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Individual-psychological characteristics and the state of psychosocial adaptation of combatants with non-psychotic mental disorders who have experienced COVID-19

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Abstract. The relevance of the studied subject lies in the importance of investigating the individual-psychological characteristics, coping behaviour, and the state of psychosocial adaptation of combatants who have recovered from COVID-19, to develop personalised therapeutic and rehabilitation measures The purpose of the study was to determine the pathocharacterological characteristics, coping repertoire features, and psychosocial maladaptation in individuals who participated in combat operations, have non-psychotic mental disorders, and have experienced the coronavirus infection, in a comparative aspect. A total of 132 male combatants with non-psychotic mental disorders who did not have COVID-19 and 120 military personnel with the same mental disorders who had experienced the coronavirus infection were examined using Standardised Multifactor Personality Inventory, questionnaire "Methods of coping behavior", and the Social-Psychological Adaptation Diagnostic Method. It was found that combatants who had experienced COVID-19 exhibited specific personality characteristics that can be considered pathocharacterological transformations and signs of neuroticism. The core pathocharacterological changes included stable anxious-depressive and hyposthenic traits, while the auxiliary psychopathological constructs included stable emotionally labile, asthenic, hypochondriacal, and dysphoric manifestations. They also exhibited changes in coping repertoire with a predominance of non-constructive strategies of escape-avoidance and confrontation, along with a deactualisation of constructive coping strategies such as problem-solving planning, seeking social support, self-control, and positive reappraisal. Furthermore, pronounced manifestations of psychosocial maladaptation were observed in all key domains. COVID-19 acts as a complicating factor, exacerbating psychopathological symptoms, contributing to the formation of pathocharacterological traits, and deepening the psychosocial maladaptation of combatants with non-psychotic mental disorders. The obtained data provide an opportunity to personalise therapeutic, rehabilitation, and preventive measures, considering the pathocharacterological individual-psychological characteristics and coping behaviour, and to improve existing programmes of social-psychological adaptation for combatants

Keywords: combatants; neurotic disorders; pathocharacterological traits; coping behaviour; psychosocial maladaptation

INTRODUCTION

In the context of Ukraine's ongoing war of liberation, the preservation and restoration of the mental health of Ukrainian soldiers are of exceptional importance. The vulnerability of military personnel (MP) to non-psychotic mental disorders is extremely high and poses a serious threat to both their mental and overall health. In recent years, another fundamental factor contributing to mental disorders is the COVID-19 pandemic. However, the combined impact of war-related stress and the coronavirus infection on the mental well-being of military personnel remains understudied, making it challenging to develop personalised therapeutic, rehabilitation, and preventive programmes aimed at preserving and restoring the mental health of soldiers who have experienced COVID-19.

Participation in war is one of the most challenging psychosocial stressors, and MP are among the populations most affected by mental disorders. War veterans often develop neuro-psychiatric conditions, including depression,

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post-traumatic stress disorder, and anxiety disorders, which tend to have a chronic nature and impact overall adaptation and functioning [1].

A. Haydabrus *et al*. [2], analysing the psychiatric consequences among Ukrainian military personnel who were hospitalised after the start of a full-scale invasion, note that armed conflicts have led to an increase in the number of mental disorders. The structure of psychopathological manifestations is polymorphic, with a predominance of anxiety, dissociative, stress-related, somatoform, and other non-psychotic mental disorders, and a significant proportion of mental and addictive disorders related to substance use.

I. Pavlova *et al.* [3] note that a considerable portion of Ukrainian MP with non-psychotic mental disorders have reached threshold levels of clinical symptoms of anxiety (44.4%), depression (43.3%), and insomnia (12.4%). The prevalence of psychopathological symptoms varies depending on constitutional-biological, social, individual-psychological, and professional factors.

The role of individual-psychological factors in the development of mental disorders in MPs was highlighted in the studies by Y. Levi-Belz *et al.* [4] and G. Zerach *et al.* [5], which showed that personal characteristics of MPs influence the level of aggression, depression, suicide risks, and resistance to war-related stress.

C. Inoue *et al.* [6] emphasise that participation in combat is associated with a high risk of psychological and social maladaptation, which mutually reinforce and aggravate post-traumatic, depressive, anxiety disorders, suicidal risks, and addictive behaviour characteristic of MPs.

With the onset of the COVID-19 pandemic, reports of acute and prolonged mental disorders associated with coronavirus infection have emerged. Studies have confirmed the presence of short -, medium -, and long-term neuropsychiatric consequences of COVID-19 [7, 8]. While some general (headache) and specific (anosmia, dysgeusia) psychiatric disorders are common in the acute phase but typically resolve within weeks or months, cognitive and affective symptoms, including attention deficit and anxiety-depressive symptoms, tend to increase over time.

Researchers state that the high prevalence of long-term mental health symptoms of COVID-19 is probably due to both biological and psychological and social factors [9, 10]. The morphological changes underlying the psychiatric post-COVID syndrome are associated with systemic inflammation, massive neuroinflammatory reactions including reactive astrogliosis and microglial activation, while psychological reactions to the illness, primarily intense fear and anxiety, considerably increase the likelihood of mental disorders in both individuals with pre-existing mental health problems and mentally healthy individuals [11-13].

However, studies investigating the impact of COVID-19 on the mental health of MPs and analysing the individual-psychological characteristics of such patients have not been found. These studies are crucial for personalising therapeutic and rehabilitation interventions, and developing comprehensive programmes for the prevention of psychiatric consequences in MPs who have recovered from COVID-19 within the context of armed conflicts.

The purpose of this study was to investigate the individual-psychological and pathocharacterological features, coping strategies, and manifestations of psychosocial maladaptation in MPs with non-psychotic mental disorders who have and have not experienced COVID-19.

MATERIALS AND METHODS

With adherence to the principles of biomedical ethics, a total of 252 male patients who had participated in military actions (confirmed by relevant documents) and sought psychiatric assistance at the Ternopil Regional Psychoneurological Hospital and received consultative and therapeutic assistance at the Department of Psychiatry, Narcology, and Medical Psychology of the I.Ya. Horbachevsky Ternopil National Medical University from 2020 to 2022 were examined. These patients were diagnosed with non-psychotic mental disorders (NMD) according to International Classification of Diseases, 10th Revision (ICD-10). The selection of psychodiagnostic methods was determined by the research objectives and ensured a comprehensive assessment of individual-typological and pathocharacterological features, including the state of socio-psychological adaptation and coping repertoire.

The examination was conducted through personal completion of test questionnaires by the participants in the presence of the researcher. Before filling out the test questionnaire, the purpose of the examination was explained to the participants, and instructions were provided according to the standard procedure outlined in the respective methodology. The psychodiagnostic tools of the study included:

1. Standardised Multifactor Personality Inventory (SMPI) [14], which is the most detailed and informative personality questionnaire based on the leading thesaurus of contemporary differential psychology, adopts a holistic approach to the examination y of personality, considering the unity of biological and social factors. Scores on each scale were determined in test scores (T-scores). Scores below 70 T-scores were interpreted as normal, scores between 70 and 80 T-scores indicated personality accentuations, and scores above 80 T-scores suggested more significant changes in mental functioning that required further clarification.

2. Questionnaire “Methods of coping behavior” by S. Folkman & R. Lazarus [15] was used to determine coping mechanisms and strategies for dealing with difficulties in various life situations and establish coping strategies. The questionnaire consisted of 50 statements, and respondents rated each statement on a four-point scale, where 0 represented “never”, 1 represented “rarely”, 2 represented “sometimes”, and 3 represented “often”. The test result was presented in points for each of the coping strategies. The strategy with the highest score was considered dominant.

3. The Social-Psychological Adaptation Diagnostic Method by C. Rogers & R.F. Dymond [16] consisted of 101 statements. Participants were asked to rate their attitudes toward each statement on a seven-point scale, where 0 represented “does not concern me at all”, 1 represented “does not concern me”, 2 represented “doubtful if it applies to me”, 3 represented “hesitant to apply it to myself”, 4 represented “similar to me, but uncertain”, 5 represented “similar to me”, and 6 represented “definitely about me”. The interpretation of the test results involved determining six indicators: adaptation, acceptance of others, internality,

self-perception, emotional comfort, and the desire for dominance. The results were compared to control values for each scale and interpreted as low below the uncertainty zone and high after reaching the maximum score in the uncertainty zone.

Among the participants, the following groups were identified: individuals who did not have COVID-19, comprising 132 individuals (Group 1 – G1), and individuals who had recovered from COVID-19, confirmed by relevant medical documents, comprising 120 individuals (Group 2 – G2).

The statistical analysis of differences was performed using the non-parametric Mann-Whitney test. Differences were considered statistically significant at a level above 95% ($p < 0.05$).

The study adhered to the ethical norms of the Helsinki Declaration for research involving human participants [17]. All individuals included in the study provided informed consent to participate.

RESULTS

The analysis of personality profiles using the SMPI revealed that the dominant positions in MP with NMD who have had COVID-19 were occupied by the scales of anxiety and pessimism, with significantly higher scores in Group 2 (G2) compared to Group 1 (G1) (Table 1). These scores indicate a greater manifestation of hyposthenic traits, a passive-suffering attitude, a tendency to intensely experience failures, worry, motivation for avoidance, elevated levels of anxiety, avoidance of active behaviour, decreased mood, heightened sense of guilt, self-doubt, and maladaptive states characterised by confusion, restlessness, intrusive fears, feelings of danger, and sleep disturbances. In combination with elevated scores on the hypercontrol scale, this indicates the formation of signs of neurotic hypercontrol with increased attention to somatic discomfort, suppression of spontaneity, inhibition of active self-realisation, and a desire for control over aggression.

Table 1. Scores based on the standardised method of personality assessment (in T-points)

SMPI scales	Indicators on scales, M ± m		p
	G1	G2	
1 (hypercontrol)	64.9 ± 9.6	65.6 ± 9.0	> 0.05
2 (pessimism)	67.0 ± 18.6	74.9 ± 10.0	< 0.05
3 (emotional lability)	59.0 ± 7.1	61.5 ± 8.3	< 0.05
4 (impulsivity)	53.6 ± 4.6	55.7 ± 6.2	< 0.05
5 (masculinity-femininity)	54.3 ± 3.5	55.1 ± 3.3	< 0.05
6 (rigidity)	56.6 ± 5.6	56.2 ± 6.1	> 0.05
7 (anxiety)	72.3 ± 10.8	76.9 ± 10.6	< 0.01
8 (individualism)	62.0 ± 6.6	61.8 ± 7.5	> 0.05
9 (optimism)	43.6 ± 10.1	40.7 ± 6.1	< 0.01
0 (introversion)	60.6 ± 6.2	62.2 ± 6.2	< 0.05

Source: [14]

In MP G2 showed elevated scores on the social introversion scale. These scores deepen hyposthenic traits and weaken sthenic traits, indicating a passive personal stance, reduced level of involvement with the social environment, difficulties in establishing relationships with microsocial surroundings, and interpersonal conflicts. The scores on the emotional lability scale were somewhat elevated (within the range of 60 T-scores) in the examined patients, indicating emotional instability and intrapsychic conflict of divergent tendencies, ambivalence, emotional sensitivity, and unstable self-esteem. Moreover, these scores were notably higher in patients in G2 compared to G1. The individualism scale, which reflects an individual's need for actualising their own identity, inclination towards fantasising, and readiness to oppose others, had slightly higher scores in patients in G1, although the difference was minor compared to patients in G2. On the other hand, the rigidity scale, which reflects increased irritability, feelings of offence, and a tendency towards dysphoria, was slightly higher in patients in G2, but the difference was not significant. The scores on the impulsivity scale, which reflects resistance to social influence, non-conformity, and the predominance of emotions over intellectual control, were generally low in MP with NMD, within the range of 50-60 points, and considerably higher in G2. The indicator on the masculinity-femininity scale was significantly higher in G2 patients; in the studied contingent, this indicator can be considered as an indicator of masculinity (rigidity of character, lack of sentimentality, tendency to polygamy). The repressive position in the personality profile of the MP with NMD was occupied by the optimism scale, which shows a positive emotional mood, activity, and extroversion, which naturally reflects the negative impact of combat stress, NMD, and COVID-19 on the psyche. The indicator on this scale in patients G2 was significantly lower compared to G1.

The obtained data show the presence of a specific individual psychological profile of MP with NMD who have had COVID-19. The core characteristics of this profile are stable anxiety-depressive and hyposthenic manifestations, which can be considered as signs of neuroticism, while the auxiliary psychopathological constructs are stable hyposthenic, emotionally labile, hypochondriacal, and dysphoric phenomena. The identified features should be considered when planning treatment and rehabilitation measures for MP with NMD who have had COVID-19.

The investigation of the structure and correlation of different types of coping behaviour in MP, depending on the previous COVID-19 disease, revealed a number of important patterns (Table 2).

Table 2. Indicators for the ways of overcoming behaviour questionnaire (in points)

Coping options	Indicators on scales, M ± m		p
	G1	G2	
Confrontational coping	41.4 ± 13.8	49.9 ± 16.8	< 0.01
Distancing	55.0 ± 14.5	48.8 ± 14.2	< 0.01
Self-control	73.1 ± 11.8	66.8 ± 10.8	< 0.01
Search for social support	68.8 ± 15.0	59.6 ± 19.5	< 0.01
Acceptance of responsibility	66.7 ± 18.6	63.0 ± 18.6	> 0.05
Escape-avoidance	37.4 ± 12.1	46.0 ± 10.7	< 0.01
Planning to solve the problem	54.0 ± 17.7	45.1 ± 15.0	< 0.01
Positive revaluation	48.1 ± 13.9	41.0 ± 11.5	< 0.01

Source: [15]

The rate of confrontational coping was significantly higher in G1 patients. Confrontational coping is aimed at solving a problem through behavioral activity, not always purposeful, projected to resolve the situation or respond to negative emotions that are associated with it. Indicators of distancing coping strategy were significantly higher in MP of G1; this strategy involves levelling the experiences associated with the current situation by subjectively reducing its value and decreasing emotional involvement in the problem. The self-control strategy, which involves attempts to alleviate negative experiences by suppressing and inhibiting emotions, reducing their influence on evaluating the situation and choosing behavioural strategies, was more characteristic of MP with NMD of G1. MP of G1 also showed higher rates for the coping strategy of finding social support, which is considered constructive and involves de-actualisation of the problem by attracting external resources and emotional, informational, and effective support from other people. There were no significant differences between the groups regarding the strategy of taking responsibility. Instead, MP with NMD of G2 were more prone to the coping strategy of escape-avoidance, which involves trying to eliminate negative experiences by avoiding, denying the problem, distracting from it, unjustified expectations, and fantasising.

The most constructive coping strategy of planning a problem solution, which is aimed at solving the situation through purposeful analysis and search for behaviour options, planning one's own actions considering experience, real conditions, and resources, was more inherent in MP of G1. The positive revaluation strategy, which involves a positive rethinking of the problem and perceiving it as an incentive for personal development, was also more typical for G1 patients.

The data obtained indicate that non-constructive coping strategies are more pronounced in MP with NMD, in particular, escape-avoidance and confrontational coping, and constructive coping strategies are less pronounced, that is planning problem solving, seeking social support, self-control, and positive revaluation. These characteristics may be associated with manifestations of neuroticism and pathocharacterological transformations, which are more pronounced in individuals who have experienced COVID-19. It is also important to consider the greater prevalence of persistent depressive and anxious symptoms in MP with NMD who have had COVID-19, as it may also have an impact on the coping repertoire of the examined patients. When interpreting the data on the coping behaviour of MP with NMD, it becomes evident that their coping repertoire combines constructive and non-constructive coping strategies, which is a reflection of both an initially satisfactory state of mental and psychological health (all individuals examined before their NMD development were mentally healthy with a fairly high level of stress tolerance) and pathological changes in the psyche that arose as a result of NMD, the impact of war stress, and COVID-19.

The examination of the state of socio-psychological adaptation of MP with NMD revealed a significantly worse state in all areas in G2 patients (Table 3).

Table 3. Indicators based on the method of diagnosis of socio-psychological adaptation by C. Rogers & R.F. Dymond (in points)

Indicator	Indicator, M ± m		p
	G1	G2	
Adaptation	33.0 ± 13.5	27.6 ± 11.3	< 0.01
Self-acceptance	28.5 ± 13.7	21.7 ± 1311	< 0.01
Accepting others	38.9 ± 15.9	34.6 ± 1611	< 0.01
Emotional comfort	33.0 ± 13.0	28.4 ± 1114	< 0.01
Internality	44.3 ± 9.2	42.5 ± 1110	< 0.05
Striving for domination	22.3 ± 16.3	17.9 ± 1512	< 0.05

Source: [16]

Thus, the rate of adaptation in MP with NMD was low, and in G2 patients, it was significantly lower compared to G1 patients. Similarly, MPS with NMD of G2 showed significantly worse indicators of self-acceptance and acceptance of others, internality, emotional comfort, and the desire for dominance.

The identified patterns show that there are pronounced manifestations of psychosocial MA in MP with NMD, who have had COVID-19, in all key areas. Notably, the contingent of examined MP with NMD is generally characterised by low indicators of psychosocial adaptation, which proves the association of NMD in MP with manifestations of psychosocial MA.

DISCUSSION

The data presented in this study coincide with the results of N. Jain *et al.* [1], which confirm the presence of persistent adverse changes in the psyche of MP, in particular, in the affective sphere, as well as with the information provided by A. Haydabrus *et al.* [2] on the change of the structure of neuroticism in Ukrainian soldiers defending against Russian aggression.

The information obtained in this study regarding specific changes in the psyche of individuals who have experienced COVID-19 is consistent with the findings of J.B. Badenoch *et al.* [7], L. Premraj *et al.* [8], M. Colizzi *et al.* [9], which demonstrated a predominance of depressive and anxiety symptoms in the structure of psychopathology. The results also support the findings of L. Steardo *et al.* [10] regarding the role of COVID-19 in the manifestation of psychopathological symptoms in individuals with a predisposition to psychopathology, and the data from M. Colizzi *et al.* [11] regarding the association between COVID-19 and pathological personality deviations.

This study also confirms and expands upon the results regarding the transformation of coping repertoire in patients who have experienced COVID-19, as presented in the work of Y. Pigaiani *et al.* [12], by identifying the structure and evaluating the quantitative expression of maladaptive coping strategies specific to the post-COVID period.

The data obtained in this experiment complement the scientific knowledge about the relationship between individual-psychological characteristics and non-psychotic mental disorders in combatants, as reported in the works of Y. Levi-Belz *et al.* [4] and G. Zerach *et al.* [5]. These studies found that psychological rigidity and elevated levels of aggression can be considered predictors of non-psychotic post-traumatic disorders. The results also align with the findings of G. Zerach *et al.* [18], which reported that pre-existing character deviations are associated with a higher risk of mental disorders and psychological problems following combat-related psychological trauma. The results obtained are also consistent with studies of D. Assonov [19], which showed that experiencing positive emotions is a significant factor in the recovery of normal mental functioning in war veterans in the long term, as well as the information provided by M. Nieto *et al*. [20] on the influence of individual psychological characteristics on psychological adaptability and the ability to resist psychological stress. The findings presented in this study contribute to the understanding of the spectrum of individual-psychological characteristics associated with combatants with non-psychotic mental disorders and provide a comprehensive characterisation of pathological personality manifestations in MP in the context of COVID-19.

These findings complement the results of studies on coping behaviour among war veterans, which have shown that maladaptive behavioural patterns contribute to the development and exacerbation of post-traumatic stress, depressive symptoms, and other psychopathological manifestations, as well as psychosocial maladjustment [21, 22]. The prevalence of psychosocial maladaptation in MP with NMD aligns with the findings of N. Jain *et al.* [1], A. Haydabrus *et al.* [2], and indicates an association between maladaptive manifestations and depressive, anxious, and post-traumatic disorders.

Furthermore, for the first time, patterns of pathological personality characteristics, coping repertoire, and psychosocial maladaptation were identified in MP patients who have had COVID-19. The association between depressive and anxious symptoms and a history of coronavirus infection has been demonstrated by several authors, which is consistent with the findings presented in this study [7-9]. The difficulties of adaptation and distortion of coping repertoire in the post-COVID period, as observed by other researchers [10-12], were also confirmed. However, the findings of these authors were obtained from the general population, while the sample in this study consisted of MP individuals characterised by specific psychopathological, pathopersonological, and individual-psychological characteristics.

Thus, this study provides the first insights into the patterns of pathological personality, individual-psychological characteristics, coping repertoire, and psychosocial maladaptation in MP patients with a history of COVID-19, offering a comparative perspective on these aspects.

CONCLUSIONS

The study identified individual-psychological and pathopersonological patterns, coping repertoire characteristics, and psychosocial maladaptation in MP with non-psychotic mental disorders who have experienced COVID-19, in a comparative aspect.

It was established that MP with NMD who have suffered from COVID-19 are characterised by specific personal changes, which can be considered as persistent pathocharacterological transformations caused by the stress of combat operations, and post-specific neuropsychiatric disorders. The core features of these pathopersonological changes include stable anxious-depressive and hyposthenic traits, while the auxiliary psychopathological constructs include stable hyposthenic, emotionally labile, hypochondriacal, and dysphoric manifestations. The experience of COVID-19 exacerbates the pathopersonological traits inherent in MP with non-psychotic mental disorders and contributes to the formation of a specific individual-psychological profile in this population.

The investigation of coping repertoire characteristics in MP with NMD who have had COVID-19 revealed their predominant use of maladaptive coping strategies such as escape-avoidance and confrontational coping. There was also deactivation of constructive coping strategies such as problem-solving, seeking social support, self-control, and positive reappraisal. In contrast, MP individuals with NMD who have not had COVID-19 were more likely to exhibit coping strategies involving self-control, seeking social support, and taking responsibility. The distortion of coping repertoire in MP with NMD who have had COVID-19 can be explained primarily by the influence of psychiatric consequences of coronavirus infection, although the negative impact of combat stress is also significant.

The examination of the state of socio-psychological adaptation revealed generally low rates of adaptation in key areas inherent in MP with NMD. Also, MP with NMD, who suffered from COVID-19, demonstrated significantly poorer indicators in the areas of adaptation, acceptance of others, self-acceptance, emotional comfort, internality, and the desire for dominance, which confirms the negative impact of coronavirus infection on the social-psychological adaptation of MP in the post-COVID period.

Individual-psychological characteristics, coping repertoire characteristics, and manifestations of psychosocial maladaptation need to be considered when developing and planning treatment and rehabilitation measures for MP with NMD. Prospects for further studies are related to psychopathological reactions in MP with NMD in the context of the experienced COVID-19 infection and the development of personalised therapeutic, preventive, and rehabilitation interventions based on the findings of these studies.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Індивідуально-психологічні особливості та стан психосоціальної адаптації учасників бойових дій з непсихотичними психічними розладами, які перенесли захворювання на COVID-19

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Анотація. Актуальність теми визначається важливістю результатів дослідження індивідуально-психологічних особливостей, копінг-поведінки та стану психосоціальної адаптації учасників бойових дій, які перехворіли на COVID-19, для розробки персоналізованих лікувально-реабілітаційних заходів. Метою дослідження було визначення патохарактерологічних характеристик, особливостей копінг-репертуару та психосоціальної дезадаптації в осіб, які брали участь у бойових діях, хворіють на непсихотичні психічні розлади, і перенесли коронавірус, у порівняльному аспекті. Обстежено 132 чоловіка-учасника бойових дій з непсихотичними психічними розладами, які не хворіли на COVID-19, та 120 таких же військових, які перенесли коронавірусну інфекцію, з використанням Стандартизованого методу дослідження особистості, опитувальника «Способи долаючої поведінки» та методики діагностики соціально-психологічної адаптації. Встановлено, що бійцям, які перенесли захворювання на COVID-19, притаманні специфічні особистісні характеристики, що можуть розглядатися в якості патохарактерологічних трансформацій і ознак невротизації. Стрижневі патохарактерологічні зміни представлені стійкими тривожно-депресивними та гіпостенічними рисами, а допоміжними психопатологічними конструктами є стійкі емоційно-лабільні, астенічні, іпохондричні та дисфоричні прояви. Для них також характерні зміни копінг-репертуару з переважанням неконструктивних стратегій втечі-уникнення та конфронтації, із дезактуалізацією конструктивних копінг-стратегій планування вирішення проблеми, пошуку соціальної підтримки самоконтролю, та позитивної переоцінки, а також виражені прояви психосоціальної дезадаптації в усіх ключових сферах. COVID-19 є чинником, що обтяжує психопатологічні прояви, сприяє формуванню патохарактерологічних рис і поглиблює психосоціальну дезадаптацію учасників бойових дій з непсихотичними психічними розладами. Одержані дані дають можливість персоналізувати лікувально-реабілітаційні та профілактичні заходи з урахуванням патохарактерологічних індивідуально-психологічних особливостей та копінг-поведінки, а також удосконалити існуючі програми соціально-психологічної адаптації учасників бойових дій

Ключові слова: комбатанти; невротичні захворювання; патохарактерологічні риси; копінг-поведінка; соціально-психологічна дезадаптація

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Experimental translocation of intestinal bacteria caused by closed abdominal trauma, acute blood loss, internal haemorrhage

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Abstract. Bacterial translocation plays an important role in the development of multiple organ failure, which develops as a result of trauma. The severity of bacterial translocation is proportional to the degree of blood loss and damage. The purpose of the study was to establish the spectrum of microorganisms involved in translocation, their population levels, and explore changes in the microecology of mesenteric lymph nodes and liver tissue in white rats subjected to closed abdominal trauma, acute blood loss, internal haemorrhage, and their combination. Experiments were performed on 36 male rats. Biological material (mesenteric lymph nodes, liver tissue) was collected at 7 and 14 hours post-experiment and the samples underwent homogenisation and were then cultured according to laboratory protocols. Isolated bacteria were identified using a Vitek-2 Compact 15 analyser (bioMérieux, France), and their population level was evaluated in $\log_{10}$ CFU/g. Bacterial translocation was considered present when a positive result was obtained simultaneously in the mesenteric lymph nodes and in the tissue of the right lobe of the liver. It was established that the main role in translocation is played by *E. coli, P. stuartii, P. mirabilis, P. aeruginosa.* The combination of abdominal trauma with acute blood loss was accompanied by translocation and *K. pneumoniae.* Trauma combined with acute blood loss causes additional translocation of *E. faecalis, E. cloacae, E. faecium;* while internal haemorrhage led to *S. aureus and S. epidermidis translocation.* The spectrum of translocating microorganisms was more diverse and multi-component when the trauma was combined with internal haemorrhage. At 14 hours post-trauma, there was a tendency for increased population levels of the isolated bacteria compared to the 7-hour data. In all experimental groups, *E. coli* strains were cultured in the highest concentrations

Keywords: gut microbiota; bacterial translocation; mesenteric lymph nodes; blunt abdominal trauma; haemorrhage

INTRODUCTION

In modern medical science, comprehensive understanding of the mechanisms underlying the interaction between the human body and its own microbiota occupies a prominent position among fundamental research tasks. It has become evident that commensal microorganisms not only reside in the body but also play a crucial role in various important functions of the host organism. Allochthonous bacteria in the gastrointestinal tract of humans serve a dual role as they contribute to digestion, participate in the defence mechanism against pathogens, and exert unique molecular mechanisms that influence the functioning of different organs and systems. However, under certain circumstances, these same bacteria can become potential pathogens. In-depth understanding and investigation of bacterial translocation (BT) help elucidate the translocation processes and facilitate the development and clinical application of new pharmaceutical preparations.

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According to modern literature sources, it is known that in the body in response to trauma and blood loss, changes in the parameters of homeostasis occur due to both pathological processes and compensatory mechanisms, and their totality is characterised by prolonged action [1, 2]. Moreover, the degree of severity of the physiological response to trauma is proportional to the degree of severity of blood loss and damage [3, 4]. A normally functioning intestinal mucosa prevents intestinal bacteria and endotoxins from entering the bloodstream and other organs [5-7]. Dysfunction of the intestinal barrier is considered the main cause of inflammation, when there is a violation of the intestinal blood supply and insufficient oxygenation of tissues. Furthermore, the loss of intestinal mucosal barrier functions and reduced intestinal blood flow lead to BT. Given the close relationship between BT and inflammatory reactions and their impact on intestinal integrity, it has become essential to explore new aspects of gut microbial ecology and various gastrointestinal and metabolic disorders [8]. The development of translocation phenomena has been demonstrated in a study conducted by Ł. Wlazło *et al.* [9], who performed a microbiological analysis of animal abscesses, revealing a significant increase in intestinal bacteria in all samples which was attributed to the migration of gut bacteria through compromised intestinal permeability and their dissemination into the bloodstream. The majority of studies indicate that the most common routes for bacterial passage from the gut to the systemic circulation and eventually to distant organs are the lymphatic and vascular pathways. It has been proven that the lymphatic collector plays a key role in the implementation of the bacterial translocation mechanism [10].

B.I. Ucar *et al.* [11] investigated the underlying molecular mechanisms of BT in sepsis, diagnosis and evaluation of BT, and ways to improve the underlying molecular mechanisms of BT in sepsis, diagnosis and evaluation of BT, and therapeutic treatments for sepsis. They determined that BT occurs more frequently in patients with intestinal obstruction, endogenous infections, endotoxemia, and immune system disorders, which further leads to the development of sepsis and subsequent multiorgan dysfunction. In some cases, the penetration of bacteria and endotoxins through the intestinal barrier can lead to blood flow infections and multiple organ failure. In the paper, Y.H. Wang [12] highlighted that in chronic kidney diseases, compromised intestinal barrier function may contribute to the translocation of gut microorganisms (endotoxins, antigens, and other microbial products) to the intestinal wall, correlating with signs of chronic inflammation in the gastrointestinal tract, endotoxemia, and systemic inflammation. Therefore, the prevention of BT can become a new important area in the treatment of various diseases. For instance, the application of specific postoperative treatment methods, which are pathogenetically justified and aimed at preventing the migration of enterobacteria into the peritoneal space in patients with acute adhesive small bowel obstruction, has shown a reduction in postoperative complications and mortality rates [13]. Normal gut bacteria have been found to migrate to the draining mesenteric lymph nodes inside host phagocytes. Clinical and experimental evidence confirms that bacteria capable of surviving within macrophages (such as *Salmonella species* and *Listeria monocytogenes*) translocate more easily compared to other bacteria, including obligate anaerobes [14]. Y. Xu *et al.* [15] identified the gut microbiota as a potential mediator of the pathogenesis of heart failure and its associated diseases. Escherichia coli, for example, can increase the permeability of the colonic walls and stimulate inflammation in intestinal tissue, leading to enhanced bacterial translocation. The development of this phenomenon in experiments on mice was established by N. Long *et al.* [16]. There is also evidence that anaerobic bacteria translocate along with facultative anaerobes in situations involving intestinal epithelial damage, such as trauma and acute mesenteric ischemia [17].

The purpose of the study was to establish the taxonomic composition and population levels of microorganisms involved in bacterial translocation and explore the changes in the microecology of mesenteric lymph nodes and liver tissue in white rats with induced closed abdominal trauma, blood loss, and their combination.

MATERIALS AND METHODS

The experimental work was conducted in the Laboratory of Microbiological and Parasitological Research at the I.Ya. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, in 2022. The study utilized 36 sexually mature male Wistar rats weighing 200-250 g, kept on a standard diet in the vivarium. Laboratory animals were divided into 4 groups: control (n = 6), the first – a group of experimental rats with simulated closed abdominal injury (n = 10), the second – a group of experimental rats with simulated closed abdominal injury and acute blood loss (n = 10), the third – a group of experimental rats with simulated closed abdominal injury, acute blood loss and internal haemorrhage (n = 10). Under anaesthesia, mesenteric lymph nodes (MLNs) and liver tissue were collected as biological material for microbiological analysis at 7 and 14 hours after the induction of pathological conditions. To avoid cross-contamination during sample collection from MLNs and liver, instruments were not reused for subsequent sampling. Each tissue sample was homogenised using a sterile 0.9% sodium chloride solution, and the homogenate was further diluted 10 and 100 times.

All diluted samples (100 µl each) were plated on blood agar to determine the population levels of isolated microorganisms. To establish the taxonomic composition of the isolated bacteria, the prepared material was also cultured on selective and differential media and incubated for 24 hours at 37°C. The staphylococci were isolated on egg yolk-salt agar, enterococci on enterococcus agar, enterobacteria on Endo, Olkenitsky, and Simmons media, bifidobacteria on bifidum medium, and lactobacilli on lactoagar. For the detection of obligate anaerobes, thioglycolate medium was used. After 24-96 hours of incubation (for facultative anaerobes) and 5 days (for obligate anaerobes) at 37°C, the number of bacteria was determined by counting colony-forming units (CFU) per gram of tissue. The bacterial population level was calculated using the formula: number of colonies in the sample (CFU/g) = average number of colonies on the medium in a Petri dish × dilution ratio, and represented as $\log_{10}$ CFU/g. Isolation of bacteria in the abdominal cavity in clinically significant concentrations (more than 5 $\log_{10}$ CFU/g) indicated the development of an inflammatory process. The isolated strains were initially

identified based on Gram staining and colony morphology, and finally confirmed using the Vitek 2 Compact 15 automated microbiological analyser (BioMerieux, France) for taxonomic identification. To assess bacterial translocation, the microbial flora of the investigated lymph nodes was compared to its presence in the liver tissue. BT was considered present when a positive result was obtained simultaneously in both mesenteric lymph nodes and liver tissue [18].

During the work with laboratory animals, international requirements for the humane treatment of animals were followed in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [19], and the methodological recommendations of the State Expert Center of the Ministry of Health of Ukraine on "Preclinical studies of medicinal products" [20]. Euthanasia of the rats throughout the experiment was performed by total exsanguination from the heart after prior propofol anaesthesia (60 mg/kg intravenously).

RESULTS

In the control group of rats (n = 6), only half of the experimental animals had cultured *Escherichia coli* from MLN. However, BT was not considered positive because the liver tissue remained sterile. This phenomenon is natural considering that the normal flora of humans trains the body's immune response. From the MLN of the first group of animals, 12 strains of facultative anaerobic rods were isolated after 7 hours of simulated closed abdominal trauma, and 18 strains after 14 hours. Among them, cultures of Enterobacteria belonging to the following species were identified: *Escherihia coli, Proteus mirabilis, Morganella morganii, Providencia stuartii*, and cultures of non-fermenting rods such as *Pseudomonas aeruginosa*. The population level of microorganisms isolated from MLN after 7 hours of trauma averaged (3.02 ± 0.84) $\log_{10}$ CFU/g (Table 1). After 14 hours, there was a tendency for an increase in bacterial concentration in MLN to (3.58 ± 0.79) $\log_{10}$ CFU/g.

Table 1. Taxonomic composition and population levels of bacteria isolated from rats with closed abdominal trauma (n = 10)

No.	Microorganism	Population level, $\log_{10}$ CFU/g				Number of rats with an existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
1	*E. coli*	4.47 ± 0.98	4.56 ± 1.02	2.92 ± 0.78	3.93 ± 0.78	60	80
2	*P. mirabilis*	3.10 ± 0.83	3.84 ± 0.81	1.98 ± 0.62	2.85 ± 0.58	40	30
3	*M. morganii*	2.32 ± 0.78	2.93 ± 0.67	0	2.56 ± 0.44	0	20
4	*P. stuartii*	2.54 ± 0.65	2.86 ± 0.85	0	2.60 ± 0.32	0	10
5	*P. aeruginosa*	3.02 ± 0.94	3.70 ± 0.62	2.32 ± 0.85	2.94 ± 0.56	20	40
Total		**3.02 ± 0.84**	**3.58 ± 0.79**	**2.21 ± 0.37**	**2.98 ± 0.54**	**100**	**100**

Source: compiled by the authors

After 7 hours of trauma simulation in the rats of this group, monospecies cultures of microorganisms were most frequently isolated from the lymph nodes, while after 14 hours with further development of the inflammatory process, most bacteria were found in two-component associations, except for 2 animals where *P. aeruginosa* was isolated as a monospecies. BT in MLN was confirmed by the isolation of the same bacterial species in liver tissues. The dominant species in the lymph nodes was *E. coli*: strains of Escherichia coli were isolated in 60% of the rats in this group after 7 hours and in 80% of the animals after 14 hours. The highest bacterial concentration in MLN was also observed for *E. coli* (4.47 ± 0.98) $\log_{10}$ CFU/g and (4.56 ± 1.02) $\log_{10}$ CFU/g (after 7 and 14 hours, respectively).

From the second group of animals, 18 and 26 strains of facultative anaerobic microorganisms were isolated (after 7 and 14 hours, respectively). After acute blood loss in rats with closed abdominal trauma, the bacterial concentration in MLN averaged (3.22 ± 0.85) $\log_{10}$ CFU/g and (3.49 ± 0.76) $\log_{10}$ CFU/g after 7 and 14 hours, respectively. Although the population level of the bacteria remained virtually unchanged, their taxonomic composition became richer. According to the results of the study, the presence of BT in this group of laboratory rats (n = 10) was caused by strains of gram-negative enterobacteria (*E. coli, Klebsiella pneumoniae*, *M. morganii, P. mirabilis*) and non-fermenting rods (*P. aeruginosa)*, and gram-positive cocci (*Enterococcus faecalis* and *E. faecium*) (Table 2). It is worth noting that gram-positive bacteria were not observed in BT after 7 hours; enterococci penetrated the mesenteric nodes only after 14 hours of the experiment. However, only one rat in this experimental group had a monospecies culture represented by *P. aeruginosa*, while in all other cases, isolated microorganisms were found in two-component associations. Unlike motile pseudomonads, enterobacteria, Proteus, and other enterobacteria, Klebsiella and enterococci are non-motile bacteria, and therefore, their translocation occurred at a slower rate. Although strains of *E. faecalis and E. faecium* were isolated from mesenteric nodes after 7 hours of the experiment, their translocation was not confirmed bacteriologically because they appeared in parenchymal organs, particularly the liver, after 14 hours. Perhaps this is why three-component bacterial associations were determined in half of the experimental rats of this group, compared with animals of the first and second groups. The deficiency of blood supply leads to impaired cell membrane function and increased permeability, which is why conditionally pathogenic Klebsiella and enterococci were not isolated from the lymph nodes of rats with only closed abdominal trauma. The highest population level in MLN was observed for *E. coli*, reaching 4.67 ± 0.7 $\log_{10}$ CFU/g and 5.03 ± 0.30 $\log_{10}$ CFU/g (after 7 and 14 hours, respectively). The lowest population level was observed for enterococci (Table 2). Strains of *E. faecalis and E. faecium* were isolated from mesenteric nodes at concentrations of 2.78 $\log_{10}$ CFU/g and 2.59 $\log_{10}$ CFU/g, respectively.

Table 2. Taxonomic composition, population level of microorganisms isolated from rats with closed abdominal trauma combined with acute bleeding (n = 10)

No.	Microorganism	Population level, $\log_{10}$ CFU/g				Number of rats with existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
gram-negative bacteria							
1	*E. coli*	4.67 ± 0.73	5.03 ± 0.30	1.75 ± 0.77	1.89 ± 1.29	90	100
2	*K. pneumoniae*	2.40 ± 0.58	3.22 ± 0.77	2.19	2.38 ± 0.93	10	30
3	*M. morganii*	2.93 ± 0.66	3.11 ± 0.42	2.94 ± 0.22	2.41 ± 0.68	10	20
4	*P. mirabilis*	4.16 ± 0.87	4.56 ± 0.49	2.76 ± 0.38	2.93 ± 0.42	40	50
5	*P. aeruginosa*	2.98 ± 0.78	4.02 ± 0.45	2.75 ± 0.87	2.93 ± 0.56	30	40
gram-positive bacteria							
6	*E. faecalis*	2.78	2.98	0	1.65	0	10
7	*E. faecium*	2.59	2.99	0	1.08	0	10
Total		**3.22 ± 0.85**	**3.49 ± 0.76**	**1.77 ± 2.18**	**1.27 ± 0.68**	**90**	**100**

Source: compiled by the authors

From the animals in the third group, 32 strains of facultative anaerobic bacteria were isolated from MLN after 7 hours of experiment simulation, and 44 strains were isolated after 14 hours. In the group of rats with closed abdominal trauma, combined with acute blood loss and internal haemorrhage, compared to the previous experimental groups, not only was the spectrum of microorganisms isolated from MLN the widest, but the population level of bacteria in the lymph nodes was also significantly higher after 14 hours of the experiment. The average concentration of microorganisms isolated from MLN in this experimental group was (3.30 ± 1.07) $\log_{10}$ CFU/g and (4.18 ± 1.54) $\log_{10}$ CFU/g after 7 and 14 hours, respectively. While the first group identified bacteria belonging to 5 species and the second group to 7 species, the bacterial spectrum in the third group included 10 species. In all experimental rats of this group, BT was confirmed by the presence of the same strains in the liver tissue.

In addition to translocation in mln of Gram-negative rods and enterococci, which were found in all experimental groups of rats, translocation of Gram-positive staphylococci was observed. Staphylococci are characterised by the secretion of enzymes such as coagulase, hyaluronidase, fibrinogen, and toxins such as leukotoxin and enterotoxin, which lead to the release of pro-inflammatory cytokines that trigger and intensify inflammatory processes, causing complications and enhancing the action of endotoxins from gram-negative bacteria. After 7 hours of the experiment, the following strains of staphylococci were isolated from MLN: *Staphylococcus aureus* and *S. epidermidis* from 30% and 40% of all animals in the third group, respectively, at a concentration of (2.56 ± 1.85)-(2.89 ± 1.12) $\log_{10}$ CFU/g. Their population level increased by an order of magnitude after 14 hours (Table 3). *E. coli* strains showed the highest population levels, as in the previous two groups. It reached (5.94 ± 1.58) $\log_{10}$ CFU/g and (8.45 ± 1.81) $\log_{10}$ CFU/g after 7 and 14 hours, respectively. This level indicates the development of an inflammatory process. They were only surpassed in concentration in MLN by cultures of *P. mirabilis*. The population level of Proteus was (4.75 ± 1.09)-(4.79 ± 1.45) $\log_{10}$ CFU/g. Cultures of enterococci were isolated at a concentration of 2.59-(2.85 ± 0.57) $\log_{10}$ CFU/g after 7 hours, and their population level after 14 hours was 2.88-(3.41 ± 0.16) $\log_{10}$ CFU/g. If the isolated microorganisms in the first and second experimental groups were found in mono- and two-component associations, the bacteria isolated from the rats in the third group were found in three-component associations. In contrast to the previous groups, the taxonomic composition of gram-positive microorganisms expanded (Table 3), with bacteria belonging to 5 species being isolated. However, the spectrum of gram-negative bacteria remained unchanged. During acute blood loss combined with internal haemorrhage, where a significant amount of blood is lost externally and accumulates in the peritoneal cavity, there is a substantial decrease in circulating blood volume (CBV). It also affects the diversity of microorganisms and their population levels.

Table 3. Taxonomic composition and population level of microorganisms isolated from rats with closed abdominal trauma combined with acute blood loss and internal haemorrhage (n = 10)

No.	Microorganism	Population level, $\log_{10}$ CFU/g				Number of rats with existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
gram-negative bacteria							
1	*E. coli*	5.94 ± 1.58	8.45 ± 1.81	4.67 ± 1.08	5.39 ± 1.56	100	100
2	*K. pneumoniae*	2.79 ± 0.22	3.92 ± 0.37	1.99 ± 0.30	2.43 ± 0.24	20	30
3	*P. stuartii*	2.87	4.41	2.56	3.55	10	10
4	*P. mirabilis*	4.75 ± 1.09	4.79 ± 1.45	3.26 ± 0.94	3.50 ± 1.02	40	60
5	*P. aeruginosa*	3.03 ± 1.52	4.02 ± 1.69	2.76 ± 1.08	2.93 ± 0.87	50	60

Table 3. Continued

No.	Microorganism	Population level, log_{10} CFU/g				Number of rats with existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
gram-positive bacteria							
6	*E. faecalis*	2.85 ± 0.57	3.41 ± 0.16	0.78 ± 0.21	1.32 ± 0.30	20	20
7	*E. cloacae*	2.59	2.99 ± 0.30	0.90	1.51 ± 0.34	10	20
8	*E. faecium*	2.61	2.88	0	1.27	0	10
9	*S. aureus*	2.56 ± 1.85	3.71 ± 1.68	1.04 ± 1.13	2.62 ± 1.52	40	70
10	*S. epidermidis*	2.89 ± 1.12	3.18 ± 1.40	1.74 ± 1.48	2.99	30	50
Total		**3.30 ± 1.07**	**4.18 ± 1.54**	**1.99 ± 1.32**	**2.86 ± 1.21**	**100**	**100**

Source: compiled by the authors

Studies have shown that in laboratory animals with closed abdominal trauma, gram-negative intestinal microflora plays a major role in the phenomenon of bacterial translocation (Fig. 1). From the rats in the first experimental group, only gram-negative bacteria were isolated from MLN. In the second experimental group, where acute bleeding was simulated only after 14 hours, gram-positive microorganisms appeared in MLN in small quantities, accounting for only 7.7% of all isolated bacteria. Internal haemorrhage stimulates an increase in bacterial translocation and its diversity, which is why gram-positive cocci accounted for 40.9% of all isolated microorganisms in the third experimental group.

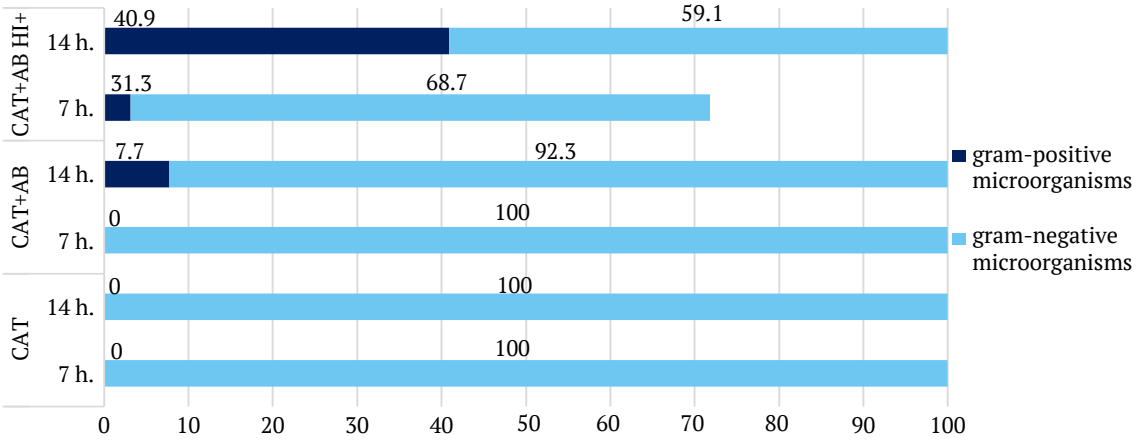

Figure 1. Ratio of gram-positive and gram-negative microorganisms isolated from mesenteric lymph nodes, %
Notes: CAT – closed abdominal trauma; CAT+AB – closed abdominal trauma + acute bleeding; CAT+AB+IH – closed abdominal trauma + acute bleeding + internal haemorrhage
Source: compiled by the authors

In a certain way, the translocation of bacteria from the intestine occurs constantly, even in 5-10% of healthy people [21]. That is why BT was not confirmed in all rats in the experiment as microorganisms from some experimental animals were isolated only from the lymph nodes, which is not a criterion for the presence of BT.

DISCUSSION

In recent years, the influence of gut microbiota on the molecular and pathological mechanisms of inflammation has been the focus of many researchers. Additionally, experiments are being conducted on new treatment methods based on the impact of gut microbiota on trauma, considering the development of BT [22].

T. Komarov *et al.* [18] emphasised that the phenomenon of microbial translocation is a key element in the development of post-traumatic complications. The data obtained in the conducted experiments also showed that closed abdominal trauma combined with blood loss leads to tissue ischemia, which, in turn, contributes to the natural migration of microorganisms inhabiting the intestinal microbiota to the MLN, peritoneal space and sinuses of the liver. This process further contributes to the development of inflammation and complications. E. Nieves *et al.* [23] found a significant statistical difference between postoperative infections in patients with signs of BT (41.6%) compared to patients without BT (12.5%; $p = 0.047$). Bacteria isolated from infection sites were the same as those cultured in MLN in 40% of cases ($n = 2$ out of 5), which allowed them to establish a causal relationship between BT and postoperative infection. M. Schietroma *et al.* [24] demonstrated that risk of bacterial translocation is associated with bleeding ⩾ 1500 ml. Moreover, a higher risk of bacterial translocation and a significantly higher incidence

of postoperative infections exist in patients requiring urgent surgical treatment (splenectomy) after blunt abdominal trauma. The researchers showed that bacterial strains isolated from infection sites were the same as those cultured in MLNs in 48.3% of cases (n = 14 out of 29). In the conducted experiments, it was determined that maximum blood loss is accompanied by the richest spectrum of bacteria involved in BT and an increase in their population level. R.I.D. da Costa *et al.* [25] investigating BT in intestinal obstruction on a rat model, demonstrated that the optimal timing for performing the operation is 24 hours. The phenomenon of bacterial translocation was observed already 7 hours after the start of the experiment. Obviously, this is due to hypoxia, additionally created by simulated blood loss. However, it should be noted that it is precisely at the 24-hour mark that the microecology of MLN and liver tissue in white rats with simulated closed abdominal trauma, haemorrhage, and their combination becomes the most pronounced and multi-component.

According to the literature, gram-negative facultative anaerobic *Enterobacteriaceae*, such as *E. coli*, *K. pneumoniae*, and *P. mirabilis*, are the fastest and easiest to migrate from the gastrointestinal tract to MLN [4, 26]. Among them, according to the data obtained by researchers E. Nieves [23] and Y. Sharapatov [27], intestinal bacteria are most frequently identified in the presence of BT. Exo- and endotoxins enhance the pathogenic action of *E. coli*. It is *this species of Enterobacteriaceae that primarily* triggers the inflammatory process in polytrauma patients [22]. In experiments on research rats, I.V. Strelbytska *et al.* [28] found that simulated limb ischemia-reperfusion, acute blood loss, and their combination lead to the translocation of gut bacteria into the abdominal cavity. These literature data are confirmed by the results of the study. Determination of the spectrum of microorganisms that migrate from the gut during experimental blunt abdominal trauma in combination with haemorrhage showed that strains of *E. coli* migrate the fastest and in greater quantities compared to other bacteria. The translocation of gram-negative bacteria is associated with inflammatory mechanisms induced by lipopolysaccharides (LPS) of their cell walls. These bacterial antigens in tissue damage areas induce an inflammatory process [29]. LPS in combination with lipid-A are mediators of white blood cell activation and inducers of macrophages. The active action of gram-negative flora endotoxins increases the secretion of pro-inflammatory antigens and decreases the production of anti-inflammatory ones. The pro-inflammatory cytokines released during this process can also disrupt tight junctions, promoting the translocation of microorganisms. For example, the presence of proteolytic endotoxin in *P. aeruginosa* often leads to the development of sepsis and septic shock [30]. The results of the study established a correlation between the increasing number of research rats in which strains of *P. aeruginosa* confirming bacterial translocation were detected and the growth of their population level with the development of inflammation. This fact is one of the indications that a closed abdominal injury leads to the development of inflammation. Therefore, the appearance of non-fermenting gram-negative rods in MLN may be a marker of complications in the inflammatory process. C. Doudakmanis *et al*. [4] demonstrated that representatives of the intestinal microbiota, which are obligate anaerobes, virtually do not have the ability to translocate. Only facultative anaerobic bacteria were isolated from rats in all groups, confirming the findings of other researchers, including S.J. Wood *et al*. [31]. According to the results of the study on the dynamics of BT in experimental abdominal trauma in combination with haemorrhage, it was found that it is enterobacteria that will appear outside the boundaries of the gastrointestinal tract.

CONCLUSIONS

As a result of simulated blunt trauma to the abdomen in combination with acute blood loss and internal haemorrhage, an inflammatory process developed in the peritoneum and bacterial translocation occurred to mesenteric nodes and liver tissue.

The main role in BT was played by representatives of the gram-negative intestinal microflora. Strains of gram-negative bacteria migrated first. These included Enterobacteriaceae such as *E. coli, K. pneumoniae, P. stuartii, P. mirabilis,* and non-fermenting rods *P. aeruginosa.* It should be noted that the *K. pneumoniae* strains were capable of migration in the presence of acute haemorrhage in rats with blunt abdominal trauma. In experimental animals with blunt abdominal trauma combined with acute blood loss, the translocation of gram-positive cocci strains, such as *E. faecalis, E. cloacae*, and *E. faecium*, was also observed. Under the condition of additional simulated internal haemorrhage, bacterial translocation of staphylococcal strains occurred: *S. aureus, S. epidermidis.* Thus, the animals with blunt abdominal trauma had the least diverse taxonomic composition, with strains from 5 species being isolated. The group of animals with blunt abdominal trauma combined with acute blood loss and internal bleeding had the richest taxonomic composition, with twice as many species being identified.

The translocation of bacteria from the intestine to the mesenteric lymph nodes and liver tissue led to an increase in the population level of the isolated and identified bacteria. Microorganisms isolated from MLN were found in the highest concentration in experimental animals with blunt abdominal trauma combined with acute blood loss and internal haemorrhage. The highest population level was observed for *E. coli* strains in all three groups of research rats. After 14 hours, the average population level of bacteria isolated from MLN in animals with blunt abdominal trauma was 3.02 ± 0.84 $\log_{10}$ CFU/g, while in the group of rats with blunt abdominal trauma combined with maximal blood loss, the average concentration of microorganisms in MLN was an order of magnitude higher and amounted to 4.18 ± 1.54 $\log_{10}$ CFU/g. In the future, it is worth continuing to investigate bacterial translocation in different pathological conditions, determining bacterial deoxyribonucleic acids.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Експериментальна транслокація кишкових бактерій спричинена закритою травмою живота, гострою крововтратою, внутрішньою кровотечою

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Анотація. Бактеріальна транслокація відіграє важливу роль у розвитку поліорганної недостатності, яка розвивається в результаті травми. Ступінь вираженості транслокації бактерій пропорційний ступеню крововтрати й ушкодження. Метою дослідження було встановлення спектру мікроорганізмів, які брали участь у явищі транслокації, їх популяційного рівня, та вивчення напрямку змін у мікроекології мезентеріальних вузлів та тканині печінки білих щурів зі змодельованими закритою травмою живота, крововтратами (гостра крововтрата, внутрішня кровотеча) та їх поєднанням. Досліди виконано на 36 щурах-самцях. Біологічний матеріал (мезентеріальні лімфовузли, тканину печінки) брали стерильними інструментами через 7 та 14 год. від початку експерименту, проводили гомогенізацію, після чого висівали на відповідні середовища згідно з лабораторними настановами. Виділені бактерії ідентифікували за допомогою аналізатора Vitek-2 Compact 15 (bioMérieux, France), їх популяційний рівень оцінювали у $\log_{10}$ КУО/г. Бактеріальну транслокацію вважали наявною, коли позитивний результат отримували одночасно в мезентеріальних лімфовузлах і в тканині правої долі печінки. Встановлено, що в транслокації головну роль відіграють *E. coli, P. stuartii, P. mirabilis, P. aeruginosa*. Поєднання травми живота з гострою крововтратою супроводжувалася транслокацією ще й *K. pneumoniae*. Травма, поєднана з гострою крововтратою, викликає додатково транслокацію *E. faecalis, E. cloacae, E. faecium;* а з внутрішньою кровотечою – транслокацію *S. aureus, S. epidermidis*. Спектр мікроорганізмів, здатних до транслокації, був різноманітнішим і багатокомпонентним, якщо травму поєднували із внутрішньою кровотечою. Через 14 год. після нанесення травми спостерігали тенденцію до зростання популяційного рівня виділених бактерій, у порівнянні з даними, отриманими через 7 год. В усіх дослідних групах штами *E. coli* висівали у найбільших концентраціях

Ключові слова: мікробіота кишечника; бактеріальна транслокація; мезентеріальні лімфовузли; тупа травма живота; кровотеча

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Regularities of structural organisation of the heart myxomas in patients with Karney complex

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Abstract. Myxomas of the heart are one of the most common primary heart tumours, which most often develop in the left atrium, are characterised by a benign nature and no relapses after surgical removal of the tumour. Karney complex is an autosomal dominant disease characterised by numerous tumours, in particular, myxomas of the heart. Insufficient coverage of this pathology in the literature leads to an erroneous diagnosis, progression of the disease and inadequate treatment. The purpose of the study was to determine the morphological features of heart myxomas in patients with Karney complex. Using light microscopy, a morphological examination of the heart myxomas was performed, which were removed during 7 operations. Histological sections were made from the operating material, which were stained with hematoxylin and eosin, according to Van Gieson, fuchselin, and Masson's Trichrome Stain in the Zerbino-Lukasevich modification. A comparison of the group of heart myxomas of patients with the Karney complex (16 tumours) with a group with myxomas of the heart that occur sporadically (278 tumours) was made to identify morphological features. It was established that myxomas of the heart are multiple, they were detected with the same frequency in the right and left chambers of the heart. It was generalised that in myxomas of the heart, signs of both alteration and high proliferative activity of endotheliocytes and fibrous connective tissue cells are noted. Secondary myxomas of the heart in patients with the Karney complex have the same structural organisation as primary tumours, but most often they are not relapses, more often they are multiple and do not have a predominant localisation in the left atrium. The analysis established an identical cellular composition of both groups of heart myxomas, which suggests the same source of tumour growth, but the presence of more complex cell formations in sporadic myxomas and the predominance of single cells in Karney heart myxomas indicate the existence of differences in their morphogenesis

Keywords: benign tumours; morphology; mitral valve; Carney syndrome; light microscopy

INTRODUCTION

According to statistics, among the pathologies of the cardiovascular system, benign heart tumours are detected with a frequency of 0.0017-0.02%. They pose a substantial problem in their detection and occupy a leading place among

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all heart diseases [1]. Myxomas of the heart are one of the most common benign tumours that develop from the vascular endothelium, can occur at any age, but most often they are diagnosed in 30-60 years, and in women, the development of the disease occurs 2-3 times more often than in men. Despite the proven benign nature of tumours, experimental data cause oncologists to be wary.

Analysis of papers by such researchers as: V.V. Popov *et al*. [1], R.M. Vitovskyi *et al*. [2], and A. Kumar *et al*. [3] indicates that the asymptomatic course of this pathology in the early stages of myxoma development, polymorphism of clinical symptoms with further development of the disease, requires correct steps in the diagnosis of heart myxomas.

Timely recognition of primary heart tumours is a difficult task, because, according to P.T. Lee *et al*. [4] and A.G. Griborio-Guzman *et al*. [5], there are no pathognomonic signs, and the disease has an asymptomatic course in the early stages. Myxomas of the heart are a pathology that is rare: from 0.002 to 0.02% and up to 0.2% of cases in autopsies. According to S. Espiard *et al*. [6] and P.B. Saputra *et al*. [7], myxomas of the heart most often develop in the left atrium, attach to the atrial septum in the area of the oval window or at the confluence of the pulmonary veins, are characterised by unicentric growth, benign nature and no relapses after surgical removal of the tumour.

For a long time, there was no doubt that in all cases clinical myxomas of the heart occur sporadically (CMH). However, in recent decades, in the scientific literature, reports of L.Y. Fedonyuk *et al*. [8] that the disease can occur in several members of the same family have appeared. Despite the fact that only 15 families with this pathology were described before 1992, according to M.I. Shved *et al*. [9], 5-10% of all heart myxomas are hereditary. K. Wei *et al*. [10] indicate that this nosological form is characterised by the fact that myxomas of the heart are part of a symptom complex that was originally called nevi, atrial myxoma, mucocutaneous myxomas, ephelides (NAME), or lentiginosis, atrial myxoma, mucocutaneous myxomas, blue nevi (LAMB). According to C.D.C. Kamilaris *et al*. [11] and S. Cherenko *et al*. [12], this syndrome was later renamed the Carney complex in honour of researcher Carney.

Modern literature reviews indicate that myxomas of the heart occur in 30-60% of patients with Karney complex. In this case, the neoplasm can damage any chamber of the heart. However, G. Pitsava *et al*. [13] in the Karney complex, localisation of the heart myxomas in the right parts of the heart is noted (67% of cases), which is more than 8 times higher than the localisation of sporadic heart myxomas. Most studies on the Karney complex and hereditary heart myxomas have a clinical focus, although some studies contain isolated data on morphological studies of the heart myxomas in patients with the clinical Karney complex (CKC). Thus, myxomas of the heart with the Karney complex continue to attract the attention of both cardiac surgeons and morphologists-oncologists. The question of the occurrence of tumours of exracardial localisation in such patients remains open.

The purpose of the study was to examine the structural organisation of the heart myxomas and determine the morphological features of tumours in patients with Karney complex.

MATERIALS AND METHODS

A retrospective morphological study of 16 heart myxomas removed during 7 operations was performed at the M.M. Amosov National Institute of Cardiovascular Surgery during the last 20 years. The first 4 follow-ups belonged to individuals from the same family: 2 cases of heart myxomas in the mother and 2 cases of heart myxomas in her son. In two cases of observation, tumours were detected in one patient. One observation was represented exclusively by a primary tumour. In one patient, mitral valve insufficiency was detected after removing the left atrial myxoma, associated with prolonged prolapse of the heart myxomas into the left ventricular cavity. In this regard, mitral valve plastic surgery was performed with resection of part of the anterior leaflet. The study was conducted in accordance with the rules of the Helsinki Declaration [14] and with the patient's consent due to the need for examination and surgical treatment. The choice of subjects was made among all patients with myxomas of the heart based on the established diagnosis – Karney complex.

The operating material was fixed in a 10% formalin solution. Histological sections were made from paraffin blocks obtained by the conventional method. The preparations were stained with hematoxylin and eosin for review microscopy, Van Gieson for investigating the state of the collagen and muscle components of the tumour, fuchselin for determining elastic fibres, and Masson's trichrome stain (MSB) in the Zerbino-Lukasiewicz modification for investigating fibrin and other blood components. The drugs were described using an algorithm scheme, which included the following characteristics of the description of sporadic heart myxomas: localisation in the heart chambers, mobility of the neoplasm, the presence of a leg in the tumour, the shape, surface, consistency of the tumour, and the cellular composition of the myxoma (Table 1).

Table 1. Algorithm for describing morphological changes by heart myxomas

Divisions of myxoma of the heart	Object	Morphological feature
Leg – the basis of the tumour	Structures from tumour cells	Single mononuclear cells
		Single multinucleated cells
		Nonperfused compact syncytia
		Nonperfused ocular syncytia
		Perfused syncytia
	Vessels	Arteries
		Veins
		Lymphatic vessels
		Small sinusoids

Table 1. Continued

Divisions of myxoma of the heart	Object	Morphological feature
Leg – the basis of the tumour	Vessels	Large Sinusoids
		Lumen obliteration
		Endothelial cell transformation
	General pathology	Dystrophy
		Fibrosis
Body (central part) of the tumour	Structures from tumour cells	Single mononuclear cells
		Single multinucleated cells
		Nonperfused compact syncytia
		Nonperfused ocular syncytia
		Perfused syncytia
	Vessels	Arteries
		Veins
		Lymphatic vessels
		Small sinusoids
		Large Sinusoids
	General pathology	Hemorrhages
		Necrosis
		Lymphocytic infiltration
		Infection
Capsule (peripheral part) of the tumour	Structures from tumour cells	Single mononuclear cells
		Single multinucleated cells
		Nonperfused compact syncytia
		Nonperfused ocular syncytia
		Perfused syncytia
	Vessels	Arteries
		Veins
		Lymphatic vessels
		Small sinusoids
		Large Sinusoids
	Subordinate sections	Hemorrhages
		Necrosis
Surface	Relief	Smooth
		Villous
	Cellular composition	Endothelium
		One row of tumour cells
		Many rows of tumour cells

Source: compiled by the authors

A comparison of the CKC group (16 tumours) with the CMH group (278 tumours) was made to identify the features of the structural organisation of the heart myxomas in patients with Karney complex.

RESULTS

When investigating patient medical histories and operating log data, it was established that the average age of patients with CKC was 18.9 ± 6.1, and the average age of patients with CMH was 46.1 ± 4.7 years, which is more than 2 times higher than the same indicator. Analysis of the localisation of neoplasms indicates that in patients of both the first and second groups, myxomas of the heart were established in all its chambers. Therewith, CKC occurred with the same frequency in the right and left chambers of the heart (1:1). In the left atrium, CKC developed in 37.5% of cases, in the left ventricle – in 12.5% of CKC. Damage to the right atrium, and to the right ventricle, was observed in 25% of CKC, respectively.

In contrast to CKC, CMH clearly showed selective localisation in the left atrium (89.3%), and in 0.4% of cases, tumours were localised in both ventricles and in the right atrium (1.4%, respectively). CKC was established to be most often multiple (2-3 tumours). In contrast, only 1 case of CMH (0.4%) showed damage to both atria.

The site of the most frequent CMH fixation was the atrial septum in the oval window area (86% left atrium myxomas and 70% right atrium myxomas). While only 25% of CKC developed from this area of the atrial septum.

Macroscopically, in each case, the myxoma was a translucent, “mottled” tumour on the incision due to areas of necrosis and haemorrhage with a wide base, was of a soft elastic consistency and fit snugly to the wall. Macroscopic examination of CKC indicates the existence of two types of tumours: compact with a smooth surface and loose with villous outgrowths on the surface. The size of the tumours ranged from a few millimetres to 15 cm, usually, they were spherical in shape, and had a gelatin-like or

dense consistency. The same differences were established in the CMH group. However, in the CKC group, almost all tumours were swollen. While among CMH, 62.9% were loose tumours and 37.1% were dense. In all myxomas of the heart of both groups, the "leg" (or base) was determined, that is the formation that provided fixation of the tumour in the heart cavity.

In 72% of cases, the surface of the myxoma is smooth and covered with a thin "false" capsule, which was formed by the endothelium of the endocardium, in the remaining 38%, the surface had the appearance of villi, which were formed by tumour valves. Myxomas with a villous surface were often macroscopically fragmented, which was due to the inability to completely remove the tumour. It was villous myxomas that created the threat of separation of certain fragments and parts of the tumour during heart contractions, followed by embolism of arterial vessels.

Only one observation in altered vessels identified the transformation of endotheliocytes in tumour cells. In this case, the vascularisation of the tumour was similar to that described in CMH cases. The bulk of the tumour was represented by an amorphous oxyphilic substance.

The CKC cell population was relatively homogeneous. The stroma of these tumours was dominated by single cells of rounded, elongated and fusiform shape with one or more centrally located nuclei. The latter was characterised, as a rule, by a rounded or oval shape and barely noticeable nucleoli. The karyolem was dominated by euchromatin, and the nuclei were slightly basophilic in colour. Areas of "enlightenment" of the pericellular matrix were noted around the cells.

Similar cellular forms are typical for CMH. However, the population of cells of the latter is more pronounced in diversity. In such tumours, in addition to individual cells, polymorphic nonperfused and perfused syncytia were observed, which consisted of the cellular structures of the tumour (Fig. 1).

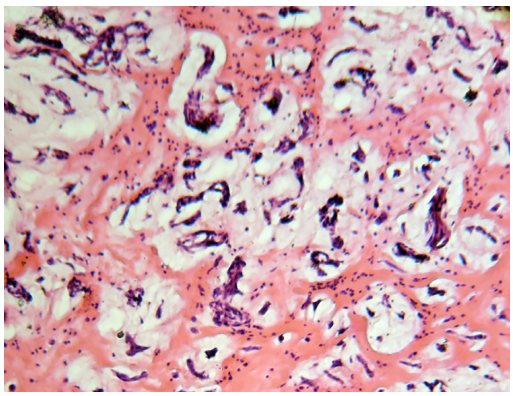

Figure 1. Single and syncytial cell structures in sporadic heart myxoma. Hematoxylin and eosin. X 400

Unlike CMH, the CKC body was characterised by a small number of syncytial structures. Only in some preparations, the presence of a small number of unanalysed compact syncytia was noted. The cells in the tumour were structurally similar to endotheliocytes and may be involved in the formation of sinusoid thin-walled blood vessels that cannot function as normal vessels. In this regard, blood-filled structures were often observed in myxomas that could not withstand the influence of the hemodynamic factor of blood flow, which led to the formation of common hematomas in the tumour body.

The surface of most CKC was represented by tumour cells in the same way as the surface of loose villous CMH. Depending on the macroscopic organisation, the surface of smooth heart myxomas was represented by the endocardial endothelium, and the surface of loose villous tumours was represented by the vascular endothelium. In areas that are located close to the base, it was possible to detect remnants of endothelial cover. Among CKC, in only one follow-up, one of the two tumours was compact and covered with endocardial endothelium in the same way as all compact CMH.

In all observations, separate single- or multinucleated cells with a more or less pronounced pericellular rim were established. Myxomal cells were located singly or in small groups in the myxomatous matrix. In addition, in patients with CKC, damage to the mitral valve leaves was observed, which was provoked by the prolonged existence of myxoma of the heart.

Histological examination of the part of the mitral valve of the heart that was removed during surgery identified that in its dense surface layer on the atrial side, there were elegant bundles of collagen fibres, sometimes homogenised or fragmented and with other signs of degenerative changes. From the outside, they were covered with an unevenly organised layer of loose fibrous unformed connective tissue, and on the damaged surface of the mitral valve leaf, signs of active proliferation of endothelial cells were noted, which formed papillary structures (Fig. 2).

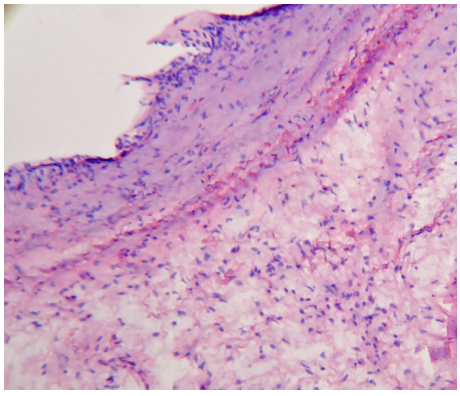

Figure 2. Mitral valve. Proliferation of endotheliocytes on the damaged leaf floor. Hematoxylin and eosin. X 400

The spongy layer of the leaf formed by loose connective tissue was very thin in a substantial area, but at the base of the chord, especially at the valvular edge, its volume increased dramatically due to oedema and fibroblast proliferation. Connective tissue cells had a typical morphological structure, which is characteristic of fibroblastic cells in mitral valve damage in patients with acquired malformations not caused by heart tumours. The described changes in the mitral valve leaf in CKC of left atrial localisation can, on the one hand, be associated with chronic mechanical injury to the valve structures by a tumour that has fallen into the left ventricle. On the other hand, it cannot be excluded that the high proliferative activity of the endothelium and connec-

tive tissue elements is a manifestation of genetic features that are characteristic of the Carney complex.

Therefore, there is reason to believe that the morphological changes described in patients with CKC are secondary to the cardiac tumours observed in patients with CMH. Microscopic examination of both groups by heart myxomas identified that the cellular composition and stroma components of these myxomic tumours are not fundamentally different, however, when investigating CKC, massive foci of necrosis and haemorrhages drew attention to themselves, which indicated a violation of the blood supply to tumours. An indirect confirmation of this was the almost complete absence of typical CKC blood vessels with signs of intima hyperplasia and middle membrane hypertrophy in the area of the CMH base. And only in a few histological preparations from the 4th observation, sharply sclerosed deformed blood vessels of the arterial type could be detected in the "leg" of the tumour.

DISCUSSION

Carney syndrome is a disease that is transmitted by an autosomal dominant type of inheritance, characterised by the development of myxomas not only of the heart but also of other organs. Clinical manifestations of this pathology can be: spotty pigmentation of the skin, the appearance of such neoplasms as tubular testicular adenoma, pituitary adenoma, and thyroid tumours in patients [15-17].

There is no clear morphological feature or several features that would allow clearly distinguishing between myxomas of the heart as part of Carney's syndrome and CMH [18]. However, according to J. Carney & R.G. Swee [19], primary heart tumours associated with Carney syndrome have no age or gender characteristics, can be either single or multiple, located in different chambers of the heart, and have a tendency to relapse despite proper surgical treatment.

In almost all CKC observations, the benign nature of the tumour was not in doubt, and this is consistent with the generally accepted view of researchers about the appearance of myxomas of the heart. In the case where signs of active proliferation were detected, but there are no atypical cells, it does not allow classifying this tumour as malignant. However, for scientific confirmation of the assumption about the malignant origin of the myxomas, additional studies are needed, in particular, a comparative immunohistochemical examination of the proliferative activity of both groups of the myxomas of the heart, and modern genetic analysis of tumours and patients [20, 21].

Researchers note that CKC recurs more often than normal heart myxomas. According to various authors, their number ranges from 7 to 20%. Complications such as embolism, acute circulatory failure, and arrhythmia, which are often observed in patients with pathology of the cardiovascular system, indicate the possibility of a malignant course of heart myxomas. Embolism is a threatening symptom in the clinical course of myxoma of the heart and occurs in 30-45% of patients with a left atrial tumour [22, 23].

According to the results of the conducted studies, it was established that relapse was considered CKC in one observation, since it occurred in the left atrium, on the atrial septum in the area of the oval window, that is, in the same place where the first tumour was localised (according to the medical institution where the first operation was performed). In the remaining observations, the tumours were primary, as evidenced by new sites of formation and growth.

CKC, in contrast to CMH, were more likely to be multiple rather than isolated and did not have a predominant localisation in the left atrium. Comparative analysis of the cellular composition of CKC and CMH allows assuming the same origin of the two groups of tumours.

According to the analysis of literature sources, with the left atrial localisation of myxoma, the tumour prevents the complete closure of the mitral valve leaves, which causes a clinical picture of a combined mitral heart defect, myxoma of the right ventricle will clinically manifest itself as a symptom complex of pulmonary artery stenosis, and in the case of left ventricular damage, it will clinically manifest itself as signs of aortic stenosis, myxoma of the right atrium imitates the syndrome of the upper or lower vena cava due to partial overlap and difficult outflow of blood from these veins, which is consistent with the clinical trials observations. Massive foci of necrosis and haemorrhage, which were diagnosed by light-optical examination of the surgically removed heart myxomas, indicated a violation of blood supply to the myxomas, which is due to insufficient tropism of the tumour. Only in a few drugs from the 4th observation, sharply sclerosed deformed arteries could be detected in the "leg" of the tumour. Detection of these structures is extremely important for the identification of tumours since they are one of the indications of the vascular origin of heart myxomas [23, 24].

However, if in CMH the development of the tumour is directed mainly towards the formation of vascular structures (perfused syncytia, sinusoid vessels), then in CKC the growth of the tumour occurred mainly by multiplying single cells without specific structure formation. That is, there is reason to assume that CKC is characterised by a more "primitive" way of development.

Microscopically, deformed arteries were often established at the base of the tumour, and in the central part – areas of necrosis, haemorrhage. Since myxomas are washed with blood located in the chambers of the heart, tumour cells multiply and grow over their necrotic areas, which allows for villous outgrowths to form. In 80% of cases, the tumour is attached to the endocardium by a long leg, less often it is fixed by a wide base, and over time, myxomas of the heart can calcify [25].

The ability to relapse and embolic complications allow classifying myxomas of the heart as benign tumours but with the possibility of a malignant course, which causes special attention from clinicians and requires timely diagnosis.

CONCLUSIONS

CKC, like CMH, are benign heart tumours, and CKC are more often multiple than separate, and unlike CMH, they do not have a predominant localisation in the left atrium. Myxomas are translucent, colourless tumours of a soft consistency that differ in the presence of an external connective tissue capsule.

The cellular composition and stroma components of these tumours do not differ fundamentally, and this allows assuming a common source of tumour growth. However, the presence of more complex cell formations in CMH and

the predominance of single cells in CKC indicate a different level of cell organisation in CKC tumours and the existence of differences in their morphogenesis.

Massive foci of necrosis and haemorrhages indicate a violation of the blood supply to tumours, which are caused by both changes in the structure of feeding vessels and newly formed sinusoid vessels. In the structures of the mitral valve that is mechanically affected by the heart myxomas in patients with CKC, there are signs of changes in the structure and activity of endothelial cells and fibrous connective tissue, which can be a sign of both alteration and high replication activity.

Further research of CKC in terms of comparative immunohistochemical studies of the proliferative activity of tumour cells with CMH, and genetic analysis of patients, are of interest. In clinical practice, the study involves conducting a taxonomy of patients and clinical manifestations of the disease, creating an algorithm for early diagnosis of heart myxomas, which will allow the development of adequate methods for treating the disease and preventing its complications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Закономірності структурної організації міксом серця у хворих із комплексом Карнея

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Анотація. Міксоми серця – одні з найпоширеніших первинних пухлин серця, які найчастіше розвиваються у лівому передсерді, характеризуються доброякісним характером і відсутністю рецидивів після хірургічного видалення пухлини. Комплекс Карнея – аутосомно-домінантне захворювання, що характеризується численними пухлинами, зокрема – міксомами серця. Недостатнє висвітлення даної патології у літературі призводить до помилкового діагнозу, прогресування захворювання та неадекватного лікування. Метою роботи було визначення морфологічних особливостей міксом серця у хворих із комплексом Карнея. За допомогою світлової мікроскопії проведено морфологічне дослідження міксом серця, видалених під час 7 операцій. З операційного матеріалу виготовляли гістологічні зрізи, які фарбували гематоксиліном та еозином, за Ван Гізон, фукселіном, а також Masson's Trichrome Stain у модифікації Зербіно-Лукасевич. Для виявлення морфологічних особливостей було зроблено порівняння групи міксом серця хворих із комплексом Карнея (16 пухлин) із групою міксом серця, що виникають спорадично (278 пухлин). Встановлено, що міксоми серця є множинними, вони з однаковою частотою визначались у правих і лівих камерах серця. Було узагальнено, що при міксомах серця відзначаються ознаки як альтерації, так і високої проліферативної активності ендотеліоцитів і клітин волокнистої сполучної тканини. Вторинні міксоми серця у хворих на комплекс Карнея мають таку ж структурну організацію, що й первинні пухлини, проте найчастіше вони не є рецидивами, частіше бувають множинними, не мають переважної локалізації у лівому передсерді. Проведений аналіз встановив ідентичний клітинний склад обох груп міксом серця, що дозволяє припустити однакове джерело росту пухлин, проте наявність у спорадичних міксомах більш складних клітинних утворень та переважання в Карнея міксомах серця поодиноких клітин свідчить про існування відмінностей в їх морфогенезі

Ключові слова: доброякісні пухлини; морфологія; мітральний клапан; синдром Карнея; світлова мікроскопія

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Cardio-respiratory endurance of individuals with different blood pressure levels

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Abstract. Evaluation of cardio-respiratory endurance in individuals with different levels of blood pressure is one of the key factors in preventing the development of diseases of the cardiovascular system. The purpose of the study was to conduct a comparative assessment of cardio-respiratory endurance, the level of maximum oxygen consumption, and the results of the Ruffier test in individuals with different blood pressure levels. 320 people were examined, of which 4 groups were formed according to the initial level of blood pressure. After measuring baseline blood pressure and heart rate, all subjects underwent a Ruffier test (30 sit-ups for 45 seconds), followed by repeated heart rate measurements at 15 seconds, 1 minute, and blood pressure measurements at 3 minutes. Determination of the level of maximum oxygen consumption was performed according to the formula. Representatives of the group with normal-low and normal blood pressure have a significantly higher level of maximum oxygen consumption. Individuals with normal-high blood pressure and first-degree hypertension had higher levels of systolic and diastolic blood pressure during daily blood pressure monitoring, both during the day and at night, compared to individuals with normal and normal-low blood pressure. It was established that people with normal-low blood pressure have a higher cardio-respiratory endurance than people with normal-high blood pressure and first-degree hypertension. The findings of the study can be used by cardiologists and general practitioners for early detection and prevention of diseases of the cardiovascular system, and physiologists in further investigation of the features of the functioning of the cardiovascular system

Keywords: Ruffier test; daily blood pressure monitoring; cardio-respiratory endurance; maximum oxygen consumption

INTRODUCTION

Prevention of the development of diseases of the cardiovascular system is an important task of modern medicine. The key step in solving this problem is to examine the features of the functioning of the cardiovascular system. One of the main indicators for this is cardio-respiratory endurance (CRE).

CRE, according to the interpretation of J. Myers *et al.* [1], is a complex indicator of the state of the body, used for early diagnosis of hypertension, coronary heart disease, and other chronic diseases. P.F. Kokkinos *et al.* [2] and M.P. Harber *et al.* [3] observed the association between CRE levels and the body's respiratory function, metabolic activity, physical activity, and anthropometric parameters. B. Mitskan *et al.* [4] confirm that individuals with low CRE levels during physical exertion develop a violation of bioenergetic processes in peripheral blood red blood cells, a decrease in the production of adenosine triphosphate (ATP), and an increase in the content of intra-erythrocyte 2.3-diphosphoglycerate (DPG). R. Ortega *et al.* [5] note that reduced CRE is also a factor in the occurrence of dys-

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lipidemia, which, in turn, contributes to the further development of cardiovascular diseases.

A simple and well-known CRE test is the Ruffier test, which analyses the dynamics of heart rate in response to physical activity. N. Kotsur & L. Tovkun [6] found that lower-than-average Ruffier test results in adolescents aged 14-16 years were associated with reduced functional reserves of the cardiovascular system. Y. Guo *et al.* [7] and G. Papini *et al.* [8] suggest that the Ruffier test with a high level of reliability allows determining the maximum level of oxygen consumption, beyond which there is no further increase in oxygen consumption with increasing load intensity (VO_{2max}).

L. Jay & X.L. Zhang [9] found that VO_{2max} it is a reliable method for estimating CRE, as it reflects the integrated ability to transport oxygen from the atmosphere to the mitochondria to perform physical work, and it quantifies the functional capacity of the human body.

A.D. Hughes & N. Chaturvedi [10] established that between the level of blood pressure (BP) and VO_{2max} there is a significant negative correlation. The studies by P. Boutouyrie *et al.* [11] confirm that an increase in BP leads to an increase in arterial stiffness and a decrease in the ability to transport blood and oxygen to working muscles.

Daily blood pressure monitoring (DBPM) provides a more complete assessment of the state of the cardiovascular system and allows predicting the health consequences better than single BP measurements in the clinic or at home [12, 13]. W.Y. Yang *et al.* [14] determined that data from 24-hour DBPM and, in particular, night BP monitoring are valuable predictors of the development of hypertension complications and overall cardiovascular mortality. I.A. Plesh *et al.* [15] found that the progression of hypertension is associated with changes in the circadian rhythm of BP. Individuals with a low level of nocturnal BP dipping had a higher degree of left ventricular wall hypertrophy and an increased discrepancy between the left ventricular (LV) myocardial mass index and the LV myocardial mass, which indicates an acceleration in the progression of hypertension.

The purpose of this study was to establish the features of CRE, the results of the Ruffier test, and the level of maximum oxygen consumption in individuals with different BP levels.

MATERIALS AND METHODS

The study was conducted in August-November 2022 at the certified laboratory of psychophysiological research (Certificate No. 055/13) of the Department of Physiology with Basics of Bioethics and Biosafety at the I.Ya. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine.

The study involved 240 individuals aged 18-22 years without cardiovascular diseases and not taking antihypertensive medications, and 80 individuals aged 18-22 years with stage 1 hypertension but not taking antihypertensive medications during the examination.

All participants underwent baseline BP measurements. The participants were divided into four groups of 80 individuals each based on their BP levels according to the recommendations of the European Society of Cardiology [16]: Group I – individuals with optimal or normal-low baseline BP (< 120/80 mmHg); Group II – individuals with normal BP (120/80-129/84 mmHg); Group III – individuals with normal-high BP (130/85-139/89 mmHg); Group IV – individuals with stage 1 hypertension (BP: 140/90-159/95 mmHg).

After measuring BP, a Ruffier test was performed. Before the test, the subjects rested for 5 minutes in a supine position. Then, the initial heart rate (HR) was determined in the standing position. After that, the subjects performed 30 squats for 45 seconds. The squat rhythm was set by a metronome (80 beats per minute). Squats were performed by bending the knees to a 90° angle, with the participant keeping their back straight and arms extended forward. After the test, HR was measured again at 15 seconds and 1 minute intervals, and BP was measured after 3 minutes. The Ruffier-Dickson index was determined using the formula [9]:

$$Index = \frac{(HR2-70)+2\times(HR3-HR1)}{10}, \quad (1)$$

where HR1 is in the initial state, HR2 is 15 seconds after the test, and HR3 is after 1 minute of rest. Heart rate is indicated in beats per minute.

VO_{2max} was determined with the formula [8]:

$$VO_{2max} = 3{,}0143 + 1{,}1585\times Gender - 0{,}0268\times \times\left(\frac{HR1}{Height}\right) + 118{,}761\times\left[\frac{(HR2-HR3)}{Age^3}\right., \quad (2)$$

where gender is encoded: 1 for men and 0 for women, HR1 – in the initial state, HR2 – 15 seconds after the test, HR3 – after 1 minute of rest. Heart rate is indicated in beats per minute, age in full years, and height in metres.

All subjects underwent daily pressure monitoring using the ABPpro device. The frequency of measurements was every 30 minutes during the day (from 08:00 to 22:00) and every hour during the night (from 22:00 to 08:00). The level of nocturnal decrease in systolic BP (%) was calculated using the formula:

$$\frac{daytime\ systolic\ BP - nighttime\ systolic\ BP}{daytime\ systolic\ BP}\times 100. \quad (3)$$

The obtained results were statistically processed using the open-source statistical package "R" [17]. The normality of the distribution of groups was assessed using the Shapiro-Wilk test. The homogeneity of variances between independent groups was determined using Levene's test. One-way analysis of variance (ANOVA) was used to assess the significance of the effects of the studied factors. The statistical significance of the differences between groups was determined using Tukey's post hoc test and Student's t-test.

Throughout the study, adherence to bioethical norms, such as the Helsinki Declaration and the World Medical Association's "Ethical Principles for Medical Research Involving Human Subjects", was ensured [18]. All subjects, before participating in the experiment, gave written informed consent to conduct the study. Confidentiality of personal data of the subjects was ensured.

RESULTS

The average values of systolic BP were as follows: Group I – 107 ± 4.93 mmHg, Group II – 125 ± 2.34 mmHg, Group III – 136 ± 2.24 mmHg, and Group IV – 145 ± 2.81 mmHg. After the Ruffier test, the systolic BP values were: Group I –

127 ± 5.38 mmHg, Group II – 143 ± 3.41 mmHg, Group III – 159 ± 3.54 mmHg, and Group IV – 173 ± 3.68 mmHg.

All groups showed a significant ($p < 0.05$) increase in systolic BP after the Ruffier test compared to baseline (Fig. 1).

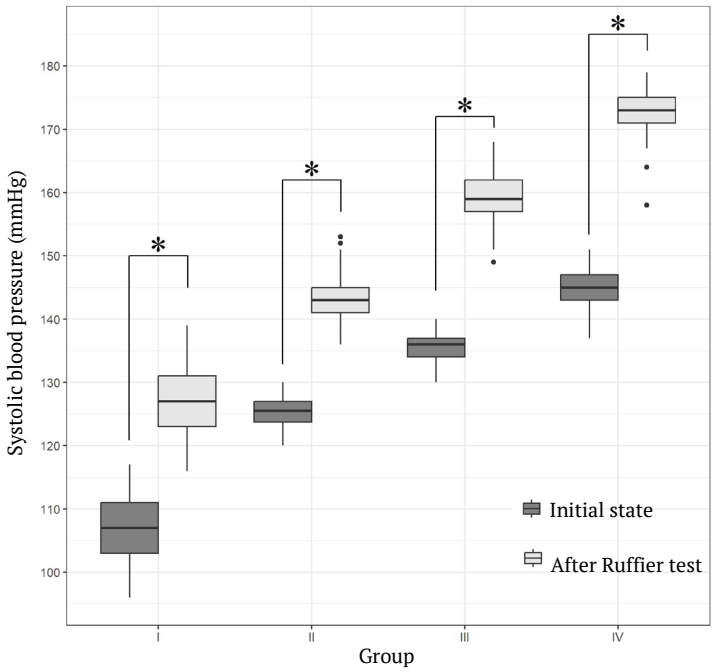

Figure 1. Dynamics of systolic BP after physical exercise
Notes: * – significantly different from the baseline value ($p < 0.05$)
Source: compiled by the authors

The increase in systolic blood pressure after physical exercise was 20.0 ± 2.46 mmHg (18.7% of the baseline value) in Group I, 17.8 ± 2.69 mmHg (14.24%) in Group II, 23.9 ± 2.40 mmHg (17.6%) in Group III, and 28.1 ± 2.15 mmHg (19.38%) in Group IV. Significantly higher increases in systolic blood pressure after physical exercise were observed in participants from Group III and Group IV compared to those from Group I and Group II (Fig. 2).

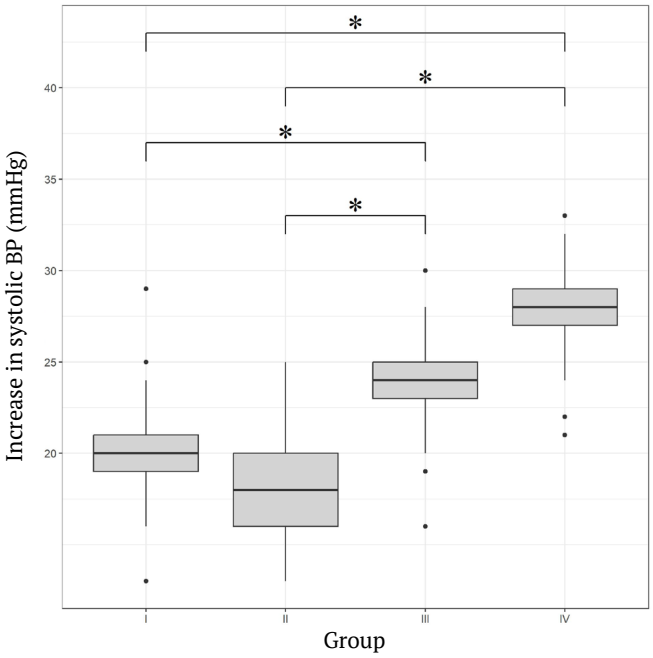

Figure 2. Increase in systolic BP after physical exercise
Notes: * – significantly different from Group I and Group II ($p < 0.05$)
Source: compiled by the authors

The average values of diastolic BP were as follows: Group I – 69.4 ± 2.17 mmHg, Group II – 79.4 ± 3.58 mmHg, Group III – 90 ± 3.43 mmHg, and Group IV – 96 ± 2.23 mmHg. After the Ruffier test, the diastolic BP values were: Group I – 79.1 ± 2.90 mmHg, Group II – 88.5 ± 4.71 mmHg, Group III – 102±3.92 mmHg, and Group IV – 109±2.96 mmHg. In all groups, a significant ($p < 0.05$) increase in diastolic BP was observed after the Ruffier test compared to the baseline level (Fig. 3).

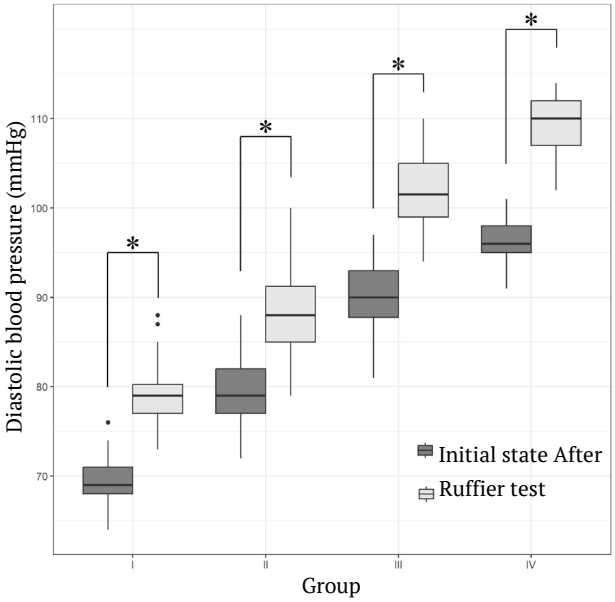

Figure 3. Dynamics of diastolic BP after physical exercise
Notes: * – significantly different from the baseline value ($p < 0.05$)
Source: compiled by the authors

The increase in diastolic blood pressure after physical exercise was 9.69 ± 2.26 mmHg (13.96% of the baseline value) in Group I, 9.05 ± 2.47 mmHg (11.4%) in Group II, 11.6 ± 2.26 mmHg (12.88%) in Group III, and 13.4 ± 2.30 mmHg (13.95%) in Group IV. Significantly higher increases in diastolic blood pressure after physical exercise were observed in participants from Group III and Group IV compared to those from Group I and Group II (Fig. 4).

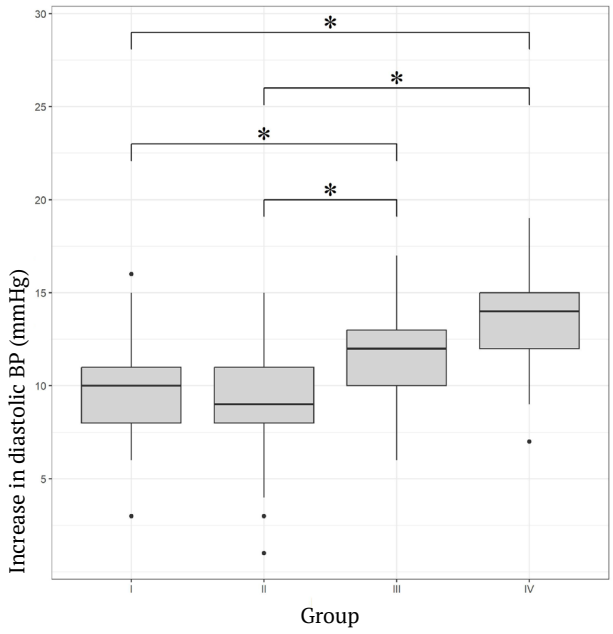

Figure 4. Increase in diastolic BP after physical exercise
Notes: * – significantly different from Group I and Group II ($p < 0.05$)
Source: compiled by the authors

The increase in HR after the Ruffier test indicates an intensified sympathetic influence on cardiac activity, as evidenced by the increase in HR at 15 seconds and 1 minute after exercise (Table 1).

Table 1. Heart rate dynamics after physical exercise

Group	Initial state, min-1	After 15 seconds, min-1	After 1 minute, min-1
I	71.8 ± 7.74	126 ± 7.90*	87.9 ± 7.87
II	70.4 ± 7.11	123 ± 7.28*	83.3 ± 7.43
III	75.4 ± 7.65	137 ± 7.55*	108 ± 7.54**, ***
IV	80.1 ± 724	145 ± 7.42*	117 ± 7.61**, ***

Notes: * – significantly different from the baseline value ($p < 0.05$). ** – Significantly different from Group I ($p < 0.05$). *** – Significantly different from Group II ($p < 0.05$)
Source: compiled by the authors

At 15 seconds after physical exercise, HR increased by 54.0 ± 0.961 beats/min^{-1} (75.2%) in Group I, 52.2 ± 1.08 beats/min^{-1} (74.14%) in Group II, 62.0 ± 0.948 beats/min^{-1} (82.23%) in Group III, and 65.4 ± 0.995 beats/min^{-1} (81.16%) in Group IV. Participants from Group III and Group IV had a significantly ($p < 0.05$) higher increase in HR at 15 seconds after exercise compared to those from Group I and Group II. 1 minute after physical activity, the increase in HR from the initial value in the subjects of Group I was 16.1 ± 2.15 min^{-1} (22.42%), Group II – 12.9 ± 1.92 min^{-1} (18.32%), Group III – 32.4 ± 2.35 (42.9%), Group IV – 36.5 ± 2.15 (45.56%). Participants from Group III and Group IV had a significantly ($p < 0.05$) higher increase in HR at 1 minute after exercise compared to those from Group I and Group II.

These findings indicate that individuals in Group III and Group IV had a significantly higher HR at 1 minute after the Ruffier test, indicating a more pronounced cardiovascular response to sympathetic influences in these groups. The significantly higher level of the Rufier-Dixon index in participants from Group III and Group IV indicates their lower endurance to physical exercise (Table 2).

Table 2. Ruffier test results and maximum oxygen consumption level

Group	Ruffier-Dixon Index	Maximum oxygen consumption level (VO_{2max}), l / min	Maximum oxygen consumption level (VO_{2max}), ml / kg / min
I	8.80 ± 0.881	2.94 ± 0.638	43.9 ± 8.63
II	7.83 ± 0.847*	3.00 ± 0.637	44.5 ± 8.24
III	13.2 ± 0.831*, **	2.79 ± 0.593	40.5 ± 6.52*, **
IV	14.8 ± 0.893*, **	2.71 ± 0.640**	39.4 ± 7.75*, **

Notes: * – significantly different from Group I ($p < 0.05$). ** – Significantly different from Group II ($p < 0.05$)
Source: compiled by the authors

Vo_{2max} level in participants from Group I and Group II corresponds to a sufficient level of CRE (42-45 ml/kg/min). In representatives of groups III and IV, this indicator was significantly lower and equal to a low level of CRE (<42 ml/kg/min).

During 24-hour ambulatory BP monitoring, the average systolic BP levels throughout the day were as follows: Group I – 109 ± 5.33 mmHg, Group II – 129 ± 3.93 mmHg, Group III – 140 ± 4.24 mmHg, and Group IV – 152 ± 4.13 mmHg. During the night, the systolic BP values were: Group I – 96.9 ± 5.38 mmHg, Group II – 116 ± 3.42 mmHg, Group III – 129 ± 3.17 mmHg, and Group IV – 139 ± 3.59 mmHg. DBPM showed a significantly higher BP in individuals of groups III and IV compared to I and II (Fig. 5).

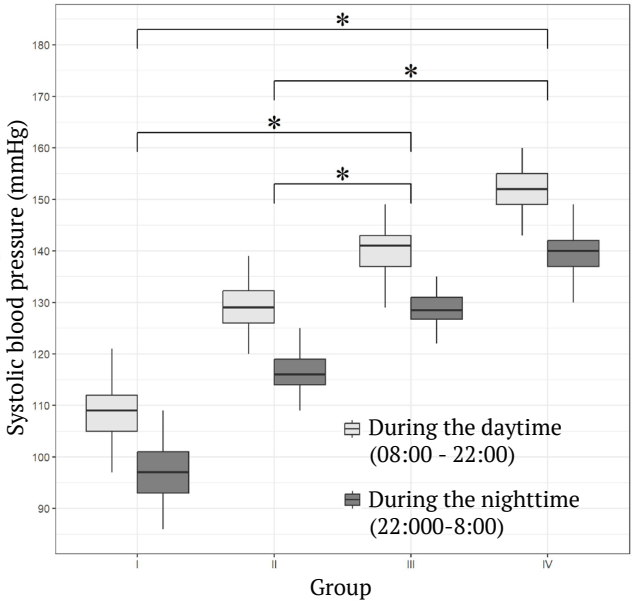

Figure 5. Daily monitoring of systolic BP
Notes: * – significantly different from Group I and Group II ($p < 0.05$)
Source: compiled by the authors

The diastolic BP during the daytime was: Group I – 70.0 ± 3.78 mmHg, Group II – 80.9 ± 5.17 mmHg, Group III – 93.0 ± 5.26 mmHg, and Group IV – 99.5 ± 3.93 mmHg. During the night, the diastolic BP values were: Group I – 60.8 ± 2.98 mmHg, Group II – 71.6 ± 4.37 mmHg, Group III – 83.6 ± 4.13 mmHg, and Group IV – 90.0 ± 3.26 mmHg. The level of diastolic BP during both daytime and nighttime was significantly higher in individuals from Group III and Group IV compared to those from Group I and II (Fig. 6).

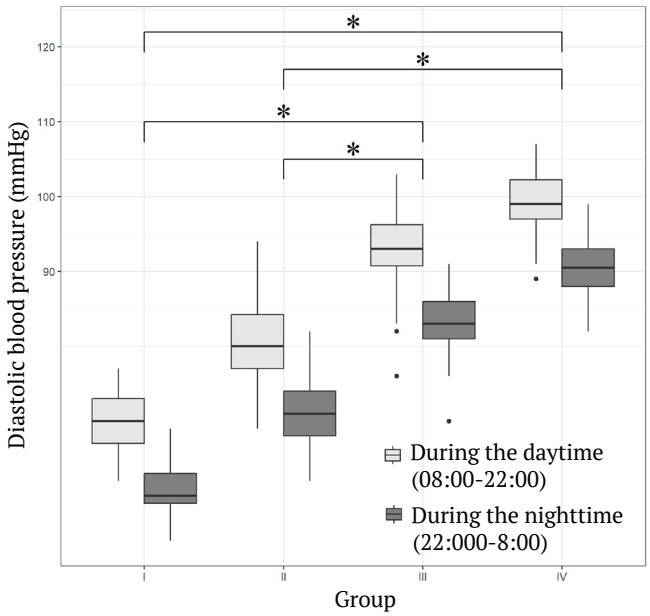

Figure 6. Daily monitoring of diastolic BP
Notes: * – significantly different from Group I and Group II ($p < 0.05$)
Source: compiled by the authors

Participants from Group III with a normal-high baseline BP level and Group IV with first-degree hypertension had significantly higher 24-hour BP values compared to individuals with normal-low BP (Group II) and individuals with normal BP (Group III). The level of nocturnal BP dipping, expressed as the percentage reduction in systolic BP, was 10.6 ± 3.38% in Group I, 9.99 ± 2.97% in Group II, 8.2 ± 2.82% in Group III, and 8.14 ± 2.81% in Group IV (Fig. 7).

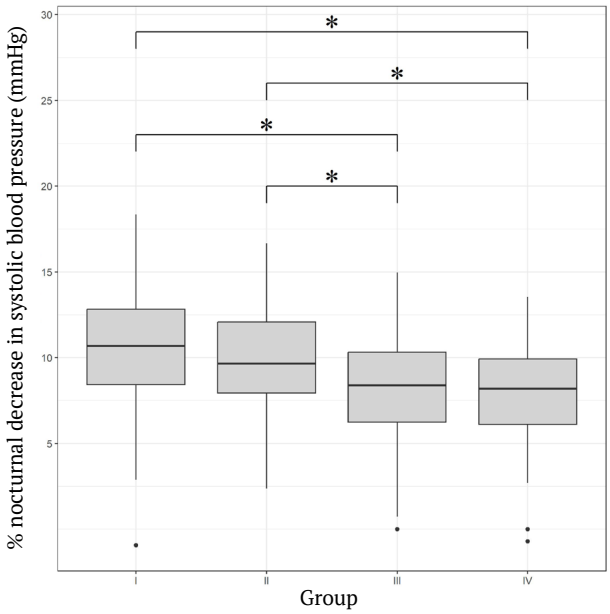

Figure 7. The level of nocturnal decrease in systolic BP
Notes: * – significantly different from Group I and Group II ($p < 0.05$)
Source: compiled by the authors

A significantly lower level of nocturnal decrease in systolic BP was established in individuals I and II Groups in comparison with representatives of III and IV Groups ($p < 0.05$).

DISCUSSION

The study revealed an increase in both systolic and diastolic BP after physical exertion in all groups of participants. A greater degree of increase in systolic and diastolic BP was observed in individuals with normal-high BP and hypertension. These results can be explained by more pronounced sympathetic responses to physical exertion in individuals with normal-high BP and hypertension. E.N. Bardsley & D.J. Paterson [19] confirm that increased sympathetic tone is an important factor in the development of hypertension. A key role in this process is played by genetically determined imbalances between intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), leading to an increase in intracellular Ca2+ levels and activation of the sympathetic system cascade. This disruption in BP regulation leads to its elevation, increased risk of arrhythmias, and cardiomyopathies.

The results of the study indicate that individuals with normal-high BP and hypertension have lower levels of VO_{2max}. Additionally, these groups showed higher HR values at the 1-minute mark after physical exertion. The findings are consistent with study by K.A. Alahmari *et al*. [20], which demonstrated a strong negative correlation between BP levels and VO_{2max}, and between heart rate and VO_{2max}. One possible physiological mechanism underlying these changes is increased vascular resistance due to increased vascular wall stiffness in individuals with normal-high BP and hypertension. This leads to impaired transportation of oxygenated blood to the muscles during physical exertion. J. Mahdiabadi [21] established a correlation between a 10.3 ml/kg/min increase in VO_{2max} and a decrease in systolic BP by 10.2 mmHg and diastolic BP by 5.9 mmHg.

In a retrospective study, T. Holmlund *et al*. [22] found that negative CRE dynamics are associated with an increased risk of pre-hypertension and hypertension. Thus, individuals who had an annual decrease in CRE from -1.0% to -2.9% had a 21% higher risk of hypertension, and individuals with an annual decrease in CRE ⩾-3% had a 25% higher risk of hypertension. Moreover, individuals with an increase in CRE by ⩾+3% had an 11% lower risk of hypertension.

In a meta-analysis of the relationship between CRE and hypertension conducted by C. Cheng *et al.* [23] concluded that the risk of hypertension was 37% lower in people with high CRE compared to people with low CRE, and 15% lower in people with moderate CRE compared to people with low CRE. In addition, in the cohort study by J. Lee *et al*. [24], involving 2 962 subjects, individuals with high CRE during 9 years of follow-up had a 29% lower risk of coronary heart disease, a 25% lower risk of acute myocardial infarction, a 46% lower risk of stroke, and a 44% lower risk of overall mortality compared to individuals with low CRE. The observed patterns are confirmed in this study, as it was found that individuals with normal to low and normal BP had higher levels of maximal oxygen consumption and, consequently, higher CRE compared to individuals with normal to high BP and hypertension.

In this study, the mean values of systolic and diastolic BP, when monitored daily, were higher both during the day and during the night in individuals with normal-high BP and hypertension compared to individuals with normal BP and normal-low BP. Furthermore, the level of nocturnal decrease in systolic BP was higher in subjects with normal-low and normal BP levels compared to people with normal-high and high BP. The results obtained are consistent with the conclusions of the EXERDIET-HTA study conducted by I. Gorostegi-Anduaga *et al*. [25], which established that individuals with low CRE have significantly lower levels of nocturnal BP dipping compared to individuals with moderate to high CRE.

Considering the data of the scientific literature and the results of this study, it can be argued that individuals with normal to high BP and those with stage 1 hypertension experience changes in the functioning of the cardiovascular system, reflected in lower CRE and a reduced nocturnal BP dipping, which in turn is evidence of a risk of further progression of arterial hypertension and the development of other cardiovascular diseases.

CONCLUSIONS

Individuals with normal-high BP and first-degree hypertension are characterised by a lower level of maximum oxygen consumption during physical exertion than individuals with normal-low BP. The lower level of endurance of the cardiovascular system to physical activity in those examined with normal-high BP and hypertension of the first degree is indicated by a lower level of the Ruffier-Dixon index in them, compared with those examined with normal-low BP and normal BP.

During daily BP monitoring, individuals with normal-high BP and first-degree hypertension had significantly higher levels of systolic and diastolic BP both during the day and at night compared to individuals with normal and normal-low BP. The level of nocturnal BP dipping was lower in subjects with normal-high BP and first-degree hypertension.

The results obtained can be used in the practice of cardiologists and family physicians for early diagnosis and prevention of hypertension, and in the work of physiologists to examine the features of the functioning of the cardiovascular system.

For further studies, a detailed analysis of fluctuations in daily BP in different groups of subjects and the development of prognostic models for clinical application of the results obtained is promising.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Кардіо-респіраторна витривалість осіб із різним рівнем артеріального тиску

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Анотація. Оцінка кардіо-респіраторної витривалості в осіб із різним рівнем артеріального тиску є одним із ключових факторів попередження розвитку захворювань серцево-судинної системи. Мета роботи полягала у проведенні порівняльної оцінки кардіо-респіраторної витривалості, рівня максимального споживання кисню та результатів проби Руф'є в осіб із різним рівнем артеріального тиску. Обстежено 320 осіб, із яких сформовано 4 групи згідно вихідного рівня артеріального тиску. Усім обстежуваним після вимірювання вихідного артеріального тиску та частоти серцевих скорочень проведено пробу Руф'є (30 присідань протягом 45 секунд), після чого виконано повторне вимірювання частоти серцевих скорочень через 15 секунд, 1 хвилину та вимірювання артеріального тиску через 3 хвилини. Визначення рівня максимального споживання кисню проводилося згідно формули. Представники групи із нормально-низьким та нормальним артеріальним тиском мають достовірно більший рівень максимального споживання кисню. У осіб із нормально-високим артеріальним тиском та гіпертонічною хворобою першого ступеня протягом добового моніторування артеріального тиску виявлено більший рівень систолічного та діастолічного артеріального тиску як в денний, так і в нічний період порівняно із особами із нормальним та нормально-низьким рівнем артеріального тиску. Проаналізовано, що особи із нормально-низьким артеріальним тиском мають більшу кардіо-респіраторну витривалість, ніж особи із нормально-високим артеріальним тиском та гіпертонічною хворобою першого ступеня. Результати роботи можуть бути використані лікарями кардіологами та лікарями загальної практики з метою раннього виявлення та попередження захворювань серцево-судинної системи, а також фізіологами при подальшому вивченні особливостей функціонування серцево-судинної системи

Ключові слова: проба Руф'є; добовий моніторинг артеріального тиску; кардіо-респіраторна витривалість; максимальне споживання кисню

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Cerebral circulation improvement method using boxing and orthodontic mouthguards

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Abstract. Due to the active hostilities in Ukraine, the rates of exacerbations of chronic diseases, trauma to the head and spinal column due to both gunshot wounds and increased force load on the musculoskeletal system have increased significantly. The issue of improving rehabilitation approaches is relevant due to the available statistics on cerebral blood supply pathologies. Given the need for affordable and effective methods of musculoskeletal rehabilitation, the study aims to investigate the effectiveness of orthodontic and boxing mouthguards on the state of cerebral blood vessels. Five patients with a history of contusion and post-traumatic stress disorder were studied at the Kyiv Regional Centre for Mental Health using a physical examination and rheoencephalography. The use of orthodontic and boxing mouthguards showed positive dynamics in the restoration of maxillofacial muscles, compensation of bite height and satisfactory blood supply to brain structures compared to the baseline data. The analysis of the encephalograms showed rheoencephalographic signs of improvement in cerebral circulation: a decrease in spasm of the arterial walls of cerebral vessels, compensation for blood supply deficit, improvement of blood circulation in the carotid basin and venous outflow in the deficit mode were detected. In the examined patients with injuries, pain syndrome and constant mechanical stress on the musculoskeletal system, a decrease in pain and improvement of muscle symmetry in the affected anatomical areas were noted while wearing orthodontic and boxing mouthguards. The obtained results can be implemented in the rehabilitation protocols for patients with concussion, brain concussion, and spinal and skull injuries. The availability and effectiveness of the proposed technique allow it to be used for patients with chronic diseases of the cardiovascular system, especially with complicated blood supply to brain structures

Keywords: medical rehabilitation; orthodontics; skull bones; rheoencephalography; aligner; post-traumatic stress disorder

INTRODUCTION

With the onset of military aggression in Ukraine, the number of stress-related diseases has increased significantly: according to current publications [1-3], there is an active dynamic of exacerbations of chronic pathologies and a sharp increase in psycho-emotional disturbances in patients. Many chronic diseases that were previously in a compensated state, such as diseases of the central nervous system (CNS) or trauma, have begun to acutely disturb patients again. According to a study by I.S. Mironyuk *et al.* [1], in Ukraine, in addition to an increase in morbidity, there are high risks of an increase in overall and premature mortality due to the war. Thus, providing affordable and effective medical correction for the population's health is an urgent issue in Ukraine [2]. In addition, as noted by M.V. Danchenko & Yu.M. Korzh [4], new direct causes of pain syndromes have emerged, such as wearing body armour, military ammunition, and prolonged intense physical activity. Therefore, the question arises of developing reliable methods of treatment, prevention of exacerbations and development of pathologies of the human musculoskeletal system, which directly affects the adequate blood supply to the CNS organs. Given the relevance of this issue for military personnel, the methods should be easy to implement, effective and consider the specifics of traumatic factors.

There are areas of medical rehabilitation not only in Ukraine, where active hostilities are taking place but also

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in many other countries. As V. Steblyuk [5], rehabilitation is focused on a quick, effective, and maximally restorative effect on the patient's musculoskeletal system to improve the quality of life. The importance of this issue is also confirmed by the statistics of the Medical Forces Command: bone defects in the military, especially with gunshot wounds, are noted in almost 12% of patients [3].

However, the question arises as to which medical tool can be used to restore the musculoskeletal system, in particular the CNS blood supply system, when a patient subjectively considers himself or herself healthy but regularly receives an atypical load on the spinal column and spinal cord, such as military personnel whose ammunition weighs up to sixty kilograms [6]. The system of medical, social, and psychological rehabilitation of veterans requires the involvement of intellectual potential and expert approach of specialists in the field of public health, medical rehabilitation and reconstructive surgery [7].

One of the areas of medical rehabilitation is devoted to the recreation of the patient's health by restoring the anatomical position of the dentition. Modern orthodontics almost completely solves the problems associated with functional disorders of the musculoskeletal system that have arisen as a result of malocclusion [8]. The methods used by orthodontists are effective and long-lasting, as changes in orthodontic treatment occur in the whole body, both at the organ and humoral levels [9]. Typically, orthodontic treatment lasts from two to three years, and changes occur not only in the oral cavity but also in the bones of the skull, gait, and posture, as well as in the cervical, thoracic, and lumbar spine. In addition, there are changes in the swallowing mechanism, resulting in changes in the innervation of the oesophagus and mediastinal organs.

Given the relevance of rehabilitation of military and civilian victims of traumatic injuries to the skull bones, cervical spine, and spinal column in general, this study aimed to experiment to investigate the effectiveness of orthodontic aligners in improving cerebral circulation by correcting the symmetry and height of the bite, as well as the anatomical load on the skull bones.

MATERIALS AND METHODS

The study was conducted at the Kyiv Regional Centre for Mental Health under the clinical supervision of neurologist Andrii Tymchenko. Five male patients (A, B, C, D, E) of the treatment centre aged 29 to 58 years were involved in the clinical trial with informed consent. The diagnosis on admission for all patients included shell shock and post-traumatic stress disorder (PTSD). During their cooperation in the research study, the patients underwent anamnestic data collection, physical examination, instruction on wearing orthodontic aligners, and rheoencephalography (REG) before and after the recommended therapy. The study included patients with shell shock and indications for orthodontic treatment of the bite. Patients with a complicated psychiatric or somatic history were excluded.

Individual boxing or orthodontic mouthguards were used for orthodontic treatment. The boxing mouthguard is a two-layer mouthguard made of ethylene vinyl acetate polymer material with a polypropylene frame. It can be adjusted to the patient's bite independently. According to the manufacturer's instructions, the mouthguard is fixed in the oral cavity by the patient at rest, and after hardening and taking on the desired shape, it is ready for daily use. During wear, the patient fixes the mouthguard in a comfortable position in the oral cavity at rest. Its anatomical position corresponded to the patient's bite morphology. The mouthguard was worn for two to three hours. Orthodontic aligners were used both in mass production and in individual production. Patients were enrolled in the study after a physical examination and general clinical examinations (complete blood count and urine test, electrocardiogram, and radiograph of the maxillofacial area). The therapeutic effect was monitored for two weeks, which was assessed by filling the occlusion and posture defect with a mouthguard. The mouthguards were worn for at least 12 hours a day by each patient.

After the patients finished wearing the mouthguard, a REG examination was performed to study cerebral blood flow by diagnosing changes in the electrical resistance of the skull structures, which is caused by volumetric fluctuations in perfusion and partially by the state of the blood filling rate in the cerebral and extracranial vessels during the passage of an alternating current of a certain frequency. Registration was performed on a rheoencephalograph with a high-frequency current of 120 kHz. The therapeutic effect of the caps on cerebral circulation was assessed by the condition of the vessels in the carotid and vertebral-basilar basins at the beginning of the study (baseline data on the condition of the vessels of patients A-E) and at the end of the application of the cap (therapeutic effect on cerebral circulation after two weeks of caps application). During registration, the electrodes were placed on the convex area of the mastoid process of the temporal bone, the upper edge of the brow arch, the occiput and the mastoid process. Hemodynamics was assessed by rheowave propagation time (Qa), fast blood filling time (α1), slow blood filling time (α2), upward wave propagation time (β), maximum fast filling velocity (V), average fast filling velocity (Vb), vascular wall tone as a ratio of anacrotic length to wavelength (α/T), rheographic index (Ri), inter-amplitude incisional index (MKi), inter-amplitude diastolic wave (MKd), brachiocephalic index (BCA).

All data obtained during the study were entered into databases built using Microsoft Excel spreadsheets and Statistica 6.0 software. The study was conducted following the requirements of Good Clinical Practice (GCP) [10] and the Declaration of Helsinki for Clinical Research Involving Human Subjects [11].

RESULTS

The research results are based on the joint efforts in the management and treatment of a sample of five patients undergoing complex rehabilitation therapy. The effectiveness of the complex orthodontic, osteopathic and rehabilitation treatment was confirmed by the indicators of the instrumental study (Table 1, Fig. 1). The rheoencephalography examination must be performed at the beginning of the study in a state of rest, without background load and medication. The next rheoencephalographic examination was performed together with the physical examination only after the use of orthodontic and boxing mouth guards for two weeks.

Table 1. Results of rheoencephalography examination of patient A before treatment (baseline)

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.21	0.26	0.19	0.26	Seconds
α1	0.01	0.26	0.04	0.23	Seconds
α2	0.16	0.07	0.14	0.02	Seconds
β	0.23	0.05	0.24	0.1	Seconds
Vb	0.1	0.01	0.1	0.01	
V	0.04	0.02	0.04	0.04	
Ri	0.09 (0.80-2.00)	0.05 (0.80-2.00)	0.1	0.05	
MKi	36.84	45.45	60	18.18	
MKd	31.58	18.18	60	54.55	
α/T	0.425	0.868	0.429	0.714	
BCA	14.29	250	28.57	80	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

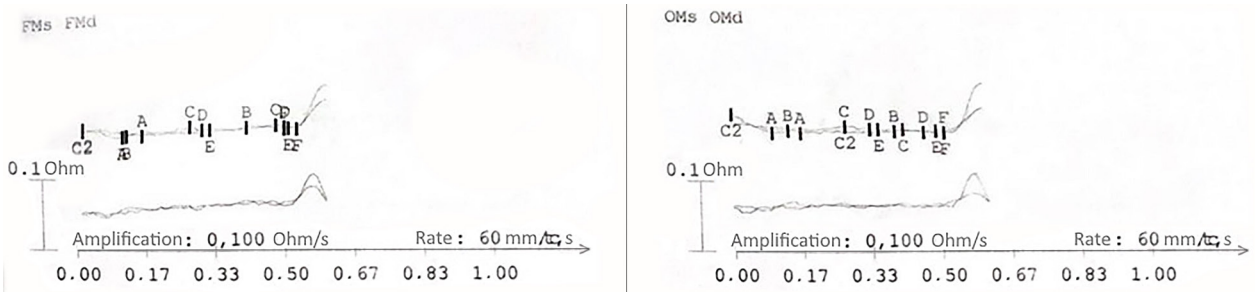

Figure 1. Rheoencephalography examination of patient A before treatment (baseline)
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

REG values were determined by FM, OM leads on the left (s) and right (d). Patient A (33 years old), at the time of the study, according to REG, was diagnosed with signs of reduced blood supply to the brain with severe hypovolaemia and hypertension of blood vessels (Table 1, Fig. 1).

Patient A's REG results after wearing the mouthguard showed moderate hypovolaemia, normal blood filling of the arterial vessels on the right, a moderate increase in cerebral blood vessel tone, and normal resistance artery tone (Table 2, Fig. 2).

Table 2. Results of rheoencephalography examination of patient A after treatment

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.15	0.17	0.08	0.07	Seconds
α1	0.07	0.06	0.13	0.16	Seconds
α2	0.08	0.05	0.04	0.04	Seconds
β	0.73	0.8	0.78	0.51	Seconds
Vb	0.4 (1.30-1.80)	0.68 (1.30-1.80)	0.2 (0.90-1.30)	0.38	
V	0.85 (0.70-1.10)	1.11 (0.70-1.10)	0.95 (0.30-0.55)	1.54 (0.30-0.55)	
Ri	0.59 (1.40-1.60)	0.76 (1.40-1.60)	0.43 (0.90-1.05)	0.93 (0.90-1.05)	
MKi	12.71	24.84	39.08	20.86	
MKd	19.49 (45.00-65.00)	28.1 (45.00-65.00)	55.17 (45.00-67.00)	33.69 (45.00-67.00)	
α/T	0.17 (0.13-0.14)	0.121 (0.13-0.14)	0.179 (0.14-0.15)	0.282 (0.14-0.15)	
BCA	24.79	6.45	525	700	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

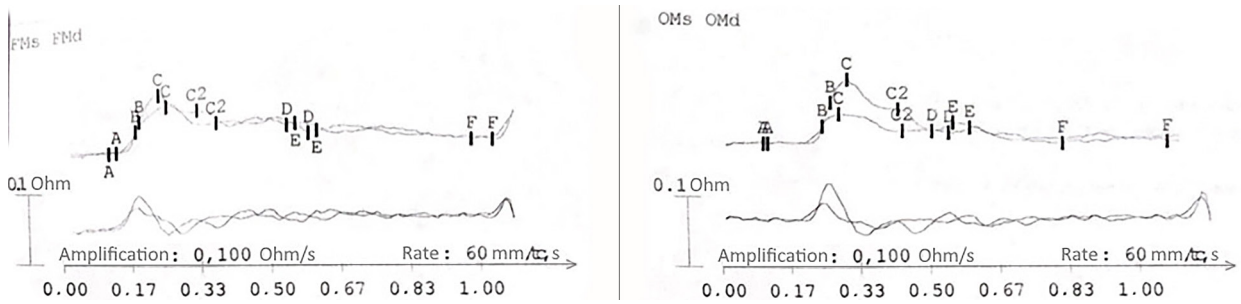

Figure 2. Rheoencephalography examination of patient A after treatment
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

Patient B (58 years old), at the beginning of the REG study, showed signs of severe hypertension of the vessel walls on the right and left, and difficult venous outflow of the deficit type (Table 3, Fig. 3).

Table 3. Results of rheoencephalography examination of patient B before treatment (baseline)

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	-0.01	0.09	-0.05	-0.05	Seconds
α1	0.2	0.1	0.24	0.24	Seconds
α2	0.04	0.04	0.03	0.03	Seconds
β	0.41	0.44	0.41	0.41	Seconds
Vb	0.28	0.55	0.23	0.23	
V	3.37	0.76	1.18	1.18	
Ri	0.88 (0.80-2.00)	0.85 (0.80-2.00)	0.69	0.69	
MKi	15.25	23.39	14.6	14.6	
MKd	15.82 (45.00-65.00)	28.65 (45.00-65.00)	18.98	18.98	
α/T	0.369	0.241	0.397	0.397	
BCA	18.64	21.02	230.77	230.77	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

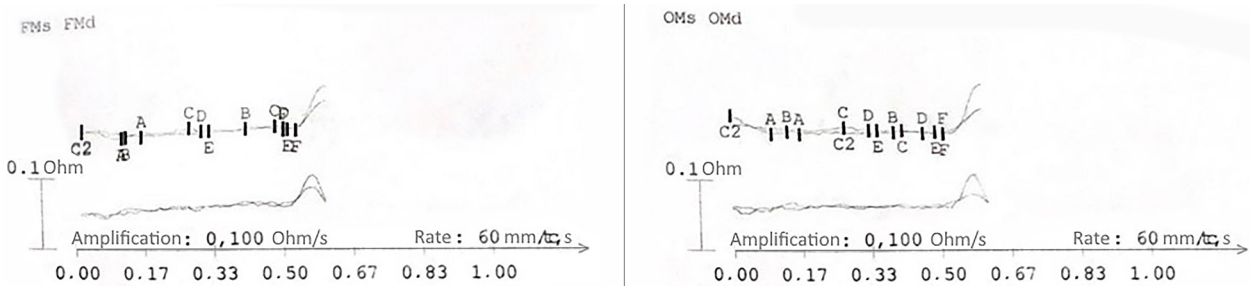

Figure 3. Rheoencephalography examination of patient B before treatment (baseline)
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

After orthodontic treatment, normal blood filling of the cerebral vessels with partial compensation of the venous outflow deficit was found (Table 4, Fig. 4). The tone of the main arteries remained slightly increased but lower than at the beginning of therapy.

Patient C (40 years old) was hospitalised with signs of decreased cerebral blood flow on the left, mild hypovolaemia, increased blood vessel wall tone on the left and significantly decreased arterial resistance tone on the left (Table 5, Fig. 5).

Table 4. Results of rheoencephalography examination of patient B after treatment

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	-0.02	-0.03	-0.03	-0.04	Seconds
α1	0.21	0.22	0.21	0.22	Seconds
α2	0.04	0.04	0.04	0.05	Seconds
β	0.39	0.48	0.49	0.48	Seconds
Vb	0.32	0.28	0.22	0.22	
V	1.45	0.89	1.67	0.67	
Ri	1 (0.80-2.00)	0.97 (0.80-2.00)	0.72	0.73	
MKi	19.4	15.38	10.42	19.05	
MKd	20.9 (45.00-65.00)	24.62	22.92	32.65	
α/T	0.391	0.351	0.338	0.36	
BCA	369.23	123.08	133.33	56.52	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

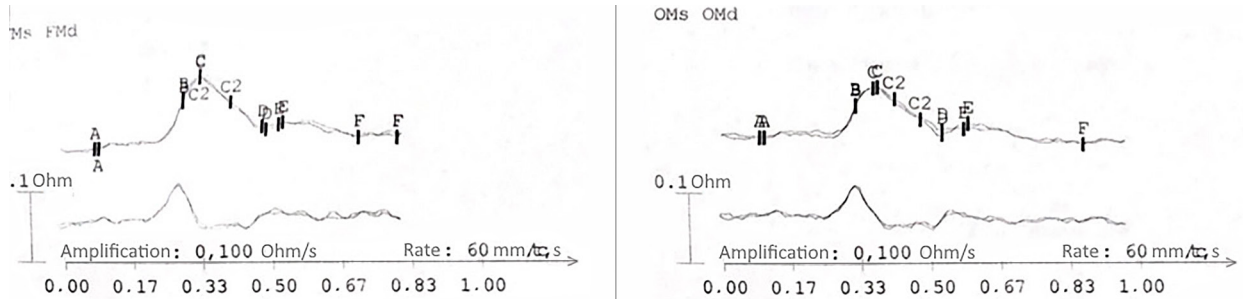

Figure 4. Rheoencephalography examination of patient B after treatment
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

Table 5. Results of rheoencephalography examination of patient C before treatment (baseline)

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.03	0.17	0.05	0.16	Seconds
α1	0.16	0.05	0.15	0.05	Seconds
α2	0.06	0.07	0.04	0.03	Seconds
β	0.76	0.68	0.55	0.66	Seconds
Vb	0.32	0.53	0.36	0.47	
V	9.58	0.42	0.6	1.02	
Ri	0.84 (0.80-2.00)	0.54 (0.80-2.00)	0.77	0.42	
MKi	-1.18	57.8	10.32	44.71	
MKd	0	66.06	12.9	60	
α/T	0.224	0.15	0.257	0.108	
BCA	-1.83	18.63	4.67	15	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

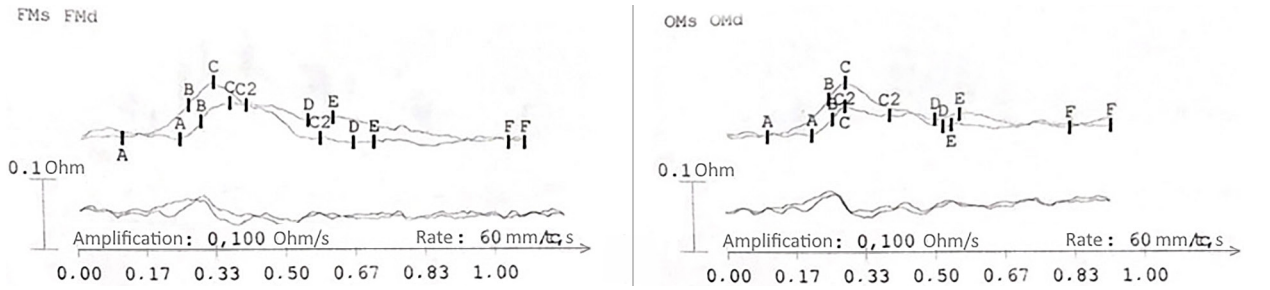

Figure 5. Rheoencephalography examination of patient C before treatment (baseline)
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

After treatment, there were no signs of impaired venous outflow; the tone of the resistance arteries on the left was satisfactory, and on the left it was slightly reduced (Table 6, Fig. 6).

Table 6. Results of rheoencephalography examination of patient C after treatment

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.19	0.14	0.01	0.02	Seconds
α1	0.07	0.12	0.24	0.23	Seconds
α2	0.05	0.16	0.04	0.04	Seconds
β	0.79	0.68	0.78	0.81	Seconds
Vb	0.66	0.24	0.22	0.12	
V	0.68	0.44	0.88	0.59	
Ri	0.79 (0.80-2.00)	0.74 (0.80-2.00)	0.77	0.46	
MKi	46.54	24.83	21.43	58.7	
MKd	52.2	25.5	22.08	60.87	
α/T	0.132	0.292	0.264	0.25	
BCA	18.35	330	21.15	371.43	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

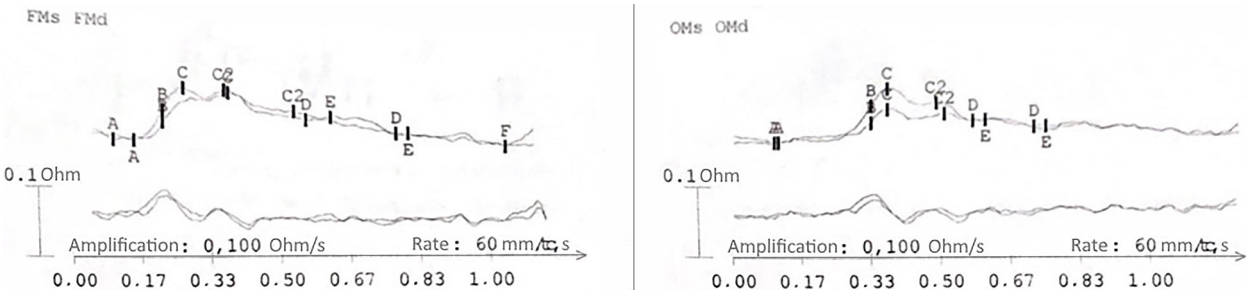

Figure 6. Rheoencephalography examination of patient C after treatment
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

At the beginning of the study, patient D (29 years old) had moderate hypovolaemia, decreased cerebral blood flow, mild hypotension, a moderate decrease in the tone of the resistance arteries on the left and an increased tone of the main arteries on the left (Table 7, Fig. 7).

After orthodontic treatment, Patient D showed signs of significant compensation of venous outflow by the deficit type, mild hypovolaemia, and a decrease in hypertension of the cerebral vessel walls on both sides according to REG (Table 8, Fig. 8).

Table 7. Results of rheoencephalography examination of patient D before treatment (baseline)

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.24	0.24	0.24	0.24	Seconds
α1	0.05	0.06	0.04	0.06	Seconds
α2	0.06	0.05	0.03	0.04	Seconds
β	0.67	0.67	0.71	0.68	Seconds
Vb	0.82	0.79	0.41	1	
V	1.74	1.26	0.39	0.56	
Ri	0.83 (0.80-2.00)	0.78 (0.80-2.00)	0.26	0.82	
MKi	31.74	27.39	62.26	32.73	
MKd	34.13	33.12	86.79	38.79	
α/T	0.141	0.141	0.09	0.128	
BCA	5.33	6.54	30	9.76	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

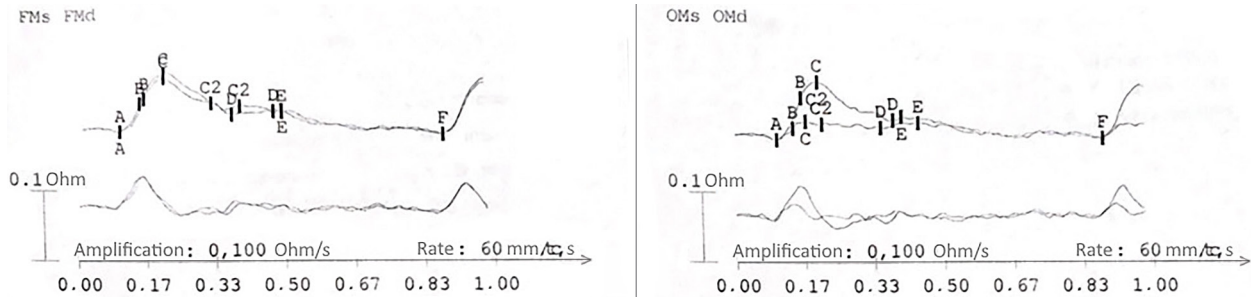

Figure 7. Rheoencephalography examination of patient D before treatment (baseline)
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

Table 8. Results of rheoencephalography examination of patient D after treatment

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.22	0.35	0.38	0.39	Seconds
α1	0.23	0.1	0.06	0.06	Seconds
α2	0.04	0.04	0.06	0.04	Seconds
β	0.68	0.49	0.67	0.5	Seconds
Vb	0.26	0.39	0.41	0.87	
V	2.57	0.94	0.75	0.76	
Ri	1.09 (0.80-2.00)	0.76 (0.80-2.00)	0.51	0.82	
MKi	24.31	30.72	20.59	35.15	
MKd	40.37	35.29	74.51	42.42	
α/T	0.284	0.222	0.152	0.167	
BCA	57.14	12.33	23.08	7.27	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

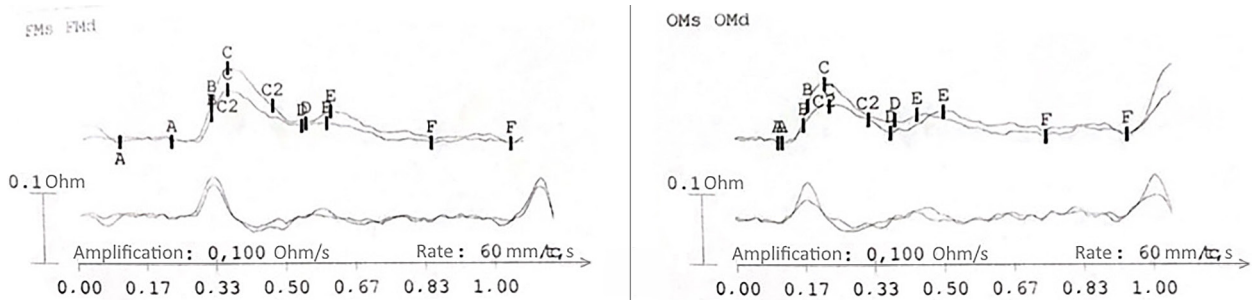

Figure 8. Rheoencephalography examination of patient D after treatment
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

Patient E (30 years old), at the initial REG, had signs of decreased blood filling of cerebral vessels on the left, increased vascular wall tone on the left, mild hypotension, and a significant decrease in the tone of resistance arteries (Table 9, Fig. 9).

Table 9. Results of rheoencephalography examination of patient E before treatment (baseline)

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	0.24	0.21	0.24	0.23	Seconds
α1	0.05	0.09	0.05	0.06	Seconds
α2	0.04	0.02	0.04	0.03	Seconds
β	0.41	0.38	0.58	0.63	Seconds
Vb	0.94	0.57	0.98	0.54	
V	2.48	1.11	1.67	1.52	
Ri	0.85 (0.80-2.00)	0.65 (0.80-2.00)	0.82	0.54	
MKi	9.41	10.77	17.68	19.44	
MKd	15.29	14.62	23.17	22.22	
α/T	0.18	0.224	0.134	0.125	
BCA	13.75	14.81	7.55	14.42	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

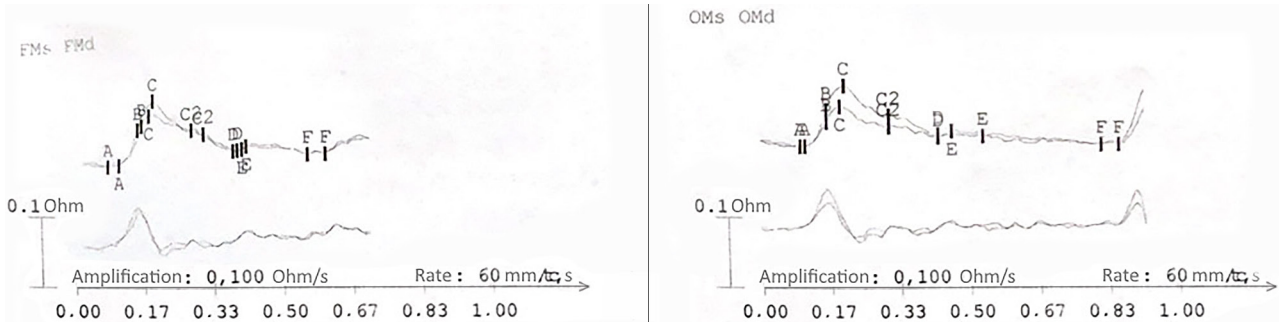

Figure 9. Rheoencephalography examination of patient E before treatment (baseline)
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

Repeated REG of Patient E showed positive dynamics of cerebral blood filling: moderate signs of deficient blood filling, but with satisfactory changes compared to the baseline data before treatment; normal vascular tone on the left and satisfactory on the right; moderate decrease in venous blood flow (Table 10, Fig. 10).

Table 10. Results of rheoencephalography examination of patient E after treatment

Indicator	Frontomastoid (FMs)	Frontomastoid (FMd)	Occipital-mastoid (OMs)	Occipital-mastoid (OMd)	Unit of measurement
Qa	-0.05	-0.05	0.13	0.14	Seconds
α1	0.24	0.24	0.06	0.05	Seconds
α2	0.04	0.04	0.04	0.03	Seconds
β	0.4	0.38	0.57	0.53	Seconds
Vb	0.21	0.21	0.82	0.85	
V	0.45	1.24	1.25	1.79	
Ri	0.69 (0.80-2.00)	0.74 (0.80-2.00)	0.76	0.62	
MKi	35.51	33.56	24.18	16.13	
MKd	36.23	38.93	28.1	23.39	
α/T	0.412	0.424	0.149	0.131	
BCA	172.73	253.85	15.58	11.3	

Notes: *Qa* – rheowave propagation time, α1 – fast blood filling time, α2 – slow blood filling time, β – upward wave propagation time, *Vb* – average fast filling velocity, *V* – maximum fast filling velocity, *Ri* – rheographic index, *MKi* – inter-amplitude incisional index, *MKd* – inter-amplitude diastolic wave, α/*T* – vascular wall tone as a ratio of anacrotic length to wavelength, *BCA* – brachiocephalic index
Source: compiled by the author

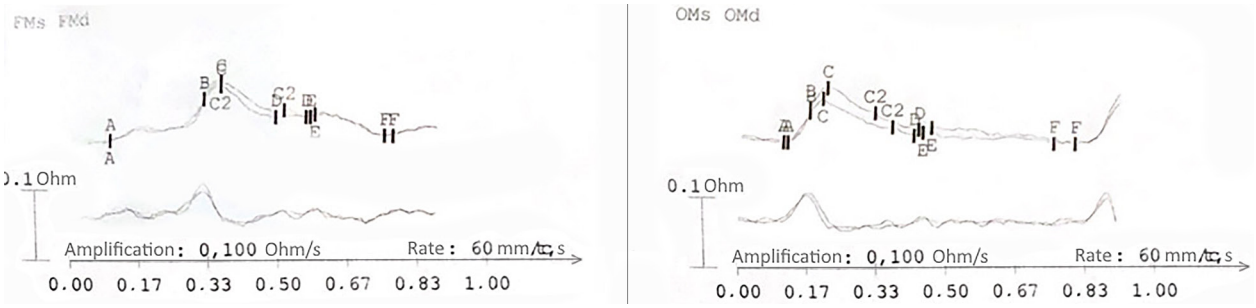

Figure 10. Rheoencephalography examination of patient E after treatment
Notes: FMs – left fronto-mastoid lead, FMd – right fronto-mastoid lead, OMs – left occipital-mastoid lead, OMd – right occipital-mastoid lead
Source: compiled by the author

In general, in the examined patients with limb or back injuries, pain syndrome and constant mechanical stress on the musculoskeletal system, a decrease in pain and improvement of muscle symmetry in the affected anatomical areas were observed with the use of orthodontic or boxing mouthguards. In patients with generalised tremors, contusions and venous stasis of the cranial region, the therapeutic wearing of orthodontic splints showed compensation of blood filling deficit and impaired venous outflow in the vertebrobasilar basin on both sides compared to the baseline data. Arterial blood filling after therapy showed satisfactory results since the restoration of venous outflow required a longer application of the orthodontic method.

DISCUSSION

A scientific and practical study has shown that the use of orthodontic and boxing mouthguards in patients with excessive musculoskeletal stress not only increases the height of the bite and stabilises physical displacements in the skull bone system, but also has a direct impact on the condition of the blood vessel walls, their tone and perfusion. As a result, the blood supply to CNS structures and venous outflow from the sinuses improves. As the results of the current study have shown, if the patient's bite is fixed in a physiological state of comfort, then after excessive or pathological load on the axial skeleton (especially on the cervical spine), it is enough to return the bite to the anatomical state in which it was adequately fixed before the loading effect, and the body's recovery is much faster. A similar emphasis in the protocol for the treatment of deformed occlusion is noted by E. Pasciuti *et al.* [12], where retention brackets were used. The authors evaluated the results of occlusion displacement using radiological examination, which should be used in the further continuation of the current work. According to the metadata of M.R. Muro *et al.* [13], in clinical conditions, orthodontic treatment with aligners should improve the initial deformed bite of patients and guarantee equivalence between the predicted and final results for the effective treatment of bite height deficiency. The authors note that the predicted results at the beginning of treatment are often overestimated and do not always coincide with the objective final positions of the jaw arches, which is due to the insufficiently long use of orthodontic constructions. This fact was partially revealed in the current study, as two patients still had encephalographic signs of

impaired outflow from the venous sinuses, while three patients had vascular spasms.

From the point of view of medical and psychological rehabilitation, restoration of the occlusion and position of the bones of the face and skull is important for the full restoration of function, the control and implementation of which occurs in the cervical spine and brain structures [14, 15]. The anatomical restoration of the occlusion and reduction of bruxism are of particular relevance in the case of maxillofacial injuries. According to K. Khalaf *et al*. [14], prosthetics and medical rehabilitation with implants and mouth guards have a great potential for restoring secondary malocclusion after maxillofacial trauma. According to G. Zanon *et al.* [16], orthodontic treatment has a positive effect on the functioning of the masticatory muscles, as, during treatment, chewing occurs evenly with the involvement of the right and left parts of the jaw structures, which in turn evens out the mechanical effect on the blood vessels. It has also been proven that orthodontic appliances reduce the number of reverse chewing cycles by improving their kinematic parameters (axis and angle of closure, maximum lateral deviation) on both sides and thus becoming symmetrical between the sides and restoring the physiological inclination of the angle of closure in the temporomandibular joint [16-18].

According to anamnestic questionnaires, during the clinical follow-up of patients, a decrease in the symptoms of muscle pain and general fatigue, improvement of posture and coordination of movements during the physical examination after the proposed treatment was noted. In addition, increasing the height of the bite and compensating for the asymmetrical position and movements of the jaw arches and facial muscles with the help of orthodontic and boxing mouthguards also leads to the restoration of the position of the vertebrae of the cervical and thoracic spine [19]. The time required for the initial recovery of muscle fatigue in the upper body is reduced to two hours after the use of orthodontic or boxing mouthguards. No differences between orthodontic and boxing mouthguards were found in the analysis of physical and rheoencephalography indicators of bite and cerebral circulation recovery. According to the results, the main difference was found only in the ease of use by patients and time savings for fitting the mouthguard. The use of boxing mouthguards is a much simpler method than the manufacture of an individual orthodontic mouthguard: it is only necessary to have hot water to soften the polymeric material of the boxing mouthguard, and the total manufacturing time does not exceed five minutes. This observation is confirmed by the results of a randomised clinical trial by A. Kalra *et al.* [20]. Thirty athletes were randomly assigned to one of the groups that were assigned to wear a specific type of mouthguard during two months of a crossover randomised clinical trial with three observation groups as part of orthodontic treatment, playing contact sports for at least two hours a day. As a result, the authors found no significant, statistically significant changes in the performance of different types of mouthguards, while respondents' data on the ease of wearing and use varied.

The clinical observations also showed that almost 70% of respondents clench their teeth when lifting heavy objects, which was revealed at the stage of the initial physical examination of patients at the Kyiv Regional Centre for Mental Health. Clenching of the teeth, i.e., bruxism, also occurs when experiencing stressful conditions, which is especially relevant for respondents in the study cohort with post-traumatic stress disorder. In this way, the body uses its compensatory resources – neuromuscular circuits are activated, which include additional muscle groups that maintain the upright position of the body, promote shock absorption, and prevent spinal injuries [21]. Post-traumatic stress disorder, according to W. Knibbe *et al.* [22], is often associated with painful temporomandibular joint disorders and can often be an etiological factor in bruxism during wakefulness and sleep. The study by S. Passardi *et al.* [23] aimed to study electromyographic changes in the activity of the facial and masticatory muscles in patients with post-traumatic stress disorder and showed that muscle activity was increased in response to negative emotional situations compared to positive ones. The authors also found increased expressive suppression and alexithymia compared to the control group. Thus, the results of the study can be applied not only to patients with objective signs of malocclusion, cerebral circulation, and cervical vertebrae displacement but also to reduce muscle tone in the maxillofacial area in post-traumatic stress disorders.

Studies conducted at the Kyiv Regional Centre for Mental Health show that the use of boxing mouthguards and orthodontic appliances aimed at increasing the height of the bite and reducing hypertension of the muscles of the maxillofacial area improves blood circulation in the brain vessels, namely in the carotid pools. In certain post-treatment rheoencephalograms, it can be seen that the spasm of the vessel walls decreases, which contributes to improved blood supply to the brain structures. In some rheoencephalograms, blood filling indices on both sides were restored to almost normal, sometimes with preserved low-grade hypovolaemia, compared to the one at the beginning of treatment. In addition, rheoencephalographic examination after the use of the caps showed normal indicators of resistance artery tone, compensation of venous outflow from the cerebral sinuses, and sufficient blood filling of the internal carotid artery and vertebrobasilar pools. Compensation for blood supply deficit has a significant therapeutic effect, since along with the normalisation of blood supply, there is a rapid restoration of lost speech, coordination of movements and functioning of organs and body systems by restoring power to the controlling structures in the CSN through endocrine, somatic and parasympathetic regulation. A literature search of open medical databases did not reveal the existence of similar scientific studies that would focus on the effect of mouth guards on cerebral circulation. Therefore, further expanded research on this topic involves the involvement of a larger cohort of subjects with possible concomitant cardiovascular diseases, which would show the feasibility of using mouthguards for patients with various combined nosologies.

CONCLUSIONS

According to the sample study, a decrease in the vertical axial load on the spine was detected, which is especially relevant for military personnel. The load in the current realities of the war in Ukraine occurs when wearing body armour, improper lifting of loads, injuries when disembarking and boarding vehicles with military equipment, and

jumping into a trench with heavy weapons. With the help of orthodontic or boxing mouthguards, effective restoration of the muscles of the upper and lower jaw, bite height, and thus blood supply to adjacent anatomical structures was observed. The acceleration of the body's recovery processes after exertion, trauma, and neuropsychological functions (in the examined patients, these were contusion and post-traumatic stress disorder) was noted. Rheoencephalography signs of cerebral circulation improvement were objectively demonstrated: a decrease in arterial-type spasm of the cerebral vessel walls, improvement of blood circulation in the carotid basin system, compensation for deficit blood filling and venous outflow by deficit type. Restoration of blood supply to the structures of the brain and spinal cord (mainly the cervical spine) has a significant therapeutic effect both immediately after treatment and in the long-term management of patients. Adequate blood supply to the structures of the central nervous system contributes to the rapid and complete restoration of lost functions, such as speech, sensitivity, and coordination of movements.

An important aspect of the proposed therapy is the saving of financial and time resources, as the patient's stay in a rehabilitation facility is reduced, which, accordingly, reduces the cost of patient care. It can also be concluded that, according to the proposed method of improving cerebral circulation and medical rehabilitation, it is more convenient to use boxing mouthguards than orthodontic mouthguards. Boxing mouthguards are more affordable in terms of time and money and are more convenient from a practical point of view compared to orthodontic mouthguards. The results of the study are relevant for implementation in the protocols of patient management in rehabilitation and medical institutions of the country, as well as for the prevention of cerebrovascular complications. Further research on this topic involves the involvement of a larger cohort of patients with concomitant cardiovascular diseases.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Метод покращення мозкового кровообігу за допомогою боксерських та ортодонтичних кап

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Анотація. У зв'язку з активними бойовими діями на території України значно зросли показники загострень хронічних хвороб, травматизації ділянки голови та хребтового стовпа за рахунок як вогнепальних поранень, так і за рахунок підвищеного силового навантаження на опорно-руховий апарат. Питання удосконалення реабілітаційних підходів є актуальним у зв'язку з наявною статистикою патологій кровопостачання головного мозку. Враховуючи потребу в доступних та ефективних методах відновлення опорно-рухового апарату, дане дослідження мало за мету дослідити ефективність впливу ортодонтичних та боксерських кап на стан кровоносних судин головного мозку. На базі Київського обласного центру ментального здоров'я досліджено п'ять пацієнтів з контузією та посттравматичним стресовим розладом в анамнезі за допомогою фізикального обстеження та реоенцефалографії. Застосування ортодонтичних та боксерських кап показало позитивну динаміку у відновленні роботи м'язів щелепно-лицевої ділянки, компенсацію висоти прикусу і задовільне кровопостачання структур головного мозку у порівнянні з вихідними даними. Аналіз реоенцефалограм показав реоенцефалографічні ознаки покращення мозкового кровообігу: виявлено зниження спазму артеріальних стінок мозкових судин, компенсацію дефіциту кровопостачання, поліпшення кровообігу у каротидному басейні та венозного відтоку в дефіцитному режимі. У обстежених осіб з перенесеними травмами, больовим синдромом та постійними механічними навантаженнями на опорно-руховий апарат відмічено зниження больових відчуттів та покращення симетричності роботи м'язів в уражених анатомічних ділянках на фоні носіння ортодонтичних та боксерських кап. Отримані результати можуть бути впроваджені у протоколи реабілітаційного відновлення пацієнтів із контузією, струсом мозку, травмами хребта та черепа. Доступність та ефективність запропонованої методики дозволяє застосовувати її для пацієнтів із хронічними хворобами серцево-судинної системи, особливо ускладненим кровопостачанням структур головного мозку

Ключові слова: медична реабілітація; ортодонтія; кістки черепа; реоенцефалографія; елайнер; посттравматичний стресовий розлад

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Cultivation and cryopreservation of rat stem cells and their interaction with lyophilised acellular matrix

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Abstract. With the rapid development of regenerative medicine in the 21st century, the study of the therapeutic potential of stem cells in both preclinical research and clinical trials has become particularly relevant. Preclinical studies on animals allow for a detailed understanding of the mechanisms of action of allogzeneic cell preparations, exploring their regenerative activity, pharmacodynamics, and potential side effects. The purpose of the study was to select optimal conditions for obtaining, cultivating, and cryopreserving mesenchymal stem cells from rats and analyse their interaction with the lyophilised acellular matrix. The enzymatic method was applied to obtain primary cell cultures from the umbilical cord, dermis, and muscles of *Rattus norvegicus* fetuses. Cell cultures were cultivated *in vitro*, and cell line proliferation rates were analysed using an inverted microscope. In addition, cryopreservation was performed to store cellular materials. The interaction of mesenchymal stem cells with an acellular matrix and cryopreservation of the obtained cells was at the 4 and 5th passages. It was shown that the optimal nutrient medium for cultivating the obtained lines of mesenchymal stem cells from the umbilical cord and dermis of rat fetuses is DMEM/F12 Advanced. It was established that the method of thawing the cell suspension by 10-fold dilution of dimethyl sulfoxide is more effective than the alternative method of immediate

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removal of cryoprotectant by centrifugation. The lyophilised acellular dermal matrix was found to have a cytotoxic effect on all cultured rat cells, while the pericardial matrix showed a positive effect on the growth of the investigated cell lines. Thus, the optimal nutrient medium and conditions for freezing/thawing of rat stem cells were selected, and the effect of lyophilised acellular matrix, planned for therapeutic use, on the obtained cell lines was determined

Keywords: i*n vitro* culture; cryopreservation; umbilical cord; muscles; dermis; lyophilised matrix; pericardium

INTRODUCTION

The last 20 years have witnessed increased interest from biomedical researchers in the use of stem cells (SCs) as an innovative personalised biotechnological method in regenerative medicine. However, before the widespread implementation of cell therapy in clinical practice, it is necessary to comprehensively study the characteristics and mechanisms of action of allogeneic SCs in animal models of various pathologies. Preclinical *in vivo* studies using laboratory animals allow for the collection and analysis of data on therapeutic effects, pharmacodynamics, and potential adverse effects of local or systemic administration of SCs.

Both Ukrainian scientists and researchers from many other developed countries have been exploring the properties of SC for more than 10 years. In particular, H.V. Budash & N.M. Bilko [1], S.K. Ray & S. Mukherjee [2], O. Redko *et al.* [3] described the ability of different types of SCs to differentiate into various cells of the body, which is considered a unique tissue repair system. Other authors revealed the powerful paracrine mechanism of SC secretome in stimulating regeneration and exhibiting immunomodulatory and anti-inflammatory effects [4-6]. In investigating the interaction of allogeneic mesenchymal stem cells (MSCs) with genetically foreign immune cells of recipients, M. Lopes-Pacheco *et al.* [5] and S.S. Chaleshtori *et al.* [6] have shown low immunogenicity of these cells since they weakly express surface human leukocyte antigen (HLA) class I proteins, while HLA class II is only intracellularly expressed and completely absent on the plasma membrane surface. Due to the above characteristics, MSCs evade recognition by alloreactive T lymphocytes. The immunomodulatory effect of MSCs is mainly manifested due to their ability to inhibit the function of various types of pro-inflammatory immune effector cells and activate anti-inflammatory immune responses. According to studies conducted by Chinese scientists, therapeutic effects of MSCs are manifested through their ability to migrate to damaged areas [7-9] and through the production of numerous extracellular vesicles filled with cytokines, growth factors, and microRNAs (miRNAs). It has been shown that molecules secreted by MSCs play an effective role as mediators of regeneration, either by directly activating target cells or by stimulating neighbouring cells to release active repair factors. They are involved in regulating important processes in the recipient's body, such as immune response, maintaining homeostasis, coagulation, inflammation, cancer progression/regression, angiogenesis, and antigen presentation. Thus, substances from MSC-derived extracellular vesicles exhibit their activity in both physiological and pathological conditions [10-12].

Proper and long-term preservation of biological materials remains an important task for medicine and biology. Cryopreservation has been established as a means to provide such capabilities [13]. Scientists have accumulated significant information on preserving the viability of SCs over extended periods of time and the influence of different cryoprotectants on cell survival after deep freezing [14, 15]. Cryopreservation of MSCs can also reduce the constant need for fresh tissues, enable quality control and standardisation of the same cell product at different times when the cellular product is needed.

One important area in regenerative medicine is the development and modification of materials for controlling the viability of SCs, which (re)construct the stromal microenvironment of various human and animal organs and tissues. In addition to the selection of artificial materials capable of biomimetic reproduction of the cellular and tissue microenvironment, the use of decellularised (cell-free) organs and tissues is also relevant for bioengineering purposes [16]. A promising way to treat skin injuries of various etiologies is the use of decellularised pig skin. The main advantages of this biological material are the composition and structure of the pig dermis, which is similar to the human dermis. Skin substitutes have previously been shown to improve wound healing quality and functional treatment outcomes, so they are a useful tool in plastic and reconstructive surgery [16].

This study was conducted as part of the state-funded fundamental research project "Investigation of the Regenerative Potential of Cell Therapy Agents in Acute Respiratory Distress Syndrome" (2021-2023, state registration number 0121U100159) [17].

The purpose of the study was to develop effective techniques for obtaining, culturing, cryopreserving, and thawing rat MSCs and analysing their interaction with dermal and pericardial lyophilised acellular matrices, which were created for the treatment of patients with burn injuries.

MATERIALS AND METHODS

The experiment was conducted in 2021 on the basis of the laboratory of cell cultures of the Ternopil National Medical University of the Ministry of Health of Ukraine. 3 female rats *Rattus norvegicus* were used to obtain primary SC cultures in the late stages of gestation. The animals were kept in standard vivarium conditions (12-hour day/night cycle; t = 20-25°C; humidity 50-55%) with free access to water and food. Euthanasia was performed using thiopental in compliance with bioethical requirements. Umbilical cords, pieces of fetal skin and muscle were taken for experiments. Using a sterile HBSS buffer solution (Gibco) with the addition of 1% penicillin-streptomycin (Sigma), the material was washed from the blood. An enzymatic method using 0.1% collagenase I (Sigma) diluted in DMEM/F12 Advanced nutrient medium (Gibco) was used to dissociate cell mass and produce viable MSCs. Then the material was pipetted and centrifugal for 5 minutes, at a speed of 3000 rpm. The resulting precipitate was resuspended in

a nutrient medium with the addition of 10% fetal bovine serum (FBS)(Gibco) and sown in culture vials, cultured in a CO2 Incubator at a temperature of 37°C and a concentration of CO_2 – 5%. The resulting primary cultures were assigned a zero passage (P_0). Visual assessment of the formation of the monolayer density (confluence) was performed using an inverted Delta Optical NIB-100 microscope.

Cell lines were transported by warm trypsinization (Triple enzymatic solution (Gibco)) when the confluence reached 90-100%. Cell counting was performed using a hemocytometer (Goryaev chamber) using the vital dye Trypan blue.

Cryopreservation of MSCs of the umbilical cord, fetal muscles and skin was performed in passages 4 and 5 after reaching 90% of the confluence. 50% of the cell suspension (2 000 000 cells each) was added to cryoprobes in a DMEM nutrient medium. Then, in 2 steps, a freezing medium was added (30% DMEM/F12 Advanced, 40% FBS, 20% conditioned medium (CS), 10% dimethyl sulfoxide (DMSO) (Sigma)). Further storage of cellular material was conducted at a temperature of -80°C (intermediate stage) and liquid nitrogen (-196°C).

The umbilical cord, dermal, and muscle MSCs at Passages 4 and 5 were used to study the interaction of MSCs with the acellular Matrix. Lyophilised and decellularised pieces of pig dermis and pericardium, 0.5 cm^2 in area, were added to the wells of 6-well culture plates with seeded SCs at the rate of 50,000 cells/ml. The experiment was laid according to the following scheme: control – the cell suspension itself; option 1 – cell suspension + a piece of the dry pericardium; option 2 – cell suspension + a piece of the soaked pericardium in the CS for 1 hour; option 3 – cell suspension + a piece of the dry dermis; option 4 – suspension + a piece of the soaked dermis in the CS for 1 hour. During soaking of the lyophilised material, the CS was changed three times. Each experiment was performed in three repetitions, 6 wells for each point. Microscopic analysis of cell culture growth was performed after 1, 3, and 7 days of the experiment using an inverted microscope. Statistical data analysis was performed using Excel.

All interventions were conducted in compliance with the principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" and the law of Ukraine No. 3447-IV "On the protection of animals from ill-treatment" [18, 19].

RESULTS

Obtaining a primary MSC culture. Cell suspensions obtained by fermentation of pieces of the umbilical cord, skin, and muscle of rat fetuses were seeded on culture vials to create conditions for cell adhesion and activation before proliferation. Microscopic analysis of the state of primary cell cultures was performed after 48 hours after sowing. Partial adhesion of polygonal cells was observed in umbilical cord MSC culture (Fig. 1). The cell population density was less than 10%, and there was a slight change in the pH of the medium towards acidic (the acidity indicator phenol red – a mandatory component of the DMEM/F12 Advanced nutrient medium – turned orange). In the SC variants, more cells were attached from the muscle and skin of rat fetuses than in the umbilical cord MSCs variant. The nutrient medium was also slightly acidified. In the primary culture of myogenic MSC cells, the cells were elongated with two diametrically opposite processes, evenly attached to the bottom of the culture plastic, and actively proliferated, covering approximately 70% of the flask area (confluent, accordingly, = 70%). In the case of dermal SCs, the cells were highly flattened, polygonal in shape, forming clones, and reached 50% confluence within the first 48 hours of cultivation (Fig. 2).

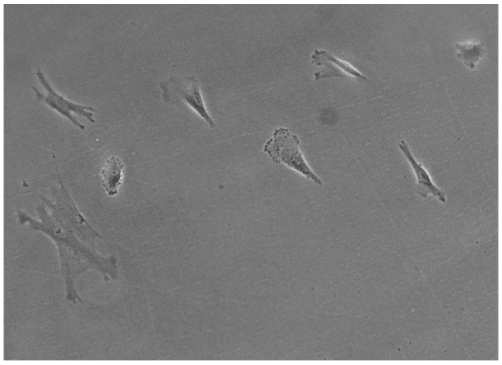

Figure 1. Primary culture of umbilical cord MSCs 2 days after the start of the experiment. Magnification ×100

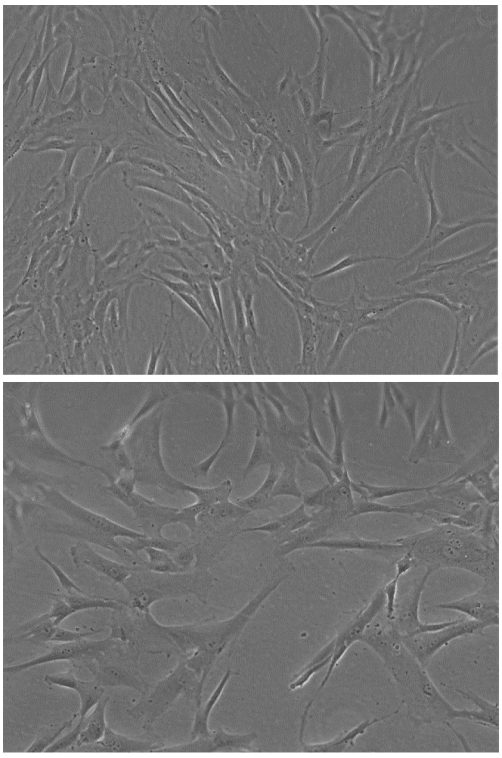

Figure 2. Primary culture of MSCs of muscles and dermis on the third day of the experiment. Magnification ×100

On the third day of cultivation, complete replacement of the nutrient medium was performed in all cell lines while maintaining a 10% FBS concentration. The subsequent analysis of the primary cultures was conducted four days after seeding the material. In the muscle and skin cell lines, the formation of a confluent monolayer (confluence = 100%) was observed, so at this stage, passaging was performed to avoid contact inhibition. In the myogenic cell

variant, occasional myospheroids began to form among the typical fibroblast-like MSCs (Fig. 3).

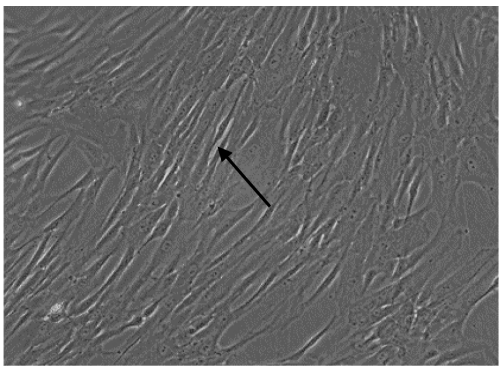

Figure 3. Fusion of myogenic cells with the formation of myospheroids (indicated by an arrow). Magnification ×100

In the umbilical cord MSC culture, after four days of cultivation, the confluence reached approximately 50%, while 100% confluence of umbilical cord cells was achieved only on the 8th day of cultivation. The slow growth rate of this cell population was associated with a lower initial cell seeding density. However, during the first passage, these MSCs increased their proliferation rate to levels comparable to the myogenic cell line. Throughout the first to third passages, the cell lines were cultured in a medium with a reduced FBS concentration of 2%.

Selection of optimal conditions for MSC culture growth. To compare the effects of different nutrient medium compositions on cell growth intensity, two types of culture media, DMEM/F12 and DMEM/F12 Advanced, were used. The experiment was performed on passages 2 and 3. Microscopic analysis of the proliferation rates of cell lines was performed before the formation of 100% confluence. The results of the experiment are presented in Table 1.

Table 1. Dependence of the influence of nutrient media on the intensity of cell proliferation rates, (cell population density, %)

Cell culture	Experimental group	1 days	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
MSC umbilical cord	DMEM/F12 Advanced	35.0±3	50.0±5	85.0±5	100					
	DMEM/F12	30.0±5	40.0±5	60.0±8	80.0±5	95.0±3	100			
MSC of muscles	DMEM/F12 Advanced	35.0±8	55.0±5	85.0±5	100					
	DMEM/F12	35.0±10	50.0±5	65.0±5	85.0±7	100				
MSC of dermis	DMEM/F12 Advanced	20.0±5	35.0±5	40.0±5	50.0±5	65.0±8	80.0±5	95.0±3	100	
	DMEM/F12	25.0±8	30.0±7	35.0±5	45.0±5	55.0±10	65.0±5	80.0±5	90.0±3	100

Source: compiled by the authors

As evident from the data presented in Table 1, the DMEM/F12 Advanced medium proved to be more effective for the growth of MSC cell populations compared to DMEM/F12. In the umbilical cord MSC line, the first variant reached 100% confluence on the 4th day of cultivation, while the second variant formed a 100% cell monolayer only on the 6th day. In the muscle MSC line, the DMEM/F12 Advanced variant achieved 100% confluence after 4 days of cultivation, while the DMEM/F12 variant reached it on the 5th day. The skin MSC line proliferated significantly slower than the previous two, as indicated by the results. In the DMEM/F12 Advanced variant, 100% confluence was achieved only on the 8th day after the start of the experiment, while in the DMEM/F12 experiment, it occurred on the 9th day.

In addition to examining the influence of nutrient media, the impact of various serum concentrations in the culture medium (DMEM/F12 Advanced + 2%, 5%, 10% FBS, and serum-free medium containing a protein similar to plasma albumin) on the activity of cell lines regarding their growth and proliferation was also investigated. The study was conducted during the 2nd and 3rd passages of the cell lines until 100% confluence was reached.

In the serum-free medium, the peak growth of umbilical cord MSCs was observed on the 7th day, reaching a maximum confluence of 40%. However, the cell line gradually started to die off without reaching 100% monolayer formation. When 2% FBS was added, the cells proliferated smoothly and evenly, achieving a monolayer on the 4th or 5th day of cultivation. Higher serum concentrations in the growth medium promoted accelerated cell proliferation but also induced the formation of spheroids on the 3rd day of cultivation (Fig. 4).

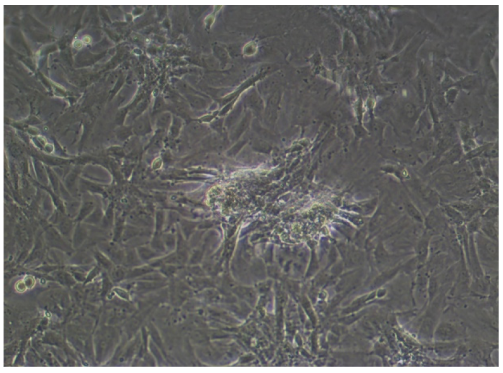

Figure 4. Formation of spheroids in umbilical cord MSC culture under high FBS concentration (10%). Magnification ×100

For muscle MSCs, the optimal serum concentration for culture was also found to be 2% FBS. At 5% and 10% serum concentrations in the growth medium, the proliferation

results were similar to those observed in umbilical cord MSCs at equivalent concentrations. In the serum-free medium, the cell line exhibited unexpected results. On the 11th day of cultivation, it reached 80% confluence, which remained unchanged for 5 days. These findings suggest that the muscle MSC cell line is relatively insensitive to the stimulating effects of serum additives in the growth medium.

The analysis of dermal MSC cell line growth showed that the optimal serum concentration was 5%. At 2% FBS, the cell line proliferated too slowly, with a monolayer forming only on the 9th day. At a concentration of 10%, rapid cell differentiation and acquisition of mature fibroblast morphology were observed.

In the serum-free medium, the cell line paused its proliferation on the 9th or 10th day of cultivation, reaching 60% confluence, and eventually started to die off. Therefore, it can be considered that the optimal FBS concentration is 2% for umbilical cord and muscle MSCs, while it is 5% for dermal MSCs.

Analysis of MSC viability after cryopreservation. One day after thawing samples of the three rat cell lines, it was observed that many cells got attached to the flask surface and acquired the typical morphology characteristic of MSCs. Thawing was performed using two methods: a 10-fold dilution of the cell suspension with freezing medium or immediate removal of the DMSO by centrifugation. In the first thawing method, the cell population density after 24 hours was 15% in all cell lines. At this time, a medium replacement was performed to fully neutralise the effect of the cryoprotectant on the cell populations. In the second thawing variant, the confluence of the umbilical cord MSC line reached approximately 5%, which was the lowest confluence rate. The confluence of the muscle and skin MSC lines was 8% and 10%, respectively. On the 5th day after thawing, the confluence in the case of 10-fold dilution of the cryoprobe content was: 75% for dermal SCs, 55% for skeletal muscle MSCs, and 35% for the umbilical cord MSCs line, and in the variant with centrifuged cells of the same lines – 70%, 60% and 30%, respectively. In the case of 100% diluted cells, a confluence was observed: for skin MSCs cell lines – on the 7th day after defrosting; and for umbilical cord and muscle MSCs – on the 9th day of the experiment. In the variant with centrifuged cells, the dermal MSC population reached a monolayer on the 8th day of cultivation, while the muscle MSCs reached it on the 9th day, and umbilical cord MSCs on the 11th day of the experiment. Thus, the method of thawing cryopreserved cells affects the proliferation rates of MSC cell populations.

Interaction of MSCs with acellular matrix. Analysis of the interaction between extracellular matrix (ECM) and umbilical cord MSCs showed that in the control and pieces of soaked dermis variants, only a small fraction of cells attached on the first day of cultivation. On the third day, cell growth continued in the control wells, and it was observed that cells began to proliferate in the variants with pieces of dry, soaked pericardium and dry dermis. On the 7th day of the experiment, active cell growth was observed in the control, dry pericardium, and soaked pericardium variants. However, in the case of pieces of dry and soaked dermis, the umbilical cord cell line died off (Table 2).

Table 2. Interaction of matrix with MSC cell lines of rats (cell density in wells, %)

Cell line	Experiment variants	Control, %	Dry pericardium, %	Soaked pericardium, %	Dry dermis, %	Soaked dermis, %
MSC umbilical cord	1st day	15 ± 2.7	no attached cells	no attached cells	no attached cells	5 ± 2.8
	3rd day	30 ± 4.2	18 ± 3.0	25 ± 3.0	10 ± 2.0	15 ± 5.1
	7th day	44 ± 3.6	42 ± 3.3	51 ± 3.9	cells are dead	cells are dead
MSC of muscles	1st day	15 ± 2.4	10 ± 2.1	15 ± 3.0	no attached cells	10 ± 4.2
	3rd day	30 ± 4.5	25 ± 3.9	35 ± 5.1	7 ± 2.3	15 ± 2.7
	7th day	51 ± 3.4	47 ± 4.3	58 ± 4.7	cells are dead	cells are dead
MSC of dermis	1st day	23 ± 3.6	15 ± 2.7	20 ± 3.2	5	5 ± 3.2
	3rd day	36 ± 3.7	25 ± 3.8	35 ± 5.6	cells are dead	8 ± 3.6
	7th day	52 ± 6.3	33 ± 3.7	47 ± 3.9	cells are dead	5 ± 2.5

Source: compiled by the authors

In the variant with muscle MSCs, the experiment with pieces of dry and soaked pericardium also showed a relatively favourable effect on the cell line. On the first day of the experiment, cell proliferation began in the control and experimental variants with pericardium and soaked pieces of dermis. By the third day of cultivation, cells proliferated in both the control and all experimental wells. The best result was observed on the 7th day in the variant with soaked pericardium pieces, followed by the control variant and wells with dry pericardium. However, in the case of pieces of dry and soaked dermis, the muscle SC cell line also died off by the end of the experiment (Table 2).

Unlike the previous two MSC cell lines, fetal dermal cells showed a positive result in the variant with soaked pieces of dermis. On the first day after the experiment was initiated, cell proliferation was observed in both the control and all experimental wells. The only difference was that in the presence of acellular dermal matrix, cells were located at the edges of the wells. By the third day, cells in the dry dermis variant had completely died off and detached. The best result was obtained on the 7th day of cultivation in the control and soaked pericardium variants.

DISCUSSION

The obtained results indicate that both types of tested nutrient media, DMEM/F12 and DMEM/F12 Advanced, have a favourable impact on the proliferation of rat MSC cell lines. The difference lies only in the fact that at the same concentration of FBS, cell lines grown in DMEM/F12 medium exhibited slightly slower growth compared to those

cultivated in DMEM/F12 Advanced. This result is obviously due to the special components that are present in the composition of the latter. DMEM/F12 Advanced additionally contains insulin, transferrin, glutathione, ethanolamine, ascorbic acid, and additional protein sources, so it can be used with minimal concentrations of xenogeneic embryonic serum, providing optimal conditions for MSC cultivation. For the cultivation of rat MSCs, an increase in FBS concentration is required when using DMEM/F12 medium. Since the growth rates of dermal SCs were slower compared to umbilical cord and muscle MSCs, it was decided to cultivate dermal cells using DMEM/F12 Advanced supplemented with 5% FBS, while 2% FBS was found to be optimal for myogenic and umbilical cord MSCs.

V.G. Dzhyvak *et al.* [20] outline that blood serum serves as a source of hormones, hormone-like growth factors, and transport proteins for their transfer. In addition, it contains molecules necessary for the formation of the extracellular matrix, facilitating cell adhesion to the culture vessel in *in vitro* cultures [21]. Therefore, for cell cultivation in *in vitro* conditions, the addition of embryonic bovine serum rich in biologically active substances is required [22].

The experiment aimed at determining the optimal FBS concentration in the growth medium revealed that at 2% FBS, cell line proliferation occurred smoothly and gradually. 5% of the serum showed faster cell proliferation and differentiation. This concentration can be used for rapid scaling of MSCs since it accelerates the mitotic division process under the influence of growth factors present in the serum. However, a concentration of 10% FBS proved to be less suitable as, despite achieving full confluence quickly, the investigated SCs started forming specific structures – spheroids (Fig. 4). Similar spheroid formation under the influence of human blood serum was also observed in another study [21]. It is known that elevated concentrations of FBS promote SC differentiation towards adipogenic, osteogenic, or chondrogenic lineages. Therefore, if the goal is to obtain a stable and long-lasting MSC cell line, the use of blood serum should be minimised [23-25]. However, it has been demonstrated that low concentrations of added FBS to the culture medium contribute to the formation of flattened cell monolayers on the culture plastic and prevent spheroid formation [21].

Based on the analysis of scientific sources regarding the cryopreservation of rat MSCs, a freezing medium was selected consisting of 30% DMEM/F12 Advanced, 40% FBS, 20% CS, 10% DMSO, and containing minimal amounts of cryoprotectant [13, 14]. From a detailed review by M. Awan *et al.* [15], it is known that although DMSO is a widely used and effective cryoprotectant, it exhibits toxic effects on cultured cells during prolonged exposure in the thawed state. However, a study by A. Mitchell *et al.* [26] demonstrated that a high concentration of FBS in the freezing medium mitigates the harmful effects of DMSO. CS also possesses protective properties for MSCs as it contains exosomes with SC secretome in addition to the products of cell metabolism [27]. Since the freezing medium was mixed with the cell suspension at a 1:1 ratio, the final concentration of cryoprotectant decreased to 5%, and its potential toxic effects were minimised by the higher concentration of FBS. As a result, the survival rate of rat MSCs in this study exceeded 85-90% after thawing. The method of thawing cell suspensions by 10-fold dilution of the cryoprotectant was found to be more effective than the alternative method of immediate removal of DMSO by centrifugation. Consequently, centrifugation of MSCs in the presence of DMSO proved to be more detrimental to the cells than their cultivation for 24 hours in the presence of a highly diluted cryoprotectant solution.

The analysis of the interaction between xenogeneic lyophilised acellular matrix and umbilical cord, muscle, and dermal MSCs revealed that for all cell lines, pericardium exhibited the most favourable interaction, particularly when it was rinsed three times in the growth medium beforehand. However, in the variants with the addition of decellularised dermal tissue fragments to the culture medium, the survival of rat SCs was observed to decline. This indicated a toxic effect of this material on the cell cultures. Studies by D.D. Mathew *et al.* [28] and R. Nassiri Mansour *et al.* [29] have shown that for the creation of bioengineered constructs for tissue and organ regeneration, autologous, allogeneic, or xenogeneic MSCs can be successfully cultured in acellular matrices. Such acellular matrices typically provide the necessary support for cell adhesion, proliferation, phenotypic differentiation of SCs, and offer favourable biochemical and biophysical conditions for tissue modelling and formation of neotissues. Therefore, based on a careful analysis of the method of obtaining the used acellular dermal matrix, it was presumed that its cytotoxic effect arises from the presence of detergents [16]. The conducted experiment revealed the necessity of modifying the protocol for obtaining acellular dermal matrix before its application in burn care practice.

CONCLUSIONS

Thus, this study demonstrates that the enzymatic method using 0.1% collagenase I is suitable for obtaining primary cultures of MSCs from umbilical cords, muscles, and dermal tissue of rat fetuses. For the cultivation of the obtained umbilical cord and dermal MSC lines, DMEM/F12 Advanced medium is optimal due to the presence of effective additives that are absent in DMEM/F12 medium. Since the culture of rat myogenic cells exhibited higher proliferative potential compared to other investigated MSC lines, it can also be cultivated in DMEM/F12 medium. It was also determined that a 2% concentration of FBS is optimal for efficient proliferation of umbilical cord and muscle MSCs, while a 5% FBS concentration is optimal for the dermal MSC line. In some cases, muscle MSCs can be cultured in a growth medium without the addition of serum.

Thawing the cell suspension by 10-fold dilution of DMSO was found to be a more effective method, as the alternative method of immediate removal of the cryoprotectant by centrifugation slowed down the growth of rat MSCs. Thus, centrifugation of MSCs in the presence of DMSO was more harmful to the cells than their cultivation for 24 hours in the presence of a highly diluted cryoprotectant solution. The analysed lyophilised acellular matrix in the form of fragments of dry dermal tissue exhibited a toxic effect on all investigated MSC cell lines. Conversely, dry and rehydrated lyophilised pericardium, when cultured in a growth medium, not only had no harmful effect but sometimes even stimulated the growth of SCs. Therefore,

lyophilised pericardium is recommended for the treatment of skin defects.

For the clinical use of decellularised porcine dermal matrix, it is necessary to improve the methodology of obtaining this material by increasing the number of washes after detergent treatment to reduce or eliminate its cytotoxic effects.

The next stage of comprehensive research is planned to investigate the mechanisms of the therapeutic effects of systemically administered allogeneic MSCs in rats with experimentally induced acute respiratory distress syndrome.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Особливості культивування та кріоконсервування стовбурових клітин щурів та їх взаємодія із ліофілізованим ацелюлярним матриксом

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Анотація. Через стрімкий розвиток регенеративної медицини у XXI столітті особливої актуальності набувають вивчення терапевтичного потенціалу стовбурових клітин як у доклінічних дослідженнях, так і в клінічних трайлах. Доклінічні дослідження на тваринах дають змогу в деталях з'ясувати механізми дії алогенних клітинних препаратів, вивчити їхню регенеративну активність, фармакодинаміку та можливі побічні ефекти. Метою дослідження був підбір оптимальних умов для отримання, вирощування та кріозберігання мезенхімальних стовбурових клітин щурів та аналіз їхньої взаємодії з ліофілізованим ацелюлярним матриксом. У ході дослідження було застосовано ферментативний метод для отримання первинних культур клітин з пуповини, дерми та м'язів плодів *Rattus norvegicus*. Культури клітин були культивовані *in vitro*, а темпи проліферації клітинних ліній були аналізовані за допомогою інвертованого мікроскопа. Крім того, здійснювалося кріоконсервування для зберігання клітинних матеріалів. Взаємодію мезенхімальних стовбурових клітин із ацелюлярним матриксом та кріоконсервацію отриманих клітин проводили на 4 та 5 пасажах. Показано, що для вирощування отриманих ліній мезенхімальних стовбурових клітин з пуповини та дерми плодів щурів оптимальним є поживне середовище DMEM/F12 Advanced. Встановлено, що спосіб розморожування клітинної суспензії шляхом 10-кратного розведення диметилсульфоксиду є ефективнішим за альтернативний спосіб негайного усунення кріопротектора за допомогою центрифугування. З'ясовано, що ліофілізований ацелюлярний дермальний матрикс цитотоксично впливає на усі культивовані щурячі клітини, в той час, як перикардіальний матрикс проявляв позитивний ефект на ріст досліджуваних клітинних ліній. Таким чином, підібрано оптимальне живильне середовище та умови для заморожування/розморожування стовбурових клітин щурів, а також визначено вплив на отримані клітинні лінії ліофілізованого ацелюлярного матриксу, що планувався для терапевтичного використання

Ключові слова: культура *in vitro*; кріоконсервація; пуповина; м'язи; дерма; ліофілізований матрикс; перикард

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Functional diagnostics of the respiratory system in patients with Long COVID

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Abstract. Given the considerable number of people with persistent respiratory symptoms more than 4 weeks after COVID-19, it is important to determine which examinations are most informative for assessing respiratory function, prognosis, and monitoring the effectiveness of treatment in such patients. The purpose of this study is to cover the functional diagnosis of the respiratory system in patients who survived Long COVID. Bibliosemantic and analytical research methods were applied. According to the world guidelines for patients with persistent respiratory symptoms after COVID-19, all of them should undergo respiratory system examinations: chest X-rays, spirometry, blood saturation measurements, lung diffusion capacity for carbon monoxide, walk tests, and others. The study confirmed that people who have had COVID-19, even after 1-6 months, show a decrease in diffusing capacity of the lung for carbon monoxide, which has a direct correlation with total lung capacity, the severity of clinical manifestations, and the results of rapid tests. Changes in spirometric parameters are observed much less frequently, mainly in patients hospitalised with severe pneumonia, which subsequently leads to post-cystic pulmonary fibrosis. Gradual improvement in functional respiratory tests occurs 3 months, 6 months, and 12 months after acute infection. The most informative tests for assessing respiratory function include the measurement of carbon monoxide diffusion capacity, total lung capacity, and blood gas composition. In conditions of limited access to the aforementioned diagnostic tests, and as a screening tool, quick tests such as the 3-minute/6-minute walk test (3MWT/6MWT) or the 1-minute sit-to-stand test (1-MSTST) can be utilised based on the patient's capabilities

Keywords: spirometry; pulmonary function tests; COVID; walk test

INTRODUCTION

The coronavirus disease (COVID-19) pandemic caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) has become a real challenge for doctors around the world. Despite advances in treatment, thousands of people continue to experience illness, isolation, and mortality. It has become evident that even successful recovery from

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the acute phase of the disease does not guarantee individuals protection from delayed manifestations or complications. Reflecting the growing number of such patients, the World Health Organization (WHO) introduced a separate code, U09.9, in January 2021 to classify the post-COVID-19 condition. Such prolongation of clinical symptoms after COVID-19 requires new approaches to their monitoring and treatment, and innovative solutions from the medical community of the world.

The term "Long COVID" was initially used by Italian physician Elisa Perego (Lombardy, Italy) on social media to describe her own experience of battling the coronavirus, which had a fluctuating course with various and changing symptoms lasting for several months. Subsequently, it appeared in an article in the British Medical Journal (BMJ) by E. Mahase [1], noting that SARS-CoV-2 is a novel virus, leaving many questions unanswered for healthcare professionals and researchers, requiring studies that evaluate the long-term consequences of COVID-19. This term was used by other publications, and "Long COVID-19" became rooted in the medical and scientific communities. The United Kingdom National Health Service (NHS) [2] proposed the following definitions: acute COVID-19 – a condition where signs and symptoms of the disease last for four weeks; ongoing symptomatic COVID-19 describes symptoms lasting for 4-12 weeks; post-COVID-19 is used when signs and symptoms that develop during or after COVID-19 infection persist for more than 12 weeks and cannot be explained by an alternative diagnosis. Post-COVID-19 typically encompasses a range of symptoms that can vary and affect various body systems. Long COVID-19 is a term proposed to describe signs and symptoms that develop after acute COVID-19 and last for more than 4 weeks. Therefore, it includes both the ongoing symptomatic variants of COVID-19 (acute and persistent) and post-COVID-19 manifestations. There is a diversity and variability of clinical symptoms observed in a cyclical pattern of COVID-19. A considerable portion of patients did not have pre-existing health issues before contracting SARS-CoV-2. In some patients, persistent systemic manifestations persist for quite a long time, even after the fever and respiratory symptoms decrease. In others, after a full recovery, some symptoms return after a few days with greater intensity and duration. A number of patients report a pronounced cyclicity, with several days of improvement and deterioration [3-5].

In the pathogenesis of the viral disease, the sensitivity of viruses to the receptors of the host cells plays a crucial role, followed by their proliferation, and the variable expression of these receptors in different organs. W. Ni *et al.* [6] in their paper provide a rationale for one of the mechanisms explaining the absence of specific symptoms in COVID-19 due to the tropism of SARS-CoV-2 for angiotensin-converting enzyme 2 (ACE2) receptors present on the cell membranes of many human organs. This partially explains the wide spectrum of clinical symptoms and their development, independent of the route of infection, severity of the disease course, and variants of the acute phase outcome.

The purpose of the study was to identify the possibilities of functional diagnostics of the respiratory system in Long COVID based on the global experience by analysing available information and literature sources.

PREDICTED SYMPTOMS, CONSEQUENCES, AND PRIMARY DIAGNOSIS OF CORONAVIRUS DISEASE

The study by D.D. Lutchmansingh *et al.* [7] provides a basis for understanding possible complications and consequences following a COVID-19 infection. The researchers proposed a model (Fig. 1) of acute COVID-19 complications with predicted symptoms thereafter and ultimate multi-organ consequences that may arise as a result of direct viral damage through angiotensin-converting enzyme 2 (ACE2) receptors, hypoperfusion, systemic inflammation, toxic effects of treatment, or a multi-organ vascular component.

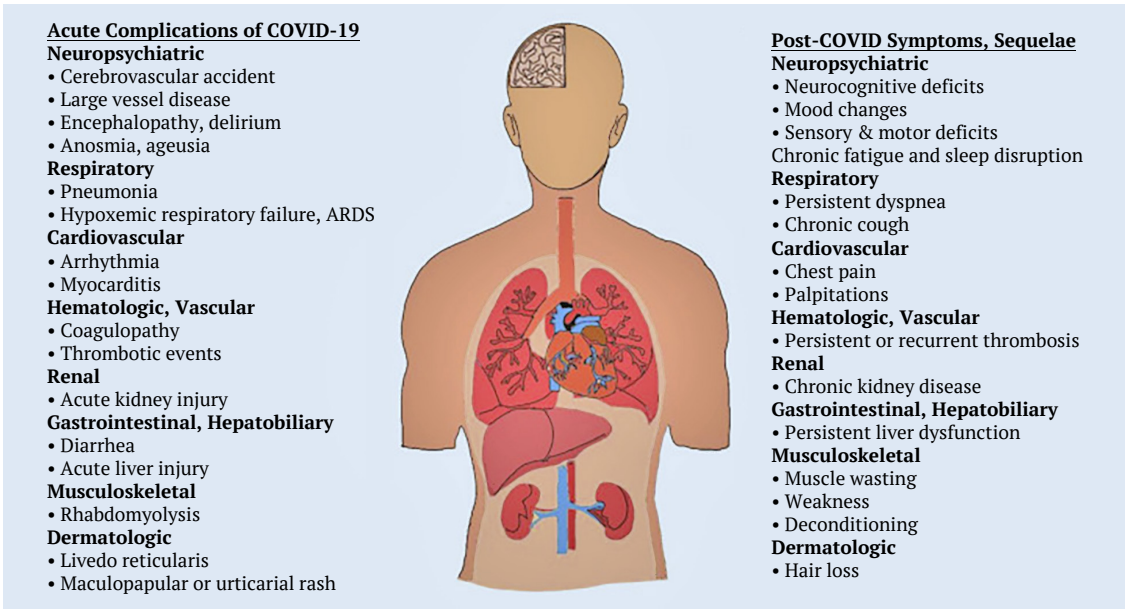

Figure 1. Model of acute pulmonary and extrapulmonary complications of coronavirus disease 2019 with predicted symptoms after COVID-19 and ultimate organ consequences
Source: [7]

Among the multifaceted long-term consequences of COVID-19, E.S. Izmailova & T.F. Reiss [8] outline three groups: pulmonary, neurological, and neuropsychiatric symptoms, including depression and cognitive dysfunction. The authors focus specifically on respiratory system changes, methods of their monitoring, and emphasise the need for further research dedicated to monitoring the condition of patients with prolonged respiratory manifestations both in hospital settings and at home.

According to a recent meta-analysis cited by D. Radovanovic & E. D'Angelo [9], 43% to 62% of recovered patients from acute COVID-19 have at least one residual symptom, with fatigue and dyspnea being the most prevalent. Numerous attempts to determine the cause of prolonged dyspnea and identify its possible associations with phenotype, patient age, and severity of the acute period are still incomplete and require a comprehensive approach to this complex condition.

K. Guziejko *et al.* [10] also provide similar observations: 24 weeks after recovery, 46% of patients report coughing, 23% – shortness of breath, and 13% – weakness. These symptoms occur not only after hospitalisation due to a severe onset of the disease but also after a period of home isolation with mild initial manifestations. Subsequently, many individuals who have had COVID-19 undergo routine examinations by general practitioners, infectious disease specialists, cardiologists, pulmonologists, and neurologists. However, the pronounced changes needed to explain all symptoms are not always detectable, and for such patients, the dominant diagnosis becomes "functional impairments".

As COVID-19 does not have strictly specific symptoms, according to global standards, the initial diagnostic workup begins with a complete blood count, blood biochemistry with a focus on urea, electrolytes, liver function tests, thyroid-stimulating hormone (TSH) levels, glycated hemoglobin (HbA1c), ferritin, vitamin D, and lipid profile [7, 11, 12] to exclude alternative diagnoses. Other examinations are prescribed according to the symptoms. For example, if a patient experiences dyspnea and chest pain, chest radiography, oxygen saturation determination, electrocardiography (ECG), echocardiography, and brain natriuretic peptide (NT-BNP) testing are included in the necessary examinations.

All individuals with persistent respiratory symptoms after COVID-19 should undergo respiratory system examinations that can visualise structural changes (chest X-ray, CT angiography of the pulmonary arteries, or high-resolution computed tomography) and assess its functional capacity: determining blood saturation, spirometry, body plethysmography, measuring lung diffusion capacity, and determining blood gas composition. It should be noted that a consensus has been reached on this recommendation [9, 11, 13].

The potential risk of SARS-CoV-2 infection considerably limited the use of respiratory diagnostic functional tests worldwide at the start of the pandemic. However, modern spirometry techniques, conducted according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards and with adherence to all conditions for preventing cross-infection, allow for a qualitative assessment of respiratory function [14-16]. Their results are most valuable in the presence of airway obstruction [14, 17]. Therefore, this examination is considered the gold standard for diagnosing chronic obstructive pulmonary disease (COPD) according to the global initiative for chronic obstructive lung disease (GOLD) report 18, 19].

In the majority of patients with mild and moderate symptoms, forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and the FEV1/FVC ratio were within normal limits [11, 19]. It was found that changes in spirometry indicating obstructive or restrictive impairments were detected in 17% of cases, mainly in patients hospitalised with severe pneumonia. Subsequently, they were only observed in individuals with post-COVID lung fibrosis confirmed by X-ray. A similar trend was observed in other studies [20, 21].

More information about respiratory function can be obtained through body plethysmography. Residual lung volume, total lung capacity (TLC), and maximal voluntary ventilation are measured using body plethysmography. It also determines the maximum expiratory and inspiratory pressures, which allow for the assessment of the strength of respiratory muscles, which is crucial in the presence of hidden forms of respiratory insufficiency [19].

ASSESSMENT OF LUNG FUNCTION AFTER COVID-19

The effectiveness of gas exchange in the lungs can be judged by changes in carbon monoxide diffusing capacity (DLCO) and blood gas composition. According to many researchers, DLCO most often reflects respiratory system impairments in Long COVID. It has been confirmed that even 1 month after discharge from the hospital, individuals who have had COVID-19 commonly experience a decrease in DLCO, which is the most common lung function abnormality. The more severe the disease, the more frequent and significant the decrease in DLCO and TLC compared to non-severe cases [10, 11, 22]. A similar correlation was observed in patients over 60 years of age and those who received invasive ventilation, with a considerable portion of them having low DLCO even 4 months after discharge [23]. However, there are conflicting statements regarding the association between the decrease in DLCO and FEV1 – some studies show a correlation [24-26], while others do not find a clear dependence [11, 19].

Lung damage in COVID-19 is associated with diffuse destruction of alveolar epithelium, capillary damage and bleeding, formation of hyaline membrane, fibrotic proliferation of the alveolar septum, and lung consolidation. However, the changes observed during its prolonged course may indicate that impairment of membrane diffusion capacity is the dominant pathophysiological mechanism compared to a decrease in lung volume [27]. Its gradual regression contributes to the fact that most individuals who have recovered from COVID-19 demonstrate progressive improvement in functional respiratory test results at 3 months, 6 months, and 12 months after acute infection [28-30].

It should be noted that the most informative examination of respiratory function was for patients who, after acute COVID-19 affecting the respiratory system, have complaints such as prolonged coughing. Conversely, in individuals who only have shortness of breath, functional respiratory tests often do not reveal deviations from normal. Since shortness of breath is not necessarily associated with impaired pulmonary function caused by SARS-CoV-2, and may be a manifestation of concomitant pathology, patients require proper differential diagnosis [10].

RAPID TESTS TO ASSESS THE FUNCTIONAL ABILITY OF THE RESPIRATORY SYSTEM

In situations with limited access to the aforementioned investigations [13, 31], rapid tests such as the 3- or 6-minute walk test (3/6MWT) or the 1-minute sit-to-stand test (1MSTST) can be used to evaluate the functional capabilities of the respiratory system in Long COVID and other chronic conditions, depending on the patient's abilities [32, 33].

E.S. Izmailova & T.F. Reiss [8] propose including diagnostic methods in a comprehensive package of dynamic monitoring, both in the clinic and at home, for individuals with slow or incomplete recovery. The scheme of this comprehensive monitoring package is presented in Figure 2. According to the authors, remote monitoring is more convenient for patients and creates more complete data sets.Remote monitoring can help answer numerous questions about the nature of this condition and aid in assessing treatment effectiveness, thereby mitigating its consequences and reducing mortality. For example, computed tomography (CT) scans and DLCO assessments are performed in clinical settings, while other lung function parameters such as FVC and FEV1, pulse oximetry results, and walking tests can be obtained remotely.

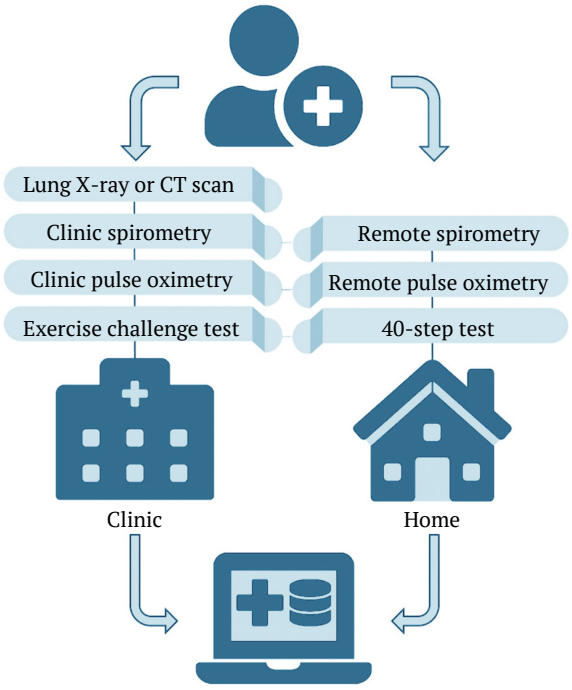

Figure 2. Examinations performed in the clinic and at home

Source: [8]

Tests such as the 3- or 6-minute walk test (3MWT or 6MWT) [20, 34, 35] play a crucial role in assessing the functional capacity of the respiratory system, determining physical exercise tolerance, evaluating prognosis, and treatment response in various respiratory conditions. In a medical facility, the patient is asked to walk as far as possible along the internal corridor in 3 or 6 minutes. If necessary, one can stop during the test. Before and after the test, measurements are taken of respiratory rate (RR), heart rate (HR), arterial oxygen saturation (SpO_2), blood pressure, shortness of breath, and subjective fatigue. The additional distance that the patient can cover compared to the previous trial is also recorded. During the test, SpO2 and HR are measured using a pulse oximeter.

In the conditions of deep desaturation development (reduced SpO_2 up to 80%), the test stops. If SpO_2 recovers to 85% during the 6MWT, the patient may be encouraged to continue walking. The test is also stopped if the patient experiences chest pain, severe shortness of breath, leg cramps, staggering, excessive sweating, or a change in skin colour to pale or ashen [36, 37]. Parameters within the test (pre- and post-test) are analysed and compared to previous results.

Proper conditions are required for the accurate execution of the 6-minute walk test (6MWT), such as a route length of 30 m or ideally longer [32]. Alternatively, the 1-minute sit-to-stand test (1-MSTST) can be used, where the patient performs the test with a chair of standard height (46 cm) without armrests placed near a wall. The person being assessed sits on the chair with a straight back, knees bent at a 90° angle, feet flat on the floor hip-width apart, and hands resting on the thighs without movement. The patient is asked to stand up (move to an upright position), and then sit back on the chair in the starting position at a speed that will be safe and comfortable. The use of hands for support should be avoided. As many repetitions as possible are performed within 1 minute, with rest allowed if needed. The position of the hands during the test (crossed on the chest, hanging by the sides, or resting on the thighs), the number of completed exercises, and the number of repetitions are recorded [37, 38]. The modified Borg scale (0-10) is used to assess breathlessness and fatigue immediately before and after the test [39, 40]. HR and peripheral oxygen saturation (SpO_2) are measured using a pulse oximeter before and after the test, along with the time taken for

the parameters to return to baseline. A desaturation level of ⩾4% is considered clinically significant for this study.

Comparative analysis of the dynamics of changes during rapid tests with walking or squatting reveals a direct correlation between their results and spirometry and X-ray examinations [21, 39], pO values$_2$ and pCO$_2$ in venous blood [34], severity of clinical symptoms, SpO$_2$, and DLCO levels which may indicate that desaturation during exercise is associated with parenchymal and/or pulmonary vascular phenomena [36]. Considering this, physical exertion tests can be a reliable, valid, and sensitive alternative or complement in situations with limited access to instrumental investigations.

The post-COVID-19 condition is relatively new, and there is a lack of experience in choosing the best management strategy for it. The presence of persistent respiratory symptoms, which occur in almost half of these patients 1 month after COVID-19 and later, necessitates the search for optimal methods to diagnose respiratory dysfunction and expedite their implementation into daily practice.

CONCLUSIONS

The COVID-19 pandemic has undoubtedly become an unprecedented case in history, despite a fairly high awareness of the mechanisms of development of viral processes, experience in combating them, and the high-tech capabilities of modern medicine. The efforts of doctors and researchers were aimed at finding effective quarantine measures and innovative methods of treating acute diseases. Unlike previous viral epidemics, SARS-CoV-2 has the ability to prolong clinical manifestations, particularly in the respiratory system. In the vast majority of survivors, at least one symptom persists for over 3-6 months, requiring the exploration of new approaches in both treatment and diagnosis since timely and effective detection of changes is unquestionably crucial for considerably reducing the risk of complications.

The most informative investigation for assessing respiratory function is the measurement of DLCO. Decreased DLCO directly correlates with changes in total lung capacity and blood gas composition. However, these assessments can only be performed in a hospital setting. Therefore, in conditions of limited access to them, rapid tests can be used as outpatient monitoring to assess the prognosis and response to treatment: the 3-x/6-minute walking test (3mwt/6MWT) or the 1-minute sit-up test (1-MSTST).

The pronounced multiorgan involvement and nonspecific symptomatology in Long COVID necessitate the adaptation of analysis algorithms and the correlation of data obtained under different conditions, and the coordination of communication channels between patients and specialists from various fields, which may be crucial in effectively countering future epidemics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Функціональна діагностика дихальної системи у пацієнтів при Long COVID

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Анотація. Враховуючи значну кількість осіб, що мають персистуючі респіраторні симптоми понад 4 тижні після перенесеного COVID-19, важливо визначити, які обстеження є найінформативнішими для оцінки респіраторної функції, прогнозу та контролю ефективності лікування у таких пацієнтів. Мета статті полягала у дослідженні функціональної діагностики дихальної системи у пацієнтів, які пережили Long COVID. Застосовано бібліосемантичний, аналітичний методи дослідження. Відповідно до світових рекомендацій щодо пацієнтів з персистуючими респіраторними симптомами після COVID-19, всі вони повинні пройти обстеження дихальної системи: рентгенографію органів грудної клітки, спірометрію, вимірювання сатурації крові, дифузійної здатності легень для монооксиду вуглецю, тести з ходьбою та інші. Дослідженням підтверджено, що в осіб, які перенесли COVID-19, навіть через 1-6 місяців виявляється зниження дифузної ємності легень для монооксиду вуглецю, що має прямий кореляційний зв'язок із загальною ємністю легень, важкістю клінічних проявів та результатами швидких тестів. Зміни спірометричних показників спостерігаються значно рідше, переважно у пацієнтів госпіталізованих з тяжкою пневмонією, яка згодом призводить до постковідного легеневого фіброзу. Поступове покращення показників функціональних дихальних тестів відбувається через 3 місяці, 6 місяців та 12 місяців після гострої інфекції. Найінформативнішими обстеженнями для оцінювання функції дихальної системи є визначення дифузійної здатності за монооксидом вуглецю, загальної ємкості легень та газового складу крові. В умовах обмеженого доступу до перерахованих обстежень та у якості скринінгового дослідження можна використати швидкі тести: тест з 3-х/6-хвилинною ходьбою (3MWT/6MWT) або 1-хвилинний тест «сісти-встати» (1-MSTST) відповідно до можливостей пацієнта

Ключові слова: спірометрія; функціональні легеневі тести; COVID; тест із ходьбою

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Spread of antibiotic-resistant microorganisms and mechanisms of their transmission from animal to human

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Abstract. Antimicrobials are essential for the treatment and prevention of infectious diseases in both humans and animals. However, the emergence and spread of antibiotic-resistant microorganisms has become a global problem of the World Health Organisation. Transmission of antibiotic-resistant macroorganisms from animals to humans is conducted directly or indirectly through the environment. This spread affects the epidemiology of resistant bacterial infections in humans. Thus, the purpose of the study was to establish and analyse the methods of distribution and mechanisms of transmission of antimicrobial-resistant microorganisms from animal to human, analyse the experience of different countries in solving problems of antibiotic resistance. Through methods of analysis and systematisation of scientific research of researchers from different countries of the world, it was established that antimicrobial agents have been used in agriculture as feed additives and maintain the health and productivity of animals since the middle of the 20th century. Animal products at all stages of food processing also often contain large amounts of antibiotic-resistant microorganisms. A substantial relationship was established between drugs used in humane and veterinary medicine. It is noted that difficult socio-economic conditions, limited laboratory facilities, and lack of regulatory authorities in developing countries also create favourable conditions for the spread of antibiotic-resistant pathogens. As a result, the treatment of many infectious diseases of people is substantially complicated or becomes impossible. The application of antibiotics should be accompanied by constant and enhanced monitoring of their spread in the animal–human–environment chain, a rational prescription in humane, veterinary medicine, and the food industry to minimise the risks of unjustified use of them

Keywords: bacteria; antimicrobials; antibiotic resistance; medicine; animal husbandry; mobile genetic elements; multi-resistance

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INTRODUCTION

Although antimicrobial agents have the ability to inhibit the growth or kill microorganisms, their excessive use has led to a substantial increase in the rate of elimination and release into the environment, and therefore an increase in the resistance of bacterial strains to drugs. In recent years, substantial progress has been made in the field of medicine and pharmacy in the development of a wide range of antibiotics, but the issue of antibiotic resistance is still not fully understood, since antimicrobials are widely used not only for the treatment of bacterial infections in humans and animals but also for non-medical purposes.

Antibiotics, or antibacterial agents, are drugs that can kill or inhibit the growth of bacteria that cause infections in humans, animals, and plants [1]. According to T.T.H. Van *et al.* [2], a substantial advance in medical science is the discovery of antimicrobials since the mid-20th century and the creation of more than 100 such compounds over the past 60 years. As S. Reardon [3] claims, the use of antibiotics in animal husbandry, despite various measures to limit their use, will increase between 2020 and 2030.

Antibiotic resistance is the ability of microorganisms to survive and multiply not only within a single species but also within bacterial genera, despite the presence of antibiotics [1, 4]. As C. Morel [1] notes, bacteria adapt quickly to environmental changes. The emergence of pathogen resistance to antibiotics is an adaptive property. Some bacteria have a natural resistance to certain antibiotics. However, when bacteria that are normally antibiotic-sensitive become resistant due to genetic changes, i.e. acquired resistance is formed, this becomes a serious problem for both public health and animal health. In a study by N.A. Lerminiaux & A.D.S. Cameron [4] it was indicated that horizontal gene transfer contributes to the rapid spread of resistance. According to the authors, plasmid conjugation, bacteriophage transduction, and natural transformation of extracellular deoxyribonucleic acid (DNA) allow genetic material to migrate between bacterial strains and species. Thus, horizontal gene transfer is important in the development of infectious diseases. In addition, the antibiotic resistance gene can be a causative agent of infections, transmitting resistance to several unrelated pathogenic microorganisms.

After a detailed examination of the molecular mechanisms responsible for both innate and acquired antimicrobial resistance, E. Palma *et al.* [5] reviewed the contribution of veterinary medicine to the overall problem of antibiotic resistance formation. Researchers describe the main sources of antibiotic reactions available in veterinary medicine, drawing attention to the indissoluble crosstalk that exists between different ecosystems and sectors, and future prospects for preventing the spread and/or development of bacterial resistance to antibiotics.

According to many researchers, resistant bacteria reduce the effectiveness of antibiotics in the treatment of animals and humans. Infectious diseases caused by such pathogens are difficult, and sometimes even impossible to treat. In such a situation, recovery often depends only on the state of the body's immune system. In addition, this trend in animal husbandry leads to substantial economic costs [1, 6]. I.I. Fohel *et al.* [7] emphasise that resistant microorganisms pose a danger not only to adults but also to children. This trend requires a special approach to prescribing antibiotics to children. Treatment of childhood infections caused by antibiotic-resistant microorganisms is more difficult to treat and requires special methods and approaches, so the choice of antibiotic drugs is limited.

Ukrainian researchers S. Tymoshchuk & L. Symochko [8] note that resistant pathogenic and opportunistic bacteria that can directly or indirectly infect humans can transfer the determinants of resistance to the human body in various ways, namely: directly through contact with animals and indirectly through the food chain, water, air, and soil fertilised with manure, or wastewater. Ultimately, in the soil (water)–microorganisms–plants chain, the soil (water) microbiota is an inseparable component. In particular, researchers in the microbiome of the water ecosystem of the Uzhgorod district detected, even after treatment in sewage treatment plants, antibiotic-resistant *Klebsiella pneumoniae* and *Enterococcus faecium*.

N.O. Vrynchanu & T.A. Bukhtiarova [9] note that the pronounced antimicrobial activity of agents of various pharmacological groups contributes to the activation of antibiotic resistance mechanisms in bacteria, which is the basis for resolving the question of the feasibility of their further use in clinical practice. L. Serwecińska [10] states that the antibiotic resistance of microorganisms has reached a critical level on a global scale. Therefore, the examination of antibiotic resistance issues is an important and urgent problem in relation to preserving human and animal health, which is caused by the transfer of bacterial resistance from animals to humans through the animal–human–environment relationship.

The purpose of the study was to summarise data on the formation of antibiotic resistance in bacteria and how they are transmitted between humans and animals, and potential solutions to this global problem.

SPREAD OF MICROORGANISMS IN THE ENVIRONMENT

There are many relationships between animals, humans, and the environment that contribute to the migration of not only bacteria, but also their mobile genetic elements. In the context of investigating microbial resistance to antibiotics, this interaction between ecological niches is particularly important [11, 12].

Since the middle of the 20th century, antimicrobial agents have been massively used for the treatment and Prevention of animal diseases. This contributed to an increase in meat production and a reduction in its prime cost, which in turn ensured an intensive increase in livestock production from year to year. However, the massive and sometimes uncontrolled use of antibiotics in animal husbandry has become one of the reasons for the ineffective treatment of infectious diseases in humans [11, 13]. Given the constant emergence of new antimicrobial-resistant pathogenic bacteria in animals, researchers point to the need to strengthen monitoring of the spread of resistant strains of microorganisms in the animal–human–environment chain [5, 14, 15].

Specialists in the medical industry are mainly engaged in investigating the mechanisms of the development of resistance of pathogenic strains of microorganisms to antibiotics in humans. However, in all living habitats, the total number of bacterial species is extremely large. As noted in

the study by G. Chala *et al.* [16] and V. Oswaldi *et al.* [17], most of the bacteria that live in the human body are part of its normal microbiome. Only a small part of them, for example, *Mycobacterium tuberculosis* or *Staphylococcus pneumoniae*, *Salmonella typhi*, are obligate human pathogens. The vast majority of microorganisms are conditionally pathogenic. They lead to the development of opportunistic infections in humans only under certain circumstances, such as: *Listeria* spp. and *Campylobacter* spp. they enter the human body with food or water and can cause diseases mainly in children, pregnant women, the elderly, or people with weakened immune systems.

According to researchers, some types of microbes accidentally enter the human body and then are eliminated. Such microorganisms, as a rule, do not lead to the emergence and development of the disease. However, many pathogenic bacterial species and assumably many commensals are found not only in humans but also in the body of livestock and wild animals, causing anthoropozoonotic infections. Thus, representatives of the Enterobacteriaceae family: *Escherichia coli*, *Klebsiella* spp., and *Salmonella* spp. (which often cause urinary tract and circulatory system infections in humans) are established in the intestines and in animal meat [18-20].

After performing a sensitivity analysis of *Escherichia coli* to antibiotics in poultry excrement in Sierra Leone, A.H.D. Mansaray *et al.* [21] established that the prevalence of multi-resistant strains among these bacteria was 95.6%. Therewith, a high index of multiple antibiotic resistance was observed – from 0.5-0.7, with an optimal <0.2. The authors note that the data obtained indicate a high pre-effect of antibiotics in these birds and such high levels of resistance to *Escherichia coli* isolated from poultry excrement can pose a serious threat to human health.

C. Chen & F. Wu [19] conducted a systematic review and meta-analysis of the characteristics of colonisation and infection of animals, livestock workers, and veterinarians with methicillin-resistant *Staphylococcus aureus* (LA-MRSA). They confirm that *Staphylococcus aureus* is the most common cause of skin infections and the second most common cause of blood infections in humans, and is also established on the skin and in animal meat. These microorganisms easily migrate between pets and humans and spread in public places and healthcare facilities. They are responsible for the spread of antibiotic-resistant infections, leading to deaths worldwide.

H.C. Lepper *et al.* [22] note that micro-organisms are repositories of genetic information that encodes bacterial resistance to antibiotics, spreading not only within one species but often between others, including intergenerational transmission. Authors argue that the environmental potential for spreading antibiotic-resistant microorganisms has recently been increasingly recognised, for example, as a result of their abundance in wastewater discharged into natural reservoirs and soils. Researchers developed a compartment model of resistance transmission between bacteria, humans, animals, and the environment to examine the importance of the environment for the long-term dynamics of the spread of resistant bacteria and the cause of infections in humans, which is shown in Figure 1.

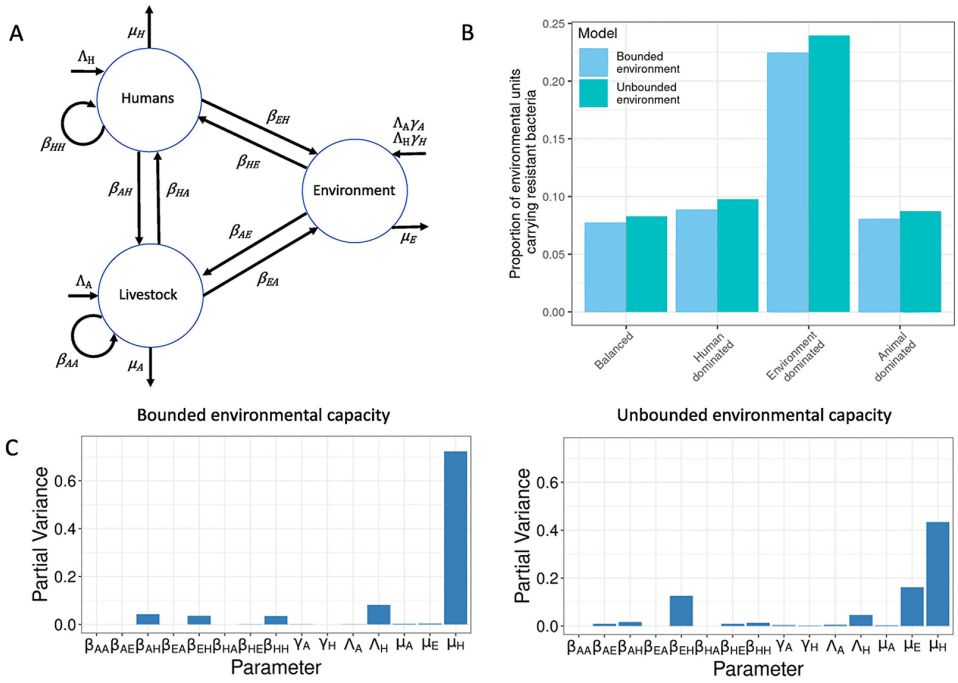

Figure 1. Model of environmental impact on human resistance levels and the role of measures aimed at reducing antibiotic consumption by animals

Notes: A – flowchart indicating the model structure. B – RE value in all transmission methods and both model structures. C – Fourier amplitude sensitivity test (FAST), which indicates how much the change in RH is explained by each parameter of the model. On the left is the FAST for the model version where RE is limited to 1. On the right is the FAST for the version of the model in which RE was unlimited

Source: [22]

According to J. Kim & J. Ahn [11], antibiotic-resistant bacteria can get directly or indirectly from animals to humans through contaminated food, water, or organic waste used as fertiliser. In addition, waste from hospitals, farms, and homes containing antibiotic-resistant microbiota is often disposed of openly without proper monitoring and treatment, leading to the spread of antibiotic resistance genes in the environment. Researchers note that animal food products at all stages of food processing contain a large number of antibiotic-resistant microorganisms.

APPLICATION OF ANTIMICROBIAL AGENTS IN ANIMAL HUSBANDRY AND FISHING INDUSTRY

According to the results of research by a number of researchers, antimicrobial agents are widely used to treat animals. Most often, antibiotics are used to treat respiratory and intestinal infections during the period of intensive growth of broiler chickens, pigs, and calves. The issue of using antibiotics in dairy cattle breeding for the treatment of sick dairy cows is relevant. A substantial part of antibiotics is used to prevent diseases of animals that come into contact with sick individuals [1, 14]. This practice provides certain economic benefits to animal producers and consumers in general [2].

In the paper by F. Ma *et al.* [14], it is reported that in the United States of America and other developed countries of the world, antimicrobials, as growth stimulants for animals, have been intensively used since the middle 20th century. Thus, in the 50s, researchers first reported that the addition of antibiotics (sulfasuxidin, streptotricin, and streptomycin) to the feed for chickens and pigs had a positive effect on increasing their productivity. Since then, the use of antibiotics has become an integral part of modern animal husbandry.

A group of researchers note that to stimulate the growth of animals, antimicrobial agents are added to the feed in doses ranging from subtherapeutic to therapeutic concentrations. Thus, an increase in meat production is ensured and it is possible to prevent the occurrence of local outbreaks of infectious diseases and their spread on a global scale [12]. However, according to F. Ma *et al.* [14], in many countries in Europe and the USA, the amount of antibiotics added to animal feed substantially exceeds the need for their use. In addition, a large number of antimicrobials are common in the treatment of humans and animals, including the latest classes of drugs such as third- and fourth-generation cephalosporins, fluoroquinolones, glycopeptides, and streptogramins.

Along with the positive effect of using antibiotics, in the early 50s of the 20th century, there were reports of their negative impact. Thus, in 1951, after streptomycin was used in turkey feed, the first cases of resistance of the bird body to this antibiotic were registered. Over time, the resistance of domestic animals to tetracycline, sulfonamides, penicillin, and other beta-lactams increased [23-25]. Based on the study, researchers established that with the daily care of infected animals, farm workers, slaughterhouses, and veterinary doctors are easily infected with antibiotic-resistant bacteria. They reported that back in 1957, researchers established that a week after feeding chickens a dietary supplement with tetracycline, microorganisms resistant to this drug were established in the gut microbiome of birds, and five to six months after the experiment was completed, more than 80% of bacteria resistant to this drug were established in 31.3% of their faecal samples. Other researchers selected 36 strains between 2004 and 2007 *E. Soli,* resistant to apramycin. They were randomly selected from biological material from pigs, chickens, and humans on six farms in areas of northeastern China. As a result, apramycin resistance genes of aminoglycoside acetyltransferase (AAC) (3)-IV type from humans and animals were established to have 99.3% homology [14].

The production of antibiotics continues to increase, and their total annual consumption in the world has increased from 100 000 to 200 000 tonnes [26]. In the European Union countries, the mass of antimicrobials used in animal husbandry in 2018 was 8 927 tonnes. In the United States, subtherapeutic doses of antimicrobials added to animal food in the same year reached approximately 14 600 tonnes. China, the world's largest producer and consumer of antibiotics, used 29 774.09 tonnes of antimicrobial agents for livestock needs in 2018, and 53.20% of total consumption was used to stimulate animal growth [14].

According to the WHO (World Health Organisation), as of 2019, the average annual global use of antimicrobials per volume of animal products is estimated at 45 mg/kg for cattle, 148 mg/kg for chickens, and 172 mg/kg for pigs. Based on this baseline, global antimicrobial consumption is projected to increase by 67% by 2030 [27].

R. Mulchandani *et al.* [28] analysed the use of antimicrobials in animal husbandry in 42 countries in different parts of the world in 2020. Researchers note that this year the use of antimicrobials worldwide was estimated at 99 502 tonnes. Antimicrobial use prevailed in Asia (67%), while it was at <1% in Africa. According to researchers' forecasts, the use of antibiotics will increase by 8.0% (to 107 472 tonnes) by 2030 [28]. The results of the study and forecasting of results are presented graphically (Fig. 2).

Along with the development of animal husbandry, the fishing industry is developing intensively. According to the Food and Agriculture Organisation of the United Nations (FAO) [29], global fish production in 2018 reached approximately 179 million tonnes. Of the total volume, 156 million tonnes were used for human consumption, which is equivalent to the estimated annual supply of 20.5 kg per capita. The remaining 22 million tonnes were intended for non-food purposes, and mainly for the production of fish meal and fish oil. Aquaculture support accounts for 46 per cent of total production and 52 per cent of fish for human consumption. And in 2020, as the FAO notes, the total production of fish and aquaculture products reached a record 214 million tonnes, including 178 million tonnes of aquatic animals and 36 million tonnes of algae. These results were achieved mainly due to the growth of aquaculture, especially in Asia. The amount intended for human consumption (excluding algae) was 20.2 kg per capita, more than double the average of 9.9 kg per capita in the 1960s [30].

However, as noted by F. Ma *et al.* [14], increased fish population density, poor sanitation, and the inability to separate healthy fish from infected ones contribute to the rapid spread of infections. The high population density in coastal areas and environmental pollution are well-known

immunosuppressive factors that contribute to the high vulnerability of fish to protozoal infectious agents. Thus, at the beginning of the 21st century in China, more than 200 bacterial infections were detected among commercial fish species, which accounted for about 15-20% of losses from their total annual catch.

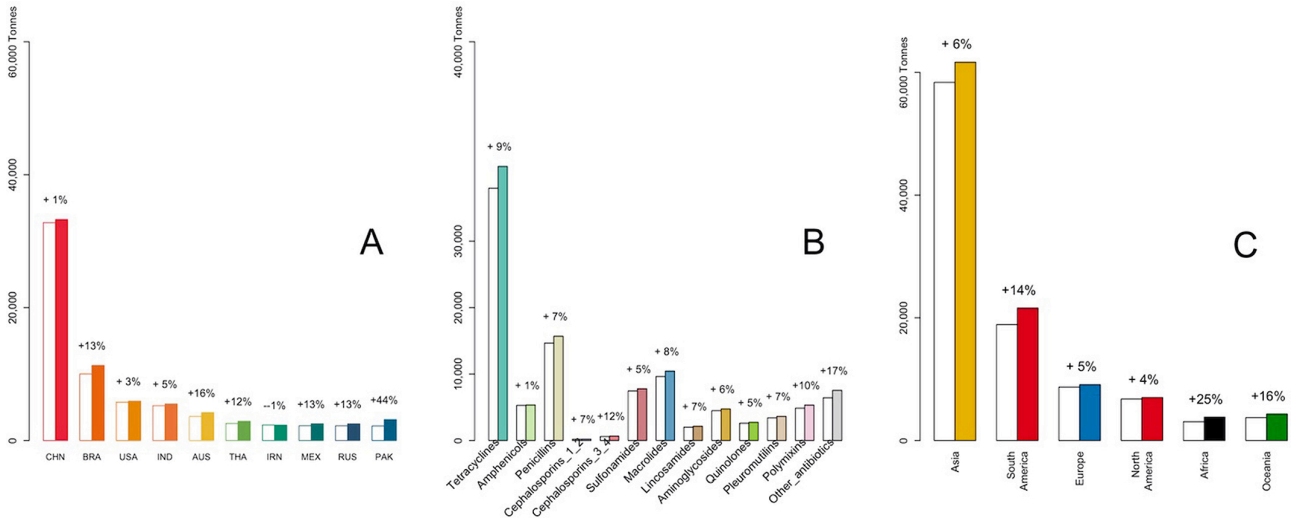

Figure 2. Antimicrobial consumption in Veterinary Medicine in 2020 (white columns) and their projected consumption in 2030 (colored columns)
Notes: A – by country (Top 10 consumers (CHN, China; BRA, Brazil; IND, India; USA, USA; Aus, Australia; IRN, Iran; Tha, Thailand; PAK, Pakistan; JPN, Japan; MEX, Mexico.)). B – by antimicrobial class, C – by continent
Source: [28]

Due to the erroneous belief that the use of a large number of antimicrobials in fish farming will ensure a large industrial production of fish products, various types of antibiotics continue to be widely used. In particular, in the paper by S. Chowdhury *et al.* [31], it is indicated that fish and other aquaculture products often contain various antimicrobial substances in concentrations that exceed the permissible norm. AMong these, oxytetracycline, ciprofloxacin, and amoxicillin are the most common. Researchers report that most antibiotics are used for both therapeutic and preventive purposes.

Residues of veterinary antimicrobials can be established in the waste products of fattening livestock, pigs, and chickens. Their high concentration in the urine and faeces of animals is very dangerous, because, in the urine and faeces, antimicrobial residues enter the water systems, pollute them, and enter the body of fish. The high concentration of antibiotics in animal droppings is also a cause for concern due to the fact that it is often used to feed water bodies to stimulate fish growth. Thus, the consumption of fish containing antibiotics becomes risky for human health [14].

WAYS TO TRANSMIT ANTIBIOTIC-RESISTANT MICROORGANISMS FROM ANIMALS TO HUMANS

There is a substantial relationship between drugs that are necessary for human use and those that are currently used in animal husbandry. World Health Organization (WHO) [27] classifies fluoroquinolones, third- and fourth-generation cephalosporins, macrolides, glycopeptides, and polymyxins as “top priority critical” antibiotics for human treatment. C. Morel [1] noted in a paper that macrolides and fluoroquinolones are most commonly used to treat diseases of the human body, and tetracyclines and sulfonamides are the most common drugs in animal husbandry. The use of antibiotics in animal husbandry continues to stimulate animal growth and critical top-priority antimicrobials needed for human treatment. According to the results of the researcher’s study, Table 1 shows a list of classes of antimicrobial agents licensed for veterinary use in the European Union countries and their main indications for use.

Table 1. List of the main classes of antimicrobial agents licensed for use in veterinary medicine in the European Union

Class of antibiotics	Veterinary use (types)	Main indications	Risk to human health	Against what microorganisms are used	Probability of transmission of antibiotic-resistant microorganisms
Aminoglycosides (gentamicin, neomycin, etc.)	cattle, sheep, goats, horses, dogs, and cats	• Septicemia • Digestive, respiratory, and urinary infections	Requires additional research	Enterobacteriaceae *Enterococcus* spp.	High
Cephalosporins (3rd and 4th generation)	Cattle, pigs, horses, dogs and cats	• Septicemia • Respiratory infections • Mastitis	High	Enterobacteriaceae	High

Table 1. Continued

Class of antibiotics	Veterinary use (types)	Main indications	Risk to human health	Against what microorganisms are used	Probability of transmission of antibiotic-resistant microorganisms
Fluoroquinolones	Cattle, pigs, chickens, turkeys, rabbits, dogs, and cats	• Septicemia • Infections (e.g. colibacteriosis)	High	*Campylobacter* spp. Enterobacteriaceae	High
Macrolides (including ketolides)	Cattle, sheep, pigs, and poultry	• Infections caused by mycoplasmas (pigs and poultry) • Hemorrhagic digestive disease and proliferative enteropathies associated with *Lawsonia intracellularis* (pigs) • Respiratory infections (cattle and sheep) • Liver abscess (cattle)	Low and short-lived	*Campylobacter* spp. *Salmonella* spp.	High
Penicillins (naturally sensitive to lactamases)	Cattle, sheep, poultry, turkeys, horses, dogs, and cats	• Septicemia • Respiratory infections • Mastitis	Low or short-lived	Non-specific	High
Penicillins (broad spectrum beta-lactam sensitivity) Aminopenicillin	Cattle, sheep, pigs, poultry and dogs	• Pasteurellosis and colibacteriosis (poultry) • Streptococcal infections (pigs) • Respiratory infections (cattle and pigs)	Further risk analysis is needed	Enterobacteriaceae *Enterococcus* spp.	High
Penicillins (narrow spectrum of beta-lactam resistance)	Cattle and sheep	• Metrit • Mastitis	Low or short-lived	Non-specific	High
Penicillins (protected by broad-spectrum beta-lactamase) – co-amoxiclav	Cattle, pigs, dogs, and cats	• Respiratory infections • Metrit • Mastitis • Colibacteriosis (cattle and pigs)	Further risk analysis is needed	Enterobacteriaceae *Enterococcus* spp.	High
Polymyxins (including colistin or polymyxin E)	Cattle, sheep, pigs, and poultry	• Septicemia • Colibacteriosis • Urinary infections • Infections caused by Gram-negative bacteria	Currently evaluated	Enterobacteriaceae	Low
Rifamycin (rifampicin)	Cattle	• Metrit • Mastitis	Low or short-lived	Non-specific	High
Tetracyclines	Cattle, sheep, goats, pigs, horses, and poultry	• Respiratory diseases • Bacterial enteritis • Urinary system infections • Metrit • Mastitis • Chlamydia • Actinomycosis • Escherichiosis • Resistant strain *Staphylococcus aureus*	Low or short-lived	*Drucella* spp.	High

Source: adapted according to [1]

According to researchers, residues of veterinary antimicrobials such as ciprofloxacin, enrofloxacin, oxytetracycline, and chlortetracycline can be detected in the waste products of fattening livestock, pigs, and chickens [14, 32].

Based on the analysis, a schematic representation of possible methods of spreading antibiotics is proposed, which is shown in Figure 3. Excessive and irrational use of various antimicrobials has led to a rapid increase in bacterial resistance to several types of antibiotics simultaneously, that is, to the formation of multi-resistance. A bacterial cell can become resistant to multiple, unrelated groups of antimicrobials, even with a single mutation. The main mechanisms of bacterial resistance formation are based on a decrease in the permeability of the bacterial cell membrane to antibiotic molecules and the absence of specific molecules or inactivation of antimicrobial compounds. In addition, the genetically determined resistance created by these bacteria is effectively transmitted during clonal reproduction and/or to other bacteria through mobile genetic elements such as plasmids, transposons, and integrons [5, 11, 33]. C. Morel [1] and E. Palma *et al*. [5] focus on the fact that multi-resistance of microorganisms leads to a violation of the health status of animals and humans and reduces the effectiveness of treatment of patients according to the protocol.

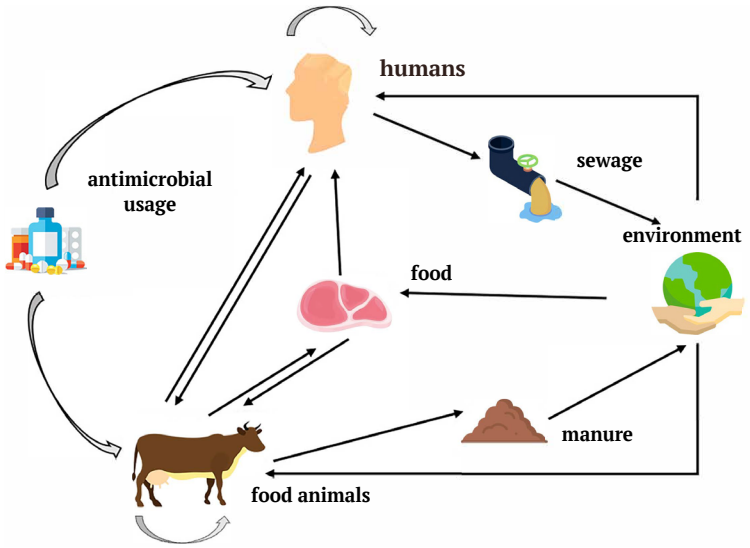

Figure 3. Potential pathways of antibiotic distribution and transmission between animals, the environment, and humans
Source: compiled by the authors

Many researchers have concluded that antibiotic resistance in the modern world is becoming one of the main problems in the field of health, animal husbandry, and food industry [32, 34]. According to the study by X.C. Monger *et al*. [32], in 2019, there were almost 5 million deaths worldwide related to antibiotic resistance of pathogenic bacteria. The authors identified six leading monoresistant and polyresistant pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii,* and *Pseudomonas aeruginosa*), which caused fatal consequences.

Researchers often attribute the increase in the prevalence of antibiotic-resistant microorganisms and, accordingly, their resistance genes to selective pressure on the use of antibiotics in both clinical and agricultural settings. Independent use of medications also strongly affects their distribution. People are at risk of contact with new resistant infectious agents or their genetic determinants through livestock products and water. One of the main factors for increasing the number of resistant microorganisms transmitted in each medium is the intensive and irrational use of antibiotics in it [13, 35].

K. Iskandar *et al.* [35] draw attention to the main mechanisms by which antibiotic-resistant animal microorganisms can pose a threat to human health. Infection can occur through: water or food that has been contaminated with antibiotic-resistant bacteria and is not properly cooked; improper hand hygiene after caring for dirty animals; interaction with contaminated surfaces. After that, the transmission of infectious agents to other people continues, and some infected people develop diseases. This variant of spread is a violation of the species barrier by a microorganism, which can be either directly pathogenic to humans or a commensal with the ability to cause opportunistic infections. Microbial resistance genes that occur when animals are infected become pathogenic to humans due to their horizontal transfer.

The spread of antibiotic-resistant microorganisms can also be affected by human activity. Thus, a study by E. Rousham *et al.* [36], which was conducted in Canada on clinical strains *Salmonella* of human and strains *E. coli* and *Salmonella* of chickens showed that stopping injections of ceftiofur, which belongs to the third generation of cephalosporins, substantially reduced the resistance of microorganisms in the examined birds and strains of these pathogens in the people who cared for them. Other researchers analysed the papers confirming the increased prevalence of resistant gut bacteria among farm workers compared to the population or farm workers who do not use antibiotics in animal feed.

Socioeconomic conditions can also affect the transmission of resistant microorganisms through human–animal communication. Inadequate sanitation in low-and middle-income countries, where people live in close proximity to animals, is considered to create ideal conditions for the interspecific spread of antibiotic-resistant pathogens. In addition, patients with antibiotic-resistant infections may not be able to provide or afford effective second-and third-line antibiotic treatment. The situation in these regions is complicated by pollution of available water sources, civil conflicts, and an increase in the number of people with weakened immune systems [13, 35]. As noted by A.H.D. Mansaray *et al.* [21], limited laboratory capacity and lack of regulatory authorities in these countries are serious problems in monitoring the impact of antibiotic use in animal husbandry, investigating the mechanisms of formation of antibiotic resistance of microorganisms, how they enter and affect the human body.

POTENTIAL SOLUTIONS TO THE PROBLEM OF ANTIBIOTIC RESISTANCE

Researchers argue that to reduce the migration of antibiotic-resistant microorganisms between animals and humans, the use of antibiotics in animal husbandry should be limited by including possible alternative substances, including probiotics, prebiotics, and various plant extracts for the treatment and prevention of diseases [36].

Understanding and knowledge of the dynamics of bacterial spread, horizontal transfer of antibiotic resistance genes between them, regardless of whether they belong to

the same bacterial species or not, the use of antimicrobial drugs and the mechanisms of resistance formation in the host body and the environment, is the basis for a possible solution to the problem of increasing antibiotic resistance [1, 37]. In a study conducted by B.A. Wee *et al.* [38], the possibility of using phylogenetic analysis of bacterial genome sequences together with epidemiological data is indicated. This approach allows visualising characteristics such as the profile of antibiotic-resistant microorganisms and the host species for more information.

According to researchers, state governments, in cooperation with health authorities, should conduct scrupulous and versatile monitoring of antibiotic consumption in animal husbandry. In addition, the government and the public health sector should provide research and analysis services for data obtained from observational studies to track antibiotics and their residue levels in most environmental samples [36, 39].

According to L. Munkholm & O. Rubin [40], in 2013, The WHO Strategic Technical Advisory Group on Antimicrobial Resistance recommended the development of a global action plan for antimicrobial resistance. This recommendation was adopted in 2014 as a resolution of the World Health Assembly (WHA), and WHO began developing a global plan of action together with the FAO and the World Organisation for Animal Health (WOAH). The plan was approved by 194 WHO member states, which were called upon to develop and implement national action plans on antibiotic resistance. As a result, in a relatively short period of time, this tripartite committee was able to create the basis for a global antimicrobial resistance management regime. More than 120 member states, through self-reporting, have developed national action plans and established a monitoring regime. The authors recommend global governance initiatives based on individual responsibilities, some of which should be legally binding to strengthen the coherence of national antimicrobial resistance policies. They also note that regional governance agencies (such as WHO regional offices) should act as intermediaries between global and local requirements to strengthen the global governance regime.

Based on the fact that the problem of antibiotic resistance has acquired a global scale, in 2019 the Cabinet of Ministers of Ukraine approved the "National Action Plan to combat antimicrobial resistance" [9, 41], which includes a number of measures aimed at ensuring the rational use of antimicrobial drugs in the healthcare, veterinary medicine, and food industry. It is proposed to limit the use of antimicrobial drugs as growth stimulants in animal husbandry, poultry, and crop production. Doctors and veterinarians should follow clear instructions to minimise the risks of unjustified use of antimicrobials.

As a result of the analysis of scientific papers, it was established that antimicrobial resistance in the modern world is a global WHO problem. The accumulated results of studies show that improper and excessive use of antibiotics in animal husbandry is one of the main factors of acquired resistance to these drugs in humans. There is an urgent need to reduce the overall use of antibiotics in agriculture and aquaculture around the world. The experience of various developed countries shows that reducing their use will not have a substantial negative impact on the health or productivity of animals, and, in some cases, will benefit the health of people, animals, and the preservation of the environment. A number of effective measures aimed at reducing antibiotic resistance have been documented in different countries of the world.

CONCLUSIONS

There are many connections between animals, humans, and the environment that promote migration not only of bacteria but also of their mobile genetic elements. Antibiotic-resistant bacteria can enter the human body from the environment, namely: from water bodies, soil, air, contaminated vegetables and fruits, etc. Many pathogens present in the normal microbiome of agricultural, domestic and wild animals, and fish enter humans, causing complex anthropozoonotic infections.

Some critical classes of antibiotics used in medicine continue to be used to treat and stimulate growth in livestock. This misuse and overuse is one of the main factors of acquired antimicrobial resistance in animals and humans.

The global emergence and spread of antibiotic-resistant bacteria in the environment requires the promotion of a coordinated interstate and regional approach to reduce risks to human and animal health. The use of antibiotics should be accompanied by constant and enhanced monitoring of their spread and rational use in humane, veterinary medicine, and the food industry. Doctors and veterinarians should follow the procedure for using antimicrobials to minimise the risks of unjustified use. Active education is also an important aspect, especially in developing countries.

The use of various measures to prevent the spread of animal and human diseases without the use of antibiotics is an important factor in reducing the spread of antibiotic-resistant microorganisms. This can be facilitated by ensuring greater access to veterinary services for farmers. It is extremely important that antibiotics are sold only by prescription. Veterinarians should have a high level of professional knowledge in the fields of preventive medicine, effective biosecurity, and vaccinology.

In the future, it is necessary to improve understanding of the factors that contribute to the emergence, spread and permanent presence of antibiotic-resistant microorganisms in the biosphere, and monitor the rational use of antibiotics and alternative medications for the treatment and prevention of animal and human infections.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Поширення антибіотикорезистентних мікроорганізмів та механізми їх передачі від тварини до людини

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Анотація. Антимікробні засоби мають важливе значення для лікування та профілактики інфекційних захворювань як людей, так і тварин. Однак, поява та поширення мікроорганізмів, резистентних до антибіотиків, стала глобальною проблемою Всесвітньої організації охорони здоров'я. Передача антибіотикорезистентних макроорганізмів від тварин до людини здійснюється прямо чи опосередковано через навколишнє середовище. Таке їх поширення впливає на епідеміологію резистентних бактеріальних інфекцій у людей. Отже, мета дослідження полягала в тому, щоб за даними наукової літератури встановити та проаналізувати способи поширення та механізми передачі стійких до антимікробних засобів мікроорганізмів від тварини до людини, з'ясувати досвід різних країн щодо вирішення проблем антибіотикорезистентності. За допомогою методів аналізу та систематизації наукових досліджень вчених різних країн світу з'ясовано, що антимікробні засоби ще із середини XX сторіччя часто використовують в сільському господарстві як добавки в кормах, так і для підтримки здоров'я та покращення продуктивності тварин. Продукти харчування тваринного походження на всіх стадіях харчової обробки також часто містять велику кількість стійких до антибіотиків мікроорганізмів. Виявлено значну спорідненість між лікарськими засобами, які використовуються в гуманній медицині та у ветеринарії. Зазначено, що складні соціально-економічні умови, обмежені лабораторні потужності та відсутність контролюючих органів в країнах, що розвиваються, також створюють сприятливі умови для поширення антибіотикорезистентних збудників. Внаслідок чого значно ускладнюється або стає неможливим лікування багатьох інфекційних захворювань людей. Застосування антибіотиків повинно супроводжуватися постійним і посиленим контролем за їх розповсюдженням у ланцюгу «тварина–людина–навколишнє середовище», раціональним призначенням у гуманній, ветеринарній медицині та харчовій промисловості для того, щоб мінімізувати ризики невиправданого їх використання

Ключові слова: бактерії; антимікробні засоби; стійкість до антибіотиків; медицина; тваринництво; мобільні генетичні елементи; мультирезистентність

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Patophysiological basis of folate cycle disorders and vitamin D deficiency in the development of syncope in childhood

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Abstract. There are many reports about the role of vitamins B6, B9, B12, and D in the development of cardiovascular diseases. However, most of them relate mainly to the adult population and are limited relative to grades in children with syncope. Understanding the role of these vitamins in the pathogenesis of syncope will help expand the range of therapeutic and preventive care for children. The purpose of the study was to analyse current scientific achievements regarding the role of the folate cycle and vitamin D in the genesis of syncope in childhood. The PubMed Medline and Scopus databases were used and the following search terms were used: "syncope" and "vitamin B"; "syncope" and "homocysteine"; "syncope" and "vitamin D". The paper summarises the role of vitamin B12 deficiency in delayed myelination and nerve conduction, increased serum norepinephrine levels, and possible pathogenetic mechanisms for the development of non-cardiogenic syncope. Scientific facts of the effect of vitamins B1, B6, and B9 on the functioning of the cardiovascular and nervous systems in children are described. The prevalence of vitamin D deficiency in 60-73% of children with vasovagal syncope and its relationship with the symptoms of the disease was established. Probable pathogenetic mechanisms of vitamin D deficiency in the development of syncope, namely a decrease in peripheral vascular resistance, a violation of neuronal conduction of the baroreflective mechanism, and heart muscle dysfunction, are analysed. The findings will allow doctors and researchers to better approach the diagnosis, prevention, and treatment of syncope in childhood and can serve as a basis for developing new strategies to manage the condition and improve medical practices

Keywords: vitamins; homocysteine; vasovagal syncope; syncope due to orthostatic hypotension; cardiogenic syncope; children

INTRODUCTION

Recent studies give every reason to believe that vitamins B6, B9, B12, and D affect the functioning of the cardiovascular and nervous systems, although the pathogenetic mechanisms of such effects are still poorly understood and unclear. The folate cycle enzymes methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) are vital in the formation of cellular homeostasis due to their key functions in the single-carbon cycle, which includes the metabolism of methionine and folate, and the synthesis of protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). The role of polymorphism of these genes in the development of diseases of the cardiovascular system (CVS) – metabolic syndrome, vascular atherosclerosis, arterial hypertension, myocardial infarction, stroke, thrombosis and thromboembolism – has been proven. In addition, there is no doubt that vitamin D deficiency is associated with various risk factors for CVS diseases associated with high-

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er mortality and the incidence of cardiovascular events. Considering the above facts, studies of folate cycle disorders and vitamin D deficiency are scientifically based and relevant from the standpoint of a better understanding of the pathogenetic mechanisms of transient loss of consciousness in children.

Vitamins B6 (pyridoxine), B9 (folic acid), and B12 (cyanocobalamin) are important cofactors in the folate and single-carbon cycles. Low levels of these vitamins are often consequences of hyperhomocysteinemia and independent risk factors for CVS, dementia, and depression [1-3]. Serum vitamins B6, B9, B12, and homocysteine levels depend on actual B vitamin intake and polymorphism of the MTHFR, MTR, and MTRR genes [4, 5]. A.D. Kaye *et al.* [6], A. Ueno *et al.* [7] proved the effectiveness of pyridoxine, folic acid, and cyanocobalamin preparations in reducing homocysteine levels in hyperhomocysteinemia. However, W. Herrmann *et al.* [8] were unable to demonstrate a reduction in the risk of CVS disease against the background of reduced homocysteine with vitamin supplements. Although the treatment of hyperhomocysteinemia with high doses of B vitamins does not have a positive effect on the secondary prevention of CVS diseases, the role of homocysteine in the primary prevention of diseases is poorly understood.

Vitamin D receptors have been shown to be found in most human cells and tissues, which indicates numerous non-intestinal effects of the vitamin, and highlights its special role in the functioning of the cardiovascular system. Vitamin D deficiency is associated with various risk factors for cardiovascular disease associated with increased mortality and the incidence of cardiovascular events in adults. Thus, A. Nitsa *et al.* [9] substantiated the role of vitamin D in the regulation of blood pressure by its effect on endothelial and smooth muscle cells of the vascular wall. N. Cosentino *et al.* [10] summarised several mechanisms that link vitamin D deficiency to cardiovascular risk factors, namely activation of the renin-angiotensin-aldosterone system, abnormal nitric oxide regulation, oxidative stress, and altered inflammatory pathways. However, the results of recent randomised controlled trials of J.E. Manson *et al.* [11, 12] do not confirm any benefit of vitamin D supplementation in the treatment of CVS diseases.

Considering all the above facts, the purpose of the study was to analyse current scientific achievements regarding the possible role of homocysteine and vitamins B6, B9, B12, and D in the pathogenetic mechanisms of syncopal states in children.

THE PROCESS OF SELECTING SCIENTIFIC PAPERS BY RESEARCH SUBJECT

The following search terms were used for the review: "syncope" and "vitamin B"; "syncope" and "homocysteine"; "syncope" and "vitamin D" in the PubMed Medline and Scopus databases. The full texts of papers in English published over the past 10 years were included in the study (January 2013 – December 2022). The results of the literature search are presented in Figure 1 as a flowchart Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [13].

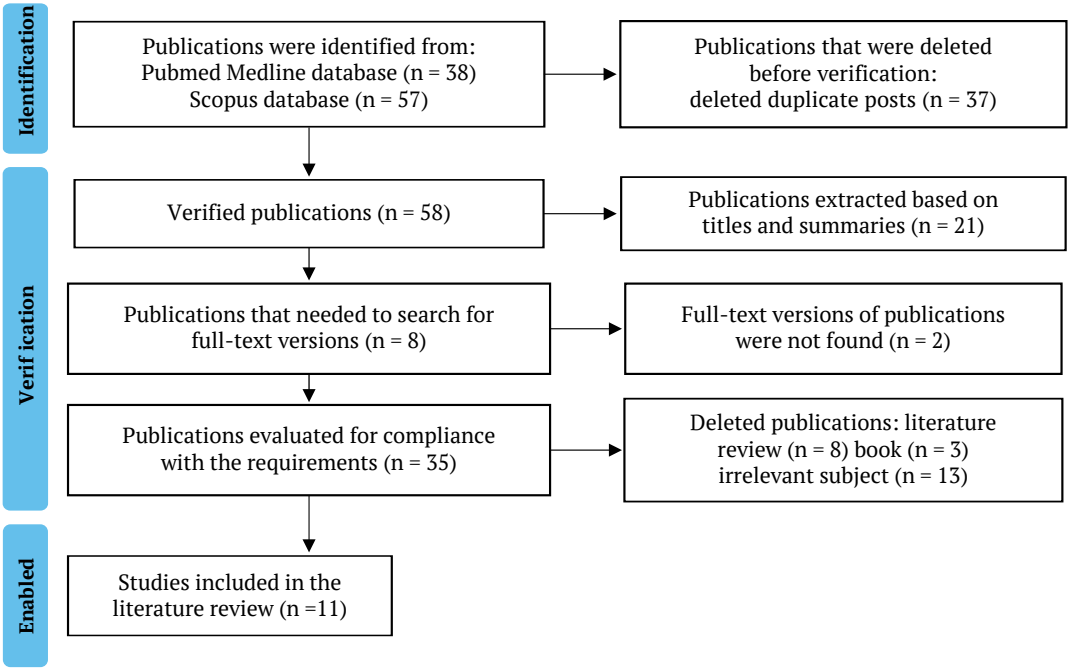

Figure 1. Prisma flowchart of the selection process for scientific publications on the research subject
Source: [13]

A total of 95 papers were identified – 38 in PubMed Medline and 57 in Scopus. Due to duplicates, 37 sources were excluded from the study. After the verification procedure of 58 publications, 47 of them were removed from the search based on irrelevant research subjects, the lack of full-text versions of study's and the results of their own experiments. Only 11 scientific sources were checked for compliance with the search subject and are reflected in Table 1. These publications have become key in the current understanding of the state of the problem of the role of homocysteine and vitamins B6, B9, B12, and D in the pathogenetic mechanisms of syncope formation in children.

Table 1. Studies that examined folate cycle and vitamin D scores in patients with syncope

Study	Country, year	Patient group, n	Diagnosis	The parameter that was defined	Characteristics of the indicator
A. Pektas *et al.* [14]	Turkey, 2018	children, 160	VS	vitamin B12	reduced
T. Öner *et al.* [15]	Turkey,2014	children,125	VS	vitamin B12 vitamin B9	reduced not changed
Y. J. Kong *et al.* [16]	China, 2022	children, 122	VS	vitamin D	deficit
Y. Xiao *et al.* [17]	China, 2022	children,142	VS SPOT OH	vitamin D	reduced
R. Zou *et al.* [18]	China, 2021	children, 130	VS	vitamin D	deficit
Q. Zhang *et al.* [19]	China, 2021	children, 76	VS	vitamin D	reduced
A. Gilani *et al.* [20]	United Kingdom, 2021	adults,7 735	OH	vitamin D	unchanged reduced deficit
S. Usalp *et al.* [21]	Cyprus, 2020	adults,75	VS	vitamin D vitamin B12	reduced reduced
R.R. Hesselbrock *et al.* [22]	USA, 202	adult, 1	syncope	vitamin B12	deficit
H.M. Serin *et al.* [23]	Turkey, 2019	children, 6	syncope	vitamin B12	deficit
Y. Li *et al.* [24]	China, 2018	children, 35	SPOT	homocysteine	increased

Notes: VS – vasovagal syncope, SPOT – Syndrome of Postural Orthostatic Tachycardia, OH – orthostatic hypotension
Source: developed by the authors based on the results of a literary search

INDICATORS OF THE FOLATE CYCLE IN CHILDREN AFTER SYNCOPE

The results of the search suggest that only two studies examined vitamin B12 levels in children with vasovagal syncope (VS). Thus, A. Pektas *et al.* [14] showed that serum vitamin B-12 concentrations in tilt-positive children were substantially lower than in tilt-negative children with VS. Overall, the prevalence of vitamin B12 deficiency was 100.0% in cardioinhibitory, 45.5% in vasodepressive, 91.7% in mixed VS types, and 92.3% in postural orthostatic tachycardia syndrome (SPOT). Furthermore, T. Öner *et al.* [15] examined plasma concentrations of vitamin B12 in patients with VS and healthy children and established the prevalence of their deficiency in 47.2% and 18.2% in the groups, respectively. In patients with SPOT, vitamin B12 deficiency was reported in 62.8%, and in children with syncope and without SPOT – in 47.2 %. As a result, both groups of authors suggested that cyanocobalamin may be involved in the pathogenetic mechanisms of syncope development.

Vitamin B12 has been shown to be a cofactor of at least three enzymes: (1) methylmalonyl-coenzyme A (CoA) mutase, which catalyses the isomerisation of methylmalonyl-CoA to succinyl-CoA during myelin synthesis; (2) phentolamine-N-methyltransferase, which is involved in the conversion of norepinephrine to epinephrine; and (3) catechol-O-methyltransferase, which is necessary for the breakdown of catecholamines [25, 26]. Thus, vitamin B12 deficiency is pathophysiologically characterised by delayed demyelination and nerve conduction, and increased serum norepinephrine levels [27]. As is known, it is the pathological Bezold-Jarisch reflex with a decrease in sympathetic impulses and an increase in vagal effects against the background of inadequate norepinephrine release that is one of the key mechanisms for the development of non-cardiogenic syncope [28, 29]. Therefore, it is logical to assume that vitamin B12 deficiency can affect the autonomic regulation of cardiac activity, just as it occurs with VS [15]. In addition, vitamin B12 deficiency is often diagnosed in patients with impaired autonomic control of CVS activity against the background of diabetes mellitus, atherosclerosis, and multiple sclerosis [30, 31].

Y. Liu *et al.* [32] have shown that both low and high levels of vitamin B12, and low serum folic acid levels, are associated with an increased risk of CVS mortality among people with diabetes mellitus. X. Xu *et al.* [33] established that dietary folic acid deficiency negatively correlates with all-cause mortality, cardiovascular disease mortality, and cancer mortality in men, and all-cause and cardiovascular disease mortality in women, and proved that increased dietary folic acid intake can reduce the risk of mortality in adults with diabetes in the United States. An analysis of 884 randomised controlled intervention studies among 883 627 participants found that folic acid supplementation reduced the risk of stroke [34]. In another UK cohort study involving 115 664 respondents, folic acid intake was found to be associated with a 5% reduction in the risk of cardiovascular events and a 10% reduction in the risk of mortality from cardiovascular diseases [35].

The beneficial effect of folic acid on the human body can be explained by several likely mechanisms. Firstly, folic acid is a cofactor of methionine synthase, which catalyses the conversion of homocysteine, so a decrease in vitamin B9 is always associated with hyperhomocysteinemia. Second, the polymorphism of 5.10-methylenetetrahydrofolate reductase, which is a critical component in folic acid metabolism due to its role in directing folic acid metabolites to the DNA methylation pathway and from the DNA synthesis pathway, can modulate subjects' susceptibility to numerous birth defects, cancer, cardiovascular, and neurological diseases [36, 37].

Studies have also shown that folic acid can prevent the development and eliminate endothelial dysfunction, which is an important risk factor for CVS. Vitamin B9 has the ability to increase the bioavailability of nitric oxide by increasing endothelial no synthase binding and nitric oxide production, and by direct uptake of superoxide radicals. By increasing the bioavailability of nitric oxide, folate improves endothelial function, thereby preventing or stopping the progression of CVS diseases [38-40].

Analysis of literature sources on the study of vitamin B9 in children with syncope identified only one study in this area. Thus, T. Öner *et al.* [15] identified no differences in folate scores in the groups of children with VS and healthy respondents. Given the important role of folate in the functioning of the cardiovascular and nervous systems, the need for further research on vitamin B9 in children with syncope is quite relevant and justified. Pyridoxine is another B vitamin that, as a cofactor of two cystathionine synthase enzymes, plays an important role in homocysteine metabolism, namely the conversion of homocysteine to cystathionine and cystathionase and the synthesis of cysteine from cystathionine. The absence of this cofactor leads to an increase in homocysteine levels [40].

The existence of a direct link between the level of circulating pyridoxal-5-phosphate and the development of cardiovascular diseases has been actively debated in recent decades, but the established evidence is still contradictory. While some researchers suggest that this relationship is direct [41], others claim that it is mediated and implemented through inflammatory mechanisms [42, 43]. One of the proposed pathways is kynurenine, which is involved in tryptophan metabolism. Along this pathway, pyridoxal-5-phosphate acts as a cofactor for enzymes that convert kynurenine into various compounds, including kynurenic acid, anthranilic acid, xanthurenic acid, and 3-hydroxyanthranilic acid with anti-inflammatory effects. Vitamin B6 can also inhibit inflammation through mechanisms involving Nuclear Factor-kappa B (NF-kB) and NOD-like Receptor Family Pyrin Domain Containing 3 (NLRP3) inflammation [44]. Inhibition of oxidative stress is another likely cardioprotective mechanism of vitamin B6 [45].

A prospective cohort study by J. Jeon *et al.* [46] showed that increased dietary intake of vitamin B6 was associated with a reduced risk of heart disease in Korean men, but not in Korean women. In another study involving two prospective cohorts in the Chinese population, high dietary intake of vitamin B6 was inversely proportional to the risk of all-cause mortality, including cardiovascular disease [47]. Another national population-based cohort study found that consuming higher doses of vitamin B6 in combination with folic acid is associated with lower mortality from CVS diseases [48]. On the other hand, a recent meta-analysis of several randomised controlled trials has shown that vitamin supplements, including vitamin B6, are ineffective in preventing cardiovascular diseases and their complications [49, 50].

The results of the conducted literature search give every reason to say that no studies on the study of vitamin B6 levels in children with syncope have yet been conducted. S. Blitshteyn [51] documented vitamin B1 deficiency in 6% of 65 patients with SPOT and described a substantial improvement in symptoms in one in four deficient patients after oral vitamin B1 administration. Thus, a much wider range of B vitamins than previously described may be associated with the development of syncope.

Hyperhomocysteinemia has been shown to be an independent risk factor for many diseases, including neurodegenerative and cardiovascular diseases. Despite a large body of evidence for the involvement of homocysteine in these diseases, pathophysiological mechanisms are still poorly described, complex, and multifactorial. Numerous experimental studies have shown that homocysteine can cause cellular and molecular oxidative stress through the formation of reactive oxygen forms [52, 53]. Disruption of epigenetic mechanisms to control gene expression, such as DNA methylation, histone modification, and non-coding RNA, is another possible mechanism for homocysteine toxicity. Homocysteine can alter the structure and function of proteins by binding to their lysine or cysteine residues. These mechanisms of homocysteine-mediated damage are not mutually exclusive since altered expression and post-translational modification of proteins involved in pro-oxidant/antioxidant pathways can lead to increased cellular oxidative stress, and conversely, free radicals can cause changes in gene expression and oxidative post-translational modifications of proteins [54, 55]. There is more than enough evidence that homocysteine affects mitochondrial homeostasis, including energy metabolism, mitochondrial apoptotic pathway, and mitochondrial dynamics [56].

Y. Li *et al.* [24] established that elevated plasma homocysteine concentrations in children with SPOT, which correlated with the severity of symptoms and indicated that homocysteine may be involved in the pathogenesis of SPOT. No other studies to confirm or refute the likely role of homocysteine in the development of syncope in children have yet been conducted.

THE ROLE OF VITAMIN D DEFICIENCY IN THE DEVELOPMENT OF SYNCOPE

Unlike the folate cycle, the problem of vitamin D deficiency in children with syncope is more well understood. Q. Zhang *et al.* [19] found low vitamin D levels in 60% of children with VS, which correlated with root mean square of successive differences (rMSSD) heart rate variability. The authors suggested that children with VS may experience autonomic CVS dysfunction and a decrease in vagal tone against the background of a drop in vitamin D levels in the blood. R. Zou *et al.* [18] described a high incidence of insufficient nocturnal blood pressure reduction (non-dipper) in children with VS on the background of vitamin D deficiency, which indicates the role of the latter in the violation of the circadian rhythm of blood pressure.

In another study, the prevalence of vitamin D deficiency in the group of children with VS was 73%, and substantial differences in syncope symptoms were found depending on serum levels of 25-hydroxyvitamin D (25(OH)D). The authors showed that syncope, nausea, and heavy sweating are more common in deficient children, while dizziness and darkening of the eyes are more common in children with vitamin D deficiency [16]. Y. Xiao *et al.* [17] showed that compared to healthy children, 25(OH)D levels were reduced in patients with orthostatic insufficiency, while parathyroid hormone levels did not differ. In addition, vitamin D was the only factor associated with orthostatic insufficiency – with an increase of 25(OH)D per unit, the probability of orthostatic insufficiency decreased by 77.7%.

The role of serum 25(OH)D deficiency in the development of syncope may be due to several likely mechanisms. The active form of vitamin D is thought to be one of the key factors in the proliferation and development

of vascular wall smooth muscle cells, endothelial cells, and immune system cells. The vitamin D receptor present in these cells regulates the relaxation and contraction of vascular wall smooth muscle cells through the synthesis of nitric oxide and calcium-mediated pathways [57, 58]. Thus, 25(OH)D deficiency in the blood can contribute to the development of syncope due to a decrease in peripheral vascular resistance during the pathological Bezold-Jarisch reflex.

One of the causes of syncope is a violation of the function of the heart muscle. It has been proven that strong contractions of the empty ventricle of the heart cause activation of cardiac C-fibres during the Bezold-Jarisch reflex, while vitamin deficiency is accompanied by a violation of the autonomic activity of the heart due to inhibition of vagus nerve tone [21]. In addition, vitamin D deficiency increases the risk of cardiovascular disease, heart failure, and sudden cardiac death [59, 60]. As a result, a lack of this vitamin can be considered an independent risk factor for developing heart muscle dysfunction.

Another important pathophysiological link of syncope is a violation of the neuronal conduction of the baroreflector mechanism. Vitamin D, which is also present in the central and peripheral nervous system, plays an important role in maintaining the neurotrophic and neuroprotective effects of growth factors involved in neurotransmitter conduction and nerve cell growth [61, 62]. Therefore, the risk of syncope may increase with hypovitaminosis D due to the indirect effect of 25(OH)D on the central nervous system, smooth muscle cells, and baroreceptor zones.

Therefore, all the above facts indicate the involvement of vitamin B6, B9, B12 deficiency, hyperhomocysteinemia, and 25-OH-D in the pathogenetic mechanisms of transient loss of consciousness of syncopal origin. Determination of the levels of vitamins B6, B9, B12, 25-OH-D and homocysteine in children with syncope will allow not only a better understanding of the nature of syncope but also conducting timely medical correction of detected disorders.

CONCLUSIONS

Thus, the results of recent studies suggest that homocysteine, vitamins B6, B9, B12, and D may play an indirect and in some cases direct effect in the pathogenesis of syncope. Vitamin B12 deficiency appears to play a role in the development of syncope due to the effects of delayed myelination and nerve conduction, and increased serum norepinephrine levels. It is the pathological Bezold-Jarisch reflex with a decrease in sympathetic impulses and increased vagal effects against the background of inadequate release of norepinephrine into the blood serum that is one of the key mechanisms for the development of non-cardiogenic syncope. Therefore, it is logical to assume that vitamin B12 deficiency can affect the autonomic regulation of heart activity in the same way as it occurs in VS.

Pathophysiological features of the effect of vitamin B6 and B9 deficiency on the development of syncope in children are still not examined. Probable pathogenetic mechanisms of vitamin D deficiency in the genesis of syncope are a decrease in peripheral vascular resistance, a violation of the neuronal conduction of the baroreflective mechanism, and dysfunction of the heart muscle. Scientific reports on the effects of vitamins B1, B6, and B9 on the functioning of the cardiovascular and nervous systems in children indicate that other vitamins may also be involved in the pathogenetic mechanisms of syncope development.

Despite this, data on the causal relationships between folate cycle and vitamin D indicators and syncope are mixed, contradictory, and ambiguous, are observational in nature, relate mainly to the adult population and require further research to find new pathogenetically sound methods of treatment and prevention.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Патофізіологічні основи порушень фолатного циклу та дефіциту вітаміну D у розвитку синкопе в дитячому віці

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Анотація. Існує чимало повідомлень щодо ролі вітамінів B6, B9, B12 та D у розвитку серцево-судинних захворювань. Однак, більшість із них стосуються головним чином дорослого населення і є досить лімітованими щодо оцінок у дітей із синкопе. Розуміння ролі цих вітамінів в патогенезі непритомності допоможе розширити комплекс лікувально-профілактичної допомоги дітям. Метою дослідження був аналіз сучасних наукових досягнень щодо ролі фолатного циклу та вітаміну D у генезі синкопе в дятячому віці. Були використані бази даних PubMed Medline і Scopus та застосовані наступні пошукові терміни: «синкопе» та «вітамін B»; «синкопе» та «гомоцистеїн»; «синкопе» та «вітамін D». В роботі узагальнено роль дефіциту вітаміна B12 у сповільненій мієлінізації та нервовій провідності, підвищенні рівня норадреналіну в сироватці крові, як імовірних патогенетичних механізмів розвитку некардіогенних синкопе. Описані наукові факти впливу вітамінів B1, B6, B9 на функціонування серцево-судинної та нервової систем у дітей. Було встановлено поширеність дефіциту вітаміна D у 60-73% дітей із вазовагальними синкопе та його взаємозв'язки з симптомами хвороби. Проаналізовані ймовірні патогенетичні механізми нестачі вітаміна D у розвитку синкопе, а саме зниження периферичного опору судин, порушення нейрональної провідності барорефлекторного механізму, дисфункція серцевого м'яза. Отримані результати дозволять лікарям та науковцям більш ефективно підходити до діагностики, профілактики та лікування синкопе у дитячому віці та можуть служити основою для розробки нових стратегій управління цим станом та вдосконалення медичної практики

Ключові слова: вітаміни; гомоцистеїн; вазовагальні синкопе; синкопе внаслідок ортостатичної гіпотензії; кардіогенні синкопе; діти

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Biological therapy of severe bronchial asthma

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Abstract. Bronchial asthma is a major public health problem in the world. A considerable proportion of patients suffer from severe asthma, which is manifested by a decrease in the quality of life, an increase in the frequency of exacerbations, hospitalisations, and mortality. The ineffectiveness of conventional therapy in such patients contributes to the development of biological treatment methods with higher specificity, aimed at the pathogenetic links of the disease. The purpose of the study was to analyse the effectiveness of the treatment of severe bronchial asthma with monoclonal antibodies based on literature data. The study examines publications over the past 5 years that are available on the Internet. The following terms were used for the search: monoclonal antibodies, endotype, phenotype. Five monoclonal antibody biological agents targeting IgE, IL-5, IL-4, and IL-13, which are approved for use in patients with severe asthma, were analysed: omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab. The use of these medications has led to progress in the treatment of bronchial asthma. It was found that determining disease endotypes based on the assessment of biomarkers such as eosinophil count in blood and sputum, fractional exhaled nitric oxide, and serum periostin contributes to the greater effectiveness of biological therapy. It was investigated that monoclonal antibody treatment improves lung function, reduces exacerbation frequency, and decreases the need for additional medications. Many other biological agents, particularly those targeting key cytokines, are in the clinical development stage. Approved monoclonal antibodies targeting IgE, IL-5, and IL-4/IL-13 demonstrate high efficacy in the treatment of severe bronchial asthma. The use of these agents in patients with severe asthma and high Th2 levels considerably improves lung function, symptom control, and reduces the frequency of disease exacerbations

Keywords: biological agents; monoclonal antibodies; endotype; phenotype; biomarkers

INTRODUCTION

Bronchial asthma (BA) is one of the current healthcare challenges worldwide due to the prevalence of this disease and its negative impact on the quality of life of people of all ages in all parts of the world. BA is a frequent cause of temporary work disability and impairment at any age. Despite the available treatment methods, severe bronchial asthma remains a problem for many patients who do not experience sufficient relief from symptoms. Biological therapy, which utilises specific agents to modulate the immune response, shows potential for improving disease control and enhancing the quality of life for patients.

According to the Global Initiative for Asthma (GINA) in 2022, it affected 262 million people. The main goal of modern treatment of asthma patients is to achieve and maintain complete control over the symptoms for a long time, minimising the risks of future exacerbations, fixed bronchial obstruction, and undesirable side effects [1, 2]. In the majority of patients, high control of the disease can

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be achieved using standard inhalation treatment methods. However, up to 10% of patients suffer from severe asthma, which is characterised by a decrease in quality of life, increased frequency of exacerbations, hospitalisations, and mortality [3].

I. Sulaiman *et al.* [4] note that unsatisfactory control of the disease is due to non-compliance with the prescribed treatment and incorrect technique of using a pocket inhaler. However, other studies indicate that many patients have daily symptoms and frequent exacerbations, despite maximum inhaled glucocorticosteroid (IGCS) therapy and high adherence to treatment. They often require maintenance therapy with systemic glucocorticosteroids (SGS) to avoid life-threatening conditions [5]. Although SGS remain important for exacerbation treatment, several studies highlight that their repeated or continuous use is associated with an increased risk of osteoporosis, infections, and type 2 diabetes. The use of SGS in patients with BA is more frequently linked to kidney function impairment, sleep apnea, and weight gain. It has also been established that the use of these drugs in the treatment of BA leads to an increase in the use of health system resources in the future. For these reasons, the priority is to reduce the use of SGS in such patients [6-8].

A. Rask-Andersen *et al.* [9] investigated the negative effects of BA on emotional and mental health. Insomnia symptoms in asthma have been found to be associated with poor disease control and exposure to characteristic comorbidities such as chronic rhinosinusitis, gastroesophageal reflux, obesity, anxiety, and depression. Other studies show that the disease complicates the family and work life of patients. BA symptoms have been shown to negatively affect daily physical activity and limit social opportunities [10-12]. The ineffectiveness of step-by-step treatment approaches in such patients indicates the heterogeneity of severe asthma and requires alternative methods of therapy with higher specificity aimed at the pathogenetic links of the disease. W.W. Busse [13] notes that advances in understanding the etiopathological mechanisms of various phenotypes and endotypes of severe asthma have contributed to the development of new biological treatments and personalised therapies for this group of patients.

The purpose of the study was to analyse and compare the effectiveness of the treatment of severe BA with monoclonal antibody preparations based on literature data.

PHENOTYPING AND DETERMINATION OF ENDOTYPES IN THE TREATMENT OF SEVERE BA

Recently, the use of biological therapy has led to progress in the treatment of BA. The advantage of this method is its selective effect on the immune system without considerable systemic effects on the body [13]. The use of biological methods in the treatment of severe BA that is resistant to standard therapy requires the use of phenotyping and determination of disease endotypes [14, 15]. The concept of "phenotype" characterises the clinical manifestations of the disease without considering the pathophysiological mechanisms. Severe asthma includes several clinical phenotypes that differ in the age of onset, presence or absence of other allergic conditions, degree of airflow limitation, frequency of exacerbations, and response to treatment [16].

The establishment of the BA endotype is based on the cellular and molecular mechanisms of airway inflammation, considering biomarkers. Their determination and dynamic assessment contribute to a better understanding of the pathological process, allow for individualised patient treatment, and help predict the course of the disease and response to therapy [17]. There are two main endotypes of BA: with high and low levels of T helper 2 (Th2) [18].

Asthma with a high Th2 level is characterised by eosinophilic airway inflammation with the secretion of interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) and is determined using the following biomarkers: eosinophil count in blood and sputum, fractional exhaled nitric oxide (FeNO), and serum periostin [17]. M.R. Edwards *et al*. [18] found that patients with this endotype are more likely to develop virus-induced exacerbations of BA. Recent data indicate that this susceptibility to viruses may be secondary to insufficient interferon production. Approximately 50% of mild to moderate asthma and a large proportion of severe asthma cases have been shown to be characterised by inflammation with high Th2 levels [19]. Depending on the age of onset of the disease, the presence or absence of other allergic conditions, and additional clinical characteristics, the following asthma phenotypes belonging to this endotype are distinguished: allergic asthma, late-onset asthma, and aspirin-induced respiratory disease [20].

Allergic BA is characterised by early onset, positive skin allergy tests, and elevated IgE levels in the blood. It is important to note that only the presence of elevated total or specific IgE is a biomarker for this asthma phenotype, as allergy skin tests can be positive in 50% of the general population [21].

The main characteristics of late-onset asthma include significant blood and sputum eosinophilia, resistance to treatment with inhaled and systemic corticosteroids, frequent exacerbations, and a severe clinical course with fixed airflow obstruction. The vast majority of these patients have comorbid chronic rhinosinusitis, which usually precedes the development of BA. High FeNO levels and normal or elevated serum total IgE levels are also detected in these individuals. Determination of this phenotype may be an indication of an earlier escalation of therapy [22].

Aspirin-exacerbated respiratory disease (AERD) is caused by non-allergic hypersensitivity to nonsteroidal anti-inflammatory drugs such as aspirin, which inhibit cyclooxygenase, a synthetic enzyme of prostaglandins. The most important clinical characteristic of AERD is eosinophilic rhinosinusitis with nasal polyps, which often leads to hyposmia. The majority of patients with this phenotype suffer from a severe disease course, and they typically develop persistent airflow obstruction that minimally improves after inhalation of β2-agonists. Only 10% of people with aspirin-induced asthma have mild symptoms [23].

The low Th2 asthma endotype is characterised by neutrophilic and paucigranulocytic inflammation. Unlike eosinophilic asthma, the specific biomarkers for neutrophil asthma that would help determine it have not yet been clearly defined. As noted by A. Matucci *et al.* [24], sputum neutrophils can serve as the only biomarker of this endotype. The mechanisms underlying neutrophilic airway inflammation are still understudied. Severe neutrophilic asthma has been linked to chronic infection caused by

atypical bacteria, obesity, smoking, and smooth muscle abnormalities. BA with low Th2 levels includes the following phenotypes: non-allergic asthma and asthma associated with obesity [25].

The main signs of non-allergic asthma are the absence of allergic sensitisation, detection of neutrophils in sputum, and onset in adulthood. This phenotype is found in 10-33% of patients with BA, is more common in women, and has a later onset than allergic BA. In many cases, non-allergic asthma is more severe than allergic asthma and may be less susceptible to standard therapy [26].

The pathophysiological mechanisms of BA associated with obesity are complex and multifaceted, but most studies suggest non-eosinophilic inflammatory changes at the molecular level. This phenotype is characterised by both early and late onset and severity of clinical symptoms with moderately preserved lung function [27]. These patients have worse asthma control, lower quality of life, and resistance to IGCS therapy. The mechanisms of the inadequate response to standard therapy are associated with increased production of inflammatory cytokines in obesity [28].

Assessing clinical symptoms, disease severity, determining biomarkers, and establishing phenotypes help select the most appropriate biologic agent for individualised treatment in each patient.

BIOLOGICAL PREPARATIONS AND THEIR MAIN CHARAC-TERISTICS

Five biologic therapy drugs belonging to different monoclonal antibody groups are officially approved for use in patients with severe asthma: omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab [29].

Omalizumab was the first biologic therapy drug approved for asthma treatment, receiving approval for use in the United States in 2003 and in European countries in 2005. It is a representative of humanised anti-IgE monoclonal antibodies. Its action aims to block and neutralise IgE in the blood, thus preventing the activation of mast cells, the release of pro-inflammatory cytokines and leukotrienes, and the development of eosinophilic inflammation [30]. Omalizumab is approved for subcutaneous administration to individuals over 6 years of age who have been diagnosed with severe allergic asthma, the symptoms of which are not controlled by IGCS. S. Rojo-Tolosa *et al*. note that a pronounced clinical effect of anti-IgE monoclonal antibodies is observed in patients with high levels of FeNO, peripheral blood eosinophils, and periostin [31]. Clinical studies have shown that the use of omalizumab improves lung function and reduces the need for additional medications. Moreover, when combined with inhaled corticosteroid and long-acting beta-agonist therapy, omalizumab reduces the frequency of exacerbations by 25% [32]. A revolution in the treatment of severe eosinophilic BA was caused by the use of monoclonal antibodies to block IL-5, which is responsible for cell differentiation, maturation, and activation of eosinophils [33].

Mepolizumab is a humanised anti-IL-5 monoclonal antibody of the IgG1/κ isotype. Studies have shown that its use in BA patients with eosinophilia notably reduces the eosinophil count in the blood, bronchoalveolar lavage fluid, and bone marrow, and reduces the frequency of exacerbations and the use of SGS in these individuals by approximately 50%. Therapy with this drug has been shown to improve patients' quality of life and control of disease symptoms [34].

Another biological agent targeting IL-5 is reslizumab, a humanised monoclonal antibody of murine origin. Its clinical effects have been demonstrated in several randomised trials. The impact of this medication on reducing eosinophil counts in sputum, improving lung function, and increasing forced expiratory volume in 1 second (FEV1) has been examined in patients with severe refractory eosinophilic asthma, particularly with late-onset disease [35].

Benralizumab – another anti-IL-5 monoclonal antibody that induces eosinophil apoptosis through antibody-dependent cellular cytotoxicity, leading to deeper and faster eosinophil depletion. This agent is approved as an add-on therapy for inadequately controlled severe asthma with eosinophilia in patients aged 12 and older [36]. Overall, studies on IL-5-targeted monoclonal antibodies demonstrate clinical improvement in over half of patients with refractory asthma and eosinophilia. Recent data have shown better efficacy of benralizumab in patients previously treated with omalizumab and mepolizumab, attributed to its unique mechanism of action compared to other anti-IL-5 agents [37, 38]. Key components of BA pathogenesis are also IL-4 and IL-13, which regulate cell proliferation, apoptosis, and expression of lymphocytes, macrophages, fibroblasts, epithelial and endothelial cells, and are involved in the regulation of Th2 functions and the synthesis of IgE with B lymphocytes [39].

Dupilumab, a human monoclonal antibody, specifically recognises and blocks the α-subunit of the IL-4 receptor, thus suppressing the biological activity of both IL-4 and IL-13. Treatment with dupilumab without maintenance therapy provides long-term symptom control, significant improvement in lung function, and reduction in Th2-related biomarkers [40]. Significant reduction in sinusitis symptoms and improvement in olfaction have been observed with the use of this medication in patients with aspirin-exacerbated asthma [41].

In addition to these agents already available in clinical practice, many other biological therapies are in various stages of clinical development. One area of the studies focuses on alarmins, key cytokines involved in the mechanisms of airway inflammation in asthma, such as thymic stromal lymphopoietin (TSLP), IL-33, and IL-25. These molecules are released by the respiratory tract epithelium against the harmful effects of microbes, pollutants, allergens, and cigarette smoke. Studies are being conducted that evaluate various drugs targeting these cytokines [42].

Tezepelumab is a monoclonal antibody that targets TSLP, an epithelial alarmin that plays a significant role in asthma pathogenesis. In the presence of tezepelumab, TSLP is unable to bind to its receptor. A number of studies have clearly shown that patients with severe uncontrolled BA treated with tezepelumab experienced a reduction in the frequency of exacerbations, increased asthma control, improved lung function, and health-related quality of life. Regarding the safety profile of this medication, no anaphylactic reactions associated with tezepelumab or the development of neutralising antibodies have been reported [43, 44].

Ipetekimab is a monoclonal antibody targeting IL-33, which leads to the activation of the high Th2 inflammatory

pathway in asthma. Phase 2 trials of this biological agent are ongoing, but preliminary results have shown a reduction in blood eosinophils in patients with severe asthma [45].

As for potential molecular targets in the biological treatment of low Th2 asthma, current research focuses on the pathogenic link connecting IL-1β, IL-23, and IL-17. Medications such as canakinumab, secukinumab, and brodalumab are under investigation in clinical trials [46].

Canakinumab is a humanised monoclonal antibody that can induce prolonged and selective blockade of IL-1β, thereby interrupting the inflammatory cascade in certain autoimmune diseases. A randomised double-blind clinical study evaluating the safety and tolerability of canakinumab in patients with mild allergic asthma, assessing its anti-inflammatory action on the late asthmatic response after allergen inhalation, showed symptom improvement compared to the pre-treatment state [47]. Despite these encouraging findings, there are no further studies of this asthma medication. Secukinumab is a monoclonal antibody targeting IL-17A, which has demonstrated symptom reduction in other diseases such as psoriasis and rheumatoid arthritis. Phase II clinical trials involving patients with uncontrolled asthma have been completed, but results are not yet available. Brodalumab, a monoclonal antibody drug directed against IL-17RA, has also recently been tested in Phase II clinical trials for patients with moderate to severe asthma. The results showed no differences in the dynamics of asthma control between those who received brodalumab and those who received placebo, but there was a clinically significant improvement in lung function [48].

These data indicate that advances in understanding the pathophysiological mechanisms underlying different asthma phenotypes and endotypes have contributed to the development of effective monoclonal antibody therapies. The practical application of phenotyping and biomarker identification allows for individualised treatment of patients and improves therapy response.

CONCLUSIONS

In most patients, asthma control can be achieved with standard therapy. However, some individuals experience severe and persistent symptoms despite appropriate treatment. Recent clinical discoveries in the pathogenesis of asthma and the application of phenotyping and endotyping allow for personalised therapy in individuals with severe asthma, selecting the most suitable biological agent for treatment. Phenotyping based on disease severity and other clinical characteristics, along with the assessment of biomarkers such as blood and sputum eosinophil counts, fractional exhaled nitric oxide (FeNO) levels, and serum periostin, contribute to the improved effectiveness of biological therapy and help choose the appropriate medication for each patient.

Approved biological therapies such as omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab demonstrate high efficacy in the treatment of severe asthma. Overall, studies on anti-IgE, anti-IL-5, and anti-IL-4/IL-13 monoclonal antibodies show clinical improvement in the majority of patients, reducing symptoms and positively impacting functional indicators. The use of anti-IgE monoclonal antibodies in individuals with high levels of FeNO and peripheral blood eosinophils improves lung function and reduces the need for additional medications. The application of anti-IL-5 monoclonal antibodies in patients with severe asthma and high Th2 levels significantly enhances symptom control, reducing the frequency and severity of exacerbations. The use of anti-IL-4/IL-13 monoclonal antibodies without maintenance therapy provides long-term symptom control and decreases biomarker levels.

In addition to the medications approved for use in clinical practice, many other biological agents targeting cytokines such as IL-1β, IL-23, and IL-17 are under investigation and demonstrate symptom reduction and improved asthma control. Patients with severe asthma and low Th2 levels lack the advantage of biological therapy due to the absence of approved agents for such individuals. Therefore, there remains a need for further research to develop new biological treatment methods to enhance therapy for all types of severe asthma.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Біологічна терапія важкої бронхіальної астми

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Анотація. Бронхіальна астма є важливою проблемою охорони здоров'я в світі. Значна частка хворих страждають від важкої астми, яка проявляється значним зниженням якості життя, збільшенням частоти загострень, госпіталізацій та смертності. Неефективність стандартної терапії у таких хворих сприяє розробці біологічних методів лікування з більш високою специфічністю, спрямованих на патогенетичні ланки захворювання. Метою дослідження було проаналізувати ефективність лікування важкої бронхіальної астми моноклональними антитілами на основі даних літератури. У дослідженні опрацьовано наукові публікації за останні 5 років, які доступні у мережі «Інтернет». Для пошуку було використано терміни англійською мовою: monoclonal antibodies, endotype, phenotype. Було проаналізовано п'ять біологічних препаратів моноклональних антитіл, спрямованих на IgE, ІЛ-5, ІЛ-4 та ІЛ-13, які дозволені для використання пацієнтам із важкою астмою: омалізумаб, меполізумаб, реслізумаб, енралізумаб та дупілумаб, застосування яких зумовило прогрес у лікуванні бронхіальної астми. Виявлено, що більшій ефективності біологічної терапії сприяє визначення ендотипів захворювання, що базується на оцінці таких біомаркерів, як: кількість еозинофілів в крові та харкотинні, фракція оксиду азоту у видихуваному повітрі та сироватковий періостин. Було досліджено, що лікування моноклональними антитілами покращує функцію легень, знижує частоту загострень та зменшує потребу в додаткових лікарських засобах. Встановлено, що багато інших біологічних препаратів, зокрема спрямованих на ключові цитокіни, знаходяться на стадії клінічної розробки. Схвалені до використання в світі, анти-IgE, анти-ІЛ-5, анти-ІЛ-4/ІЛ-13 моноклональні антитіла показують високу ефективність у лікуванні важкої бронхіальної астми. Застосування цих препаратів у пацієнтів з важкою астмою та високим рівнем Th2 значно покращує функцію легень, контроль над симптомами та знижує частоту загострень захворювання

Ключові слова: біологічні препарати; моноклональні антитіла; ендотип; фенотип; біомаркери

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