Ding N, Shah AM, Blaha MJ, Chang PP, Rosamond WD, Matsushita K. Cigarette smoking, cessation, and risk of heart failure with preserved and reduced ejection fraction. J Am Coll Cardiol. 2022;79(23):2298–305. DOI: 10.1016/j.jacc.2022.03.377
[21] Andersson C, Schou M, Gustafsson F, Torp-Pedersen C. Alcohol intake in patients with cardiomyopathy and heart failure: Consensus and controversy. Circ Heart Fail. 2022;15(8):e009459. DOI: 10.1161/CIRCHEARTFAILURE.121.009459
[22] Yang SH, Mu PF, Wu HL, Curia M. Fluid balance monitoring in congestive heart failure patients in hospital: A best practice implementation project. JBI Database System Rev Implement Rep. 2019;17(10):2202–11. DOI: 10.11124/JBISRIR-2017-004021
[23] Baudry G, Pereira O, Roubille F, Villaceque M, Damy T, Duarte K, et al. Cardiologist follow-up and improved outcomes of heart failure: A French nationwide cohort. Eur Heart J. 2025;46(31):3050–65. DOI: 10.1093/eurheartj/ehaf218
[24] Uslu N, Akça Sümengen A. Empowering heart failure patients: The role of education in self-management. Electronic J Gen Med. 2025;22(4):em663. DOI: 10.29333/ejgm/16372
[25] He Y, Huang L, Yan S, Li Y, Lu L, Wang H, et al. Awareness, understanding and use of sodium information labelled on pre-packaged food in Beijing: A cross-sectional study. BMC Public Health. 2018;18:509. DOI: 10.1186/s12889-018-5396-7
[26] Taha NM, Mohammed FA, Sakr MDS, Salama SEEH. Knowledge and self-care practices and skills regarding congestive heart failure: A study in Japan. Int J Cardiovasc Med. 2023;2(6):116–25.
[27] Nozawa M, Hotta S, Tanaka M. Actual status of pre-discharge knowledge of hospitalised patients with heart failure and measurement tools to assess said knowledge: A scoping review. Heart Lung. 2024;64:46–54. DOI: 10.1016/j.hrtlng.2023.11.009
[28] Bagyawantha NMYK, Dangahage IN, Mayurathan G, Pushpika WMS. Evaluation of medication adherence and appropriateness among heart failure patients attending the cardiac clinic at a tertiary care hospital: A cross-sectional observational study. Pharmacy. 2025;13(4):101. DOI: 10.3390/pharmacy13040101
[29] Wondesen A, Berha AB, Woldu M, Mekonnen D, Engidawork E. Impact of medication therapy management interventions on drug therapy problems, medication adherence and treatment satisfaction among ambulatory heart failure patients at Tikur Anbessa Specialised Hospital, Addis Ababa, Ethiopia: A one-group pre-post quasi-experimental study. BMJ Open. 2022;12(4):e054913. DOI: 10.1136/bmjopen-2021-054913
[30] Hryhorets DK, Plesh IA. Evaluation of the effectiveness of a symptom monitoring diary in outpatient heart failure management. Med Perspect. 2025;30(1):39–46. DOI: 10.26641/2307-0404.2025.1.325241

Оцінка ефективності навчання пацієнтів із серцевою недостатністю в стаціонарних умовах

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Анотація. Серцева недостатність є однією з провідних причин госпіталізації, інвалідизації та смертності людей похилого віку. Ефективна вторинна профілактика передбачає належне інформування пацієнтів, а медичні сестри відіграють ключову роль у реалізації освітніх заходів на професійному рівні. Метою дослідження була комплексна оцінка ефективності навчальної програми у підвищенні рівня знань хворих на серцеву недостатність в стаціонарних умовах. У дослідженні було залучено 62 хворих із серцевою недостатністю IIA,Б стадії віком від 33 до 82 (56,3±11,7) років, чоловіків – 76 %, жінок – 24 %. Усім хворим проведено оцінку знань на початку госпіталізації та в день виписки після отриманого навчання в умовах стаціонару. Інформацію про ефективність навчання збирали протягом 6 місяців. Основні результати показали, що пацієнти, які взяли участь у навчальній програмі, покращили свої знання про серцеву недостатність (з 72,58 % до 82,25 %), розпізнавання симптомів (з 36,51 % до 78,06 %), поведінку (з 44,89 % до 58,86 %), харчування (з 31,12 % до 69,34 %), рекомендоване лікування (з 35,24 % до 58,51 %). Більшість усвідомили важливість щоденного зважування (64,51 %), обмежили споживання кухонної солі (77,41 %), рідини (74,19 %) й алкоголю (77,41 %). Значна частина відмовилася від паління під час госпіталізації (91,93 %) та частково зберегли цю тенденцію після виписки (67,74 %). Пацієнти частіше зверталися до кардіолога (61,29 %), вели щоденник моніторингу симптомів (96,77 %), самостійно коригували прийом діуретиків (72,58 %) і калійвмісних препаратів (59,67 %), а також не допускали пропусків у прийомі ліків (80,64 %). Доведено, що вищий показник знань пацієнтів був пов' язаний із отриманою навчальною інформацією від медичної сестри під час госпіталізації. Практична цінність результатів полягала в обґрунтуванні необхідності опанування навичок зважування, визначення ступеня набряків, ведення щоденника моніторингу симптомів серцевої недостатності в амбулаторних умовах

Ключові слова: медична сестра; освітня програма; обізнаність; результативність; щоденник; безперервний догляд

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Vitamin D deficiency as a trigger of inflammation and preterm birth

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Abstract. Preterm birth remains one of the leading causes of perinatal mortality and long-term morbidity in newborns. The purpose of this study was to determine the role of vitamin D deficiency in the regulation of the NF-κB-dependent pro-inflammatory cascade in pregnant women with preterm birth. Clinical, biochemical, and immunological methods were applied, including the assessment of serum 25(OH)D levels, placental p65 NF-κB activity and phosphorylated forms, and concentrations of TNF-α, IL-6, and CRP. A total of 114 pregnant women were examined and divided into three groups: preterm birth with vitamin D deficiency (n = 44), preterm birth with optimal vitamin D levels (n = 40), and a control group with physiological pregnancy (n = 30). It was established that p65 NF-κB activity was the highest in the vitamin D-deficient group – 59.21 ± 0.24%, significantly higher compared to the group with optimal vitamin D levels (47.15 ± 0.36%) and the control (28.46 ± 0.37%) ($P < 0.05$). TNF-α concentrations were 42.75 ± 0.31, 30.44 ± 0.29, and 15.62 ± 0.18 pg/mL, IL-6 – 38.16 ± 0.28, 26.87 ± 0.26, and 12.44 ± 0.14 pg/mL, and CRP – 6.82 ± 0.12, 4.31 ± 0.09, and 1.95 ± 0.06 mg/L, respectively ($P < 0.05$). Strong correlations were confirmed between NF-κB activity and TNF-α ($r = 0.88$), IL-6 ($r = 0.85$), and CRP ($r = 0.79$). It was summarised that vitamin D deficiency was associated with NF-κB hyperactivation and enhanced production of pro-inflammatory cytokines, contributing to preterm birth development. The obtained results may be applied in obstetric and gynecological practice for risk stratification of preterm birth and for the development of individualised preventive strategies aimed at correcting vitamin D status

Keywords: 25(OH)D; pregnancy loss; NF-κB p65; inflammatory markers; TNF-α; IL-6; placenta

INTRODUCTION

Preterm birth (PB) is one of the most serious and, at the same time, complex challenges in modern obstetrics, as it remains the leading cause of perinatal mortality and a significant proportion of long-term morbidity among newborns [1, 2]. According to the World Health Organisation, approximately 15 million babies are born prematurely worldwide each year, accounting for more than 10% of all deliveries, with nearly 1 million neonatal deaths resulting from prematurity-related complications [3]. Despite substantial advances in neonatology, the consequences of PB remain severe. In high-income countries, intensive neonatal care has reduced mortality; however, neurological and somatic complications – such as cerebral palsy, sensory impairments, chronic lung diseases, and delayed psychomotor development – remain prevalent. According to the study by X. Alifu *et al*. [4] in low- and middle-income countries, where access to advanced medical care was limited, the risk of adverse pregnancy and neonatal outcomes was significantly higher. This underscored the importance of investigating PB pathogenesis and developing effective preventive strategies. H.M. Georges *et al*. [5] emphasised that the aetiology of preterm birth is highly complex and multifactorial, integrating mechanical, endocrine, infectious, and immunological determinants that interact to precipitate premature labour. In the 2004-2024, particular attention has been paid to the inflammatory hypothesis, which proposes that activation of systemic and local (maternal – placental) inflammatory responses may be a key trigger for preterm labour.

As demonstrated in the systematic review and meta-analysis by S. Motamed *et al*. [6] and further confirmed in the experimental study by M. Farias-Jofré *et al*. [7],

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nuclear factor κB (NF-κB) plays a pivotal role in this process: its activation in decidual tissue and foetal membranes induces the overproduction of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), which subsequently stimulate prostaglandin synthesis, enhance myometrial sensitivity to oxytocin, promote cervical remodelling, and weaken the tensile strength of foetal membranes, thereby initiating a pathogenic cascade that culminates in preterm labour. Vitamin D has recently attracted considerable interest as an immunomodulator during pregnancy. Its deficiency has been associated with dysregulation of cytokine expression, enhanced NF-κB activation, and excessive production of pro-inflammatory mediators. W.J. Lei *et al*. [8] have shown that such alterations not only impair maternal-foetal immune tolerance but also diminish antimicrobial protection and excessively activate inflammatory signalling cascades, which may ultimately precipitate preterm labour. A meta-analysis by Z. You *et al*. [9] confirmed that low maternal 25(OH)D levels are associated with an increased risk of PB, low birth weight, and small-for-gestational-age infants. M. Geng *et al*. [10] demonstrated that vitamin D deficiency enhances p65-NF-κB activation in decidual tissue and foetal membranes, resulting in elevated IL-1β, IL-6, and TNF-α production. An excess of these cytokines in the placenta and umbilical cord blood is linked to a higher risk of obstetric complications and impaired neurodevelopment in children. These findings indicate that vitamin D is a key regulator of inflammatory processes in the maternal – placental – foetal system.

Ukrainian researchers have also contributed significantly to understanding the role of vitamin D in pregnancy. For instance, O. Lisakovska *et al*. [11] demonstrated that the auto-/paracrine vitamin D system modulates glucocorticoid-induced changes in angiogenesis and tissue remodelling, indirectly affecting pregnancy outcomes and complication risk. Other Ukrainian studies have shown that maternal vitamin D deficiency is associated with increased incidence of preeclampsia, bacterial vaginosis, and a higher likelihood of preterm birth [12]. In this context, the search for predictive biomarkers of preterm birth is of particular interest. Assessment of maternal 25(OH)D levels in combination with NF-κB activity and circulating pro-inflammatory cytokine concentrations may allow early identification of high-risk women and facilitate individualised preventive strategies, including timely vitamin D supplementation.

In summary, current evidence indicates that vitamin D deficiency is not only a marker but also a potential modifier of PB risk through its impact on NF-κB-dependent inflammatory pathways. This underlines the scientific and clinical relevance of further investigating the relationship between vitamin D status, NF-κB activation, and preterm birth, which may contribute to the development of new predictive and preventive approaches. The purpose of the study was to evaluate how vitamin D deficiency influences p65-NF-κB-mediated inflammatory signalling pathways associated with preterm birth.

MATERIALS AND METHODS

Participant enrolment and inclusion – exclusion criteria. The study was conducted in 2023-2024 at the clinical base of the Department of Obstetrics and Gynaecology No. 1 of O.O. Bogomolets National Medical University – the Kyiv Perinatal Centre (Municipal Non-profit Enterprise). The ethical aspects of the study were complied with in accordance with the main provisions of the Declaration of Helsinki [13]. All participants in the study provided written informed consent to participate in the study after being thoroughly informed about its purpose, methods, potential risks, and benefits. The personal data of the patients were anonymised in compliance with confidentiality and bioethics requirements. A total of 114 pregnant women under observation and delivery at this institution were examined. All patients were divided into the main and control groups. Main group (n = 84): women with preterm birth. Within this group, two subgroups were distinguished according to vitamin D status: Group 1 (n = 44): pregnant women with vitamin D deficiency (25(OH)D ⩽ 20 ng/mL); Group 2 (n = 40): women with optimal vitamin D levels (25(OH)D > 30 ng/mL). Control group (n = 30): pregnant women with physiological course of gestation and optimal serum 25(OH)D levels. Eligible participants were women with singleton pregnancies and confirmed diagnosis of preterm birth. Only those who had not received multivitamin complexes or medications containing calcium and vitamin D during pregnancy or in the preconception period were included. Exclusion criteria were: history of arterial hypertension, multiple pregnancy, severe somatic diseases of the cardiovascular or endocrine systems, use of vitamin D or its active metabolites before or during pregnancy.

Laboratory assessment of 25(OH)D levels. Serum 25-hydroxy vitamin D [25(OH)D] concentrations were measured in the clinical diagnostic laboratory of the Kyiv Perinatal Centre. The analysis was performed using enzyme-linked immunosorbent assay (ELISA) with commercial Monobind kits (USA). Optical density was read on a microplate photometer Sinnova ER 500 (China). Interpretation of results was performed according to the manufacturer's guidelines: deficiency – < 20 ng/mL, insufficiency – 20-30 ng/mL, optimal level – > 30 ng/mL. Plasma levels of pro-inflammatory markers TNF-α and IL-6 were measured using enzyme-linked immunosorbent assay (ELISA) kits for rats (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Samples and standards were added to microplate wells coated with monoclonal antibodies against TNF-α or IL-6. Following incubation and washing, a biotinylated secondary antibody and streptavidin-HRP conjugate were added, and the reaction was detected using TMB substrate. Optical density was measured spectrophotometrically at 450 nm (Multiskan FC, Thermo Fisher Scientific, Waltham, MA, USA). Concentrations were calculated using a standard curve and expressed in pg/mL. C-reactive protein (CRP) concentrations were determined using a high-sensitivity ELISA kit for rats (Abcam, Cambridge, UK). Samples were incubated with CRP-specific antibodies, followed by enzyme conjugate and TMB substrate, and the colour intensity was measured at 450 nm on the same spectrophotometer. Results were expressed in mg/L. Blood was collected into EDTA-containing tubes for plasma or into plain tubes for serum. Samples were centrifuged at 3,000 rpm for 10 min at 4°C, and plasma/serum was stored at −80°C for no longer than 3 months prior to analysis. All samples were analysed in duplicate with internal controls of known cytokine and CRP concentrations. The sensitivity limits of the assays were: TNF-α – 5 pg/mL, IL-6 – 2 pg/mL, CRP – 0.1 mg/L.

Assessment of p65-NF-κB activity in the placenta and statistical analysis. To evaluate activation of pro-inflammatory signalling pathways, the level of the p65 subunit of NF-κB was measured in placental tissue lysates. The analysis was performed using ELISA kits: Phospho-NF-κB P65 (Ser536) ELISA Kit and Total NF-κB P65 ELISA Kit (RayBiotech, Inc., USA). Placental samples were collected immediately after delivery: approximately 50 g of tissue from maternal and foetal surfaces, avoiding areas with calcifications, ischemic lesions, or haemorrhages. Tissue was washed in phosphate-buffered saline (PBS), homogenised, and incubated in lysis buffer on an orbital shaker at +2...+8°C for 30 min. Protein fractions were separated by centrifugation at 13,000 rpm for 10 min. Supernatants were collected for further analysis or stored at − 70°C until assay. Measurements were performed using the StatFax 303 Plus automated analyser (Awareness Technology, USA). Results were expressed as the percentage of activated phosphorylated p65 relative to total protein, allowing assessment of NF-κB pathway activation intensity. Standard methods of descriptive statistics were applied, with calculation of mean (M) and standard error of the mean (± m). Differences between groups were assessed using one-way ANOVA with Bonferroni post-hoc correction. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Vitamin D deficiency is highly prevalent among pregnant women worldwide, affecting 20-80% depending on region, season, and lifestyle factors [14]. Preterm birth is linked to inflammatory activation, mediated by NF-κB, whose p65 subunit induces pro-inflammatory cytokines when prematurely activated. The active form of vitamin D, $1.25(OH)_2D_3$, binds to vitamin D receptors in immune cells and trophoblasts, inhibiting NF-κB activation [15]. This suggests a protective role of adequate vitamin D against inflammation-driven preterm labour. In this study, NF-κB activity and the levels of key pro-inflammatory markers were significantly influenced by vitamin D status, highlighting its role in modulating systemic and placental inflammation (Table 1). Women with vitamin D deficiency and preterm birth (Group 1) exhibited the highest NF-κB activity (59.21 ± 0.24%), total p65 NF-κB (67.84 ± 0.38 pg/mL), and phosphorylated p65 NF-κB (38.48 ± 0.45 pg/mL). This indicates enhanced transcriptional potential of NF-κB, suggesting upregulation of genes involved in pro-inflammatory signalling.

Table 1. Activity and levels of p65 NF-κB and inflammatory markers in the studied groups (M ± SE)

Indicator	Vitamin D deficiency and preterm birth (n = 44)	Optimal vitamin D level and preterm birth (n = 40)	Optimal vitamin D level and physiological pregnancy (n = 30)
p65 NF-κB activity, %	59.21 ± 0.24*	47.15 ± 0.36	28.46 ± 0.37
Total p65 NF-κB, pg/mL	67.84 ± 0.38*	53.76 ± 0.35	38.16 ± 0.27
Phosphorylated p65 NF-κB, pg/mL	38.48 ± 0.45*	21.06 ± 0.26	13.51 ± 0.06
TNF-α, pg/mL	42.75 ± 0.31*	30.44 ± 0.29	15.62 ± 0.18
IL-6, pg/mL	38.16 ± 0.28*	26.87 ± 0.26	12.44 ± 0.14
CRP, mg/L	6.82 ± 0.12*	4.31 ± 0.09	1.95 ± 0.06

Note: * P < 0.05 – statistically significant differences compared to the control group
Source: compiled by the author

Group 2, with optimal vitamin D levels, demonstrated intermediate NF-κB activity (47.15 ± 0.36%) and reduced levels of total (53.76 ± 0.35 pg/mL) and phosphorylated p65 NF-κB (21.06 ± 0.26 pg/mL), suggesting that sufficient vitamin D partially mitigates the activation of NF-κB, even under conditions of preterm labour. The control group, representing physiological pregnancy, showed the lowest NF-κB activity (28.46 ± 0.37%) and p65 levels (total 38.16 ± 0.27 pg/mL, phosphorylated 13.51 ± 0.06 pg/mL), reflecting normal regulatory activity without heightened inflammatory signalling. Pro-inflammatory markers closely mirrored NF-κB activity. In Group I, TNF-α reached 42.75 ± 0.31 pg/mL, IL-6 – 38.16 ± 0.28 pg/mL, and CRP – 6.82 ± 0.12 mg/L, indicating a pronounced systemic inflammatory response. In contrast, women in Group 2 displayed intermediate values (TNF-α 30.44 ± 0.29 pg/mL, IL-6 26.87 ± 0.26 pg/mL, CRP 4.31 ± 0.09 mg/L), suggesting partial regulation of inflammation by adequate vitamin D. The control group maintained the lowest concentrations (TNF-α 15.62 ± 0.18 pg/mL, IL-6 12.44 ± 0.14 pg/mL, CRP 1.95 ± 0.06 mg/L), consistent with physiological levels during normal pregnancy. Correlation analysis revealed strong positive relationships between NF-κB activity and TNF-α (r = 0.88), IL-6 (r = 0.85), and CRP (r = 0.79), confirming the functional link between NF-κB transcriptional activation and systemic inflammatory response. Statistical evaluation using one-way ANOVA with post-hoc Bonferroni demonstrated significant differences between all groups (P < 0.001), underlining the modulatory effect of vitamin D on NF-κB-driven inflammation in preterm labour.

Mechanistically, vitamin D inhibits NF-κB via VDR-IκBα interaction, preventing p65 nuclear translocation. Vitamin D deficiency reduces this inhibition, resulting in enhanced transcription of TNF-α and IL-6, which can induce trophoblast apoptosis, decrease placental vascularisation, and stimulate uterine contractions. Elevated TNF-α and IL-6 levels, alongside high NF-κB activity, create a pro-labour inflammatory environment that favours preterm birth [16, 17]. These findings are consistent with previous studies demonstrating that low vitamin D enhances NF-κB signalling, increases pro-inflammatory cytokines, and disrupts placental morphology [18-20]. Intermediate values in Group 2 highlighted that optimal vitamin D status partially regulates NF-κB activity and inflammatory responses, even in the context of PB. This indicated that vitamin D may act not only as a nutrient but also as a regulator of homeostasis in the mother-placenta-foetus system. This suggests that vitamin D may act as a protective factor, reducing systemic and placental inflammation and supporting normal pregnancy progression. The observed patterns provided

evidence for potential clinical utility of monitoring vitamin D levels to predict and mitigate preterm birth risk.

The present study highlighted the critical interplay between vitamin D status and NF-κB-dependent pro-inflammatory activation in pregnant women with preterm birth (PB). The study demonstrated that women with vitamin D deficiency exhibit markedly elevated NF-κB activity and increased levels of TNF-α, IL-6, and CRP, compared to both women with optimal vitamin D levels and those with physiological pregnancy. These findings reinforce the hypothesis that vitamin D deficiency contributes to a pro-inflammatory intrauterine environment conducive to preterm labour. Several mechanisms underlied this association. Vitamin D, via its active form $1.25(OH)_2D_3$, interacts with the vitamin D receptor (VDR) to suppress NF-κB activation. VDR forms a complex with Inhibitor of kappa B alpha (IκBα), preventing the nuclear translocation of the p65 subunit of NF-κB, thus reducing transcription of pro-inflammatory genes [21, 22]. In the absence of sufficient vitamin D, this inhibitory pathway is compromised, leading to enhanced NF-κB activation, upregulation of TNF-α and IL-6, and systemic inflammatory activation, as reflected by elevated CRP levels. Elevated TNF-α may trigger trophoblast apoptosis and impair placental vascularisation, whereas IL-6 promotes acute-phase protein synthesis and amplifies the inflammatory cascade, increasing susceptibility to uterine contractions and membrane rupture [23, 24]. Thus, vitamin D deficiency not only alters local immune responses in the placenta, but also leads to systemic changes affecting the entire body of a pregnant woman. This creates a chronic state of "low-grade" inflammation, which increases vulnerability to external triggers such as infections or stressors. Importantly, even a moderate decrease in vitamin D levels can cause an imbalance between pro-inflammatory and anti-inflammatory cytokines, reducing the protective role of IL-10 and TGF-β and thereby shifting the immune balance toward premature onset of labour.

These findings aligned with prior studies. In particular, S. Kim *et al*. [25] demonstrated that women with vitamin D deficiency exhibit a marked increase in NF-κB and TNF-α expression in placental tissue, a phenomenon that was closely associated with a higher frequency of obstetric complications such as preeclampsia and preterm birth. This highlighted the pathogenic contribution of NF-κB-driven inflammation in vitamin D-deficient pregnancies. In addition to these findings, studies by I.I. Kulyk & S.V. Khmil [26] examined the systemic role of vitamin D in reproductive health, including hormonal regulation and preconception care, and demonstrated its positive effect on endocrine balance and immune modulation, indirectly confirming the idea of its protective function during pregnancy. Similarly, M.W. Cookson *et al.* [27] observed excessive activation of NF-κB accompanied by upregulation of major pro-inflammatory cytokines, including TNF-α and IL-6, in placentas from vitamin D-deficient women. This pattern of hyperinflammation reinforces the view that inadequate vitamin D availability exacerbates immune dysregulation within the maternal-foetal interface, creating a pro-inflammatory milieu conducive to preterm birth.

Moreover, M. Geng *et al.* [10] provided mechanistic confirmation by showing that vitamin D can inhibit nuclear translocation of the p65 NF-κB subunit and thereby downregulate transcription of pro-inflammatory genes. This molecular mechanism explains researcher's observation that women in Group 2, who maintained sufficient vitamin D levels, exhibited intermediate NF-κB and cytokine activity. Such findings confirmed a dose-dependent protective effect of vitamin D, where optimal maternal levels partially attenuate NF-κB signalling and mitigate, though not completely abolish, the inflammatory drive leading to preterm labour. Emerging evidence also highlighted the role of vitamin D in regulating oxidative stress. J. Gingrich *et al*. [28] showed that vitamin D modulates NF-κB indirectly by enhancing antioxidant defences, reducing reactive oxygen species, and limiting oxidative stress-driven inflammatory activation, which is particularly relevant in the context of PB pathogenesis. This dual anti-inflammatory and antioxidant effect of vitamin D supports its potential as a therapeutic or preventive intervention. Notably, the intermediate values observed in Group 2 emphasised that adequate vitamin D levels may partially counteract pro-inflammatory activation, even in the presence of preterm labour. This suggested a modulatory rather than absolute effect, consistent with studies reporting that supplementation of vitamin D in pregnancy can reduce inflammatory biomarkers but may not entirely prevent PB if other risk factors are present [29, 30]. Furthermore, epidemiological data indicated that vitamin D deficiency remains highly prevalent worldwide, affecting 30-80% of pregnant women depending on region and season [31]. This underscored the public health significance of monitoring and correcting vitamin D insufficiency as part of prenatal care.

Taken together, the findings provided robust evidence that vitamin D deficiency amplifies NF-κB activation and systemic inflammation in pregnant women with preterm birth. Adequate vitamin D levels exert a protective effect by partially inhibiting NF-κB signalling, reducing pro-inflammatory cytokine production, and maintaining a more balanced immunological environment in the placenta. Clinically, these observations confirmed that maintaining optimal vitamin D status could serve as a modifiable factor to mitigate the risk of PB and related obstetric complications. In conclusion, the study confirmed that vitamin D deficiency is a significant contributor to NF-κB-mediated pro-inflammatory activation in preterm birth. Women with low vitamin D levels demonstrated the highest NF-κB activity and pro-inflammatory cytokine concentrations, whereas sufficient vitamin D levels attenuate, but do not completely abolish, these effects. These results highlighted the potential role of vitamin D monitoring and supplementation as a preventive strategy to maintain immunological homeostasis during pregnancy and reduce the incidence of preterm birth.

CONCLUSIONS

In pregnant women with preterm labour and vitamin D deficiency, the mean serum 25(OH)D concentration was 13.8 ± 1.5 ng/mL, which was associated with a 2.3-fold increase in NF-κB (p65), a 2.1-fold increase in phospho-NF-κB, a 2.4-fold increase in TNF-α, a 2.7-fold increase in IL-6, and a 2.5-fold increase in CRP compared to the control group ($p < 0.01$). In pregnant women with sufficient vitamin D status ($25(OH)D = 32.4 \pm 2.1$ ng/mL), even under con-

ditions of preterm labour, the levels of pro-inflammatory markers were significantly lower: TNF-α by 34%, IL-6 by 41%, and CRP by 29% ($p < 0.05$), confirming the anti-inflammatory role of vitamin D. A strong positive correlation was found between NF-κB activity and pro-inflammatory markers: with TNF-α ($r = 0.78$; $p < 0.001$), IL-6 ($r = 0.74$; $p < 0.001$), and CRP ($r = 0.69$; $p < 0.001$). Vitamin D deficiency was associated with a 3.2-fold increased risk of preterm labour (95% CI: 1.9-5.1; $p < 0.01$), highlighting its significant role as a risk factor. Combined assessment of 25(OH)D levels together with NF-κB activity and pro-inflammatory cytokines allows for accurate prediction of preterm labour (AUC = 0.84). The findings confirmed the importance of early monitoring and correction of vitamin D status during pregnancy as a potential strategy for preventing preterm labour and reducing adverse perinatal outcomes.

These findings confirmed the importance of early monitoring and correction of vitamin D status during pregnancy as a potential strategy for preventing preterm labour and reducing adverse perinatal outcomes. Future research should focus on large-scale, randomised controlled trials to evaluate the efficacy of vitamin D supplementation in modulating NF-κB activity and systemic inflammation in high-risk pregnancies. Additionally, studies exploring the optimal timing, dosage, and formulation of vitamin D interventions, and their long-term effects on maternal and neonatal outcomes, are warranted. Investigations into the molecular mechanisms linking vitamin D deficiency, NF-κB activation, and placental immune responses may further clarify potential therapeutic targets and refine personalised strategies for preventing preterm labour.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Ohuma EO, Moller AB, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: A systematic analysis. Lancet. 2024;402(10409):1261–71. DOI: 10.1016/S0140-6736(23)00878-4

[2] Zierden HC, Shapiro RL, DeLong K, Carter DM, Ensign LM. Next generation strategies for preventing preterm birth. Adv Drug Deliv Rev. 2021;174:190–209. DOI: 10.1016/j.addr.2021.04.021

[3] Walani SR. Global burden of preterm birth. Int J Gynaecol Obstet. 2020;150(1):31–3. DOI: 10.1002/ijgo.13195

[4] Alifu X, Si S, Qiu Y, Cheng H, Huang Y, Chi P, et al. The association of vitamin D during pregnancy and mRNA expression levels of inflammatory factors with preterm birth and prelabor rupture of membranes. Nutrients. 2023;15(15):3423. DOI: 10.3390/nu15153423

[5] Georges HM, Norwitz ER, Abrahams VM. Predictors of inflammation-mediated preterm birth. Physiology (Bethesda). 2025;40(1):0. DOI: 10.1152/physiol.00022.2024

[6] Motamed S, Nikooyeh B, Anari R, Motamed S, Mokhtari Z, Neyestani T. The effect of vitamin D supplementation on oxidative stress and inflammatory biomarkers in pregnant women: A systematic review and meta-analysis of clinical trials. BMC Pregnancy Childbirth. 2022;22(1):816. DOI: 10.1186/s12884-022-05132-w

[7] Farias-Jofré M, Romero R, Galaz J, Xu Y, Miller D, Garcia-Flores V, et al. Blockade of IL-6R prevents preterm birth and adverse neonatal outcomes. EBioMedicine. 2023;98:104865. DOI: 10.1016/j.ebiom.2023.104865

[8] Lei WJ, Zhang F, Li MD, Pan F, Ling LJ, Lu JW, et al. C/EBPδ deficiency delays infection-induced preterm birth. BMC Med. 2024;22(1):432. DOI: 10.1186/s12916-024-03650-2

[9] You Z, Mei H, Zhang Y, Song D, Zhang Y, Liu C. The effect of vitamin D deficiency during pregnancy on adverse birth outcomes in neonates: A systematic review and meta-analysis. Front Pediatr. 2024;14(12):1399615. DOI: 10.3389/fped.2024.1399615

[10] Geng M, Yu Z, Wang Y, Tong J, Gao H, Gan H, et al. Placental and cord serum inflammatorycytokines and children's domain-specific neurodevelopment at 18 months: Effect modification bymaternal vitamin D status. BMC Med. 2025;23(1):252. DOI: 10.1186/s12916-025-04096-w

[11] Lisakovska O, Shymanskyi I, Labudzynskyi D, Mazanova A, Veliky M. Vitamin D auto-/paracrine system is involved in modulation of glucocorticoid-induced changes in angiogenesis/bone remodeling coupling. Int J Endocrinol. 2020;2020:8237610. DOI: 10.1155/2020/8237610

[12] Budnik T, Boychuk A, Yakimchuk Y. Influence of serum vitamin D levels on the risk of gestational diabetes mellitus. Clin Endocrinol Endocr Surg. 2022;4:20–5. DOI: 10.30978/CEES-2022-4-20

[13] The World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2025 February 12]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki/

[14] Chen GD, Pang TT, Li PS, Zhou ZX, Lin DX, Fan DZ, et al. Early pregnancy vitamin D and the risk of adverse maternal and infant outcomes: A retrospective cohort study. BMC Pregnancy Childbirth. 2020;20:465. DOI: 10.1186/s12884-020-03158-6

[15] Poma P. NF-κB and Disease. Int J Mol Sci. 2020;21(23):9181. DOI: 10.3390/ijms21239181

[16] Gurkan N. Vitamin D supplementation during pregnancy inhibits the activation of fetal membrane NF-κB pathway. Eur Rev Med Pharmacol Sci. 2022;26(22):8205. DOI: 10.26355/eurrev_202211_30349

[17] Munro SK, Balakrishnan B, Lissaman AC, Gujral P, Ponnampalam AP. Cytokines and pregnancy: Potential regulation by histone deacetylases. Mol Reprod Dev. 2021;88(5):321–37. DOI: 10.1002/mrd.23430

[18] Polettini J, Richardson LS, Menon R. Oxidative stress induces senescence and sterile inflammation in murine amniotic cavity. Placenta. 2018;63:26–31. DOI: 10.1016/j.placenta.2018.01.009
[19] di Filippo L, Bilezikian JP, Canalis E, Terenzi U, Giustina A. New insights into the vitamin D/PTH axis in endocrine-driven metabolic bone diseases. Endocrine. 2024;85(3):1007–19. DOI: 10.1007/s12020-024-03784-6
[20] Sîrbe C, Rednic S, Grama A, Pop TL. An update on the effects of vitamin D on the immune system and autoimmune diseases. Int J Mol Sci. 2022;23(17):9784. DOI: 10.3390/ijms23179784
[21] Olmos-Ortiz A, Avila E, Durand-Carbajal M, Díaz L. Regulation of calcitriol biosynthesis and activity: Focus on gestational vitamin D deficiency and adverse pregnancy outcomes. Nutrients. 2015;7(1):443–80. DOI: 10.3390/nu7010443
[22] Monika, Khillan S, Garg R, Kaur P, Singh J. Serum 25 (OH) vitamin D and calcium levels and adverse maternal and perinatal outcomes in pregnancy induced hypertension. Asian J Pharm Clin Res. 2024;17(6):118–21. DOI: 10.22159/ajpcr.2024.v17i6.50510
[23] Kiely ME, Wagner CL, Roth DE. Vitamin D in pregnancy: Where we are and where we should go. J Steroid Biochem Mol Biol. 2020;201:105669. DOI: 10.1016/j.jsbmb.2020.105669
[24] Shatylo S, Bogomaz V, Babych O. Vitamin D deficiency in Ukraine: A multicentre cross-sectional study. Glob Epidemiol. 2024;8:100170. DOI: 10.1016/j.gloepi.2024.100170
[25] Kim S, Shim S, Kwon J, Ryoo S, Byeon J, Hong J, et al. Alleviation of preeclampsia-like symptoms through PlGF and eNOS regulation by hypoxia- and NF-κB-responsive miR-214-3p deletion. Exp Mol Med. 2024;56(6):1388–400. DOI: 10.1038/s12276-024-01237-8
[26] Kulyk II , Khmil SV. Endometriosis-associated infertility: The role of hormones and its correction. Int J Med Med Res. 2020;6(2):5–10. DOI: 10.11603/ijmmr.2413-6077.2020.2.12011
[27] Cookson MW, Ryan SL, Seedorf GJ, Dodson RB, Abman SH, Mandell EW. Antenatal vitamin D preserves placental vascular and fetal growth in experimental chorioamnionitis due to intra-amniotic endotoxin exposure. Am J Perinatol. 2018;35(13):1260–70. DOI: 10.1055/s-0038-1642033
[28] Gingrich J, Ticiani E, Veiga-Lopez A. Placenta disrupted: Endocrine disrupting chemicals and pregnancy. Trends Endocrinol Metab. 2020;31(7):508–24. DOI: 10.1016/j.tem.2020.03.003
[29] Lian RH, Qi PA, Yuan T, Yan PJ, Qiu WW, Wei Y, et al. Systematic review and meta-analysis of vitamin D deficiency in different pregnancy on preterm birth: Deficiency in middle pregnancy might be at risk. Medicine (Baltimore). 2021;100(24):e26303. DOI: 10.1097/MD.0000000000026303
[30] Yates N, Crew RC, Wyrwoll CS. Vitamin D deficiency and impaired placental function: Potential regulation by glucocorticoids? Reproduction. 2017;153(5):163–71. DOI: 10.1530/REP-16-0647
[31] Pérez-López FR, Pilz S, Chedraui P. Vitamin D supplementation during pregnancy: An overview. Curr Opin Obstet Gynecol. 2020;32(5):316–21. DOI: 10.1097/GCO.0000000000000641

Роль дефіциту вітаміну D у стимуляції запальної реакції та ризику передчасних пологів

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Анотація. Передчасні пологи залишаються однією з провідних причин перинатальної смертності та довготривалої захворюваності новонароджених. Метою роботи було з'ясування ролі дефіциту вітаміну D у регуляції NF-κB-залежного прозапального каскаду у вагітних із передчасними пологами. У дослідженні були використані клініко-лабораторні, біохімічні та імунологічні методи, включно з визначенням рівня 25(OH)D, активності та фосфорильованих форм p65 NF-κB у плаценті, а також концентрацій TNF-α, IL-6 і CRP у сироватці крові. Було обстежено 114 вагітних жінок, які були розподілені на три групи: з передчасними пологами та дефіцитом вітаміну D (n = 44), з передчасними пологами та оптимальним рівнем вітаміну D (n = 40) та контрольну групу з фізіологічною вагітністю (n = 30). Було встановлено, що активність p65 NF-κB була найвищою у групі з дефіцитом вітаміну D – 59,21 ± 0,24 %, що перевищувало показники групи з оптимальним рівнем (47,15 ± 0,36 %) та контролю (28,46 ± 0,37 %) (P < 0,05). Концентрації TNF-α становили відповідно 42,75 ± 0,31, 30,44 ± 0,29 і 15,62 ± 0,18 пг/мл, IL-6 – 38,16 ± 0,28, 26,87 ± 0,26 та 12,44 ± 0,14 пг/мл, CRP – 6,82 ± 0,12, 4,31 ± 0,09 і 1,95 ± 0,06 мл/л (P < 0,05). Було підтверджено сильні кореляції між активністю NF-κB та рівнями TNF-α (r = 0,88), IL-6 (r = 0,85) і CRP (r = 0,79). Було узагальнено, що дефіцит вітаміну D асоціювався з гіперактивацією NF-κB та посиленням продукції прозапальних цитокінів, що сприяло розвитку передчасних пологів. Отримані результати можуть бути використані в акушерсько-гінекологічній практиці для стратифікації ризику передчасних пологів і розробки індивідуалізованих профілактичних заходів, спрямованих на корекцію вітамін-D статусу

Ключові слова: 25(OH)D; невиношування вагітності; NF-κB p65; запальні маркери; TNF-α; IL-6; плацента

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Histological and histochemical changes in the treatment of wounds with an injectable implant based on high-molecular hyaluronic acid and sodium succinate: Experimental study

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Abstract. The aim of the study was to evaluate the effectiveness of an injectable implant combining hyaluronic acid and sodium succinate for accelerating the reparative processes of donor wounds after autodermoplasty in patients with deep burns. The study was conducted from 1 January to 30 June 2014 in the Department of Combustiology of the Kharkiv Medical Academy of Postgraduate Education at the City Clinical Emergency and Urgent Care Hospital named after Prof. O.I. Meshchaninov, where in 50 patients with donor wounds after autodermoplasty the effectiveness of the hyaluronic acid and sodium succinate injectable implant and standard therapy was compared according to clinical, morphological and biochemical parameters. The results showed that by the 10th day, complete healing was observed in 78% of patients in zone A (39 out of 50), while in zone B this indicator was only 6% (3 out of 50). By the 14th day, epithelisation was completed in 100% of cases in zone A, compared with 42% in zone B (21 out of 50). Morphological analysis showed a higher mitotic index in zone A – 16.1% versus 6.8% in zone B on the 10th day, which reflected more active cell renewal. Biochemical studies confirmed a more pronounced increase in catalase activity in zone A (from 28.2 to 36.6) compared with the control zone (from 25.8 to 27.4). Scars in zone A formed delicate and elastic, while in zone B dense and less organised structures predominated. The injectable implant of hyaluronic acid with sodium succinate accelerated healing and improved scar quality compared with standard therapy. The results obtained may be used by burn specialists, plastic surgeons and clinical pharmacologists in the practice of burn centres and departments of reconstructive surgery for optimising the treatment of donor wounds after autodermoplasty

Keywords: donor site; regeneration; cell proliferation; collagen fibres; scars; antioxidant enzymes; tolerability

INTRODUCTION

According to the World Health Organisation, about 180,000 deaths caused by burn injury are registered worldwide each year [1]. The survival of patients with deep burns directly depended on the timely and adequate closure of wound surfaces, including through autodermoplasty. However, performing this procedure was accompanied by the formation of donor wounds, which became an additional site of injury. The presence prolonged the duration of inpatient treatment, intensified the pain syndrome, increased the risk of secondary infection, and created prerequisites for the development of pathological scars. The use of traditional approaches (gauze dressings, air-drying or local antiseptics) allowed epithelisation to be achieved, but did not ensure rapid restoration of full skin cover or reduce the risk of complications. For this reason, the problem of optimising the treatment of donor wounds in combustiology remained unresolved and required the introduction of modern biocompatible materials and implants capable of accelerating reparative processes and improving the quality of newly formed tissue.

International data confirmed the effectiveness of hyaluronic acid in the treatment of burn wounds and in improving the quality of tissue regeneration. In the study by Y. Dong *et al.* [2], the use of a conformal hydrogel based on hyaluronic acid with adipose tissue stem cells was integrated with cell therapy, which led to accelerated epithelisation

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and enhanced angiogenesis compared with the control group. The study combined the biopolymer and cells into a single therapeutic system, demonstrating the promise of this approach. The analysis conducted by E. Alemzadeh *et al.* [3] showed that the inclusion of stem cells in a hyaluronic acid-based hydrogel modulated the expression of interleukin-1β (IL-1β), transforming growth factor β1 (TGF-β1), and basic fibroblast growth factor (bFGF), resulting in faster wound healing. The authors confirmed that regulation of the cytokine profile was a key mechanism for improving the morphology of the regenerated skin. In the study by Á. Sierra-Sánchez *et al.* [4], the use of dermal substitutes containing hyaluronic acid, led to faster epithelisation and restoration of skin structures. The researchers emphasised that the use of such materials reduced the severity of inflammation and promoted a more ordered process of regeneration. In the study by S.V. Korkunda *et al.* [5], it was noted that the use of hyaluronic acid in the complex treatment of patients with wound defects contributed to faster epithelisation and a reduction in the severity of inflammatory reactions, which highlighted its therapeutic potential in regenerative medicine.

Numerous studies were aimed at creating modified materials based on hyaluronic acid capable of combining biocompatibility with additional functional properties. In the study by Z. Wang *et al.* [6], the development of films based on chitosan and derivatives of hyaluronic acid demonstrated increased strength and biodegradability, which ensured faster healing of skin defects compared with unmodified materials. The authors emphasised that the combination of polymers improved both the mechanical characteristics and the biological activity of the coating. The study conducted by Z. Hussain *et al.* [7] showed that the functionalisation of hyaluronic acid polymeric nanoparticles improved the delivery of active substances to deeper layers of the skin, resulting in significantly higher wound-healing effectiveness. It was proven that such an approach provided increased bioavailability and a pronounced anti-inflammatory effect. In the work by S. Zhang *et al.* [8], the creation of hybrid hydrogels based on arginine derivatives and hyaluronic acid led to the formation of materials with antioxidant properties, which contributed to the reduction of oxidative stress in the area of injury. The authors confirmed that these properties promoted more harmonious skin recovery and a reduction in the intensity of the inflammatory response. In the study by M.V. Balanenko [9], the creation of biomedical dressings based on a chitosan nanocomposite demonstrated the prospects of combining national biotechnologies with innovative approaches of tissue engineering. It was established that the created materials exhibited antibacterial activity and contributed to the course of regenerative processes, which was important for the treatment of donor wounds in clinical practice.

Studies devoted to improving the treatment of donor wounds confirmed the effectiveness of modern biopolymeric dressings and hyaluronic acid-based materials. In the study by N.M. Nor [10], the use of film wound dressings for the treatment of donor sites in patients with deep burns was evaluated clinically, and the results demonstrated a reduction in epithelisation time and a decrease in the frequency of infectious complications. It was emphasised that the choice of dressing materials directly influenced the speed of regeneration and the quality of the formed skin. U.Ye. Sobina [11] investigated the possibilities of creating a medical device based on hyaluronic acid, designed for use in regenerative medicine. The author showed that the combination of natural polymers with bioengineering technologies made it possible to create a product with the potential to optimise wound healing. In the study by G. Papa *et al.* [12], a comparison of hyaluronic acid- and silver-based dressings in patients after autodermoplasty was conducted. The results demonstrated that the use of hyaluronic acid-containing materials improved scar quality and contributed to better functional characteristics of donor sites.

Despite the significant volume of experimental and clinical studies, scientific research still lacked sufficient data regarding the evaluation of the effectiveness of injectable implants based on hyaluronic acid in combination with sodium succinate in the treatment of donor wounds in combustiology. The aim of the study was to determine the clinical outcomes of using an injectable implant based on hyaluronic acid in combination with sodium succinate in patients with burn injury.

MATERIALS AND METHODS

Study design, patient characteristics and ethical aspects. The study was conducted as an open comparative single-group design with intra-individual control: the donor site of each patient was conditionally divided into two equal parts, where one half received an injectable implant based on hyaluronic acid and sodium succinate, and the other – standard local treatment. The period of implementation lasted from 1 January to 30 June 2014, which provided sufficient time for recruiting the planned sample of patients, carrying out the 14-day treatment and observation cycle, and performing laboratory and statistical processing of the results. The study was conducted in the Department of Combustiology, Reconstructive and Plastic Surgery of the Kharkiv Medical Academy of Postgraduate Education on the basis of the City Clinical Emergency Hospital named after Prof. O.I. Meshchaninov (Kharkiv, Ukraine).

The study included 50 patients with donor wounds after autodermoplasty for thermal burns of IIIA-IIIB degree with an area of up to 5% of body surface. Among the participants, there were 28 men and 22 women aged 18 to 65 years. The localisation of burns was distributed as follows: hand – 8 patients, forearm – 8, shoulder – 10, torso – 9, thigh – 9, shin – 6. Patients with donor sites corresponding to the indicated parameters were included; in women of reproductive age, a negative pregnancy test was a mandatory condition. Individuals with burn disease, hypersensitivity to the components of the study drug, active inflammatory processes at the site of administration, a tendency to form hypertrophic scars, blood coagulation disorders, diabetes mellitus, as well as those who received thrombolytic or anticoagulant therapy within two weeks prior to inclusion, were not allowed to participate. In addition, patients with severe decompensated diseases or acute conditions that could affect the treatment results, as well as those who required medications not provided for by the protocol or were already participating in other clinical trials, were excluded.

The ethical principles of the study complied with the provisions of the Declaration of Helsinki of the World Medical Association [13], which defined the priority of patient

safety, voluntariness of participation, and mandatory informed consent. In addition, the protocol was developed taking into account the requirements of the international guideline on Good Clinical Practice [14], which guaranteed the reliability of the obtained clinical data and the protection of participants' rights. All participants signed written informed consent, which included confirmation of voluntary participation in the study and acceptance of the conditions regarding the processing of personal data in compliance with the principles of confidentiality and anonymisation of the obtained information.

Treatment interventions and therapy protocol. Before the start of therapy, all patients underwent screening, which included clinical examination and laboratory tests. Treatment was started immediately after autodermoplasty. The donor site was conditionally divided into two equal parts. In one zone an injectable implant (zone A) containing hyaluronic acid and sodium succinate (Hyalual, "Yuria-Pharm", Ukraine) was used. The drug was injected directly under half of the wound in a dose of 1-2 ml, depending on its area, immediately after surgery and again after three days. The other half of the donor surface was treated according to the standard protocol (zone B): a sterile gauze napkin without additional impregnation was applied to the entire area, after which drying was carried out with a specialised medical heat fan WarmTouch WT 6000 (Covidien, USA), certified for use in burn surgery departments. Patients were examined daily, which included an assessment of general condition, visual characterisation of the donor site, and registration of subjective complaints. Dressings in the control zone were carried out once a day, synchronously with daily examinations. Treatment lasted until complete epithelialisation, and the main criterion of effectiveness was the assessment on the 14th day. Advanced clinical and laboratory examinations were performed on the specified control days – on days 1, 3, 7, 10 and 14, which included general clinical examination (body temperature, auscultation of the heart and lungs, palpation and percussion of the abdomen, condition of the skin and mucous membranes), laboratory tests (complete blood count, general urine analysis, biochemical blood parameters), as well as visual assessment of the donor wound. Histological and histochemical studies, including determination of the mitotic index and cellular activity, were performed on the 3rd and 10th days. The activity of antioxidant enzymes – superoxide dismutase (SOD) and catalase – was determined on the 3rd and 14th days. Tolerability of therapy was assessed after the completion of the treatment course, while all adverse reactions were recorded daily. In cases where complete epithelialisation of the donor site occurred earlier than 14 days, the final clinical and laboratory examination was carried out on the day of healing.

Methods of assessing clinical and morphological treatment results and statistical analysis. The assessment of effectiveness was carried out according to primary and secondary criteria. The primary criteria included the duration of treatment (in days) until complete epithelialisation of the donor site and the morphological characteristics of tissues determined by the results of histological and histochemical studies in dynamics. The drug was considered effective if complete epithelialisation of the donor surface was achieved without complications, while partial epithelialisation was qualified as an insufficient therapeutic result. The secondary criteria were the average healing time of the donor site in the observation groups, changes in histological parameters in biopsies during treatment, and the results of histochemical studies reflecting the features of proliferative and reparative processes in the control and experimental zones.

For clinical assessment, a categorical scale from 0 to 3 points was used. The degree of epithelialisation was defined as complete (0 points), more than 50% of the surface (1 point), 25-50% (2 points) or less than 25% (3 points). The character of epithelialisation was assessed as continuous (0 points), marginal and insular (1 point), marginal only (2 points) or absent (3 points). Inflammatory changes in the wound and adjacent tissues were classified from the absence (0 points) to mild (1 point), moderate (2 points) or severe (3 points). Skin itching in the healing area was also assessed gradually: from absence (0 points) to mild (1 point), moderate (2 points) and severe (3 points). Morphological characteristics of tissue quality included relief smoothness, where 0 points corresponded to absence of irregularities, 1 point – mild, 2 points – moderate and 3 points – severe changes. Tissue colour was defined as normal (0 points), with mild hyperaemia (1 point), marked hyperaemia (2 points) or purplish-cyanotic shade (3 points). Tolerability of therapy was assessed comprehensively, taking into account patients' subjective complaints, clinical data and laboratory test results, as well as the frequency and nature of adverse reactions. For the integrated assessment a three-level scale was used: good tolerability was defined by the absence of pathological changes or deviations in clinical and laboratory parameters and patients' complaints; satisfactory corresponded to temporary and minor deviations that did not require therapy modification or additional interventions; unsatisfactory was established in cases of severe pathological changes requiring discontinuation of the drug and the appointment of alternative treatment.

Statistical data processing was carried out using MS Excel (Microsoft Office, USA). For quantitative variables the mean value (M), median (Me), standard deviation (SD), minimum (Min) and maximum (Max) values were calculated. For qualitative indicators, absolute (n) and relative (%) frequencies were determined. Normality of distribution was checked using the Shapiro-Wilk test. In cases of normal distribution a paired Student's t-test was used, and in its absence – the Mann-Whitney test. Categorical data were compared using Pearson's χ^2 test or Fisher's exact test. Differences were considered statistically significant at $p < 0.05$ (for the Shapiro-Wilk test – $p < 0.01$). The limitation of the study was that it did not provide for the assessment of long-term quality of formed scars and long-term functional results, which reduced the possibility of a comprehensive analysis of therapy effectiveness.

RESULTS

Comparative characteristics of clinical outcomes in zones A and B. Clinical results showed significant differences in the speed and quality of healing of donor wounds depending on the treatment approaches used. In patients in whom an injection implant based on hyaluronic acid and sodium succinate was administered in zone A, the recovery processes took place more dynamically and were accompanied

by distinct positive changes from the very first days of treatment. This was manifested both in faster epithelialisation of the wound surface and in a reduction in the severity of inflammatory reactions and in a more orderly formation of tissues. For a detailed reflection of the process dynamics during the observation period on days 1, 3, 7, 10 and 14, the results of the clinical assessment of the condition of donor wounds in zone A were summarised in Table 1.

Table 1. Dynamics of donor wound healing in zone A (n=50)

Indicator	Expression	Day 1	Day 3	Day 7	Day 10	Day 14
Degree of epithelialisation	0 – complete	–	–	12	39	50
	1 – more than 50%	–	–	15	11	–
	2 – 25-50%	–	–	23	–	–
	3 – <25%	50	50	–	–	–
Character of epithelialisation	0 – continuous	–	–	12	36	50
	1 – marginal and insular	–	–	30	14	–
	2 – marginal	–	50	8	–	–
	3 – absent	50	–	–	–	–
Inflammatory changes	0 – none	–	–	12	19	27
	1 – slight	–	10	15	28	22
	2 – moderate	35	36	23	3	1
	3 – severe	15	4	–	–	–
Skin itching	0 – none	–	–	–	–	–
	1 – slight	–	–	–	–	41
	2 – moderate	–	–	–	–	9
	3 – severe	–	–	–	–	–
Relief irregularity	0 – none	50	–	–	–	21
	1 – slight	–	–	–	–	25
	2 – moderate	–	–	–	–	4
	3 – severe	–	–	–	–	–
Colour intensity	0 – normal	–	–	–	–	–
	1 – moderate hyperaemia	–	–	–	–	40
	2 – severe hyperaemia	50	–	–	–	10
	3 – purple-cyanotic	–	–	–	–	–

Source: compiled by the author

The analysis of Table 1 data demonstrated that at the initial stage (1-3 days) all patients had minimal epithelialisation (<25% of the surface), which corresponded to the expected course of the early postoperative period after autodermoplasty. Such a result indicated the adequacy of the research model and the absence of initial deviations between patients. By the 7th day, in 24% of cases (12/50) complete epithelialisation was noted, in another 30% restoration of more than half of the surface was observed, and in 46% – from 25 to 50%. Such dynamics demonstrated the activation of reparative processes under the influence of the injection implant, which allowed a significant number of patients to enter the phase of full recovery much earlier than usually expected with standard therapy. By the 10th day, in 78% of participants (39/50) complete epithelialisation was observed, while the remaining 22% reached this indicator by the 14th day. Thus, the absolute majority of patients completed the healing process within the first ten days, which clinically meant a significant reduction in the duration of inpatient treatment. The character of epithelialisation also confirmed the high quality of recovery: by the 7th day in 24% of cases the newly formed epithelium was already continuous, and by the 14th day this indicator was 100%, which indicated the uniformity and stability of regeneration.

The inflammatory component, typical for postoperative wounds, gradually decreased. At the start of the study, severe inflammation was noted in 15/50 patients, but by the 10th day only isolated moderate manifestations remained, and by the 14th day severe cases completely disappeared. This meant that the implant had a pronounced anti-inflammatory effect, which contributed to a more stable course of the reparative process and reduced the risk of infectious complications. Regarding subjective complaints, itching was recorded only on the 14th day: in 82% of cases it was slight, and in 18% – moderate. Such dynamics indicated that itching appeared as a symptom of tissue remodelling and did not negatively affect the overall clinical result. Morphological markers of skin quality showed gradual improvement: by the 14th day in 42% (21/50) of patients the relief irregularity completely disappeared, while the rest had only slight or moderate manifestations; no severe cases were recorded. This demonstrated a reduced risk of hypertrophic or keloid scars. Hyperaemia, which at the beginning was present in 100% of patients (50/50), gradually decreased: by the 14th day only 10 cases of moderate changes remained. Such a result confirmed the normalisation of microcirculation and the adequacy of vascularisation of the restored tissues, which, together with morphological indicators, pointed to the high quality of regeneration. Unlike zone A, where an injection implant was used, zone B used a standard approach to the treatment of donor wounds, which included gauze dressings and drying with a specialised heat blower. The dynamics of recovery in this group proved to be less intensive: the process of epithelialisation proceeded more slowly, with a larger proportion of cases of pronounced

inflammatory manifestations and uneven structure of the newly formed tissue. Detailed indicators of the clinical evaluation of the condition of donor sites in zone B during the entire observation period were presented in Table 2.

Table 2. Dynamics of donor wound healing in zone B (n=50)

Indicator	Expression	Day 1	Day 3	Day 7	Day 10	Day 14
Degree of epithelialisation	0 – complete	–	–	–	3	21
	1 – more than 50%	–	–	2	29	24
	2 – 25-50%	–	–	22	16	5
	3 – <25%	50	50	26	2	–
Character of epithelialisation	0 – continuous	–	–	–	3	21
	1 – marginal and insular	–	–	6	16	29
	2 – marginal	–	31	44	31	–
	3 – absent	50	19	–	–	–
Inflammatory changes	0 – none	–	–	5	12	19
	1 – slight	–	6	15	21	20
	2 – moderate	35	38	30	17	11
	3 – severe	15	6	–	–	–
Skin itching	0 – none	–	–	–	–	–
	1 – slight	–	–	–	–	24
	2 – moderate	–	–	–	–	22
	3 – severe	–	–	–	–	4
Relief irregularity	0 – none	50	–	–	–	11
	1 – slight	–	–	–	–	23
	2 – moderate	–	–	–	–	16
	3 – severe	–	–	–	–	–
Colour intensity	0 – normal	–	–	–	–	–
	1 – moderate hyperaemia	–	–	–	–	23
	2 – severe hyperaemia	50	–	–	–	23
	3 – purple-cyanotic	–	–	–	–	4

Source: compiled by the author

Analysis of the results in zone B showed a significantly slower course of reparative processes compared to the experimental area. Already on the 7th day, there were no recorded cases of complete epithelisation, while most wounds (52%, 26/50) remained at the level of 25-50% restored surface, and in 26 patients (<25%) only initial signs of healing were observed. Such a delay indicated insufficient stimulation of cellular and vascular repair mechanisms under standard treatment, which caused the prolonged persistence of the defect. Only on the 10th day did 3 patients show complete epithelisation, whereas in zone A, this figure reached 39 cases, which made the difference fundamental. On the 14th day only 42% (21/50) achieved complete healing, while the rest continued to demonstrate incomplete tissue restoration, which in the clinical context meant the risk of infectious complications, the need for continued inpatient treatment and increased cost of therapy. The nature of epithelisation also confirmed the delay in recovery: even on the 14th day, in the majority of patients (29/50) the marginal or island type prevailed, which reflects the fragmentary closure of the wound surface. Complete restoration was recorded in less than half of the cases, which indicated insufficient integration of the cellular matrix and uneven epithelial growth. This created the prerequisites for the formation of scar changes, especially under conditions of repeated injuries or additional infectious load.

Inflammatory reactions persisted much longer than in zone A. While at the beginning 15 patients had pronounced changes, even on the 14th day 11 individuals retained moderate manifestations, and complete absence of inflammation was recorded in only 19/50. Such dynamics indicated insufficient control over the exudative phase of the wound process and the likelihood of chronic inflammation, which is often associated with the formation of coarse pathological scars. Subjective symptoms were also more pronounced. On the 14th day, almost all patients reported itching: in 44% it was moderate, and in 8% severe. Such intensity of itching is an indirect marker of active remodelling with increased proliferative activity of fibroblasts, which is associated with the risk of hypertrophic scar formation and impaired tissue elasticity. Morphological parameters of the quality of the restored skin were also less favourable. In 32% of cases, significant surface unevenness was recorded, whereas in zone A no such manifestations were observed. This meant that standard treatment did not ensure sufficient restoration of the dermal structure and left a risk of cosmetic and functional defects. Hyperaemia proved to be more persistent and pronounced. On the 14th day, 23 patients still had pronounced hyperaemia, and in 4 more cases signs of purplish-cyanotic colouring were observed, indicating stagnant phenomena in the microcirculatory bed and incomplete normalisation of tissue vascularisation. This indicator reflected the insufficient maturity of the newly formed vessels and the risk of secondary complications, in particular hypoxia of the regenerated areas.

The overall analysis of the results in zones A and B revealed significant differences in the speed and quality of reparative processes. In zone A, where the Hyalual injectable implant was used, epithelisation occurred much more

dynamically: already on the 7th day, 24% of donor surfaces were fully restored, whereas in zone B none of the wounds reached this level. On the 10th day, 78% of patients in zone A had complete healing, which strongly contrasted with 6% in zone B. By the end of the 14-day observation, 100% of cases in zone A were completely epithelised, while in zone B this result was recorded in only 42% of patients. An important factor was also the reduction of the inflammatory component: in zone A, by the 14th day there remained only one case of moderate inflammation, while in zone B these manifestations persisted in 11 patients. Subjective symptoms also differed: in zone A, itching was mild in 82% and moderate in 18% of patients, whereas in zone B almost half (44%) reported moderate and another 8% severe itching, indicating a risk of scarring. Morphological signs confirmed the advantages of the implant: in zone A, most patients had a smooth surface, only 29% had minor or moderate changes, while in zone B more than 78% retained deformations. Skin colour normalised more quickly in zone A, where hyperaemia persisted in 20%, whereas in zone B pronounced redness was recorded in 46% of patients, and another 8% had a bluish tint. This indicates a higher quality of recovery in zone A. The obtained results indicate that the use of an injectable implant based on hyaluronic acid and sodium succinate in the treatment of donor wounds after autodermoplasty ensured faster and higher- quality epithelisation compared with the standard approach. The drug contributed to a more ordered course of regeneration, reduced inflammation intensity, limited itching and improved morphological characteristics of the restored skin. This makes it possible to consider it a promising means for optimising the recovery of postoperative wounds in clinical combustiology.

Morphological and biochemical bases of therapy effectiveness. To assess the biochemical mechanisms underlying the regenerative effect of the injectable implant, the activity of key antioxidant enzymes – SOD and catalase – was analysed. These enzymes play a leading role in neutralising reactive oxygen species, reducing oxidative stress in tissues and creating favourable conditions for cell proliferation. The results of the study in zone A, where the preparation based on hyaluronic acid and sodium succinate was used, are presented in Table 3.

Table 3. Dynamics of antioxidant enzyme activity in zone A (n=50)

Indicator	Evaluation point	M	Me	SD	Min	Max
SOD	Day 3	9.11	9.1	0.79	7.8	10.5
	Day 14	10.03	9.8	0.66	9.2	11.3
Catalase	Day 3	28.2	28.2	1.36	26.2	30.8
	Day 14	36.6	36.9	1.64	33.2	38.8

Source: compiled by the author

The analysis of the data in Table 3 showed that in zone A the activity of antioxidant enzymes increased during the course of treatment, reflecting the biochemical prerequisites for accelerated wound healing. The SOD level rose from 9.11 ± 0.79 on the 3rd day to 10.03 ± 0.66 on the 14th day. This meant that tissues more actively utilised the superoxide radical, reducing oxidative stress and preventing damage to cellular structures. At the same time, a marked increase in catalase activity was observed – from 28.2 ± 1.36 to 36.6 ± 1.64, which indicated the strengthening of detoxification mechanisms for hydrogen peroxide, one of the main by-products of metabolism. Such dynamics were typical for tissues with a high level of proliferative processes, since the effective functioning of the antioxidant system created conditions for preserving cell viability, active collagen synthesis and the ordered formation of new connective tissue. The obtained results explain why in zone A regeneration occurred faster: the reduction of oxidative stress and the increase of antioxidant protection contributed to a more harmonious course of reparative processes. In zone B, where standard treatment was applied, the dynamics of these enzyme changes reflected less intense reparative processes. Detailed indicators are presented in Table 4.

Table 4. Dynamics of antioxidant enzyme activity in zone B (n=50)

Indicator	Evaluation point	M	Me	SD	Min	Max
SOD	Day 3	7.24	7.1	0.75	6.1	8.8
	Day 14	8.68	8.8	0.6	7.2	9.8
Catalase	Day 3	25.8	25.8	1.33	23.2	27.8
	Day 14	27.4	27.4	1.12	25.2	29.4

Source: compiled by the author

The analysis of the data in Table 4 showed that in zone B the activity of antioxidant enzymes also increased, but it was less pronounced compared with zone A. The SOD level increased from 7.24 ± 0.75 on the 3rd day to 8.68 ± 0.6 on the 14th day, which indicated some activation of superoxide radical neutralisation mechanisms. At the same time, the values remained lower than in zone A, which reflected less intensive metabolic processes and weaker protection against oxidative stress. Catalase rose from 25.8 ± 1.33 to 27.4 ± 1.12, but such an increase was minimal and did not ensure a sufficient level of hydrogen peroxide detoxification. This meant that in the control zone, tissue regeneration was accompanied by less effective antioxidant protection, which could lead to more prolonged inflammation and slower formation of mature granulation tissue. In general, the dynamics in zone B confirmed the lag in the quality of biochemical recovery mechanisms compared to the experimental zone. To com-

prehensively characterise reparative processes, indicators of the mitotic index and mitotic activity in donor wound biopsies were analysed. These parameters reflect the intensity of cell division and proliferation, which are key for restoring damaged tissues. The obtained results are presented in Table 5.

Table 5. Indicators of mitotic index and mitotic activity

Indicator	Evaluation point	Zone A (n = 50)	Zone B (n = 50)
Mitotic index, %	Day 3	10.6±1.92	8.81±1.77
	Day 10	16.1±4.71	6.8±1.68
Mitotic activity, %	Day 3	5.95±1.91	4.1±0.78
	Day 10	7.28±2.54	5.1±1.63

Source: compiled by the author

The analysis of the data demonstrated that the dynamics of the mitotic index and mitotic activity directly reflected the differences in the morphological mechanisms of tissue restoration between zone A and zone B. In zone A, an increase of the mitotic index to 16.1% on the 10th day meant active proliferation of epithelial cells and fibroblasts, which contributed to faster formation of granulation tissue and subsequent organisation of mature connective tissue. The increase of mitotic activity to 7.28% indicated stable involvement of cells in division processes, providing a sufficient pool of cells for collagen synthesis and dermal structure restoration. This explains the more orderly organisation of collagen fibres and the reduced risk of pathological scarring in this group. In zone B, where indicators tended to decrease (mitotic index – 6.8%, mitotic activity – 5.1% on the 10th day), the cell cycle was less active. This meant that recovery processes proceeded more slowly, with less intensive formation of new cells and collagen synthesis. Morphologically, such dynamics could lead to less mature and less organised connective tissue, with a higher likelihood of coarse fibrous structures, which are a prerequisite for pathological scar formation. Thus, the data confirmed that the implant ensured a favourable microenvironment for cell proliferation and morphogenesis of new tissues, whereas standard therapy did not create sufficient conditions for such activity. At the stage of fibrous connective tissue formation, morphological differences between zones A and B were identified. In biopsies from zone A, already on the 3rd-5th day, intensive formation of thin collagen fibres was recorded in combination with numerous fibroblasts and a branched network of capillaries. This indicated active remodelling of granulation tissue and the creation of conditions for a rapid transition to the stage of mature recovery. In the control zone B, the number of vessels was lower by 15-20%, and the appearance of signs of fibrous tissue formation occurred later (after the 6th day), which potentially increased the risk of delayed reparative processes and pathological scar formation. Morphological characteristics of this stage are shown in Figure 1.

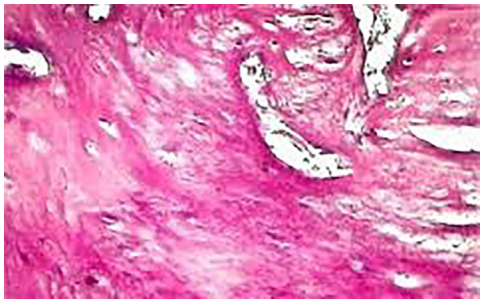

Figure 1. Formation of fibrous connective tissue in zone A (3rd-5th day of treatment)
Source: obtained by the author

On the 10th-14th day in zone A, the final stage of the reparative process was observed, characterised by the ordered formation of scar tissue. Collagen fibres had a more mature morphological structure, were tightly oriented in parallel bundles, which provided tissue with mechanical stability. The vascular network was represented by individual capillaries with signs of stabilisation of the walls, and the cellular infiltrate was minimised, which indicated the completion of the inflammatory stage and the transition to tissue remodelling. The obtained changes reflected favourable conditions for the formation of a thin and elastic scar without a tendency to hypertrophy. In zone B, the tissue structure remained less organised compared with zone A. Collagen fibres were arranged chaotically, were predominantly thin and immature, which indicated an unfinished process of the remodelling. The vascular component was poorly developed: isolated vessels with signs of lumen reduction were recorded, which reduced the quality of tissue nutrition. In the intercellular substance, a significant number of mesenchymal cells remained, and compaction of the interfibrillar matrix was noted, which created prerequisites for the formation of a denser and more rigid scar. Such a combination of morphological characteristics indicated a slower nature of regeneration and less favourable conditions for tissue recovery. In contrast, in zone A the process ended with the formation of a delicate and elastic scar, which corresponded to higher-quality repair (Fig. 2).

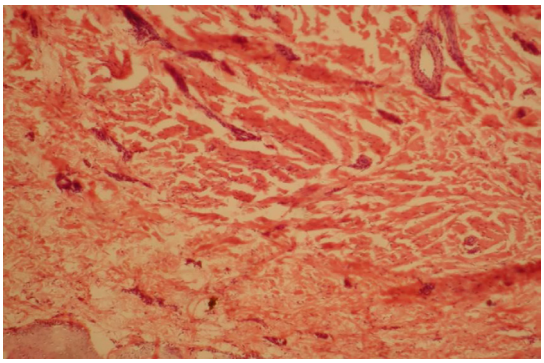

Figure 2. Formation of scar connective tissue in zone B (10th-14th day of treatment)
Source: obtained by the author

In addition, the tolerability of therapy was satisfactory in both zones: throughout the entire treatment period, no unforeseen side effects, acute complications or exacerbations of chronic diseases were detected. In zone A, the injectable implant was evaluated as well tolerated: no clinically significant deviations in laboratory parameters or pathological changes during objective examination were recorded, and subjective complaints were limited to mild itching, which was transient and did not require correction of therapy. In zone B, tolerability also remained satisfactory, but on the 14th day 22 patients reported moderate itching, and in 4 cases it was severe, indicating a less favourable course of the reparative process. The obtained data indicated that the use of an injectable implant based on hyaluronic acid and sodium succinate contributed to the activation of antioxidant enzymes, stimulation of cell proliferation and more orderly formation of connective tissue. In zone B, similar processes were significantly slower and accompanied by a less pronounced antioxidant response. This confirmed the important role of the preparation in accelerating regeneration and improving the quality of donor wound healing.

DISCUSSION

The obtained results confirmed that the use of an injectable implant based on hyaluronic acid and sodium succinate ensured faster and higher-quality healing of donor wounds compared to the standard approach. The use of the preparation was accompanied by more organised epithelialisation, a reduction in the severity of inflammation, and the formation of more elastic scar tissue. Morphological and biochemical studies indicated the activation of reparative processes and the normalisation of tissue homeostasis. The tolerability of therapy remained good, without the development of clinically significant complications or adverse reactions.

In zone A, complete epithelialisation was achieved in 78% (39/50) of patients as early as on the 10th day and in 100% (50/50) by the 14th day, whereas in zone B the corresponding figures were 6% (3/50) and 42% (21/50). Inflammatory manifestations disappeared more quickly in zone A, where on the 14th day these manifestations persisted only in isolated cases, while in zone B moderate changes were recorded in 22% of patients. This correlated with the data of S.F. Forghani *et al*. [15], who demonstrated that hyaluronic acid-based gels accelerated the healing of burn wounds in animal models. The results in zone A confirmed these data and indicated the universality of reparative mechanisms under the influence of hyaluronic acid. Similarly, G. Huerta-Ángeles & E. Mixcoha [16] noted that hyaluronic acid acted as a modulator of inflammation and stimulated reparative mechanisms, which explained the faster normalisation of tissue homeostasis in the present study. The analysis of biopsy samples showed an increase in the mitotic index in the experimental zone, indicating the activation of proliferative activity of cells. This was consistent with the concept of stimulating cell migration by biopolymer matrices [17]. In the review by G. Kaur *et al.* [18], this very mechanism, crucial for rapid recovery of damaged tissues, was highlighted. The quality of the scar formed was characterised by a more uniform structure and less pronounced relief defects. A similar effect was demonstrated in the studies of A.M. Jorgensen *et al.* [19], where the inclusion of hyaluronic acid in composite biomaterials accelerated wound closure and improved the quality of regenerated tissue.

The results of morphological analysis showed that in zone A on the 10th day the mitotic index reached 16.1 ± 4.71%, whereas in zone B it was only 6.8 ± 1.68%. Mitotic activity was also higher in zone A (7.28 ± 2.54%) compared to the control (5.1 ± 1.63%), which indicated a pronounced stimulation of cell proliferation. These data correlate with the results of the clinical study by H. Kim *et al*. [20], which showed that topical formulations with active amino acid- and hyaluronic acid-based components contributed to reduced scarring and the normalisation of cellular activity in patients with burns. Similar effects were also observed in the present clinical observations, where in zone A the formation of higher-quality granulation tissue and a reduced risk of hypertrophic changes were recorded. The study by M. Miastkowska *et al*. [21] demonstrated that the gel form based on hyaluronic acid in combination with nanostructured carriers provided controlled release of active substances, enhancing cell regeneration. In the cohort of patients studied, an increase in proliferative activity of cells was also noted, which was confirmed by morphological indicators and was consistent with the mechanism of controlled release. In the clinical work of R. Yildirim *et al.* [22], it was shown that the use of hyaluronic acid-based dressings for the treatment of superficial facial burns reduced healing time and improved the quality of newly formed tissue, which was consistent with the higher indicators of mitotic activity in zone A. This gave

grounds to consider that the effectiveness of the implant in the treatment of donor wounds had common pathogenetic bases with the successful results of using dressings in clinical practice. Similarly, A.F. Kamdem *et al.* [23] noted the effectiveness of care protocols using hyaluronic acid in children with second-degree burns, where faster recovery and better tissue organisation were observed. In the context of the obtained results, this indicated that Hyalual contributed not only to rapid epithelialisation but also to high-quality tissue remodelling, which was confirmed by histological indicators.

The results of histochemical analysis demonstrated increased activity of antioxidant enzymes in zone A, where the average indicators of SOD increased from 9.11 to 10.03, and catalase – from 28.2 to 36.6 conventional units between the 3rd and 14th day. In zone B, these changes were less pronounced: SOD increased from 7.24 to 8.68, and catalase – from 25.8 to 27.4, which confirmed enhanced antioxidant protection in the implant introduction zone. The increase in antioxidant enzyme activity recorded in zone A reflected more favourable conditions for tissue regeneration. This effect was consistent with the data of S. Zhang *et al.* [24], who emphasised that hyaluronic acid stabilised the cellular microenvironment and helped reduce oxidative stress, creating optimal conditions for cell proliferation. The comparison of these results with clinical observations confirmed that the antioxidant potential of hyaluronic acid was a key factor in forming a favourable course of reparative processes. Similar results were obtained by J.L. Soriano-Ruiz *et al*. [25], who, when creating a thermosensitive gel based on hyaluronic acid, found a significant decrease in the level of reactive oxygen species, which explained faster recovery of tissue structure. In the clinical work of M. Maruccia *et al*. [26], the use of a dermal substitute based on hyaluronic acid in patients with intermediate-deep hand burns provided not only faster epithelialisation but also a lower complication rate, which corresponded to the results obtained in zone A. A similar dynamic indicated the universality of the effect of hyaluronic acid, which was not limited only to donor areas but was also manifested in more complex clinical cases. The study of D. Yoon *et al.* [27] showed that the inclusion of hyaluronic acid in collagen matrices in patients with deep burns reduced inflammatory manifestations and contributed to the orderly formation of collagen fibres. The recorded increase in SOD and catalase activity in zone A was directly related to the well-known antioxidant properties of hyaluronic acid, which determined higher-quality healing results.

The results showed that in zone A by days 10-14 a delicate and elastic scar was formed, with a predominance of mature collagen fibres and minimal cellular infiltration. In contrast, in zone B a less organised structure with thin, chaotically arranged fibres, isolated vessels, and a tendency to rougher scarring was observed. The obtained morphological data were consistent with the conclusions of H. Yang *et al*. [28], who proved that hyaluronic acid contributed to a more ordered organisation of collagen fibres and reduced the likelihood of pathological scar formation in the wound healing process. These results confirmed the observations of M.F. Graça *et al*. [29], who noted that the use of hyaluronic acid-based dressings ensured tissue microrelief alignment, reduced hyperaemia and the risk of hypertrophic changes, which corresponded to even scarring in zone A. Similar results were demonstrated by R. Yang *et al*. [30], who reported that injectable hyaluronic acid hydrogels stimulated angiogenesis, activated fibroblast proliferation, and contributed to the formation of more mature and elastic tissue. In addition, Y. Kawano *et al*. [31] showed that medium-molecular-weight fractions of hyaluronic acid exhibited the greatest effectiveness in regulating the expression of genes associated with collagen synthesis and extracellular matrix remodelling, which ensured high-quality remodelling and reduced the risk of fibrosis. The obtained data confirmed that therapy with the addition of hyaluronic acid and sodium succinate contributed to the formation of a delicate and functionally complete scar, which had better morphological characteristics than in the control zone.

In the study, the tolerability of therapy was assessed as good: in the zone with the use of the injectable implant, no clinically significant deviations in laboratory indicators, pathological changes during objective examination, or severe complications were recorded. Subjective complaints of patients were limited to mild transient itching, which did not require changes in the treatment regimen. In zone B, the tolerability of therapy was assessed as satisfactory, but in a significant proportion of patients on the 14th day itching persisted: moderate in 22 cases and severe in 4, whereas in the experimental zone most participants reported only mild transient sensations. This difference may indicate a longer course of inflammatory processes and less favourable regeneration conditions in the control group. The obtained results are consistent with the data of W. Baranska-Rybak *et al*. [32], who in the work emphasised the need for careful monitoring of delayed reactions after the use of hyaluronic acid preparations. In clinical practice, this was of great importance, since even in the absence of serious complications, monitoring of patients was required for the timely detection of undesirable effects. The authors A. Janovskiene *et al.* [33] in a systematic review also noted that in most cases therapy with the use of fillers was safe, and serious complications were rare. These observations confirmed the general conclusion about a favourable safety profile, which coincided with the data obtained in the experimental sample. The analysis of T. Tamura *et al.* [34] on a large sample (more than 290 thousand cases) confirmed that the frequency of severe complications was low, although attention was required in the case of non-compliance with the administration technique. At the same time, in the work of F. De Francesco *et al*. [35], it was shown that hyaluronic acid when applied topically not only promoted faster healing but also demonstrated a high safety profile without significant side effects.

The obtained results of the clinical study confirmed that the use of an injectable implant based on hyaluronic acid and sodium succinate ensured significantly faster epithelialisation of donor wounds, contributed to the formation of mature connective tissue, and reduced the intensity of inflammatory manifestations. Compared to the control zone, better organisation of collagen fibres, higher levels of antioxidant enzymes, and more pronounced proliferative processes were observed. The tolerability of therapy remained good in both groups, but in the control zone complaints of itching were recorded more often, which reflected less favourable recovery conditions. Taken together, these

data indicate the high clinical and biological effectiveness of the proposed therapeutic strategy in combustiology.

CONCLUSIONS

In the course of the analysis of clinical results, it was established that the use of an injectable implant based on hyaluronic acid and sodium succinate contributed to significantly faster epithelialisation of donor wounds compared to traditional treatment. By the 7th day in zone A, complete epithelialisation was recorded in 12 (24%) patients, while in zone B no case of complete healing was observed. By the 10th day, in the experimental group 39 (78%) wounds had completely healed, and by the 14th day this figure reached 100%, whereas in the control zone complete epithelial recovery occurred in only 21 (42%) patients. The morphological characteristics of wounds in zone A reflected a more favourable course of the process due to faster organisation of collagen fibres and a reduction of the inflammatory component: pronounced manifestations, which at the start were detected in 15 patients, completely disappeared by the end of treatment, while in zone B moderate inflammation persisted in 11 cases. The presence of itching in zone A was mainly mild (41 cases slight and 9 – moderate), whereas in zone B itching was slight in 24 patients, moderate in 22, and severe in 4, which indicated a less qualitative course of reparative processes.

The evaluation of morphological and biochemical indicators confirmed the differences between the groups. In zone A, an increase in the activity of antioxidant enzymes was noted: the level of SOD increased from 9.11 to 10.03 units, and catalase – from 28.2 to 36.6 units during the observation period, while in zone B the increase was less pronounced (SOD increased from 7.24 to 8.68 units, catalase – from 25.8 to 27.4 units). A similar tendency was observed also for the mitotic index: in zone A it increased from 10.6% to 16.1%, whereas in zone B it decreased from 8.8% to 6.8%. Mitotic activity in zone A increased from 5.95% to 7.28%, while in zone B only from 4.1% to 5.1%. Histological studies showed that in the experimental group the formation of mature connective tissue occurred faster: by days 10-14 collagen fibres had an ordered structure, the vascular network was sufficiently developed, and the number of mesenchymal-origin cells had significantly decreased. In group B, immature tissue predominated with chaotically arranged collagen fibres, reduced capillaries, and pronounced cellular infiltration. The tolerability of therapy in both zones was assessed as good, but in the control zone subjective complaints of itching were recorded more frequently, while in zone A no adverse reactions, acute complications, or exacerbations of chronic diseases were observed. Overall, the results confirmed the higher clinical and biological effectiveness of the use of the implant, which ensured complete and high-quality tissue recovery in the shortest possible time. Further studies should be aimed at prospective investigation of the long-term effects of therapy and its comparison with other modern methods of treating donor wounds.

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REFERENCES

[1] World Health Organisation. Burns [Internet]. 2023 October 13 [cited 2025 January 30]. Available from: https://www.who.int/news-room/fact-sheets/detail/burns
[2] Dong Y, Cui M, Qu J, Wang X, Kwon SH, Barrera J, *et al*. Conformable hyaluronic acid hydrogel delivers adipose-derived stem cells and promotes regeneration of burn injury. Acta Biomater. 2020;108:56–66. DOI: 10.1016/j.actbio.2020.03.040
[3] Alemzadeh E, Oryan A, Mohammadi AA. Hyaluronic acid hydrogel loaded by adipose stem cells enhances wound healing by modulating IL-1β, TGF-β1, and bFGF in burn wound model in rat. J Biomed Mater Res B Appl Biomater. 2019;108(2):555–67. DOI: 10.1002/jbm.b.34411
[4] Sierra-Sánchez Á, Fernández-González A, Lizana-Moreno A, Espinosa-Ibáñez O, Martinez- Lopez A, Guerrero-Calvo J, et al. Hyaluronic acid biomaterial for human tissue-engineered skin substitutes: Preclinical comparative *in vivo* study of wound healing. J Eur Acad Dermatol Venereol. 2020;34(10):2414–27. DOI: 10.1111/jdv.16342
[5] Korkunda SV, Grigorieva TG, Oleinik GA. Clinical results of the use of injectable hyaluronic acid and sodium succinate implants in burn treatment. Emerg Med. 2014;63(8):64–73.
[6] Wang Z, Li K, Xu Q, Fu G, Li H, Yang W. Preparation and evaluation of chitosan- and hyaluronic acid-grafted pullulan succinate films for skin wound healing. Int J Biol Macromol. 2022;223(A):1432–42. DOI: 10.1016/j.ijbiomac.2022.11.100
[7] Hussain Z, Pandey M, Thu HE, Kaur T, Jia GW, Ying PC, *et al*. Hyaluronic acid functionalization improves dermal targeting of polymeric nanoparticles for management of burn wounds: *In vitro*, *ex vivo* and *in vivo* evaluations. Biomed Pharmacother. 2022;150:112992. DOI: 10.1016/j.biopha.2022.112992
[8] Zhang S, Hou J, Yuan Q, Xin P, Cheng H, Gu Z, *et al*. Arginine derivatives assist dopamine- hyaluronic acid hybrid hydrogels to have enhanced antioxidant activity for wound healing. Chem Eng J. 2020;392:123775. DOI: 10.1016/j.cej.2019.123775
[9] Balanenko MV. Biotechnology of creation and application of biomedical dressings based on a chitosan nanocomposite [Master's thesis]. Kyiv: National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"; 2024.
[10] Nor NM. Treatment of donor wounds in patients with deep burns using film wound dressings. Sci Collect InterConf. 2021;54:429–34.
[11] Sobina UYe. Bioengineering project for the production of a medical device (reagent) based on hyaluronic acid [Bachelor's thesis]. Kyiv: National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"; 2023.

[12] Papa G, Mosella F, Meshini G, Sidoti GB, Zaffiro A, Rega U, et al. Improvement of scar quality in split-thickness skin graft donor sites: A single blind randomized clinical trial comparing Rigenase® and polyhexanide versus hyaluronic acid and silver sulphadiazine based dressings. Plast Reconstr Regen Surg. 2023;2(1–2):40–8. DOI: 10.57604/PRRS-188
[13] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2025 January 30]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[14] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use ICH Harmonised Guideline: Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) [Internet]. 2016 [cited 2025 January 30]. Available from: https://www.ema.europa.eu/en/ich-e6-good-clinical-practice-scientific-guideline
[15] Forghani SF, Bagheri T, Naderi Gharahgheshlagh S, Hoveidamanesh S, Ghadimi T, Ghorbanian Kelachayeh S, et al. The effect of hyaluronic acid and iodine complex gel compared to Vaseline on deep second-degree burn wound in rats. Int Wound J. 2024;21(2):e14738. DOI: 10.1111/iwj.14738
[16] Huerta-Ángeles G, Mixcoha E. Recent advances, research trends, and clinical relevance of hyaluronic acid applied to wound healing and regeneration. Appl Sci. 2025;15(2):536. DOI: 10.3390/app15020536
[17] Chornyi O, Fedorenko V, Yaremenko V, Ishchenko O, Gureyeva S. Development of a chitosan polymer based composite haemostatic agent and a method for evaluation of performance. Technol Eng. 2025;26(1):67–78. DOI: 10.30857/2786-5371.2025.1.6
[18] Kaur G, Narayanan G, Garg D, Sachdev A, Matai I. Biomaterials-based regenerative strategies for skin tissue wound healing. ACS Appl Bio Mater. 2022;5(5):2069–106. DOI: 10.1021/acsabm.2c00035
[19] Jorgensen AM, Mahajan N, Atala A, Murphy SV. Advances in skin tissue engineering and regenerative medicine. J Burn Care Res. 2022;44(1):33–41. DOI: 10.1093/jbcr/irac126
[20] Kim H, Kwak I, Kim M, Um J, Lee S, Chung B, et al. Evaluation of a cosmetic formulation containing arginine glutamate in patients with burn scars: A pilot study. Pharmaceutics. 2024;16(10):1283. DOI: 10.3390/pharmaceutics16101283
[21] Miastkowska M, Kulawik-Pióro A, Szczurek M. Nanoemulsion gel formulation optimization for burn wounds: Analysis of rheological and sensory properties. Processes. 2020;8(11):1416. DOI: 10.3390/pr8111416
[22] Yildirim R, Guner A, Cekic AB, Usta MA, Ulusahin M, Turkyilmaz S. Outcomes of the use of hyaluronic acid-based wound dressings for the treatment of partial-thickness facial burns. J Burn Care Res. 2023;44(3):551–4. DOI: 10.1093/jbcr/irz004
[23] Kamdem AF, Parmentier AL, Mauny F, Soriano E. Assessment of care protocol using hyaluronic acid dressing in second-degree skin burns in children. Burns Open. 2021;5(3):118-24. DOI: 10.1016/j.burnso.2021.05.001
[24] Zhang S, Dong J, Pan R, Xu Z, Li M, Zang R. Structures, properties, and bioengineering applications of alginates and hyaluronic acid. Polymers. 2023;15(9):2149. DOI: 10.3390/polym15092149
[25] Soriano-Ruiz JL, Calpena-Campmany AC, Silva-Abreu M, Halbout-Bellowa L, Bozal-de Febrer N, Rodriguez-Lagunas MJ, et al. Design and evaluation of a multifunctional thermosensitive poloxamer-chitosan-hyaluronic acid gel for the treatment of skin burns. Int J Biol Macromol. 2020;142:412–22. DOI: 10.1016/j.ijbiomac.2019.09.113
[26] Maruccia M, Magistri S, Elia R, Maggio G, Giudice G. Hyaluronic acid-based dermal substitute with stromal vascular fraction vs partial thickness skin grafts for the treatment of intermediate- deep burns of the hand: A retrospective case-control study. Ann Burns Fire Disasters. 2024;37(4):305–11.
[27] Yoon D, Cho YS, Joo SY, Seo CH, Cho YS. A clinical trial with a novel collagen dermal substitute for wound healing in burn patients. Biomater Sci. 2020;8(3):823-9. DOI: 10.1039/C9BM01209E
[28] Yang H, Song L, Zou Y, Sun D, Wang L, Yu Z, et al. Role of hyaluronic acids and potential as regenerative biomaterials in wound healing. ACS Appl Bio Mater. 2021;4(1):311–24. DOI: 10.1021/acsabm.0c01364
[29] Graça MF, Miguel SP, Cabral CS, Correia IJ. Hyaluronic acid-based wound dressings: A review. Carbohydr Polym. 2020;241:116364. DOI: 10.1016/j.carbpol.2020.116364
[30] Yang R, Liu X, Ren Y, Xue W, Liu S, Wang P, et al. Injectable adaptive self-healing hyaluronic acid/poly(γ-glutamic acid) hydrogel for cutaneous wound healing. Acta Biomater. 2021;127:102–15. DOI: 10.1016/j.actbio.2021.03.057
[31] Kawano Y, Patrulea V, Sublet E, Borchard G, Iyoda T, Kageyama R, et al. Wound healing promotion by hyaluronic acid: Effect of molecular weight on gene expression and *in vivo* wound closure. Pharmaceuticals. 2021;14(4):301. DOI: 10.3390/ph14040301
[32] Baranska-Rybak W, Lajo-Plaza JV, Walker L, Alizadeh N. Late-onset reactions after hyaluronic acid dermal fillers: A consensus recommendation on etiology, prevention and management. Dermatol Ther. 2024;14(7):1767–85. DOI: 10.1007/s13555-024-01202-3
[33] Janovskiene A, Chomicius D, Afanasjevas D, Petronis Z, Razukevicius D, Jagelaviciene E. Safety and potential complications of facial wrinkle correction with dermal fillers: A systematic literature review. Medicina. 2025;61(1):25. DOI: 10.3390/medicina61010025
[34] Tamura T, Okumura K, Funakoshi Y, Teranishi H. Serious complications of hyaluronic acid fillers – a retrospective study of 290,307 cases. Ann Plast Surg. 2025;94(6):630–3. DOI: 10.1097/SAP.0000000000004327
[35] De Francesco F, Saparov A, Riccio M. Hyaluronic acid accelerates re-epithelialization and healing of acute cutaneous wounds. Eur Rev Med Pharmacol Sci. 2023;27(3):37–45. DOI: 10.26355/eurrev_202304_31320

Гістологічні та гістохімічні зміни при лікуванні ран ін'єкційним імплантом на основі високомолекулярної гіалуронової кислоти та сукцінату натрію: експериментальне дослідження

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Анотація. Метою роботи була оцінка результативності ін'єкційного імплантату, що поєднує гіалуронову кислоту і сукцинат натрію, для прискорення репаративних процесів донорських ран після аутодермопластики у пацієнтів з глибокими опіками. Дослідження проведено з 1 січня по 30 червня 2014 року у відділенні комбустіології Харківської медичної академії післядипломної освіти на базі Міської клінічної лікарні швидкої та невідкладної допомоги ім. проф. О. І. Мещанінова, де у 50 пацієнтів з донорськими ранами після аутодермопластики порівнювали ефективність ін'єкційного імплантату гіалуронової кислоти з сукцинатом натрію та стандартної терапії за клінічними, морфологічними й біохімічними показниками. Результати показали, що вже на 10-ту добу повне відновлення спостерігалося у 78 % пацієнтів у зоні А (39 із 50), тоді як у зоні В цей показник становив лише 6 % (3 із 50). До 14-ї доби епітелізація завершувалася у 100 % випадків у зоні А, проти 42 % у зоні В (21 із 50). Морфологічний аналіз показав вищий мітотичний індекс у зоні А – 16,1 % проти 6,8 % у зоні В на 10-ту добу, що відображало активніше клітинне оновлення. Біохімічні дослідження підтвердили більш виражене зростання активності каталази у зоні А (з 28,2 до 36,6) порівняно з контрольною зоною (з 25,8 до 27,4). Рубці у зоні А формувалися ніжними та еластичними, тоді як у зоні В переважали щільні й менш організовані структури. Ін'єкційний імплантат гіалуронової кислоти з сукцинатом натрію прискорив загоєння та покращив якість рубців порівняно зі стандартною терапією. Отримані результати можуть бути використані лікарями комбустіологами, пластичними хірургами та клінічними фармакологами у практиці опікових центрів і відділень реконструктивної хірургії для оптимізації лікування донорських ран після аутодермопластики

Ключові слова: донорська ділянка; регенерація; проліферація клітин; колагенові волокна; рубці; антиоксидантні ферменти; переносимість

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Advanced approaches to immunomodulation in clinical practice: Analysis of dietary supplements and medicines

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Abstract. The purpose of the study was to establish the immunomodulatory properties of herbal remedies and the prospects for their clinical implementation. The methodology was based on a theoretical and analytical generalisation of literature data on the immunomodulatory properties of preparations "Immunormalin", "Immunal", Asian ginseng, *Ginkgo biloba*, common marigold, and shiitake mushrooms. The results of the analysis showed that "Immunormalin" was marked by a wide range of mechanisms, combining antioxidant, anti-inflammatory, prebiotic, and sedative effects, which simultaneously covered immune, metabolic, and neuroendocrine pathways. This multifactorial nature distinguished it from more highly specialised products and determined a special position among nutraceuticals. In contrast, "Immunal", created based on *Echinacea purpurea*, demonstrated a directed immunomodulatory mechanism through the activation of macrophages and T-lymphocytes, which made it mainly effective in the prevention of respiratory infections. Asian ginseng combined the properties of an adaptogen and an immunomodulator, causing increased resistance of the body to infections and the formation of antitumour potential. *Ginkgo biloba*, sharing an antioxidant and anti-inflammatory effect with ginseng, had an additional vascular-regulating effect, which expanded its use in chronic pathologies and complex therapy of metabolic syndromes. *Calendula officinalis* showed the most pronounced anti-inflammatory profile among the compared agents, since it suppressed the production of pro-inflammatory cytokines and stimulated the secretion of anti-inflammatory mediators, which reduced the risk of tissue damage in long-term inflammatory processes. Shiitake mushrooms, due to their beta-glucan content, acted as natural adjuvants, enhancing the cellular immune response and combining antiviral and antitumour activity. Generalisation of the results showed that natural immunomodulators have a common antioxidant effect, but differ in multifactorial and clinical orientation, which determines the need for standardisation and differentiated use. The results obtained can be used by clinicians, pharmacologists and researchers in the field of evidence-based herbal medicine to optimise the selection of immunomodulatory agents and develop standardised approaches to their use

Keywords: inflammatory process; cytokines; macrophages; lymphocytes; interleukins; bioactive compounds; infections

INTRODUCTION

Infectious diseases and complications that occur against the background of immune dysfunction in patients with chronic pathologies substantiate the need to find new approaches to immune correction. Immune insufficiency is accompanied by a tendency to severe infections, a decrease in the effectiveness of immunoprophylaxis, and the development of prolonged inflammatory processes. Attention is paid to the use of phytopreparations and nutraceuticals that contain

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biologically active polysaccharides, saponins, and phenolic compounds that can modify cytokine regulation, activate macrophages and natural killer cells (NK cells) and maintain antioxidant balance. Such drugs are considered as an auxiliary component of complex therapy aimed at strengthening the immune response and preventing complications that occur due to weakened immune reactivity.

Phytopreparations were considered an important component of immunocorrection, as they combined the ability to influence cellular mechanisms of immunity with a relatively low level of toxicity. Administration of plant complexes has been shown to alter interleukin (IL) secretion and macrophage activity, as noted by I. Bielenichev *et al.* [1], and these effects were considered as the basis for integrating herbal remedies into complex therapeutic regimens. The researchers also noted that such approaches created conditions for personalising immune support depending on the clinical context. Generalisation by C. Burlou-Nagy *et al.* [2] showed that multicomponent phytopreparations stimulated phagocytic activity and cytokine production, which provided an increase in innate and adaptive responses. The researchers further emphasised that it was the multi-factorial nature of the action that opened up opportunities for their use in preventive strategies. Review by J.A. Valdés-González *et al.* [3] summarised the pharmacological properties of polysaccharides and ginsenosides that modified inflammatory cascades and activated the antitumour immune response. These results were interpreted as confirming the role of plant metabolites in supporting the body in cancer and inflammatory processes.

Plant bioactive complexes have a multi-vector effect on the immune system, activating cells of innate and adaptive immunity and regulating cytokine balance. It was found that polysaccharides of Asian ginseng (*Panax ginseng*) modified the function of macrophages and T-lymphocytes, altering IL secretion and stimulating proliferation, as reported by Y. Hu *et al.* [4]. These results demonstrated the potential of ginseng as a basis for the development of standardised immunotherapy drugs. Systematic analysis performed by N. Mohamed [5] combined data on polysaccharides of various medicinal plants and showed their ability to comprehensively affect immune regulation. The researcher also emphasised the lack of sufficient standardisation of these compounds, which poses a serious problem for their clinical implementation.

Extracts of *Ginkgo biloba*, as evidenced by M.A. Ibrahim *et al.* [6], reduced the expression of pro-inflammatory genes and increased the activity of effector cells in models of viral infections. The data obtained confirmed the prospects of ginkgo for the further development of antiviral immunomodulators of natural origin. The study by A.M. Samoilenko [7] proposed a technology for creating a tablet form of an antiviral agent from plant raw materials. The paper emphasised the complexity of optimising the composition and proving the effectiveness of such drugs, which remains an unsolved problem of contemporary pharmaceutical science. The study by D. Silva *et al.* [8] has shown that extracts of common marigold (*Calendula officinalis*) suppressed the production of tumour necrosis factor alpha (TNF-α) and other pro-inflammatory mediators, which reflected their anti-inflammatory and regulatory potential. These observations highlighted the possibility of using common marigold in the treatment of inflammatory pathologies with a complex immune component. The study by B.G. Oliveira *et al.* [9] demonstrated that extracts of purple coneflower (*Echinacea purpurea*) activated cytokine production and enhanced the antibacterial response, which provided a powerful defence of the body against infectious agents. The researchers showed that the use of dried extracts has little promise in both the prevention and treatment of diseases with impaired immune balance.

Complex immunocorrection includes both synthetic drugs and natural bioactive compounds that have demonstrated the ability to influence cellular pathways of immune regulation. Polysaccharides from shiitake mushrooms (*Lentinula edodes*), as shown by A. Roszczyk *et al.* [10], interacted with macrophage and dendritic cell receptors, resulting in increased cytokine production and activation of immune cascades. The researchers also proved that beta-glucans of the mushroom increase the body's ability to respond to antitumour and antiviral drugs. A systematic review by M. Vlassopoulou *et al.* [11] summarised data from clinical trials in which fungal beta-glucans showed a decrease in the incidence of infections in patients with immune deficiency. These results confirmed the promising use of fungal polysaccharides in preventive medicine. Generalisation by L.F. Popovici *et al.* [12] highlighted that extracts of *Calendula officinalis* combined anti-inflammatory and immunomodulatory effects, so they were used in pharmaceutical compositions. The researchers also emphasised the importance of this plant as a source of bioactive compounds for the creation of new phytopreparations.

Despite a significant amount of research, there are still gaps in the standardisation of plant immunomodulatory agents and evaluation of their clinical efficacy. The purpose of the study was to find out the mechanisms of immunomodulatory action of bioactive compounds and preparations of plant origin and determine their significance for clinical immunocorrection.

MATERIALS AND METHODS

The study was of a theoretical and analytical nature and was aimed at systematising literature data on the immunomodulatory properties of phytopreparations and dietary supplements. Six drugs and plant extracts were selected for analysis, combining the presence of a clinical evidence base, pharmacological prevalence, and availability in standardised forms. Among them, a separate place was occupied by "Immunormalin" (sole proprietor Sarkis Gagikovich Avakian, Ukraine), presented as a biologically active supplement developed by S.G. Avakian, which included extracts of common horsetail (*Equisetum arvense* L.), flowers of small-leaved linden (*Tilia cordata* Mill.), leaves of black currant (*Ribes nigrum* L.), wild chamomile (*Matricaria chamomilla* L.), common chicory (*Cichorium intybus* L.), common knotgrass (*Polygonum aviculare* L.), peppermint (*Mentha piperita* L.) and fruits of burnet rose (*Rosa pimpinellifolia* L.); its characterisation was based on available materials describing the mechanisms of action and efficacy in clinical applications. In the group of conventional herbal medicine preparations, the drug "Immunal" (Sandoz, Switzerland) was considered, which contained dry and liquid extracts of purple coneflower (*Echinacea purpurea)* and it was studied in various forms – from tablets of

80 mg (240-320 mg/day) to a solution of 2.5 mL three times a day [13-15]. Asian ginseng (*Panax ginseng)* was considered as an example of an adaptogenic agent based on clinical trials using encapsulated dry root extract at doses of 200-400 mg/day [16, 17]. To reflect the neuroprotective and antioxidant effects, *Ginkgo biloba* was analysed, presented in the studies in the form of tableted extracts standardised for ginkgoflavonglycoside content (120-240 mg/day) [18, 19]. The anti-inflammatory potential of herbal remedies from *Calendula officinalis* has been illustrated, which was used in clinical settings mainly in the form of alcohol tincture of 20-30 drops three times a day [20, 21]. Shiitake mushroom (*Lentinula edodes)* extract was included in the analysis described in publications as a encapsulated powder of β-glucans at doses of 1-3 g/day, which was characterised by a pronounced immunomodulatory effect [22, 23]. The choice of these products was determined by their ability to represent various groups of natural immunomodulators – from complex developments by researchers to classic herbal remedies and nutraceuticals of fungal origin. This approach allowed covering a wide range of mechanisms of action, including stimulation of the cellular and humoural immune response, anti-inflammatory effect and antioxidant protection, and also provided an opportunity to compare various pharmacological strategies in the analysis.

As part of the analytical approach, the evaluation of drugs was carried out at two levels: immunological and clinical. At the immunological level, data on macrophage activation, T-lymphocyte proliferation regulation, B-lymphocyte (bone marrow lymphocyte) functional activity, natural killer cytotoxicity, and the balance of pro-inflammatory and anti-inflammatory cytokines, in particular IL and TNF-α, were considered. The clinical level included analysis of such indicators as the frequency of infectious complications, the duration of the course of diseases, the effectiveness of immunoprophylaxis, and the severity of correction of inflammatory processes. The choice of publications under study was based on the availability of standardised forms of drugs, a sufficient evidence base in the form of randomised clinical trials or experimental models, a clear description of dosages, and the availability of safety profile data. As part of the study, natural immunomodulators were compared by their mechanisms of action, clinical effects, level of evidence and safety profile, which provided an opportunity for a comprehensive assessment of their therapeutic potential.

The choice of the publications was based on several criteria: for registered medicines and dietary supplements of international production – the availability of standardised release forms, described dosages, data from randomised clinical trials or preclinical models, and a safety profile. The inclusion of "Immunormalin" in the analysis was conditioned by its use in practice as a biologically active additive of Ukrainian production and the original design by S.G. Avakian. The authors noted that they have no commercial or personal interest in promoting the product, and the results and conclusions of the study are presented solely for scientific purposes. Despite the lack of standardised publications and open data on the composition, the drug continued to be considered in the context of potential use in clinical immunocorrection. Its consideration demonstrated the presence in national practice of drugs that claimed an immunomodulatory effect, but required further scientific verification. The limitation was that the study was theoretical in nature and was based on the analysis of already available data without conducting its own clinical trials.

RESULTS AND DISCUSSION

Immunomodulatory drugs: Clinical effects and mechanisms of action. Immunomodulatory drugs are a separate group of agents whose action is aimed at restoring or correcting the functional activity of the immune system. In clinical practice, they are used both for the prevention and treatment of infectious, inflammatory and immunodeficiency conditions. Different classes of immunomodulators differ in origin, composition and spectrum of active mechanisms, but they are united by their ability to influence cellular and humoural links of immunity. Plant complexes containing a rich set of polyphenols, polysaccharides, essential oils, and vitamins exhibit a mild, multi-vector effect, combining anti-inflammatory, antioxidant, and adaptogenic properties. The drug "Immunormalin" is a multicomponent dietary supplement in the form of an oral solution and suppositories, which includes a complex of plant extracts in combination with ascorbic acid, citric acid, and sodium benzoate. The bioactive composition includes polyphenols, anthocyanins, flavonoids, phenolic acids, essential oils, and inulin, which determines antioxidant, anti-inflammatory, prebiotic, and sedative mechanisms of action. The recommended dosage was 5-10 mL of the solution 1-2 times a day for a course of 2-4 weeks.

The results of the analysis of the scientific literature showed that black currant extracts were characterised by high concentrations of anthocyanins and phenolic acids, which neutralised free radicals, stabilised cell membranes, and maintained the viability of immunocompetent cells. Polyphenols of this plant also regulated the production of pro-inflammatory and anti-inflammatory cytokines, reduced the intensity of inflammation, and enhanced the barrier function of the airway epithelium. M. Oczkowski [24] proved that bioactive compounds of *Ribes nigrum* L. had a pronounced antioxidant and immunomodulatory effect, which corresponded to the effects of this component in the drug. It was found that rosehip fruits contained phenolic acids, ascorbic acid, and biologically active fatty acids, which enhanced antioxidant protection, activated collagen synthesis, and accelerated tissue repair. Simultaneously, they reduced the secretion of TNF-α and IL-6, which contributed to the regulation of the humoural link of immunity. These effects were confirmed by the M. Pashaei & H. Hassanpour [25], who showed that *Rosa pimpinellifolia* L. combined antioxidant and anti-inflammatory properties, which justified its importance as a basic source of antioxidants in the drug.

Literature data have shown that chamomile extracts contain flavonoids, coumarins, and essential oils that inhibit the expression of pro-inflammatory mediators (TNF-α, IL-1β, IL-6) and stimulate IL-10 secretion. This action helps to reduce the hyper-inflammatory response and restore immune homeostasis. A.I. Drif *et al.* [26] found that *Matricaria chamomilla* L. had significant anti-inflammatory and antitumour potential due to inhibition of NF-kB and reduced levels of pro-inflammatory cytokines, which confirmed its role

in normalising the immune response in multicomponent formulas. Linden and peppermint extracts provided sedative and antispasmodic effects, reduced catecholamine secretion, and stabilised the hypothalamic-pituitary-adrenal axis, thereby reducing stress-induced immunosuppression. In addition, the bioactive substances of these plants had a moderate anti-inflammatory effect due to the regulation of cytokine release. B. Kadıoğlu & S. Kadıoğlu [27] confirmed that plants with adaptogenic and sedative properties were actively used during the coronavirus pandemic to maintain resistance, which was consistent with the use of linden and peppermint in the preparation.

Analysis of publications has shown that common chicory acts as a source of inulin, which stimulates the growth of bifidobacteria and lactobacilli in the intestine. Metabolites of short-chain fatty acids formed during fermentation affect the differentiation of T-lymphocytes and the function of dendritic cells, establishing a balanced mucosal immunity. This mechanism corresponds to the current concept of "gut-immunity", which is considered as a key factor in maintaining systemic immune regulation. The analysed data also confirmed that common horsetail and common knotgrass enrich the formula with silicon compounds, phenolic acids, and flavonoids, which exhibit antioxidant and anti-inflammatory activity. This is associated with an increased cytoprotective effect and maintaining antioxidant balance in immune cells. The findings of T. Kadiyska *et al.* [28] emphasised that multicomponent phytopreparations implemented a synergistic mechanism of action, combining antioxidant, anti-inflammatory and adaptogenic effects, which fully corresponded to the role of horsetail and knotgrass in the composition of the drug.

The drug "Immunal" belongs to the immunomodulatory agents of plant origin due to the use of a standardised extract of *Echinacea purpurea*. Its creation was aimed at providing a reproducible concentration of biologically active substances, which was important for maintaining clinical effectiveness. The dosage form included both 80 mg tablets and a liquid solution in doses of 2.5 mL three times a day, which made it possible to individualise therapy and increase the convenience of use. Due to the combination of alkylamides, phenolic compounds, and polysaccharides, the drug was able to affect various parts of the immune system. The published results of experimental models showed that the intake of the *Echinacea purpurea* extract stimulates macrophage activation, increases phagocytosis, and the production of pro-inflammatory cytokines of the first line of defence. An increase in T-lymphocyte proliferation and stimulation of IL-2 secretion, which activates the cellular response against viral and bacterial pathogens, were recorded. Such data reflect the role of the drug as a regulator of the early stages of the immune response. The study by S.F. Vieira *et al.* [13] showed that it was the combination of macrophage stimulation and T-lymphocyte activation that explained the increase in non-specific resistance when using the purple coneflower. The researchers stressed that this effect was important not only for the treatment, but also for the prevention of infections, as it formed a more balanced immune response. Clinical results showed that "Immunal" significantly reduced the frequency of episodes of acute respiratory infections, reduced the intensity of symptoms and the duration of the course of diseases. Patients who used the drug during preventive courses showed faster recovery of immunological parameters and less often needed additional drug therapy. These results were confirmed in a randomised placebo-controlled trial conducted by S.K. Lee *et al.* [14], which showed that the systematic use of the *Echinacea purpurea* extract increased the effectiveness of immunoprophylaxis among the adult population. The researchers noted that the clinical effect of the drug was particularly noticeable in patients with an increased risk of respiratory diseases, and also noted that the reproducibility of these results was possible due to the standardised composition of the drug, which ensured the stability of the therapeutic effect.

Thus, an important characteristic of "Immunal" was its standardisation. While purle coneflower-based dietary supplements showed significant composition variability, the registered drug provided stable levels of active substances. A. Demyd *et al.* [15], using high-performance thin-layer chromatography, demonstrated that it was the dosage forms that had the most reproducible profile, while additives often differed in the content of bioactive components. This confirmed that clinical results can be associated primarily with the use of standardised drugs, and not with uncontrolled forms. Another important property of this drug is its ability to affect the balance of cytokines. There was a decrease in the secretion of excess pro-inflammatory mediators, including TNF-α and IL-6, and an increase in the level of anti-inflammatory IL-10. This mechanism helped not only to reduce the severity of inflammatory processes, but also to prevent excessive tissue damage in acute infections. Analysis by S. Choudhary *et al.* [29] confirmed that the immunomodulatory effect of phytoactive compounds from purple coneflower was combined with the anti-inflammatory effect, which made it suitable for both prevention and adjunctive therapy. The safety assessment showed that "Immunal" had a favourable tolerance profile. Side effects were isolated and mostly limited to mild allergic reactions. Long-term use of the drug in the recommended doses was not accompanied by the development of serious complications. This allowed using this drug in repeated courses, in particular in periods of increased seasonal morbidity.

Generalisation of literature sources showed that plant immunomodulatory complexes, in particular "Immunormalin" and "Immunal", were characterised by a combination of antioxidant, anti-inflammatory, adaptogenic, and prebiotic effects. Publications have indicated that their use is associated with reducing the frequency and duration of respiratory infections, restoring cytokine balance, and maintaining immune homeostasis. The standardised composition and favourable safety profile emphasise the potential of these drugs as a component of strategies for immunocorrection and prevention of infections in clinical practice.

Adaptogenic herbal remedies and their effect on immunity. Ginseng belongs to adaptogens because its bioactive compounds combine the ability to regulate stress responses and simultaneously affect the immune system. The plant contains ginsenosides, polysaccharides, and other metabolites that determine the multi-vector mechanism of action. As part of the analysis results, it was shown that ginseng modulated cellular and humoural links of the immune response, increased resistance to infectious agents, and maintained a balance between pro-inflammatory and

anti-inflammatory responses. It was found that water-soluble ginseng polysaccharides activate phagocytosis, stimulate the production of nitric oxide and IL by macrophages, and enhance the differentiation of T-lymphocytes. In addition, the results of the analysed experimental models showed that these biopolymers can increase the secretion of interferons, which play a leading role in antiviral protection. The findings by L. Cui e*t al.* [16] confirmed that ginseng flower heteropolysaccharides activated macrophages and T lymphocytes, and induced the secretion of IL-2 and IFN-γ (interferon-gamma), which was crucial for the development of an effective cellular response.

Data from analysed preclinical studies have shown that standardised extracts of *Panax ginseng* stimulated cytotoxic activity of NK cells and cytotoxic T-lymphocytes, which was manifested in the growth of elimination of virus-infected cells and in inhibition of tumour development in model organisms. In parallel, there was a decrease in the levels of pro-inflammatory cytokines, in particular, TNF-α and IL-6, and stimulation of the secretion of anti-inflammatory IL, such as IL-10, which ensured the maintenance of immune balance and reduced the severity of inflammatory responses. Generalisation made by Z.A. Ratan *et al.* [17] confirmed these results, indicating that the adaptogenic effects of ginseng were closely related to the ability of ginsenosides and polysaccharides to activate the cytotoxic function of T-lymphocytes and NK cells, block the activation of NF-kB and other signalling pathways. As a result, the production of pro-inflammatory mediators was simultaneously suppressed and the antitumour response was enhanced, which made ginseng a promising candidate for integration into antitumour immunotherapy regimens and correction of chronic inflammatory conditions.

The analysis showed that ginseng had a pronounced antioxidant effect, which was implemented by reducing the level of reactive oxygen species and activating antioxidant defence enzymes – superoxide dismutase and catalase. This helped to protect cells and tissues from oxidative damage, which usually accompanies chronic inflammation and immune dysfunctions. M. Balasubramaniam *et al.* [30] interpreted this mechanism as a key factor in the anti-inflammatory effect of ginseng, which allowed increasing its effectiveness in the complex therapy of inflammatory pathologies. This effect is particularly important in the context of low-grade chronic inflammation, where immune overactivation and oxidative stress act as parallel damaging factors [31]. The results of clinical trials showed that the use of standardised ginseng extracts reduced the incidence of acute respiratory infections, and reduced the duration of the course of diseases. Patients who took capsulated forms of the drug at doses of 200-400 mg per day showed faster recovery of immunological parameters compared to the control group [17]. The review by C.J.W. Liang *et al.* [32] confirmed that *Panax ginseng* was characterised by low toxicity and good tolerability, and the risk of side effects remained minimal even with prolonged use, which justified its inclusion in preventive regimens.

Ginkgo biloba is one of the most studied herbal remedies, combining antioxidant, anti-inflammatory, and immunomodulatory properties. Its standardised extracts contain ginkgoflavonglycosides and terpenoid lactones, which provide a wide range of biological activity [33]. The results of the analysis showed that the use of ginkgo extracts led to a decrease in the expression of pro-inflammatory genes and an increase in the activity of effector cells, including NK cells and cytotoxic T-lymphocytes. Simultaneously, there was a restoration of cytokine balance regulation by inhibiting TNF-α and IL-6 secretion and stimulating IL-10 production. This reflected the drug's ability to strengthen natural immune defences and reduce the severity of chronic inflammation. These results were consistent with the findings of H.M. Abdel-Latif *et al.* [18], who proved that leaf extract of *Ginkgo biloba* activated immune cells, modified the transcription of cytokine genes, and provided multi-level control of the immune response.

Data from experimental models also showed that ginkgo extracts had the ability to enhance the body's antioxidant defences. The cells showed a decrease in the level of reactive oxygen species, activation of antioxidant enzymes – superoxide dismutase and glutathione peroxidase, and stabilisation of mitochondrial membranes. This was crucial for reducing oxidative stress, which often accompanies immune dysfunctions and chronic pathologies. In this context, R. Das *et al.* [19], interpreted the antioxidant effect of *Ginkgo biloba* as one of the factors of maintaining cellular integrity and resistance of the body to immune-dependent diseases. Clinical data have shown that the inclusion of ginkgo in therapeutic regimens reduces the incidence of exacerbations of chronic diseases associated with an imbalance of the immune system and improves the overall immune status of patients. According to S.N. Mousavi *et al.* [34], using standardised extracts of *Ginkgo biloba* at doses up to 500 mg/day, IL-6 levels were reduced, which indicated their anti-inflammatory potential. The researchers emphasised that it was the suppression of pro-inflammatory cytokines that explained the clinical benefits of ginkgo in conditions associated with chronic inflammation.

In addition to the effect on the immune system, *Ginkgo biloba* had a positive effect on microcirculation and endothelial function, which created additional conditions for an optimal immune response. In clinical and experimental observations, there was an improvement in blood supply to tissues, a reduction in ischaemic damage, and normalisation of metabolic processes. This complex effect made allowed using ginkgo as a universal agent that can be integrated into therapeutic schemes of various profiles. F.G. Barbosa *et al.* [35] confirmed that the multifactorial action of *Ginkgo biloba* makes it a promising representative of plant immunomodulators with clinically significant effects. The safety of the drug also remained an important aspect. Ginkgo has been shown to be characterised by low toxicity and good tolerability even with prolonged use. Simultaneously, the need for strict compliance with standardised dosages was noted, because their excess could change the pharmacokinetic profile and reduce therapeutic efficacy, which was consistent with the conclusions of F. Buonfiglio *et al.* [36]. The researchers emphasised that it was standardised extracts that guaranteed clinical reliability and reproducibility of effects, while deviation from optimal doses could pose a risk to patients.

The results of the analysis showed that adaptogenic herbal remedies, in particular *Panax ginseng* and *Ginkgo biloba*, had a complex effect on the immune system, combining activation of cellular and humoural links with

regulation of the cytokine profile, antioxidant and anti-inflammatory effects. Ginseng was effective in stimulating the antiviral and antitumour response, while ginkgo provided an additional neuroprotective and vascular-regulating component that enhanced its immunomodulatory potential. Both drugs were characterised by good tolerability and low toxicity, which justified their use in both preventive and therapeutic regimens. Thus, their multifactorial action and the presence of a clinical evidence base make these drugs promising in strategies for maintaining and correcting immune function.

Anti-inflammatory phytonutrients and mushroom extracts with immune activity. Studies of anti-inflammatory immunomodulatory phytonutrients have led to a detailed investigation of *Calendula officinalis*, which has become a model for analysing the relationship between plant bioactive compounds and mechanisms of immune response regulation. Its extracts include flavonoids, saponins, triterpenoids, carotenoids, and essential oils, which cause a wide range of biological effects. Of particular importance is the anti-inflammatory and immunomodulatory activity associated with the regulation of the cytokine profile and inhibition of key pro-inflammatory mediators.

Analysis of the results of preclinical models showed that common marigold extracts reduced the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL 6. In parallel, an increase in the secretion of anti-inflammatory IL-10 was observed, which provided a restoration of regulation between pro- and anti-inflammatory processes. This reflected the plant's ability to reduce excessive immune activation and prevent tissue damage. Such effects were consistent with generalisation by K. Patil *et al.* [20], who stressed that *Calendula officinalis* can influence a wide range of inflammatory mediators and form a balanced immune response. The analysed results showed that the use of alcohol tinctures of common marigold in experimental models of chronic inflammation reduced neutrophil infiltration into the lesion and reduced the activity of cyclooxygenase-2, which reduced the development of prostaglandins. In clinical observations, it was recorded that regular use of standardised forms of common marigold helped to reduce the symptoms of inflammatory diseases of the mucous membranes and skin. These data were correlated with the findings by S.I. Abdelwahab *et al.* [21], who demonstrated that long-term studies of common marigold have confirmed its persistent anti-inflammatory and immunomodulatory activity in various fields of medicine.

The antioxidant effect of *Calendula officinalis* also attracted considerable attention. Its bioactive compounds have been shown to reduce reactive oxygen species levels, restore the balance of antioxidant enzymes, and protect cells from oxidative stress, which often accompanies immune dysfunctions. This is of particular importance in the treatment of chronic inflammatory processes associated with an imbalance of the immune system. Analysis by A.M. Rezende *et al.* [37], confirmed that the active ingredients of common marigold combine anti-inflammatory and antioxidant properties, thereby expanding the range of its clinical use. Clinical results also showed that the use of *Calendula officinalis* extracts in the form of tinctures or tablet forms, it was associated with a decrease in the frequency of infectious complications, a reduction in the duration of respiratory diseases, and an improvement in overall immune status. Patients showed good tolerability of the drugs, which emphasised their safety in long-term use. Comparative review by G. Dhingra *et al.* [38], generalised that biologically active compounds of common marigold were characterised by low toxicity and high biocompatibility, and therefore could be used as a basis for creating nutraceuticals and phytopreparations with proven immunomodulatory activity.

The study of mushroom extracts in the context of anti-inflammatory immunomodulatory nutraceuticals focused on beta-glucans from shiitake mushrooms. These polysaccharides can affect innate and adaptive immunity by activating macrophages, dendritic cells, and NK cells, and by regulating the cytokine profile. The results of the analysis showed that shiitake beta-glucans stimulated macrophage activity, increasing their ability to phagocytosis, nitric oxide production, and early response cytokine secretion. Simultaneously, dendritic cells were activated, which led to more efficient presentation of antigens and triggering adaptive immune responses. As noted by I. Mirończuk-Chodakowska *et al.* [22], such effects confirmed the role of β-glucans as natural adjuvants that can enhance the effectiveness of the immune system. These results showed the ability of shiitake beta-glucans to act as key modulators between innate and adaptive immunity, making them promising in the prevention and treatment of infectious diseases, in particular viral aetiology.

Analysis of published data showed that β-glucans from *Lentinula edodes* increased the secretion of IL-12, which stimulates the differentiation of T-helpers by Th1 type. This leads to the activation of cytotoxic T-cells and NK cells, which are involved in antitumour and antiviral protection. Inhibition of tumour growth and more intensive elimination of virus-infected cells were observed in the model organisms. C. Cerletti *et al.* [23] confirmed that it was the ability of β-glucans to shift the balance towards cellular immunity that was crucial for their antitumour potential. The use of shiitake extracts was accompanied by inhibition of the synthesis of TNF-α and IL-6, which are key mediators of chronic inflammation. Simultaneously, IL-10 production was increased, which helped to reduce excessive immune activation and prevent tissue damage. E.J. Murphy *et al.* [39] emphasised that even β-glucans obtained from the same mushroom samples could show differences in the strength of the effect on the cytokine profile, which highlighted the need for standardisation of extracts for clinical practice.

In addition to affecting the cytokine balance, beta-glucans of *Lentinula edodes* showed pronounced antioxidant properties. Systematisation of experimental data indicated a decrease in the level of reactive oxygen species and activation of key antioxidant defence enzymes – superoxide dismutase and glutathione peroxidase. This helped to protect cell membranes and mitochondria from oxidative damage. D. Kumar *et al.* [40] interpreted these effects as one of the links in preventing the development of immunodeficiency states and the progression of malignant processes, since reducing oxidative stress allowed increasing cell resistance to damage. The analysed experimental data confirmed that shiitake beta-glucans can reduce lipopolysaccharide-induced inflammatory changes and support

immune regulation. M. Jafari *et al.* [41] showed that extracts of *Lentinula edodes* adjusted haematological parameters and normalised immune response in the acute inflammation model, which indicates their potential as part of immunocorrective agents in immunodeficiency conditions.

Summarising the results of the literature analysis, it can be argued that *Calendula officinalis* and *Lentinula edodes* represent different but complementary areas of natural immunocorrection. *Calendula officinalis* has proven to be an effective anti-inflammatory agent capable of suppressing over-activation of cytokines and protecting tissues from damage, while shiitake beta-glucans have been characterised as universal natural adjuvants that enhance both innate and adaptive immune responses. Both agents simultaneously demonstrated antioxidant properties, due to which they provided multi-level protection of cells and the body as a whole. The combination of these effects determines their significance in complex therapeutic and preventive strategies aimed at maintaining immune homeostasis and reducing the risk of chronic pathologies.

Comparative efficacy and safety of natural immunomodulators in practice. Generalisation of information about natural immunomodulators allowed identifying common features and differences in their effect on the immune system. Despite the different nature and composition, most drugs combine antioxidant, anti-inflammatory, and immunomodulatory mechanisms, while individual drugs show additional effects – adaptogenic, prebiotic, or sedative. The most characteristic mechanisms of action and clinical effects are systematised in Table 1, which allows clearly assessing their therapeutic capabilities and potential for use.

Table 1. Mechanisms of action and clinical effects of natural immunomodulators

Agent	Basic mechanisms	Key clinical effects
"Immunormalin"	Antioxidant, anti-inflammatory, prebiotic, sedative.	Reduction of the severity of respiratory infections, maintaining immune balance, reduction of stress-induced immunosuppression.
"Immunal"	Macrophage activation, IL-2 stimulation, phagocytosis.	Prevention of acute respiratory infections, reduction of their duration.
Panax ginseng	Adaptogenic effect, activation of T-lymphocytes and NK cells.	Increase of resistance to infections, antitumour potential.
Ginkgo biloba	Antioxidant, cytokine regulation, vascular effect.	Reduction of the frequency of exacerbations of chronic diseases, immune stabilisation.
Calendula officinalis	Inhibition of TNF-α, IL-1β, IL-6; stimulation of IL-10.	Anti-inflammatory effect, reduction of symptoms of inflammatory conditions.
Lentinula edodes	Activation of dendritic cells, NK cells; increase in IL-12.	Antiviral and antitumour immune response, prevention of recurrent infections.

Source: compiled by the authors

Comparative analysis showed that a common property for all the drugs considered was the presence of antioxidant potential through polyphenols, flavonoids, anthocyanins, or polysaccharides. It was this action that established the basic level of cellular protection, prevented oxidative damage to immunocompetent cells, and created conditions for the normalisation of the immune response. However, the differences were in additional mechanisms that determined the specifics of the clinical use of each drug. "Immunormalin" was noted for its most multi-vector effect, combining antioxidant, anti-inflammatory, prebiotic, and sedative mechanisms. This set it apart from other agents because it simultaneously affected cytokine regulation, the gut microbiota, and the neuroendocrine system. No other drug has demonstrated such complexity. In contrast, "Immunal" acted mainly through the activation of macrophages and T-lymphocytes, that is, it had a more narrowly directed immunomodulatory mechanism, effective mainly for the prevention of acute respiratory infections. Ginseng occupied an intermediate position: its action went beyond purely immunomodulation and included an adaptogenic effect. A common feature with "Immunormalin" was multivariate regulation, but the difference was the predominant emphasis on antitumour and antiviral protection through activation of NK cells and T-lymphocytes.

While *Ginkgo biloba* also shared the ability to reduce inflammation with ginseng, its uniqueness was determined by the combination of immune and vascular-regulating effects, which was of little importance in chronic pathologies. Common marigold had a common anti-inflammatory effect with ginkgo, but realised the effect through a clearer regulation of the cytokine profile (a decrease in TNF-α, IL-1β, IL-6 with an increase in IL-10). Thus, it showed the most pronounced anti-inflammatory profile among all the compared agents. Shiitake mushrooms shared a common mechanism with purple coneflower and ginseng to stimulate cellular immunity, but they differed in a much wider spectrum – from antiviral to antitumour. Unlike "Immunormalin", they did not include a prebiotic or sedative effect, but acted as natural adjuvants, enhancing the immune response to an antigenic challenge. Therefore, a common property of natural immunomodulators was the antioxidant effect and the ability to regulate cytokine balance. Their differences were determined by the degree of multifactorial mechanisms: from narrowly directed activation of macrophages and T-lymphocytes (purple coneflower) to integrated effects on immune, metabolic, and neuroendocrine processes ("Immunormalin"). It was due to the combination of extracts of several plants with multidirectional bioactivity that "Immunormalin" demonstrated the most complex action profile. In contrast, other drugs occupied specific therapeutic niches – prevention of respiratory infections (purple coneflower), correction of inflammatory processes (marigold, chamomile, ginkgo) or antitumour and antiviral support (ginseng, shiitake beta-glucans). In addition to the mechanisms of action and the spectrum of clinical effects, an

important criterion is the level of evidence and safety profile. It was these parameters that determine the possibilities of integrating funds into the practice of evidence-based medicine and their clinical significance. Generalised data on the availability of evidence and safety features of individual drugs and nutraceuticals are shown in Table 2.

Table 2. Level of evidence and safety profile of natural immunomodulators

Agent	Evidence base	Safety
"Immunormalin"	Local data confirmed only by patient reviews, there are no randomised controlled trials.	Portability data are limited; standardised safety assessments are not available.
"Immunal"	Randomised controlled trials, clinical efficacy confirmed.	Generally safe; isolated cases of mild allergic reactions.
Panax ginseng	Randomised controlled trials, generalisations in reviews.	Isolated gastrointestinal disorders have been reported; toxicity is low.
Ginkgo biloba	Meta-analyses of clinical trials.	Safety depends on compliance with dosages; there may be an increased risk of bleeding.
Calendula officinalis	Preclinical data, individual clinical observations.	Data indicate low toxicity and high biocompatibility.
Lentinula edodes	Preclinical and isolated clinical data.	No serious complications were recorded; tolerability is satisfactory.

Source: compiled by the authors

Comparison of natural immunomodulators revealed significant differences in the level of evidence and safety, which determined their clinical significance. All the considered drugs were combined with low toxicity and good tolerability, but the degree of scientific confirmation of their effects was uneven. A significant evidence base was demonstrated by the purple coneflower-based preparation "Immunal" and ginseng, for which the results of randomised controlled trials were accumulated. This allowed classifying them as the "gold standard" of plant immunomodulators with proven clinical efficacy. *Ginkgo biloba* took an intermediate place: its effectiveness was supported by meta-analyses and systematic reviews, which provided a high level of confidence, but at the same time requires strict dosages to avoid undesirable effects. In contrast to these drugs, common marigold and shiitake mushrooms were characterised mainly by preclinical confirmations and a limited number of clinical trials. Although their results showed pronounced immunomodulatory and anti-inflammatory potential, the rationale for widespread clinical use remained insufficient. "Immunormalin" was distinguished among all drugs by the most complex composition, combining several plants with different mechanisms of action. This created a synergistic effect and potentially expanded the scope of application – from preventing respiratory infections to reducing stress-induced immunosuppression. In combination, the analysis showed that immunomodulators with proven clinical efficacy were characterised by a high level of standardisation and reproducibility of results, while multicomponent nutraceuticals showed promising, but not yet sufficiently confirmed potential. Thus, further development of this field requires combining the accumulated experience of application with contemporary requirements of evidence-based medicine.

Thus, the comparative analysis showed that natural immunomodulators form a wide range of opportunities for maintaining the immune system, but their clinical value differs significantly depending on the level of evidence and safety profile. Drugs with proven efficacy in randomised controlled trials (purple coneflower, ginseng, ginkgo) can be considered as the most reasonable for integration into modern practice, while drugs with a predominantly preclinical base (common marigold, shiitake) and local multicomponent formulas ("Immunormalin") remain promising, but require further standardisation and large-scale clinical trials. Therefore, the introduction of such tools into medical practice should be based on combining their multi-factor potential with the requirements of evidence-based medicine.

CONCLUSIONS

Generalisation of the results confirmed that the multicomponent drug "Immunormalin" had a complex effect on the immune system due to a combination of antioxidant, anti-inflammatory, prebiotic, and sedative mechanisms. The presence of various plant extracts allowed combining several areas of action: regulation of the cytokine profile, maintenance of barrier functions of the mucous membranes, modulation of the intestinal microbiota, and reduction of stress-induced immunosuppression. This synergistic effect caused a decrease in the frequency and severity of symptoms of respiratory infections and helped to maintain immune balance. Generalised data showed that the action of Asian ginseng and *Ginkgo biloba* was based on different but complementary mechanisms. Ginseng increases the activity of NK cells and T-lymphocytes, providing antiviral and antitumour potential, and also acts as an adaptogen, increasing the body's resistance to stress factors. *Ginkgo biloba* has an immunomodulatory and at the same time vascular-regulating effect, which is especially valuable in chronic pathologies where immune and metabolic disorders are combined. Both drugs were characterised by good tolerability and a low risk of toxicity, which created prerequisites for their use in both prevention and complex therapy.

The analysis confirmed that the common marigold has a well-defined anti-inflammatory mechanism of action, reducing the production of TNF-α, IL-1β and IL-6 while increasing the secretion of IL-10. This restores the balance between pro- and anti-inflammatory processes and prevents excessive tissue damage in response to infectious or autoimmune stimuli. The effects of the common marigold were found in both preclinical models and clinical

observations, where there was a reduction in symptoms of inflammatory diseases of the mucous membranes and skin. In turn, shiitake mushrooms implement a different mechanism – activation of dendritic cells and NK cells with increased IL-12 production. This provided powerful stimulation of the antiviral and antitumour response, and prevention of recurrent infections. Unlike the narrowly directed anti-inflammatory effect of common marigold, fungal beta-glucans act as natural immune adjuvants, strengthening the cellular link of immunity. Thus, both drugs occupy different niches: marigold – in reducing chronic inflammation, and shiitake – in maintaining an active immune defence against infections and neoplasms.

Comparative analysis showed that all natural immunomodulators were characterised by low toxicity and good tolerability, but the level of evidence base was significantly different. The most convincing results were obtained from purple coneflower ("Immunal") and ginseng, confirmed by randomised controlled trials, while ginkgo biloba was confirmed by meta-analyses and systematic reviews. Common marigold and shiitake mushrooms remained mostly at the level of preclinical confirmations. "Immunormalin" was characterised by a complex composition, but its effectiveness was based only on local data and required further thorough clinical testing. Further studies should be aimed at obtaining empirical evidence through randomised controlled trials involving representative samples of patients.

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CONFLICT OF INTEREST

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REFERENCES

[1] Bielenichev I, Gorchakova N, Harnyk T, Shumeiko O, Klymenko O, Klymenko O, et al. Immunomodulatory effect of phytopreparations. Phytotherapy. 2025;1:5–29. DOI: 10.32782/2522-9680-2025-1-18

[2] Burlou-Nagy C, Bănică F, Jurca T, Vicaș LG, Marian E, Muresan ME, et al. *Echinacea purpurea* (L) Moench: Biological and pharmacological properties. A review. Plants. 2022;11(9):1244. DOI: 10.3390/plants11091244

[3] Valdés-González JA, Sánchez M, Moratilla-Rivera I, Iglesias I, Gómez-Serranillos MP. Immunomodulatory, anti-inflammatory, and anti-cancer properties of ginseng: A pharmacological update. Molecules. 2023;28(9):3863. DOI: 10.3390/molecules28093863

[4] Hu Y, He Y, Niu Z, Shen T, Zhang J, Wang X, et al. A review of the immunomodulatory activities of polysaccharides isolated from *Panax* species. J Ginseng Res. 2022;46(1):23–32. DOI: 10.1016/j.jgr.2021.06.003

[5] Mohamed N. Pharmacological properties of polysaccharides of medicinal plants: A review of the effects on the immune system and potential applications in immunotherapy [Master's thesis]. Dnipro: Dnipro State Medical University; 2025.

[6] Ibrahim MA, Ramadan HH, Mohammed RN. Evidence that *Ginkgo biloba* could use in the influenza and coronavirus COVID-19 infections. J Basic Clin Physiol Pharmacol. 2021;32(3):131–43. DOI: 10.1515/jbcpp-2020-0310

[7] Samoilenko AM. Pharmaceutical development of an antiviral drug based on medicinal plant raw material in the form of tablets [Master's thesis]. Kyiv: Kyiv National University of Technologies and Design; 2021.

[8] Silva D, Ferreira MS, Sousa-Lobo JM, Cruz MT, Almeida IF. Anti-inflammatory activity of *Calendula officinalis* L. flower extract. Cosmetics. 2021;8(2):31. DOI: 10.3390/cosmetics8020031

[9] Oliveira BG, Santos LF, Costa MC, Bastos RW, Carmo PH, Santos DD, et al. Antimicrobial and immunomodulatory activities of dried extracts of *Echinacea purpurea*. Braz J Pharm Sci. 2022;58:e21026. DOI: 10.1590/s2175-97902022e21026

[10] Roszczyk A, Turło J, Zagożdżon R, Kaleta B. Immunomodulatory properties of polysaccharides from *Lentinula edodes*. Int J Mol Sci. 2022;23(16):8980. DOI: 10.3390/ijms23168980

[11] Vlassopoulou M, Yannakoulia M, Pletsa V, Zervakis GI, Kyriacou A. Effects of fungal beta-glucans on health – a systematic review of randomized controlled trials. Food Funct. 2021;12(8):3366–80. DOI: 10.1039/D1FO00122A

[12] Popovici LF, Oancea S. Calendula officinalis – overview on applications and pharmaceutical uses. Curr Trends Nat Sci. 2024;13(25):268–79. DOI: 10.47068/ctns.2024.v13i25.032

[13] Vieira SF, Gonçalves VM, Llaguno CP, Macías F, Tiritan ME, Reis RL, et al. On the bioactivity of *Echinacea purpurea* extracts to modulate the production of inflammatory mediators. Int J Mol Sci. 2022;23(21):13616. DOI: 10.3390/ijms232113616

[14] Lee SK, Lee DR, Kim HL, Choi BK, Kwon KB. A randomized, double-blind, placebo-controlled study on immune improvement effects of ethanolic extract of *Echinacea purpurea* (L.) Moench in Korean adults. Phytother Res. 2024;38(7):3645–59. DOI: 10.1002/ptr.8224

[15] Demyd A, Vronska L, Ivanusa I, Mykhalkiv M. Application of high-performance thin-layer chromatography for the identification of *Echinacea purpurea* in dietary supplements and medicinal products based on it. SWorldJournal. 2024;(27–2):3–18. DOI: 10.30888/2663-5712.2024-27-00-005

[16] Cui L, Chen L, Yang G, Li Y, Qiao Z, Liu Y, et al. Structural characterization and immunomodulatory activity of a heterogalactan from *Panax ginseng* flowers. Food Res Int. 2021;140:109859. DOI: 10.1016/j.foodres.2020.109859

[17] Ratan ZA, Youn SH, Kwak YS, Han CK, Haidere MF, Kim JK, et al. Adaptogenic effects of *Panax ginseng* on modulation of immune functions. J Ginseng Res. 2021;45(1):32–40. DOI: 10.1016/j.jgr.2020.09.004

[18] Abdel-Latif HM, Hendam BM, Nofal MI, El-Son MA. *Ginkgo biloba* leaf extract improves growth, intestinal histomorphometry, immunity, antioxidant status and modulates transcription of cytokine genes in hapa-reared Oreochromis niloticus. Fish Shellfish Immunol. 2021;117:339–49. DOI: 10.1016/j.fsi.2021.06.003

[19] Das R, Lami MS, Chakraborty AJ, Mitra S, Tallei TE, Idroes R, et al. *Ginkgo biloba*: A treasure of functional phytochemicals with multimedicinal applications. Evid Based Complement Alternat Med. 2022;2022(1):8288818. DOI: 10.1155/2022/8288818
[20] Patil K, Sanjay CJ, Doggalli N, Devi KR, Harshitha N. A review of *Calendula officinalis*-magic in science. J Clin Diagn Res. 2022;16(2):23–7. DOI: 10.7860/JCDR/2022/52195.16024
[21] Abdelwahab SI, Taha MM, Taha SM, Alsayegh AA. Fifty-year of global research in *Calendula officinalis* L. (1971-2021): A bibliometric study. Clin Complement Med Pharmacol. 2022;2(4):100059. DOI: 10.1016/j.ccmp.2022.100059
[22] Mirończuk-Chodakowska I, Kujawowicz K, Witkowska AM. Beta-glucans from fungi: Biological and health-promoting potential in the COVID-19 pandemic era. Nutrients. 2021;13(11):3960. DOI: 10.3390/nu13113960
[23] Cerletti C, Esposito S, Iacoviello L. Edible mushrooms and beta-glucans: Impact on human health. Nutrients. 2021;13(7):2195. DOI: 10.3390/nu13072195
[24] Oczkowski M. Health-promoting effects of bioactive compounds in blackcurrant (*Ribes nigrum* L.) berries. Rocz Panstw Zakl Hig. 2021;72(3):229–38. DOI: 10.32394/rpzh.2021.0174
[25] Pashaei M, Hassanpour H. Phenolic, amino acids, and fatty acids profiles and the nutritional properties in the fresh and dried fruits of black rosehip (*Rosa pimpinellifolia* L.). Sci Rep. 2024;14:19665. DOI: 10.1038/s41598-024-70574-5
[26] Drif AI, Yücer R, Damiescu R, Ali NT, Abu Hagar TH, Avula B, et al. Anti-inflammatory and cancer-preventive potential of Chamomile (*Matricaria chamomilla* L.): A comprehensive *in silico* and *in vitro* study. Biomedicines. 2024;12(7):1484. DOI: 10.3390/biomedicines12071484
[27] Kadıoğlu B, Kadıoğlu S. Medicinal and aromatic plants consumption habits of consumers in the coronavirus pandemic. Ataturk Univ Ziraat Fak Derg. 2021;52(3):325–34. DOI: 10.17097/ataunizfd.860913
[28] Kadiyska T, Tourtourikov I, Dabchev K, Zlatarova A, Stoynev N, Hadjiolova R, et al. Herbs and plants in immunomodulation. Int J Funct Nutr. 2023;4(1):1. DOI: 10.3892/ijfn.2023.31
[29] Choudhary S, Khan S, Rustagi S, Rajpal VR, Khan NS, Kumar N, et al. Immunomodulatory effect of phytoactive compounds on human health: A narrative review integrated with bioinformatics approach. Curr Top Med Chem. 2024;24(12):1075–100. DOI: 10.2174/0115680266274272240321065039
[30] Balasubramaniam M, Sapuan S, Hashim IF, Ismail NI, Yaakop AS, Kamaruzaman NA, et al. The properties and mechanism of action of plant immunomodulators in regulation of immune response – a narrative review focusing on *Curcuma longa* L., *Panax ginseng* C.A. Meyer and *Moringa oleifera* Lam. Heliyon. 2024;10(7):e28261. DOI: 10.1016/j.heliyon.2024.e28261
[31] Klymenko M. Progress and prospects in research on low-grade diffuse chronic inflammation: A literature review. Ukr J Med Biol Sport. 2025;10(1):16–29. DOI: 10.63341/ujmbs/1.2025.16
[32] Liang CJW, Woerdenbag HJ, Ekhart C, Vitalone A, van Hunsel FP. Safety considerations for natural products with adaptogenic and immunomodulating activities. Pharmaceuticals. 2025;18(8):1208. DOI: 10.3390/ph18081208
[33] Galkin A, Gorchakova N, Zaychenko G, Golembiovska O, Bondarenko L, et al. Efficiency and safety issues of modern multi-component herbal medicines. Kyiv: Igor Sikorsky Kyiv Polytechnic Institute; 2024. 264 P.
[34] Mousavi SN, Hosseinikia M, Yousefi Rad E, Saboori S. Beneficial effects of *Ginkgo biloba* leaf extract on inflammatory markers: A systematic review and meta-analysis of the clinical trials. Phytother Res. 2022;36(9):3459–69. DOI: 10.1002/ptr.7544
[35] Barbosa FG, de Mattos MC, Nunes FM, Mafezoli J, Oliveira MCF. Immunomodulation potential of woody plants. In: Sangwan N, Farag M, Modolo L, editors. Plants and phytomolecules for immunomodulation: Recent trends and advances. Singapore: Springer; 2022. P. 469–92. DOI: 10.1007/978-981-16-8117-2_16
[36] Buonfiglio F, Pfeiffer N, Gericke A. Immunomodulatory and antioxidant drugs in glaucoma treatment. Pharmaceuticals. 2023;16(9):1193. DOI: 10.3390/ph16091193
[37] Rezende AM, Albuquerque AL, Silva MJ, De Melo Cruvinel W, Gomes CM, Borges LL, et al. Drug-like properties and therapeutical potential of *Calendula officinalis* L. active ingredients. In: Taft C, de Lazaro S, editors. Progress in hydrogen energy, fuel cells, nano-biotechnology and advanced, bioactive compounds. Cham: Springer; 2024. P. 301–13. DOI: 10.1007/978-3-031-75984-0_12
[38] Dhingra G, Dhakad P, Tanwar S. Review on phytochemical constituents and pharmacological activities of plant *Calendula officinalis* Linn. Biol Sci. 2022;2(2):216–28. DOI: 10.55006/biolsciences.2022.2205
[39] Murphy EJ, Masterson C, Rezoagli E, O'Toole D, Major I, Stack GD, et al. β-Glucan extracts from the same edible shiitake mushroom *Lentinus edodes* produce differential *in-vitro* immunomodulatory and pulmonary cytoprotective effects – implications for coronavirus disease (COVID-19) immunotherapies. Sci Total Environ. 2020;732:139330. DOI: 10.1016/j.scitotenv.2020.139330
[40] Kumar D, Kashyap S, Gupta S. β-Glucans in mushrooms: Immunomodulatory and anticancer effects. In: Kumar D, Gupta S, Ansari M, Sangeeta, Hasan W, Kashyap S, et al., editors. Mushroom magic. London: CRC Press; 2024. P. 105–22. DOI: 10.1201/9781003570257
[41] Jafari M, Boskabaday MH, Rezaee SA, Rezaeian S, Behrouz S, Ramezannejad R, et al. Lentinan and β-glucan extract from shiitake mushroom, *Lentinula edodes*, alleviate acute LPS-induced hematological changes in mice. Iran J Basic Med Sci. 2023;26(7):836–42. DOI: 10.22038/IJBMS.2023.67669.14820

Сучасні підходи до імуномодуляції в клінічній практиці: аналіз дієтичних добавок та лікарських препаратів

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Анотація. Метою дослідження було встановлення імуномодулюючих властивостей рослинних засобів і перспектив їх клінічного впровадження. Методологія ґрунтувалася на теоретико-аналітичному узагальненні літературних даних щодо імуномодулюючих властивостей препаратів «Імунормалін», «Іммунал», женьшеню азійського, гінкго білоби, календули лікарської та грибів шиїтаке. Результати аналізу засвідчили, що «Імунормалін» відзначався найширшим спектром механізмів, поєднуючи антиоксидантні, протизапальні, пребіотичні та седативні ефекти, які одночасно охоплювали імунні, метаболічні й нейроендокринні шляхи. Така багатофакторність відрізняла його від більш вузькоспеціалізованих засобів та зумовлювала виокремлену позицію серед нутрицевтиків. На противагу, «Іммунал», створений на основі ехінацеї пурпурової, демонстрував спрямований імуномодулюючий механізм через активацію макрофагів і Т-лімфоцитів, що робило його переважно ефективним у профілактиці респіраторних інфекцій. Женьшень азійський поєднував властивості адаптогена та імуномодулятора, зумовлюючи підвищену стійкість організму до інфекцій та формування протипухлинного потенціалу. Гінкго білоба, поділяючи з женьшенем антиоксидантний і протизапальний вплив, мав додаткову судиннорегулюючу дію, що розширювало його застосування у хронічних патологіях та комплексній терапії метаболічних синдромів. Календула лікарська демонструвала найчіткіше виражений протизапальний профіль серед порівнюваних засобів, оскільки пригнічувала продукцію прозапальних цитокінів і стимулювала секрецію протизапальних медіаторів, що знижувало ризик тканинних ушкоджень при тривалих запальних процесах. Гриби шиїтаке, завдяки вмісту бета-глюканів, реалізовували функції природних ад'ювантів, підсилюючи клітинну імунну відповідь і поєднуючи противірусну та протипухлинну активність. Узагальнення результатів показало, що природні імуномодулятори мають спільну антиоксидантну дію, але відрізняються за багатофакторністю та клінічною спрямованістю, що зумовлює потребу у стандартизації й диференційованому застосуванні. Отримані результати можуть бути використані лікарями-клініцистами, фармакологами та науковцями у сфері доказової фітотерапії для оптимізації підбору імуномодулюючих засобів і розробки стандартизованих підходів до їх застосування

Ключові слова: запальний процес; цитокіни; макрофаги; лімфоцити; інтерлейкіни; біоактивні сполуки; інфекції

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