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Telerehabilitation model in operated individuals with upper limb and chest trauma due to road traffic accidents

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Abstract. Implementing telerehabilitation methods for individuals injured in road traffic accidents with upper limb and chest trauma is critically essential for improving patients' functional recovery, ensuring effective interaction between the patient and the rehabilitation specialist, and reducing overall time and costs associated with rehabilitation. The purpose of the study is to assess the effectiveness of applying telemedicine monitoring tools and the developed rehabilitation model in the functional recovery of operated patients with upper limb and chest trauma. The study included patients who underwent surgery due to upper limb and chest trauma resulting from road traffic accidents. Patients were divided into two groups: retrospective (186 patients) underwent standard rehabilitation, while the main group (62 patients) was involved in the developed telerehabilitation model programme. Functional outcomes were analysed using the qDASH scale, including the average time spent by patients on rehabilitation per day, the number of patient visits to the medical facility within 3 weeks, and the total time spent by the rehabilitation specialist per patient over 3 weeks. After rehabilitation, over 80% of patients in both groups demonstrated positive and satisfactory results, with a slight advantage of excellent results in the main group (12.90% versus 9.14%). Patients in the main group spent more time on their rehabilitation (41+3 minutes per day) and had access to progress monitoring functions and communication with the doctor, which increased their motivation and involvement. Telerehabilitation remarkably reduced the number of required doctor visits (5±2 versus 11±3 visits) and the time spent by the rehabilitation specialist on each patient over 3 weeks (132±12 minutes versus 243±17 minutes). The average time per day spent by the injured individuals on rehabilitation exercises in the main group was 41±3 minutes, while in the retrospective group, it was 31±7 minutes. The telerehabilitation model is an equivalent alternative and complement to standard rehabilitation methods. Its advantages include increased motivation for performing rehabilitation exercises and the ability to conduct training in a distance-controlled environment

Keywords: polytrauma surgery; telemedicine; rank analysis; retrospective study

INTRODUCTION

According to recent studies, road traffic accidents are a key cause of mortality from trauma [1, 2]. In Ukraine, the percentage of fatalities among all injured in road traffic accidents (RTAs) varies between 15-17%, while in European countries, this figure is 3-4%, and in the United States, it is 2-3% [3].

The majority of research on polytrauma resulting from road traffic accidents focuses on the principles of emergency care for the injured to reduce the mortality rate. However, a retrospective analysis of 9012 RTA victims by E. Berkeveld *et al.* [4] concluded that achieving optimal outcomes for this group of patients does not depend on the time of prehospital transport but requires timely implementation of individualised treatment, adherence to clinical protocols, careful dynamic observation, and professional rehabilitation after discharge. A study conducted in Croatia by J. Kovačević *et al.* [5] observed a decrease in self-care and employment opportunities among 200 out of 640 RTA victims, leading to a lower quality of life. It also caused a significant economic burden for their families.

With the development of information technologies, telemedicine can address these issues by improving access to healthcare professionals and communication in the medical field and reducing logistical costs. A study by

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K. Moulaei *et al.* [6] demonstrated that using a portable device can provide continuous monitoring of patient's physiological parameters and detect real-time changes that can be automated and less noticeable to medical personnel.

In the clinical study "MERLIN" by S.G. Rozevink *et al.* [7], the effectiveness of using portable devices for home-based rehabilitation to address economic issues, particularly by reducing costs and travel time for patients, was analysed. It also allowed rehabilitation specialists to conduct classes more effectively simultaneously with several patients since each could interact with their own device and be in a convenient place. It was noted that the effectiveness of remote rehabilitation was not inferior to conventional methods. However, such scientific observations mainly relate to the telerehabilitation of patients after strokes and do not fully reveal the importance of using portable devices for the remote rehabilitation of RTA victims [8, 9].

According to A.I. Tsvyakh *et al.* [10], one of the main advantages of using portable telerehabilitation systems is the improvement of musculoskeletal functions. In addition, it increases patients' interest and motivation for performing rehabilitation exercises. It also leads to greater commitment to rehabilitation procedures and more active participation in treatment procedures. This is particularly relevant for caring for patients with polytrauma associated with upper limb and chest injuries [11].

The main focus of most studies on RTA victims is on immediate medical care, while long-term rehabilitation remains less explored. It is important to pay attention to the quality of life issues faced by surviving victims after RTAs and their families, especially in the context of inadequate rehabilitation. In this case, telemedicine can play a key role in improving care for this category of patients. Modern research highlights the substantial potential of telerehabilitation in the treatment and recovery of patients after various types of injuries. Numerous publications demonstrate that these approaches not only improve the functional status of survivors but also provide greater accessibility and effectiveness of rehabilitation services, patient engagement in the recovery process, while reducing economic and time costs. However, the effectiveness of telerehabilitation in RTA survivors with upper limb and chest injuries is not sufficiently highlighted. The purpose of this study is to evaluate the effectiveness of telemedicine monitoring tools and the developed rehabilitation model in the functional recovery of patients with upper limb and chest injuries.

MATERIALS AND METHODS

The medical records of hospitalised patients who underwent treatment at the municipal non-profit enterprise "Ternopil City Emergency Hospital" from 2015 to 2020 were analysed, following a protocol approved by the hospital's ethics committee. The study materials included 186 medical records of patients with upper limb and chest injuries, forming the retrospective group. In the main group, 62 patients with upper limb and chest injuries were sequentially enrolled in the study over two years at the same hospital – from September 2020 to November 2022. The study included survivors aged 18 and older who were admitted to the emergency department of the hospital after road traffic accidents. Patients with injuries not related to a motor vehicle were excluded. Both groups were compared by age, gender, occupation, nature of injuries, injury severity score, and evaluated the functional status of the limb, the average duration of rehabilitation per day, number of patient visits to the medical facility over 3 weeks, and the total time spent by the rehabilitation specialist on one patient over 3 weeks.

To analyse the effectiveness of rehabilitation in both groups, the QuickDASH questionnaire [12], which is a universal tool for diagnosing functional limitations of the arm, shoulder, and hand, was used. This method includes 11 criteria that allow assessing the physical abilities and symptoms in patients with single or combined upper limb and shoulder girdle injuries. Each question has 5 possible answers, reflecting the degree of difficulty in performing tasks (1 – I can perform the task easily; 2 – I have slight difficulty; 3 – I have moderate difficulty; 4 – I have a lot of difficulty; 5 – I am unable to perform the task). The overall score of the answers is converted into points using the following formula:

$$QuickDASH = 25 * (\frac{(sum\ of\ n\ responses)}{n} - 1), \qquad (1)$$

where n – number of responses. It should be noted that the QuickDASH score cannot be calculated if more than 1 item is missing. Thus, 0 points correspond to full function recovery, while 100 points indicate severe disability.

All 186 patients from the retrospective group underwent conventional rehabilitation procedures for 3 weeks after the trauma. This group received standard methods of rehabilitation therapy. A total of 62 patients from the main group were involved in telerehabilitation for a 3-week period after the trauma and were taught a set of exercises with a developed portable device for home use. In the main group, the QuickDASH score was assessed at discharge from the hospital and after 3 weeks of telerehabilitation. In the retrospective group, the QuickDASH score was also assessed on the day of discharge from the hospital according to records in the patient's medical records. After 3 weeks, this indicator was determined based on patient surveys during visits to the doctor and using a developed Google Forms survey. All participants followed an approved rehabilitation protocol (massage, exercises, therapeutic exercises). The exercise program, which included passive and active movements – flexion, extension, and strengthening of the upper limb and chest muscles, was conducted three times a day for three weeks and aimed to improve flexibility, strength, proprioception, and neuromuscular coordination.

The conventional rehabilitation group performed the exercise program for 15 minutes, three times a day, independently at home, and visited a medical facility as needed. The exercise programme was prescribed by the same physiotherapists as in the main group, and the intensity of the exercises was determined by the physiotherapists based on the patient's symptoms. A prototype for monitoring patients with musculoskeletal disorders, developed and tested during a research project funded by the Ministry of Health of Ukraine (registration number 0119U000608, 2019-2021), was used for the study.

For the main group, a telerehabilitation model was created, which included using this prototype with sensors to track the limb's position in space and sensors to measure the angle of flexion, temperature, volume, and pulse (Fig. 1). During the study, the sensors were integrated into an orthosis fixed on the injured limb of the patients. A specially developed software complex allowed for remote monitoring of biomechanical parameters of movement, local temperature, frequency of active movements, and volume of the injured limb. During the performance of rehabilitation exercises at home, data from the patients' portable sensors were collected and transmitted to a cloud server via mobile internet and then displayed on personal smartphones or doctors' computers in digital and graphical formats. The telerehabilitation protocol included: 1) training on the use of the prototype and attachment of the portable device to the affected limb, as well as the use of special software; 2) performing home exercises, including passive flexion and extension of the upper limb; 3) active flexion and extension of the upper limb. In addition, all study participants underwent training on determining pain intensity using a 10-point visual analogue scale.

Figure 1. Developed prototype with sensors
Source: photographed by the author

In the telerehabilitation group, patients were provided with instructions for using the prototype and performing the exercise program three times a day for 15 minutes under remote monitoring. Qualified physiotherapists provided patients with consultations and recommendations regarding their progress in performing exercises, maintaining an active daily routine, and alleviating symptoms using text messages and phone calls. Rehabilitologists also monitored patients' performance of home exercises and, if necessary, adapted the load programme according to their condition and needs.

The study was conducted in accordance with the principles of good clinical practice and ethical principles as required by the Helsinki Declaration [13]. To maintain confidentiality and protect patients' personal data, information about their identity was excluded from the analysis and stored in a secure electronic format with limited access. For statistical processing and comparison of the study results between groups of patients depending on the type of data and distribution law, Student's t-test, Mann-Whitney U test, and one-way analysis of variance (ANOVA) were used.

RESULTS

In the retrospective group, there were 135 (72.58%) males and 51 (27.42%) females. In the main group, there were 44 (71.97%) males and 18 (29.03%) females. The nature of the injuries was diverse in both sexes but similar in rank in both groups. Thus, among all patients, the majority of injuries were sustained by cyclists and pedestrians, regardless of gender (Table 1).

Table 1. Distribution of victims by the type of injury and gender

No.	Type of the injury	Retrospective group						Main group					
		Men			Women			Men			Women		
		Number	%	Rank	Number	%	Rank	Number	%	Rank	Number	%	Rank
1.	Cyclists	48	35.56	1	7	13.73	3	24	38.71	1	1	1.61	2
2.	Car driver	24	17.78	3	2	3.92	-	5	8.04	3	0	0	-
3.	Motorcycle driver	15	11.11	5	0	0	-	4	6.45	4	0	0	-
4.	Animal-drawn transport	3	2.22	-	0	0	-	4	6.45	4	0	0	-
5.	Passenger of the bus	1	0.74	-	2	3.92	-	0	0	-	1	1.61	4
6.	Passenger of the car	17	12.59	4	11	21.57	2	1	1.61		4	6.45	3
7.	Pedestrian	27	20.00	2	29	56.86	1	6	9.68	2	12	19.35	1
8.	Total	135			51			44			18		

Source: compiled by the author

However, it is necessary to note the pronounced risk of injury among men in both the retrospective and main groups, both as cyclists (first rank) and pedestrians (second rank). Although women in both groups also faced risks of injury as pedestrians and cyclists, their overall frequency is lower compared to all groups where the injured individuals used transportation. Both groups show a similar percentage distribution between men and women: 72.58% in the retrospective group and 70.97% in the main group (Table 2).

Table 2. Characteristics of research groups on differences in the compared indicators

	Retrospective group	Main group	p-value
Men	135 (72.58%)	44 (70.97%)	
Women	51 (27.42%)	18 (29.03%)	

Table 2. Continued

	Retrospective group	Main group	p-value
Age			0.225829
18-24	40 (21.51%)	7 (11.29%)	
25-44	79 (42.47%)	34 (54.84%)	
45-60	38 (20.43%)	13 (20.97%)	
>60	29 (15.59%)	8 (12.90 %)	
Employment			0.957764
student	7 (3.76%)	2 (3.23%)	
works	74 (39.78%)	27 (43.55%)	
does not work	81 (43.55%)	25 (40.32%)	
pensioner	24 (12.90%)	8 (12.90%)	
ISS	21.66	21.12	

Notes: the p-value is obtained using the chi-square test for categorical variables and the analysis of variance (ANOVA test) for continuous variables

Source: compiled by the author

In terms of age distribution, the most common among patients are individuals of working age from 25 to 44 years old, comprising over 40% in both groups. Meanwhile, the smallest group consists of individuals aged 18-24 years in the retrospective group and individuals over 60 years old in the main group. When comparing the distribution by employment categories between the two groups, minor differences can be observed, but the number of injured individuals among those employed and unemployed is approximately the same and approaches 40%. Other categories, such as students and retirees, are represented in smaller numbers, with their percentage not exceeding 13% in any group. Comparing both groups by Injury Severity Score (ISS), they are practically identical. Thus, it can be argued that the retrospective and main groups were identical in such parameters as age, gender, employment, and injury severity index.

In the retrospective group, the rehabilitation programme was based on early implementation of controlled functional mobilisation, overcoming swelling using medication therapy, and prescribing physical therapy at the place of residence for three weeks. In the main group, the standard rehabilitation programme was supplemented by the use of the developed telemedicine model. Due to the remote monitoring capabilities, the physician could control the correctness of each stage of rehabilitation tasks in real time and, considering the functional state of the limb, make adjustments to the loads during exercises. At the beginning of the rehabilitation process for patients with hand injuries, a key step was the implementation of controlled functional mobilisation under telemedicine control. This played a crucial role in the remote control of the range of motion, reflected in the graph, where restrictions in bending angles up to 31 degrees and a gradual increase in local temperature to 26-27 degrees during exercises were visualised. This indicates a tendency to improve local blood circulation and enhance metabolic processes (Fig. 2).

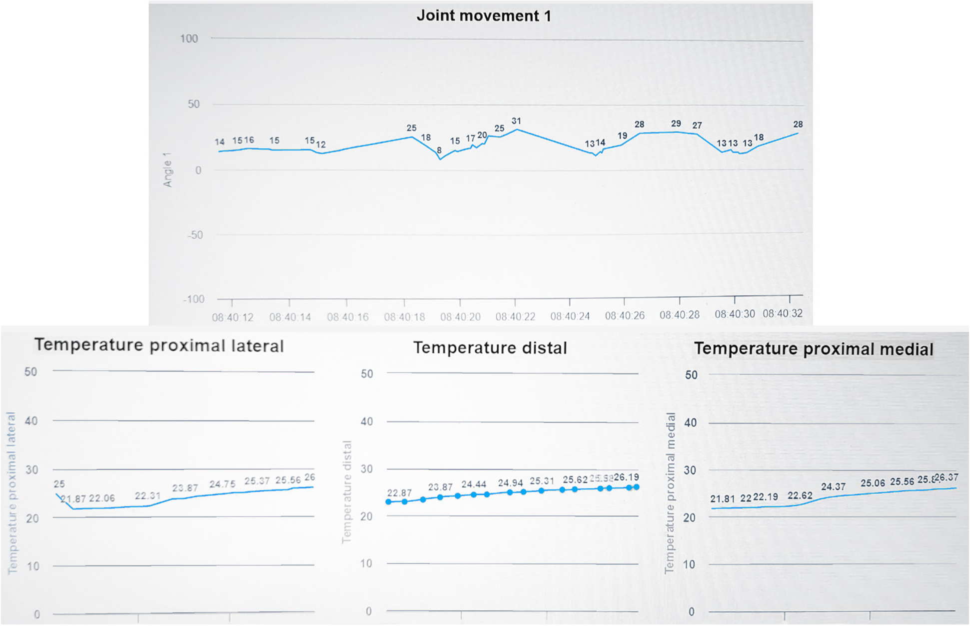

Figure 2. The first day of TV rehabilitation. Visualisation of the rehabilitation method, which makes it possible to evaluate the patient's physiological indicators using graphs

Source: photographed by the author

However, the graph clearly shows that with the increasing load during exercises on the injured arm, the local temperature does not reach the norm, and at this moment, the range of motion stops increasing. If movements are not stopped, an inflammatory process may begin. The peculiarity of the telerehabilitation programme lies in the methods of dealing with such complications, particularly with swelling due to excessive load on the limb.

On the 21st day of the telerehabilitation process, patients in the main group achieved bending angles of up to 90 degrees (Fig. 3). The graph shows the normal range of motion of the arm. It should be noted that a stable local temperature of about 31 degrees Celsius was recorded during intensive muscle work. This indicated the effectiveness of muscle work and an optimal load level, which is critically essential for restoring hand functions. Such visual data helped doctors understand the dynamics of recovery and adapt rehabilitation programmes according to each patient's individual needs. They also became a valuable tool for demonstrating patient's progress, motivating them to further active participation in rehabilitation measures.

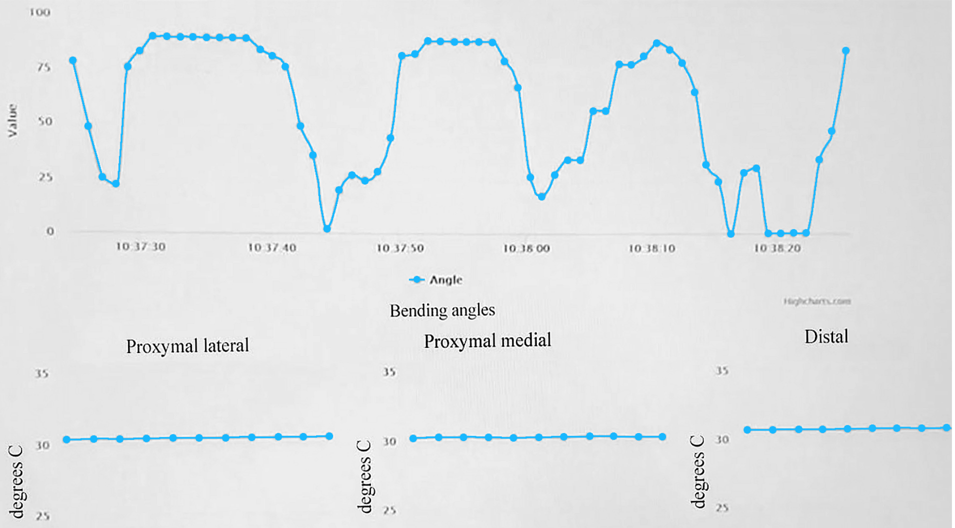

Figure 3. Changes in the range of motion and temperature on the 21st day of telerehabilitation
Source: photographed by the author

Participants in the main group also independently recorded changes in pain levels after performing exercises using a gadget. In case of increasing pain, they indicated its intensity, choosing one of three levels: mild increase in pain (1-4 points), moderate increase (5-7 points), significant increase (8-10 points). The software allowed increasing the daily load in case the pain intensity after exercises did not exceed 7 points, and there was no progressive swelling of the limb. In case of persistent increase in pain and presence of swelling, the doctor made adjustments to the rehabilitation algorithm. In the retrospective group, the patient visited a medical institution for consultation with a rehabilitation specialist in case of severe pain.

Notably, in the main group, the rehabilitation specialist systematically monitored the dynamics of the patient's clinical condition, analysing the range of motion, pain reduction, and improvement of limb function. If necessary, corrections were remotely made to the telerehabilitation protocol for optimal effectiveness. Based on the observation results of the patient's dynamics, the medical specialist could modify the rehabilitation plan, adjusting the intensity and duration of procedures, as well as integrating additional exercises to achieve progress. Functional outcomes in the retrospective and main groups were analysed using the QuickDASH scale. The average time in minutes spent by patients for rehabilitation per day, the number of patient visits to the medical facility within 3 weeks, and the total time spent by the rehabilitation specialist on one patient within 3 weeks were also evaluated (Table 3).

Table 3. Functional results in the retrospective and main groups

	Retrospective group	Main group	p-value
qDISH start of rehabilitation			
Excellent (0-25 points)	-	-	p=0.70
Good (26-50 points)	71 (38.17%)	21 (33.87%)	
Satisfactory (51-84 points)	107 (57.53%)	37 (59.68%)	
Unsatisfactory (85-100 points)	8 (4.30%)	4 (6.45%)	
qDISH 21st day of rehabilitation			
Excellent (0-25 points)	17 (9.14%)	8 (12.90%)	p=0.14
Good (26-50 points)	115 (61.83%)	45 (72.58%)	
Satisfactory (51-84 points)	46 (24.73%)	7 (11.29%)	
Unsatisfactory (85-100 points)	8 (4.30%)	2 (3.23%)	

Table 3. Continued

	Retrospective group	Main group	p-value
Average duration of rehabilitation per day	31 ± 7 min	41 ± 3 min	p < 0.005
Number of patient visits to a medical facility in 3 weeks	11 ± 3	5 ± 2	p < 0.005
The total time spent by the rehabilitator on one patient during 3 weeks	243 ± 17 min	132 ± 12 min	p < 0.005

Notes: the p-value is obtained using the chi-square test for categorical variables and the analysis of variance (ANOVA test) for continuous variables
Source: compiled by the author

The obtained data show that at the end of the rehabilitation course (after 3 weeks), in both study groups, over 80% of the injured individuals noted positive and satisfactory (26-84 points) indicators. It should be noted that about 10% of patients successfully underwent rehabilitation and achieved excellent qDASH scores (<25 points). Although these data do not differ particularly, it is necessary to note a slightly higher percentage of excellent functional outcomes in the main group – 12.90% compared to 9.14% in the retrospective group. This can be explained by the greater motivation of patients in the telerehabilitation group. It is also important to note that about 8 (4.3%) injured individuals with extremely poor functional outcomes (85-100 points) did not improve their condition in the retrospective group. In the main group, there was a slight decrease in the cohort of such injured individuals – from 4 (6.45%) at the beginning of rehabilitation to 2 (3.23%) after three weeks.

The study revealed a statistically significant difference between the two groups in the average time in minutes spent by patients on rehabilitation per day – 31±7 min in the retrospective group and 41±3 min in the main group (p<0.005). Clearly, the developed portable device has functions that allow patients to track their rehabilitation progress, set reminders for exercises, and access a rehabilitation specialist in case of complications or adverse events in real time. The feeling of constant control by the doctor helps better engage patients and motivates them. Substantial differences were also found in the number of patient visits to the medical facility within 3 weeks, which decreased by almost half in the telerehabilitation group (p<0.005). Furthermore, in the main group, there was a threefold reduction in the total time spent by the rehabilitation specialist on one patient within 3 weeks (p<0.005). Therefore, after three weeks of rehabilitation, over 80% of the injured individuals in both groups achieved positive results, with the main group showing a slightly higher percentage of excellent results, which may be due to greater motivation and continuous monitoring of patients using the developed telerehabilitation model.

DISCUSSION

Patients who have experienced road traffic accidents often face a range of health consequences that can manifest both in the short and long term. These consequences can lead to varying degrees of impairment and disability, which in turn can result in substantial economic costs. These costs not only impact the quality of life of survivors but also have substantial implications for their families.

According to a study by F. Cunha-Diniz *et al.* [14], the treatment outcomes of individuals injured in road traffic accidents vary widely and depend on several factors. The nature of the accident plays a crucial role – for example, pedestrians, cyclists, and motorcyclists are often more susceptible to serious injuries. In addition, victim characteristics such as age, gender, and health status also greatly influence outcomes. Among these factors, the type and severity of injuries sustained during the accident are considered the main prognostic factors for injury outcomes and future quality of life.

According to many researchers, after upper limb injuries in road traffic accidents, there is a noticeable decrease in quality of life during the initial acute phase, usually within 1-2 weeks after the injury, with gradual improvement over the first year of recovery [15-17]. Previous studies have highlighted the disabling nature of upper limb and chest injuries. Interestingly, patients with upper limb injuries showed significant improvement within the first three months but did not reach their pre-injury levels of quality of life until 12 months post-injury. In a study by B. Gopinath *et al.* [17] on quality of life after such injuries, it was discovered that most patients who were not hospitalised recovered within two months, except for patients with spinal injuries. These groups showed a recovery pattern similar to hospitalised patients, with progress noted up to five months but at suboptimal levels of quality of life.

R. Rissanen *et al*. [18] observed that non-hospitalised patients with upper limb injuries experienced a significant loss of quality of life at 2.5 months, which improved to general population norms at nine months post-injury. In contrast, hospitalised patients with similar injuries remained significantly below these norms even at 24 months. Their study underscored the overall impact of upper limb injuries on health, particularly noting that proximal injuries, such as shoulder fractures, demonstrate slower recovery compared to distal injuries.

The difference in the results of these studies may be related to differences in the duration of observation, data collection methodologies, diagnostic categories, severity of injuries, and cultural factors. However, they all did not consider the importance of the individual choice of limb rehabilitation method. Despite the complexity and importance of these studies, there is a lack of rehabilitation protocols that use a holistic and individual approach to analysing the severity of injuries in road traffic accidents. Most of these studies typically focus on specific aspects of injury, whereas the development of individual treatment and rehabilitation programmes should include a broader spectrum, covering not only injuries sustained in road traffic accidents but also the economic consequences and individual characteristics of the injured. This comprehensive perspective is crucial for understanding the impact of road traffic accidents on individuals and developing more effective rehabilitation techniques, including the use of modern technologies, exoskeletons, and telemedicine.

Since 2000, many research groups have also been developing complex robotic devices for rehabilitation that can be controlled remotely and worn by the injured [12, 19]. T. Ahmed *et al*. [20] developed a telerehabilitation system based on an exoskeleton device that allows the rehabilitation specialist to remotely control it as a subordinate device through the main apparatus. However, the doctor can only monitor the patient's condition visually or through a webcam. N. Singh *et al*. [21] proposed a telerehabilitation system based on an end-effector device for passive and assistive wrist and finger training. The system includes a data collection and communication module for remote monitoring, visual biological feedback.

The current landscape of rehabilitation robotics is characterised by devices that are primarily autonomous mechanical systems with limited networking capabilities. This poses a substantial problem as it restricts interaction between the rehabilitation specialist and the system, which may be located at a considerable distance. Effective rehabilitation requires the healthcare provider to carefully monitor the patient's progress and appropriately adapt exercises when needed. The advanced innovation in the developed prototype lies in its system for tracking the spatial position of the limb, autonomy, and small size. This system, in conjunction with specialised software, transmits detailed information about the hand's kinematics and performed exercises to the rehabilitation specialist via the internet, allowing for adjustments and changes in the load regime as needed. In addition, the developed prototype includes additional sensors for measuring temperature, pulse, and volume, providing essential data that the doctor can assess dynamically to prevent complications. This allows for a more qualitative assessment of whether the patient has achieved specific rehabilitation goals. From the patient's perspective, such a highly adaptive portable telerehabilitation device that can be comfortably used at home can significantly increase motivation for regular physical exercises. This is a substantial step forward in home rehabilitation, combining advanced technology with user-oriented design to improve treatment outcomes.

The proposed telerehabilitation model also allows patients and doctors to be less bound by strict work schedules. Patients can perform rehabilitation exercises at a time convenient for them, while doctors can provide services from anywhere, both online and by analysing previous records of recovery progress. The applied telemedicine system is equipped with advanced data management tools. They can be integrated with electronic medical records, stored in cloud servers, ensuring that the patient's rehabilitation history is always up-to-date, accessible, and securely protected. These data confirm the high effectiveness of the proposed telerehabilitation model for this category of patients.

CONCLUSIONS

The study highlights the variety of consequences faced by individuals who have experienced road traffic accidents, including short-term and long-term health impacts that can lead to reduced quality of life and disability. In addition to the direct health damage, substantial economic costs for rehabilitation are also important.

The use of advanced technologies, such as telerehabilitation devices that allow remote control and adaptation of the rehabilitation process, plays a key role in improving the accessibility and effectiveness of rehabilitation. These devices provide detailed monitoring of patients' physical condition, enabling doctors to promptly adjust treatment and exercises according to the patient's needs. The created telemedicine model can be used in comprehensive rehabilitation of patients with upper limb injuries, especially those associated with shoulder or chest injuries.

The study results demonstrate the advantage of telerehabilitation over conventional physiotherapy, and the proposed telemedicine model can be a viable alternative or addition to standard rehabilitation methods for individuals with upper limb and shoulder injuries. The main advantages include increased motivation for performing rehabilitation exercises and the ability to train in a remotely controlled environment and at home with greater intensity than conventional rehabilitation.

In particular, the average duration of daily rehabilitation sessions in the main group was 10 minutes longer (41±3 minutes), and the number of visits to medical facilities was almost halved. As a result, in the main group, excellent rehabilitation results (qDASH <25 points) were achieved in 12.90% of patients, compared to 9.14% in the retrospective group, demonstrating the increased effectiveness and convenience of the proposed telerehabilitation model.

In general, this study indicates the need for the development of integrated, individually adapted approaches to rehabilitating individuals injured in road traffic accidents, emphasising innovative technologies and telemedicine, which can remarkably improve the quality and accessibility of rehabilitation services. This will help improve the quality of life of injured patients and particularly reduce the costs of the rehabilitation period. Further research in this area should focus on assessing the long-term effects of telerehabilitation, developing individually adapted protocols, and integrating new technologies to ensure effective and accessible recovery for the injured.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Телереабілітаційна модель в оперованих постраждалих в дорожно-транспортних пригодах з травмою верхньої кінцівки та грудної клітки

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Анотація. Впровадження методів телереабілітації для постраждалих у дорожньо-транспортних пригодах з травмами верхньої кінцівки та грудної клітки є критично важливим для покращення функціонального відновлення пацієнтів, забезпечуючи ефективну взаємодію між пацієнтом та реабілітологом, та сприяє зменшенню загального часу і витрат, пов'язаних з реабілітацією. Мета дослідження полягала в оцінці ефективності застосування засобів телемедичного контролю та розробленої теререабілітаційної моделі при функціональному відновленні прооперованих пацієнтів з травмами верхньої кінцівки та грудної клітки. У дослідженні взяли участь пацієнти, які були прооперовані з приводу травми верхніх кінцівок та грудної клітки в результаті дорожньо-транспортних пригод. Пацієнти були розподілені на дві групи: ретроспективна (186 пацієнтів) проходила стандартну реабілітацію, тоді як основна група (62 пацієнти) була залучена до програми розробленої телереабілітаційної моделі. Функціональні результати були проаналізовані за допомогою шкали qDASH, а також оцінювалися: середній час, затрачений пацієнтами для реабілітації в день, кількість візитів пацієнта до медичного закладу за 3 тижні та загальний час, затрачений реабілітологом на одного пацієнта протягом 3 тижнів. Після реабілітації понад 80 % пацієнтів в обох групах продемонстрували позитивні та задовільні результати, з незначною перевагою відмінних результатів у основній групі (12,90 % проти 9,14 %). Пацієнти основної групи витрачали більше часу на свою реабілітацію (41+3 хвилини щодня) та мали доступ до функцій моніторингу прогресу і зв'язку з лікарем, що підвищувало їх мотивацію та залученість. Телереабілітація значно зменшила кількість необхідних візитів до лікаря (5±2 проти 11±3 візити) та час, витрачений реабілітологом на кожного пацієнта за 3 тижні (132±12 хв проти 243±17 хв). Середній час на день, витрачений постраждалими на реабілітаційні вправи в основній групі був 41±3 хв, у той час як в ретроспективній – 31± 7 хв. Телереабілітаційна модель є рівноцінною альтернативою та доповненням до стандартних методик реабілітації. Її переваги полягають у підвищенні мотивації до виконання реабілітаційних вправ та можливості проведення тренувань у дистанційному контрольованому середовищі

Ключові слова: хірургія політравми; телемедицина; ранговий аналіз; ретроспективне дослідження

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Latissimus dorsi flap in breast reconstruction following radical mastectomy and prophylactic subcutaneous mastectomy in the same patient: Case report

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Abstract. One of the most common surgical treatment options for breast cancer is radical mastectomy, which affects the quality of life of patients in remission and their psychological state. One of the solutions to this problem is the use of skin and muscle flaps from other anatomical areas, in particular the back, for plastic reconstruction of the breast after radical mastectomy. The study aimed to present the practical implementation of reconstruction of both breasts in the remote period of radical mastectomy in a patient in remission of breast cancer using a skin and muscle flap of the broadest back muscle in combination with an implant. The patient, who met the criteria for participation in the study, had previously undergone a radical mastectomy of the right breast for direct indications along with radiotherapy. Delayed breast reconstruction within the study was performed 6 years after the planned right-sided mastectomy. The first surgical intervention included marking the back and breast area, excision and harvesting of the flap, preparation of the implantation site and its placement with the implant. Three months after the operation, she underwent a prophylactic mastectomy of the left breast based on molecular genetic testing with immediate reconstruction using the second flap of the broadest back muscle. The surgery was performed using a perimammary approach. Due to the available dissection, various anatomical configurations of the flap, and low-variability vascular anatomy, the *latissimus dorsi* flap is an adequate choice of material for the reconstruction of radical postmastectomy interventions. The final result of bilateral use of the flap for breast reconstruction after mastectomy is in favour of restoration of the anatomical tissue defect and a satisfactory aesthetic option, without complications in the form of seromas, haematomas, or pain. The result has a positive impact on the quality of life of a patient in remission of breast cancer

Keywords: mammoplasty; surgical correction; implant; myofascial repair

INTRODUCTION

Breast cancer (BC) is the most common cancer among women and the second leading cause of cancer-related death in this group [1]. The high prevalence of BC is associated with modifiable risk factors, but mammography screening and surgical treatment can improve prognoses. Frequent invasive forms of breast cancer require radical methods, such as mastectomy. It is important to consider not only survival but also the quality of life of women after radical interventions. More than a third of patients do not receive a sufficient assessment of their symptoms during the remission period. Mammoplasty surgical interventions aimed at restoring anatomy and aesthetics after mastectomy can improve the quality of life and psychological state of patients. In particular, the use of skin and muscle flaps is the optimal method for aesthetic breast reconstruction after radical mastectomy.

Following D.L. Lovelace *et al.* [2] and O. Kaidar-Person *et al.* [3], patients with successful breast cancer treatment who are in remission in most cases face negative long-term consequences of treatment that affect the quality of life,

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namely the growth of scar tissue in the area of mastectomy and breast tissue deficiency. These consequences include physical, functional, emotional, and aesthetic changes. The goals of surgical treatment of different types of BC remain the same – the elimination of metaplastic tumours from the breast, but with the least degree of anatomical deformation of the topographic area, as noted by the authors of A.N. Giaquinto *et al*. [4]. Whether it is radical lumpectomy or mastectomy, the need for aesthetic improvement of the results and their significant impact on the psycho-emotional state have been studied and recognised by surgeons in both the United States (US) [5] and the European Union (EU) [6]. The use of muscle flaps from anatomical areas, in particular the back, for plastic reconstruction of the breast after radical surgical excision of the latter in the focus of breast cancer is a safe method for women in remission, as evidenced by the results of their use by H.G. Cha *et al.* [7]. A musculocutaneous flap of the *latissimus dorsi* (LD) is widely used as a method of reconstruction of postmastectomy consequences using an autologous tissue complex with or without implants. Their availability in the surgical protocol of surgery and limited contraindications have been demonstrated by Slovak scientists Z. Danková *et al.* [8] and L. Krasničanová *et al.* [9]. Flaps from the abdominal area are also used along with LD flaps, but women of reproductive age often have direct contraindications to their excision, as follows from the results of A.G. Waks & E.P. Winer [10]. Among the topical issues of the methodology for performing plastic breast reconstruction using muscle flaps is the need for lipofilling, as noted by P. Koczkodaj *et al.* [11], the parallel use of implants, assessment of autograft volume, assessment of blood supply variability and innervation of the implanted tissue complex [12].

The study aimed to present the practical implementation and results of breast reconstruction in the long term (namely, 6 years after radical mastectomy) using LD in a patient in remission of BC and prophylactic reconstruction with a muscle flap with an implant.

MATERIALS AND METHODS

A patient in remission from BC was selected for breast reconstruction using the LD muscle flap. The patient, 49 years old, had a history of BC, for which a radical right-sided mastectomy was performed with a subsequent course of radiotherapy. At the time of the preliminary examination, the right breast was absent, and the condition of the postoperative scar on the chest was satisfactory. The left breast was intact and not involved in the metastatic process of breast cancer. At the time of the study, this patient was undergoing outpatient care after radiotherapy and radical mastectomy. The surgical intervention for plastic reconstruction was performed at the Department of Plastic, Reconstructive and Aesthetic Surgery of the Pasteur University Hospital and the Faculty of Medicine of the Pavol Josef Safaryk University in Košice in 2023. The total duration of the study was 10 months.

The selection criteria for participation in the study were: remission of BC, radical mastectomy, satisfactory condition of the skin of the thoracic region (turgor, elasticity) after radiotherapy; absence of an active infectious or viral process, absence of defects in the back area (fistulas, connective tissue scars, injuries) of the back area, especially in the LD area; documented consent of the patient to excision of the skin and muscle flap for plastic reconstruction. Contraindications to the use of the LD flap were muscle damage due to damage to the neurovascular bundle; myopathy in history; congenital anomalies and defects in the development of the LD. The general contraindications for plastic reconstruction and exclusion from the study were the presence of an active inflammatory process of any localisation; diabetes mellitus and immunopathologies (especially immunodeficiencies); and anaemia.

For the surgical intervention, the patient underwent a routine clinical examination before hospitalisation, including a general and biochemical blood test, general urine test, chest X-ray, allergy tests to the implant material, blood glycaemic and coagulation profile. No additional magnetic resonance imaging was prescribed, but the conclusions of the last examination were considered. Surgical intervention, risks of complications, and previous medical history were agreed upon with the patient's attending oncologist. There were no contraindications to participation in the study.

The surgical tactics were aimed at delaying plastic surgery on both breasts. Considering the previous medical history, the left breast was remodelled using an autograft, namely an LD skin-muscle flap. An additional implant was used to restore the volume of the right breast. The selection of the specific anatomical area and the type of tissue flap was based on the objective of this research, which aimed to assess the efficacy of the LD material. For a planned prophylactic mastectomy of the right breast, an LD flap without an implant with immediate reconstruction was also used. The patient had no contraindications to excision and the use of two autografts. The surgical interventions were performed following the clinical guidelines and protocols of the Department of Plastic, Reconstructive and Aesthetic Surgery of the Pasteur University Hospital and the Faculty of Medicine of the Pavol Josef Šafárik University in Košice. The effectiveness of the surgical treatment was assessed three months after the intervention with constant monitoring of the patient's somatic condition. The experiment was conducted with the patient's voluntary consent, as indicated in the written informed consent to participate, and in compliance with the ethical principles of the Helsinki Declaration for clinical research involving human subjects [13]. The design and methodology of the study were approved by the Research Ethics Committee of Pavol Jozef Šafárik University.

RESULTS

Delayed breast reconstruction, which was performed as part of the study as the first surgical intervention, was performed 6 years after a planned right-sided mastectomy, the results of which are shown in Figure 1. For the reconstruction of the breast in this patient, a skin-muscle flap of LD with a combination of alloplastic material, namely a silicone breast implant, was used. The patient was preliminarily diagnosed with a BRCA1 gene mutation, which indicated a high risk of developing second BC. Therefore, 6 months later, she underwent a subcutaneous prophylactic mastectomy of the left breast with subsequent immediate reconstruction. Thus, the left-sided mastectomy with plastic surgery had a prophylactic purpose, which was substantiated by the results of molecular genetic testing. The proposed approach to surgical treatment included, first of all, planning before plastic reconstruction. The preliminary planning and marking of the breast reconstruction trajectory was performed in the form of drawing a pattern on the patient's skin in a standing position (Fig. 1, 2). Preoperative markings were applied on the inframammary fold on the healthy and reconstructed side, medial and lateral borders; at the points of attachment of the pectoralis major muscle, and areas of abduction; the dorsal side included markings along the edges of the LD. The markings were made along the midline of the anterior chest and on each gland along the midline regarding the middle third of the clavicle and the location of the nipple. The contours of the implant placement for the right breast after radical mastectomy were determined according to the location of the pectoral muscles and the intact right breast.

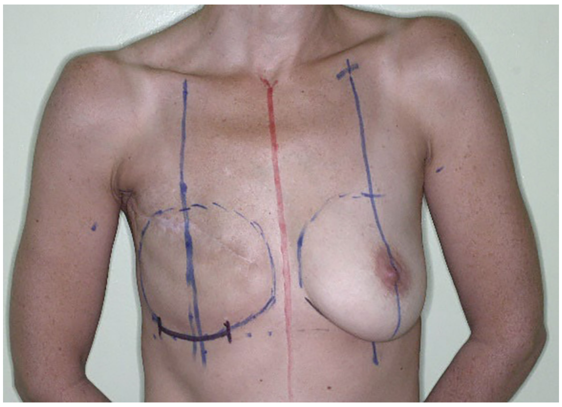

Figure 1. A 49-year-old patient with breast cancer underwent radical mastectomy of the right breast. Preoperative marking before plastic surgery
Source: photographed by the author

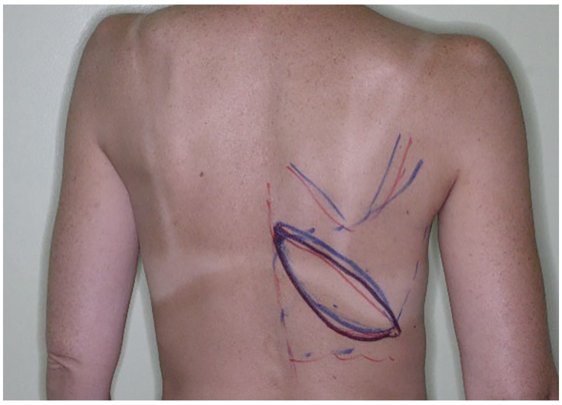

Figure 2. Preoperative marking: the boundaries of the back muscles, in particular, the *latissimus dorsi* and its blood supply
Source: photographed by the author

After a patient has undergone a modified radical mastectomy with a transverse scar, an oval-shaped skin flap is placed below and obliquely above the midline of the pectoralis major muscle. The preparation of the LD flap is accompanied by its elevation. To do this, an incision is made in the elliptical (oval) skin area, its dissection, and the actual isolation of the pectoralis major muscle, followed by dissection of the muscle by cutting off additional tissues, mainly the dense connective tissue of the fascial layers. After careful preparation of the flap, the skin-muscle complex was transferred to the patient's anterior chest wall (Fig. 3).

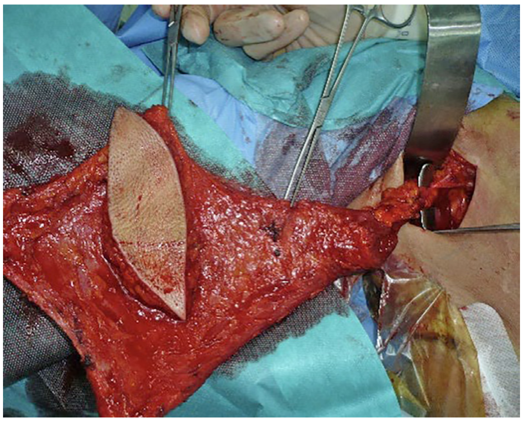

Figure 3. Muscle and skin flap from the broadest muscle of the back
Source: photographed by the author

Since the previous modified radical mastectomy involved the total excision of the breast, breast skin and subordinate pectoralis major fascia with axillary lymph nodes, an autograft with an implant was used for the right breast (Fig. 4). The separated musculocutaneous flap of the LD contains elements of blood supply and innervation, as well as a continuous skin flap with hypodermis. However, the flap does not contain fascial sheets or additional tissue complexes of adjacent topographic zones. After that, the actual reconstruction site was prepared. For this purpose, the mastectomy scar was excised, and a subcutaneous tunnel was created for the dorsal passage of the skin and muscle flap. Only after the reconstruction site was prepared, the implant was fixed and placed with the appropriate prepared material. A prophylactic subcutaneous mastectomy was performed on the left breast through a perimammary incision that preserved the nipple tissue and intact breast. The subcutaneous mastectomy was performed with immediate reconstruction using a flap of LD.

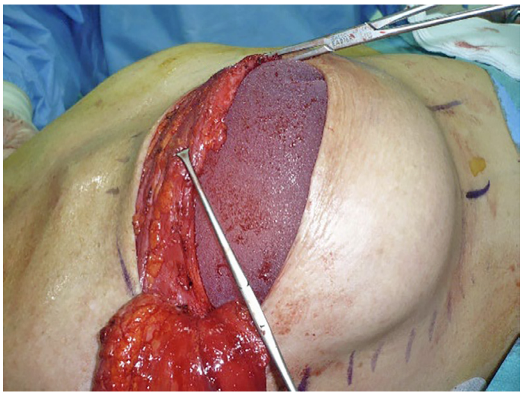

Figure 4. Reconstruction of the latissimus dorsi flap and augmentation of the implant covered by the pectoralis major and *latissimus dorsi* muscle
Source: photographed by the author

The use of the LD skin-muscle flap together with the implant allowed to restoration of the anatomical defect of the breast tissue after radical mastectomy and radiotherapy (Fig. 5). The skin flap is sufficiently elastic for plastic reconstruction and implant placement.

Figure 5. The result of the reconstruction of the *latissimus dorsi* muscle flap after radical mastectomy 3 months after plastic surgery
Source: photographed by the author

Thus, the patient's left breast was reconstructed using autogenous tissue in the form of a LD flap. This was a delayed reconstruction that showed satisfactory effectiveness in terms of restoring anatomical volume and the absence of immunological reactions to the implanted tissues and implant, which was assessed 3 months after the intervention (Fig. 5). The right breast underwent prophylactic mastectomy with immediate reconstruction along with mastectomy. Late reconstruction in this patient was possible given the satisfactory state of skin turgor and elasticity after radiotherapy (Fig. 6).

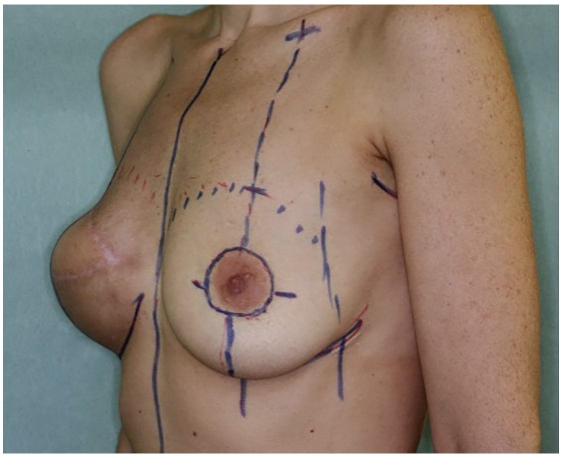

Figure 6. Preoperative markings on the left breast before prophylactic subcutaneous mastectomy
Source: photographed by the author

The perimammary approach for prophylactic mastectomy of the left breast was sufficient to place the LD flap and adequately reconstruct the volume after the removal of breast tissue (Fig. 7). The operation was performed according to the preliminary markings (Fig. 8).

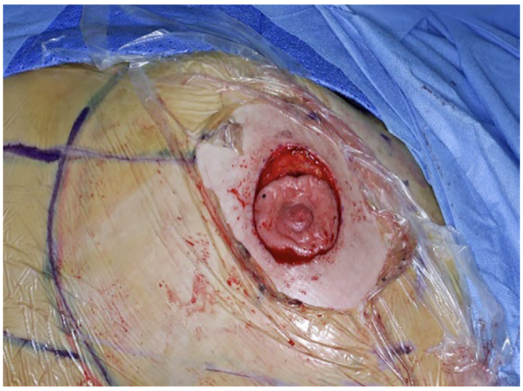

Figure 7. Prophylactic subcutaneous mastectomy through a perimammary incision that preserves the nipple and the breast itself
Source: photographed by the author

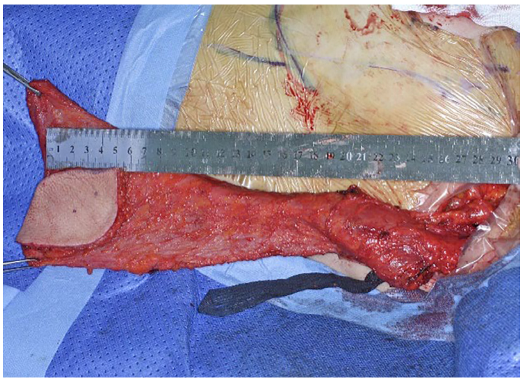

Figure 8. Preparation of a flap from the *latissimus dorsi* muscle
Source: photographed by the author

For the plastic reconstruction of the left breast immediately after prophylactic mastectomy, a flap of the LD with preserved elements of innervation and blood supply – the thoracodorsal artery and thoracodorsal nerve – was also used (Fig. 9, 10).

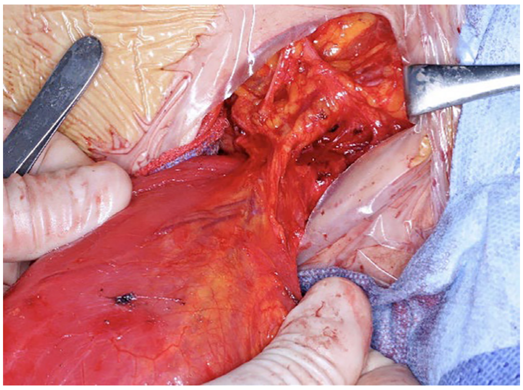

Figure 9. Muscle flap from the broadest muscle of the back together with the elements of blood supply and innervation – thoracodorsal artery and thoracodorsal nerve
Source: photographed by the author

Figure 10. The musculocutaneous flap transferred to the anterior chest wall of the left breast
Source: photographed by the author

Breast reconstruction with an LD flap using an implant is effective, especially when a patient has contraindications to abdominal tissue harvesting. In contrast to local skin flaps used in most plastic surgery protocols after mammoplasty, the LD flap provides a larger and more adequate skin flap volume together with subcutaneous tissue (Fig. 5). These properties make it suitable for patients after breast radiotherapy when the skin after aggressive radiation treatment has low turgor and elasticity and is not an option for covering the artificial implant material.

Thus, a partially autologous reconstruction was performed, as the patient's biological material was used, but with the use of an implant for the right breast. The bilateral reconstruction was performed using both LD flaps, which showed no signs of rejection or complications in the postoperative period, excluding pain, impaired upper limb function, or limited range of motion (Fig. 11).

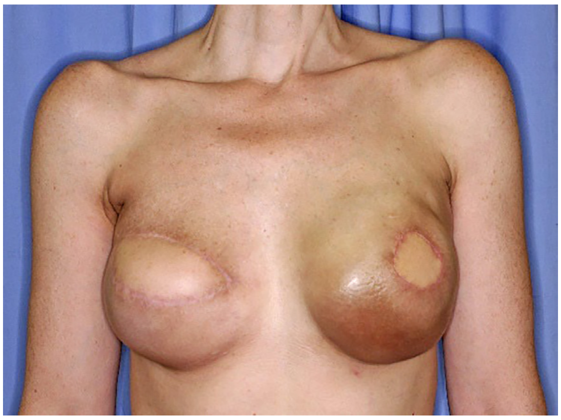

Figure 11. The final result after plastic surgery using the *latissimus dorsi* flap to reconstruct both breasts
Source: photographed by the author

Comparison of the patient's baseline data after unilateral radical mastectomy with the intermediate and final results indicates favour of restoration of the anatomical defect minus tissue and a satisfactory aesthetic option. The final result has a positive impact on the patient's quality of life in breast cancer remission. In addition, left-sided prophylactic mastectomy with plastic surgery is a preventive life-saving step in the management of this clinical case.

However, among the identified disadvantages of LD plastic surgery is the long duration of the surgery, which includes the harvesting and preparation of the skin and muscle flap, preparation of the site for implant and flap placement, and the actual plastic surgery of the breast area. Another disadvantage is the long scar on the back that remains after flap harvesting and the minor aesthetic effect on the operated breast: the transplanted skin from the back differs in colour from the surrounding thin and lighter skin on the breast.

DISCUSSION

Surgical treatment for breast cancer depends on the type of cancer, the stage of the disease, the patient's age, genetic factors, and general condition, as noted by L. Wilkinson & T. Gathani [14]. Modified radical mastectomy is the standard treatment for most patients with BC. Modern technologies allow mastectomy patients to restore the shape of the removed breast and improve their quality of life after radical and aggressive treatment. S. Char *et al.* [15] noted that mammary glands after mastectomy can be reconstructed using alloplastic materials (saline or silicone implants) or autologous tissues in the form of skin and muscle flaps. Sometimes, both implants and autologous tissue are used for breast reconstruction, as demonstrated in this study. Breast reconstruction can be performed at different times, but the patient in the study chose delayed breast reconstruction, which is a surgery performed several weeks, months or years after mastectomy according to clinical indications and informed consent.

According to S.B. Nam *et al.* [16], the advantage of using the LD flap for further breast reconstruction is that it provides for almost complete restoration of shoulder function in the long term. The thoracodorsal nerve division does not lead to volume loss or deformation of the topographic area of the shoulder and upper extremity at the site of autograft harvesting. Reliable anatomy (low variability of blood supply, innervation, and fibre course), easy dissection and relatively low complication rate, as indicated by J.B. Thomsen *et al.* [17], ensures the reliability of this technique, which is actively used by surgical schools. Long-term follow-up by G. Wattoo *et al.* [18] on a large cohort of patients who underwent reconstruction using LD showed a relatively low rate of side effects and unplanned revision surgery for inflammatory complications, as well as high patient satisfaction after breast reconstruction (namely, after radical mastectomies for BC in history), which indicates how reliable this technique is in the time aspect.

Considering all the advantages of the LD flap, the study aimed to use the flap for both reconstructions: after radical mastectomy of the right breast and for immediate reconstruction after prophylactic mastectomy of the left breast in the same patient. The patient received objectively satisfactory results of restoration of breast volume and anatomy without serious complications and with a satisfactory aesthetic effect. Randomised controlled trials conducted by M.B. Rindom *et al.* [19] show that after delayed breast reconstruction with a dorsalis pedis muscle flap or thoraco-dorsal artery graft, this group of patients is less likely to have postoperative symptoms of shoulder pain and show satisfactory shoulder function one year after surgical reconstruction. However, the harvesting of a back muscle flap carries a potential risk of deterioration in shoulder joint function, chronic pain, and reduced quality of daily function. Contrary to the proposed opinion, the study did not show the presence of pain or impairment of both limbs at the control examination three months after the interventions on both breasts.

Some clinics prefer to use a flap from the posterior shoulder muscle. This material is advisable for patients who have scars after laparotomy, have undergone abdominoplasty, do not have access to microsurgery, or are smokers or obese. On the other hand, R. Tevlin *et al.* [20] demonstrated the use of deep inferior flap material. Important parameters of the clinical outcome after autologous breast reconstruction for BC are the fact of flap loss, fatty necrosis of the reconstruction site, abdominal protrusion, and abdominal hernia at the site of skin and muscle flap harvesting, as demonstrated by S.D. Archangelo *et al.* [21]. The first two conditions are related to flap perfusion, while the latter reflect pathological abnormalities in the patient unrelated to the breast. Retrospective clinical studies on numerous groups of patients after radical mastectomies conducted by J. Beugels *et al.* [22] show that the procedure of delayed breast reconstruction with abdominal skin and muscle flaps allows restoring the physiological volume of the mammary glands due to a sufficient amount of tissue. However, among the few complications, which did not exceed 4% in total, the authors highlight seroma (as the earliest complication) and flap loss. Such complications were not observed in the course of the study, and the volume of the LD flap also had satisfactory volumetric parameters.

Due to its simple surgical access, various anatomical flap configurations (shape and volume of harvested material), possible flap orientation and consistent vascular anatomy, the LD flap has been widely used in various fields of reconstructive surgery, as shown by the study, which focused on the effectiveness of its combination with implants when necessary. However, with the advent of abdominal flaps and free flaps, interest in the LD flap for breast reconstruction has partially declined. Abdominal flap harvesting has its advantages, but in most cases is challenging due to the different specific harvesting techniques used by different surgical schools and several somatic parameters used to assess donor site morbidity and suitability for autotransplantation, as described by H. Mortada *et al.* [23]. For example, according to the study by D. Boczar *et al.* [24], obesity is linked to increased postoperative complications during abdominal flap harvesting and is also considered a contraindication for subsequent procedures involving the extraction of skin and muscle flaps from the abdomen. A frequent possible complication in obese patients is abdominal protrusion and abdominal hernia after microsurgical breast reconstruction for BC.

J.S. Palve *et al.* [25] show that the structure of complications for different skin and muscle flaps for breast reconstruction after BC is significantly different. The patients studied by the authors with an implanted LD flap had the highest rate of minor complications, in most cases seroma, and the lowest rate of postoperative complications requiring reoperation. Patients with abdominal flaps in the study showed the highest rate of complications requiring reoperation. In breast reconstructions after radical mastectomies using implants, the same number of both minor complications and complications requiring surgical intervention were found. These differences may indicate individual characteristics of the postoperative process depending on the study population, differences in protocols and patient management tactics in different surgical centres. Part of the differences can also be explained by the peculiarities of the grading of complications and the time of their registration in the postoperative period. The patient in the present study did not have early or late postoperative complications in both breasts, which can be explained by the insufficient sample of subjects to assess the risks of complications.

The need to use an implant is usually explained by the potentially unsatisfactory flap volume in a particular clinical case and contraindications to harvesting a significant amount of tissue, as indicated by H. Sousa *et al.* [26]. In the case of the right breast, the use of a combination of a skin and muscle flap with an implant was due to the need to restore the anatomical volume of breast tissue after radical mastectomy. Such a surgical choice carries potential risks of seroma and haematoma in the early postoperative period, but they were not detected in this patient.

Despite the above-mentioned controversies and potential complications, the LD flap remains particularly useful for breast reconstruction in patients who wish to avoid abdominal scars due to the use of abdominal flaps, in patients with contraindications to abdominal flap harvesting, as well as in those who have already used abdominal flaps in the past, as described by P.S. Soon *et al.* [27]. Thus, it is important to choose the optimal method of breast reconstruction, considering the individual characteristics of the patient and his or her medical history. Depending on the clinical case, it may be advisable to use a combination of different methods, such as an LD flap with an implant, to achieve optimal results.

CONCLUSIONS

Breast cancer is one of the most common malignancies in women, and its surgical treatment is an evidence-based therapy. The choice of modified radical mastectomy is the most common approach for most patients, performed according to a standard protocol. However, a significant disadvantage of radical mastectomy is the psychological trauma for the patient associated with the loss of the breast. Therefore, breast reconstruction is the best way to solve psychological problems after radical mastectomy.

This clinical case illustrates the use of a skin and fat flap to reconstruct both breasts in the same patient after radical mastectomy. The method of choice was delayed breast reconstruction, i.e., a surgical intervention performed a long time after mastectomy (from several weeks to years). The timing of delayed reconstruction is variable depending on the patient's indications and depends on the objective state of health, the stage of progression or remission of BC, the presence of direct contraindications and the duration of additional treatments (chemotherapy, radiation, or hormonal therapy).

The LD musculocutaneous flap is a reliable skin and muscle flap for the reconstruction of anatomical defects. The advantages of the *latissimus dorsi* flap include providing sufficient tissue volume for autologous remodelling, as well as complete vascular coverage for breast reconstruction with implants in case of prior radiotherapy. Thus, reconstruction of the right breast after radical mastectomy with implants and complete autogenous reconstruction of the left breast after prophylactic subcutaneous mastectomy using skin and fat flaps provided a satisfactory aesthetic and physiological result in a patient with delayed breast reconstruction. Further practical studies of this issue will focus on the effectiveness of the results of using the *latissimus dorsi* flap for delayed reconstruction of different periods after radical mastectomy.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Клапоть найширшого м’яза спини при реконструкції молочної залози після радикальної мастектомії та профілактичної підшкірної мастектомії: клінічний випадок

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Анотація. Одним із найпоширеніших варіантів хірургічного лікування раку молочної залози є радикальна мастектомія, яка впливає на якість життя пацієнток у стадії ремісії та їх психологічний стан. Одним із варіантів вирішення цієї проблеми є використання шкірно-м’язових клаптів з інших анатомічних ділянок, зокрема спини, для пластичної реконструкції молочної залози після радикальної мастектомії. Метою роботи було представити практичне виконання реконструкції обох молочних залоз у віддаленому періоді радикальної мастектомії у пацієнтки в період ремісії раку молочної залози з використанням шкірно-м’язового клаптя найширшого м’яза спини в поєднанні з імплантатом. Пацієнтка, яка відповідала критеріям участі в дослідженні, раніше за прямими показаннями разом із променевою терапією перенесла радикальну мастектомію правої молочної залози. Відстрочену реконструкцію молочної залози в рамках дослідження проводили через 6 років після запланованої правобічної мастектомії. Перше оперативне втручання включало розмітку області спини та грудей, висічення та забір клаптя, підготовку місця імплантації та встановлення його з імплантатом. Через 3 місяці після операції їй виконано профілактичну мастектомію лівої молочної залози на основі молекулярно-генетичного дослідження з негайною реконструкцією другим клаптем найширшого м’яза спини. Операцію проводили перимаммарним доступом. Завдяки доступній дисекції, різноманітній анатомічній конфігурації клаптя та низькій варіабельності судинної анатомії клапоть найширшого м’яза спини є адекватним вибором матеріалу для реконструкції радикальних постмастектомічних втручань. Кінцевим результатом двостороннього використання клаптя для реконструкції молочної залози після мастектомії є відновлення анатомічного дефекту тканини та задовільний естетичний варіант, без ускладнень у вигляді сером, гематом або болю. Результат позитивно впливає на якість життя пацієнтки в період ремісії раку молочної залози

Ключові слова: мамопластика; хірургічна корекція; імплант; міофасціальна репарація

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Computer screening of peptidomimetics and small-molecule ligands of B-cell membrane proteins for therapy of Burkitt lymphoma

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Abstract. The capabilities of molecular modelling and docking allow for the discovery of new potential drug agents to improve the treatment of diseases, which is a current concern. The objective of this study was to conduct *in silico* screening for antibody mimetics to B-cell membrane proteins for the treatment of Burkitt lymphoma through virtual screening. In this work, a standard protocol for structure-based virtual screening was employed, with the distinction that pharmacophores for screening were built not based on small-molecule ligands but on selected amino acid residues of antibodies. Based on literature data and the presence of a mechanism of direct cytotoxic action, as well as the availability of 3D structures of complexes, three monoclonal antibodies were selected: obinutuzumab, epratuzumab, and atezolizumab. The identification of biological targets was carried out by searching for 3D structures of selected complexes with target proteins in the Protein Data Bank. For virtual screening, the web service Pharmit was chosen. Using the Molecular Operating Environment program, pharmacophore models were constructed for three complexes: CD20 and obinutuzumab, CD22 and epratuzumab, and PD-L1 and atezolizumab. Docking with the CD20, CD22, and PD-L1 proteins was conducted at the binding sites recognised by the original antibody. Through *in silico* virtual screening using the Molecular Operating Environment software, a search for antibody mimetics to B-cell membrane proteins for Burkitt lymphoma treatment was conducted, resulting in the selection of 5 potential anti-lymphoma agents: CHEMBL505179 for the CD20 receptor, an antagonist of the melanocortin receptor for CD20 (PubChem-44406884), an inhibitor of blood clotting Factor Xa for CD22 (PubChem-136510605), and a blocker of epithelial Na+ channels for CD22 (PubChem-126761430), and an agonist of the melanocortin receptor for PD-L1 (PubChem-25078192). The obtained results can be applied in the pharmaceutical industry and oncological practice to enhance therapeutic outcomes in the treatment of patients with Burkitt lymphoma

Keywords: molecular docking; pharmacophores; antibodies; mimetics; immunogenicity

INTRODUCTION

The search for new potential drug agents remains a relevant task. Modern bioinformatic methods and algorithms significantly reduce the time and cost of laboratory research, streamlining the search and development of new drugs. Computational drug discovery methods are more cost-effective than standard methods and can analyse a vast number of components under conditions that would be impossible to recreate in laboratories. These methods combine three components: biological data, chemical knowledge, and modelling algorithms, offering a wide range of tools and plugins for predicting the biological activity of both molecular structures and chemical compounds with high probability. Developed and validated models can be further used for virtual screening of a vast number of substances, enabling the quick identification of promising components. These virtual methods can be combined with

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classical molecular docking methods to confirm their activity, as mentioned by S. Ai *et al.* [1] and L. Kucherenko *et al.* [2].

Molecular modelling and docking methods allow the selection and assessment of the binding strength between interacting pairs, such as antibodies and proteins. For this task, various programs exist to prepare computer models of molecules for interaction and conduct their docking. One such program is Molecular Operating Environment (MOE), known for its intuitive interface, a wide range of functions, and plugins, providing accurate computational results. In the work of Y. Zhang *et al.* [3], virtual screening of pharmacophores and molecular docking were utilised to identify potential inhibitors of Src (proto-oncogene tyrosine-protein kinase). The researchers obtained 891 pharmacophores and selected 10 for further investigation based on the highest docking scores and calculated binding energy between components. An essential component of computational drug discovery methods is the availability of information in databases about the properties of components, including absorption, distribution in the body, metabolic pathways, and elimination routes, as well as toxic properties. Evaluating all these characteristics of selected components, researchers identified two molecules as potential inhibitors of the Src kinase family.

Frequently, the atomic structure of a pharmacologically relevant receptor is unknown. Three-dimensional alignment of potential ligands can be applied to establish structural requirements for their biological activity. Another strategy is the creation of pharmacophores, aligning ligands considering the minimum number of molecular properties of components [4]. Alternatively, searching databases for 3D molecular structures and their alignment can be employed [5]. The methodology based on aligning 3D structures to determine the biological activity of ligands assumes that if two ligands have similar biological activity and bind at the same points (pockets) to the receptor (protein), the connection between these conformations is strong [6].

Burkitt lymphoma (BL) belongs to the group of non-Hodgkin lymphomas (NHL) and is the most aggressive malignancy, doubling its cell population every 24-48 hours [7]. BL is characterised by hyperinvasiveness and high lethality [8]. Intensive, prolonged combined chemotherapy regimens are used for BL treatment, demonstrating positive results (adult 5-year survival is 56-70%). However, 15% of patients develop chemotherapy-resistant or recurrent forms, requiring the development of new therapeutic agents [9].

In addition to chemotherapy, monoclonal antibody drugs are used for BL treatment, showing high effectiveness but having drawbacks such as the inability for oral intake, immunogenicity, and high production costs, as mentioned by T. Lutsenko & M. Chalenko [10]. The search for small-molecule antibody mimetics, devoid of these drawbacks, is relevant. Computer-aided screening methods, which significantly reduce the time and financial costs of finding potential therapeutic agents, are suitable for such a search.

The study aimed to search for *in silico* antibody mimetics to B-cell membrane proteins for Burkitt lymphoma treatment through virtual screening. To achieve this, virtual screening and molecular docking with BL B-cell target proteins based on known cytotoxicity properties of substances from open libraries are required. Promising complexes need to be selected, and their potential action as anti-lymphoma agents should be analysed.

MATERIALS AND METHODS

The research was based on the use of a standard protocol for structure-based virtual screening. However, the applied pharmacophores for screening were constructed not based on small-molecule ligands but on selected amino acid residues of antibodies [11-13]. The first step in computer-aided drug discovery is the identification of the biological target. 3D structures of selected complexes with target proteins were downloaded from the Protein Data Bank (PDB). Two conditions were considered for selecting a protein as the target:

1. The therapeutic antibody to the protein should act through the mechanism of direct cytotoxicity (neutralization).
2. The availability of a 3D structure of the antibody-protein complex in the PDB database. Performing computer-aided drug discovery is possible even without using the 3D structure – for example, based on the structure of homologous proteins, but it is less accurate.

An important second step in the search for a potential drug candidate is the construction of pharmacophoric hypotheses, or simply pharmacophores. The MOE software package was used to build the pharmacophore model. The "QuickPrep" function in the MOE program was used to prepare the complexes before constructing the pharmacophore. This program has several functions that allow the removal of unnecessary water molecules, addition of missing hydrogen atoms, and selection of antibody regions for further binding. For virtual screening, the Pharmit web service was chosen, containing more than 340 million compounds from databases such as CHEMBL25, ChemDiv, ChemSpace, MCULE, MCULE-ULTIMATE, MolPort, NCI Open Chemical Repository, PubChem, LabNetwork, ZINC, and three manually added libraries of peptide mimetics [14].

Pharmit uses the Pharmer technology as its search algorithm [15]. Pharmer decomposes the uploaded pharmacophore into individual triangles, then searches for matches between compounds from libraries and formed triangles, after which it combines the results. For each compound, the root-mean-square deviation (RMSD) value is computed using the formula [16] (1):

$$RMSD = \sqrt{\frac{\sum_i^n \left(\frac{d(c_i,q_i)}{r_i}\right)^2}{n}}, \qquad (1)$$

where $d(c_i, q_i)$ is the distance between the corresponding feature of the loaded pharmacophore and the compound; r_i – the permissible radius of the connection; n – the number of pharmacophore signs.

This value is a measure of the similarity between a compound and the pharmacophore. Following the execution of the Pharmer search query, a table is generated with the found compounds arranged in decreasing order of the RMSD value.

Based on literature data and the presence of a mechanism of direct cytotoxic action, as well as the availability of 3D structures of complexes, three monoclonal antibodies were selected:

1. Obinutuzumab [17] – a humanized monoclonal antibody to the CD20 protein, which is expressed on the surface of 93-100% of lymphoma cells [18, 19].

2. Epratuzumab – a humanized monoclonal antibody to the CD22 protein, which is expressed on the surface of 47-70% of lymphoma cells [18, 20].

3. Atezolizumab – a humanized monoclonal antibody to the PD-L1 protein, which is expressed on the surface of 80% of lymphoma cells [21].

The 3D structures of the complexes CD20 and obinutuzumab, CD22 and epratuzumab [22], and PD-L1 and atezolizumab [23] were downloaded from the PDB with the following codes: 6Y9A (resolution 4.20 Å), 5VL3 (resolution 3.10 Å), and 5X8L (resolution 3.10 Å), respectively (Fig. 1).

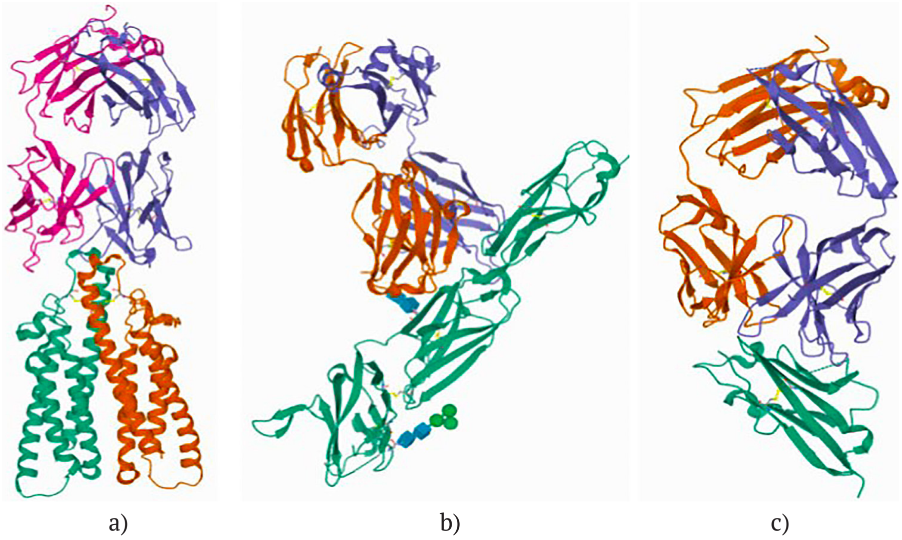
a) b) c)

Figure 1. 3D structures of the proteins used in this work

Notes: a) 3D structure of CD20 and obinutuzumab complex; b) 3D structure of CD22 and epratuzumab complex; c) 3D structure of PD-L1 and atezolizumab complex

Source: [22, 23]

To prepare structures for docking in the MOE program, files in PDB format were loaded, and heavy and light chains of processed antibodies were removed. Using the "Dock" function, docking was performed with compounds loaded in SDF format with selected regions of the target protein. The choice of the program is explained by its broader and more convenient set of tools compared to other programs such as LigandScout and Maestro. The virtual screening method based on the pharmacophore involves comparing compounds from a molecular library with a pharmacophore hypothesis, allowing the automated identification of compounds likely to have biological activity in a relatively short time. The obtained activity will be like the activity of compounds used to model the current pharmacophore. Obinutuzumab was chosen because it has a Fc fragment with increased affinity for FcγRIIIa receptors on the surface of effector cells. These target cells include neutrophils and macrophages, so this antibody has an enhanced ability for antibody-dependent cellular cytotoxicity in the immune response. Its direct cytotoxic action also has a lysosomal nature and does not depend on additional apoptotic pathways [24]. As for epratuzumab, it initiates phosphorylation of CD22 with subsequent internalization of CD22 and the CD79α protein, which is part of the B-cell receptor and disrupts intracellular signalling during immune reactions. The action of epratuzumab almost entirely relies on direct cytotoxicity through negative regulation of BCR signalling and to a tiny extent on complement-dependent cytotoxicity (CDC) and Antibody-dependent cellular cytotoxicity (ADCC) [25]. Atezolizumab, in turn, blocks the interaction of PD-L1 with PD-1 and CD80, reactivating the immune response against tumour cells [26, 27]. The choice of Pharmit is explained by the fact that among all web applications for virtual screening, it contains the most comprehensive compound database and allows users to add their own compound libraries. The exclusion of the use of desktop programs is explained by the limited computational capabilities during the research; it is indicative that Pharmit works 10-1000 times faster than MOE [16].

RESULTS AND DISCUSSION

Using the MOE computer program, pharmacophore models were constructed for three complexes. For the CD20 and obinutuzumab complex, a pharmacophore model was built based on the amino acid residues Ser30, Tyr31, Trp33, Arg52, Gly54, and Asp55 in the heavy chain of obinutuzumab. The obtained model contains a total of 17 features, including two aromatic groups, five hydrogen bond acceptors, six hydrogen bond donors, and five hydrophobic groups. The pharmacophore model for the CD22 and epratuzumab complex was constructed based on the amino acid residues Ser31, Trp33, Arg53, Tyr56, Glu58 in the heavy chain of epratuzumab. The resulting model contains a total of 8 features, including one aromatic group, three hydrogen bond acceptors, and four hydrogen bond donors. The pharmacophore model for the PD-L1 and atezolizumab complex was constructed based on the amino acid residues Trp50, Ser52, Tyr54, Gly55, Ser57, Thr58, Tyr59 in the heavy chain of atezolizumab. The obtained model contains only nine features, including one aromatic group, four hydrogen bond acceptors, and four hydrogen bond donors (Fig. 2).

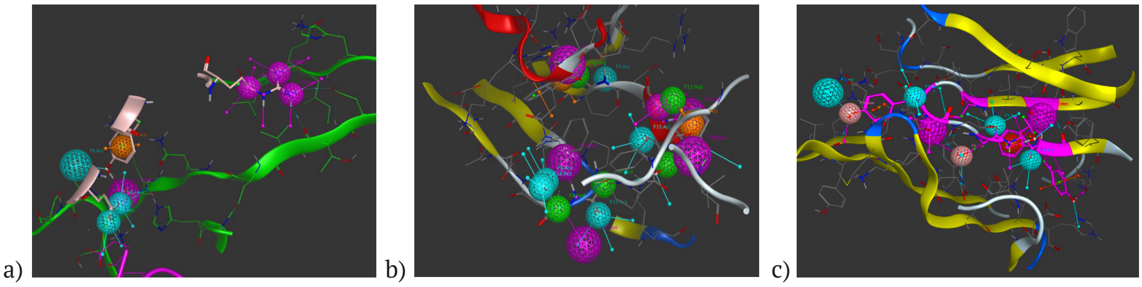

Figure 2. Pharmacophore protein-binding site of antibodies to (a) CD20; (b) CD22; (c) PD-L1
Notes: a) the CD20 pharmacophore model consists of amino acid residues Ser30, Tyr31, Trp33, Arg52, Gly54 and Asp55 of the heavy chain of obinutuzumab; b) the CD22 pharmacophore model consists of amino acid residues Ser31, Trp33, Arg53, Tyr56, Glu58 of the heavy chain of epratuzumab; c) PD-L1 pharmacophore model consists of amino acid residues Trp50, Ser52, Tyr54, Gly55, Ser57, Thr58, Tyr59 of the heavy chain of atezolizumab
Source: compiled by the authors

As a result of the library screening, the following results were obtained: for the CD20 and obinutuzumab pair – 157 compounds, for the CD22 and epratuzumab pair – 117 compounds, and for the PD-L1 and atezolizumab pair – 205 compounds. Guided by the threshold value of the RMSD indicator (0.8 Å), 15 compounds were selected for the CD20 protein, 15 compounds for CD22, and 35 compounds for PD-L1 for further work. Obinutuzumab was chosen based on its Fc fragment with increased affinity for FcγRIIIa receptors on the surface of effector cells. Epratuzumab was selected based on its ability to initiate phosphorylation of CD22 with subsequent internalization of CD22 and CD79α, which is part of the B-cell receptor and disrupts intracellular signalling during immune reactions.

Docking with the CD20 protein was performed at the binding site recognised by the original antibody. The binding site consists of amino acids in chain A, namely Ala170, Asn171, Pro172, Ser173, Glu174, Kys175, Asn176, Ser177, and Pro178. Docking with the CD22 protein was performed with the amino acids Ile 145, Ser 211, His 213, Gly 214, Asp 232, Thr 233, Gln 235, Val 267, Asn 271, and Tyr 274. Docking with the PD-L1 protein was conducted with the amino acids Ala18, Glu45, Asp49, Ala51, Ala52, Ile54, Tyr56, Glu58, Glu60, Asp61, Asn63, Qln66, Val68, His69, Val111, Arg113, Met115, Ser117, Tyr118, Gly119, Ala121, Tyr123, and Arg125. Based on the docking results with the minimum score (S) values, which characterize the binding affinity, 5 potential anti-lymphoma agents were selected (Fig. 3).

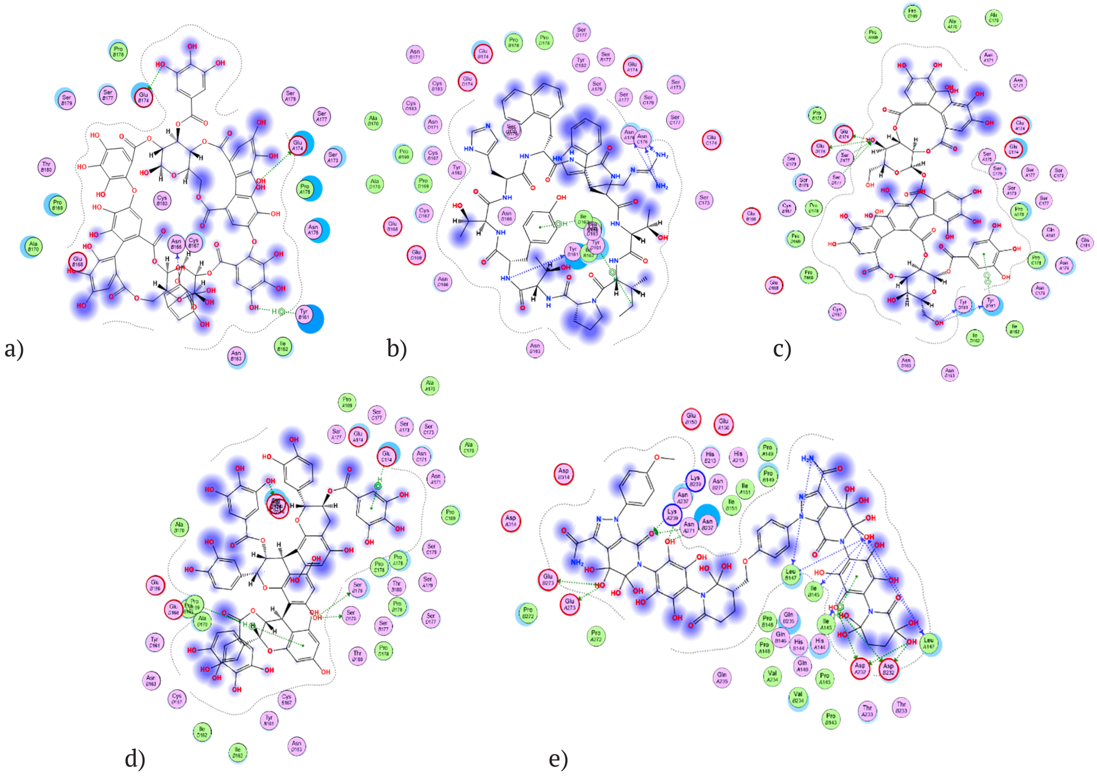

Figure 3. Agents obtained as a result of docking
Notes: a) CHEMBL505179 in the binding site of obinutuzumab with CD20; b) PubChem-44406884 in the binding site of obinutuzumab to CD20; c) PubChem-101933623 in the binding site of obinutuzumab with CD20; d) PubChem-44249983 in the binding site of obinutuzumab to CD20; e) PubChem-136510605 in the CD22 binding site of epratuzumab
Source: compiled by the authors

According to the standard parameters of Pharmit, it is recommended to filter compounds based on a value of 2 Å, or vary it in the range of 1.5-2.5 Å. By filtering at 0.8 Å, the authors applied more stringent computational constraints to select potential compounds. Among all the selected compounds suitable for testing *in vitro* anti-lymphoma activity are the following:
CHEMBL505179, PubChem-101933623,
PubChem-16135635, PubChem-44249983,
PubChem-16209234, PubChem-135854913,
PubChem-44256795, MolPort-046-788-300,
PubChem-45359413, MCULE-5553462135.
MolPort-046-857-137 (Table 1).

Their activity can be assessed on BL cell lines such as Raji, Ramos, and Akata. This can be achieved by evaluating cell viability and survival using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) after incubating cells with the selected compounds. The analysis of these potential anti-lymphoma agents was conducted using PubChem data, revealing that five of the selected compounds already exhibit documented biological activity. Four of them, namely PubChem-44406884, PubChem-136510605, PubChem-126761430, and PubChem-25078192, are experimentally confirmed *in vitro* ligands for proteins distinct from the chosen biological targets typical for B-cell BL.

Table 1. Summarized data on the results of molecular docking of formed formacophores with ligands

White	Ligand	S score, kcal/mol	A/c with which it forms connections
CD20	CHEMBL505179	-9.9295	Glu A174, Tyr B161, Asn B166, Glu B174
	PubChem-44406884	-9.5939	Tyr B161, Ile B162, Asn B176
	PubChem-101933623	-9.3220	Tyr B161, Glu B174, Ser B177
	PubChem-16135635	-8.8296	Tyr B161, Glu B174, Glu B168, Cys B183, Asn A176
	PubChem-44249983	-8.4617	Pro B169, Glu B174, Ser B179
CD22	PubChem-16209234	-10.807	Asn 235, Asn 271
	PubChem-136510605	-10.5678	Ile 145, Leu 147, Asp 232, Lys 239, Asn 271, Glu 273
	PubChem-135854913	-10.1344	Ile 145, Leu 147, Lys 215, His 213, Ser 269, His 213, The 233, Asn 237
	PubChem-44256795	-10.0038	His 213, Val 234, Gln 235, Glu 266, Val 267, Glu 273
	PubChem-126761430	-9.9341	Asp 232, Ser 269, Ser 270
PD-L1	MolPort-046-788-300	-10.953	Val 76, Arg 113, Asp 122
	PubChem-45359413	-10.8218	Gln 66, Arg 125
	MCULE-5553462135	-9.9989	Glu 58, Arg 113, Ser 117, Arg 125
	MolPort-046-857-137	-9.8211	Glu 58, Met 59, Glu 60, Arg 113, Arg 125
	PubChem-25078192	-9.207	Asp 26, Glu 60, Lys 124, Arg 125

Source: compiled by the authors

The compound PubChem-44406884 (Table 2) acts as an antagonist to the melanocortin receptor. This receptor is specific to the heptapeptide core, which is common to adrenocorticotropic hormone and alpha-, beta-, and gamma-MSH. PubChem-44406884 plays a central role in energy homeostasis and somatic cell growth in the body. The melanocortin 4 receptor (MC4R) is expressed in hypothalamic cells and is essential for regulating appetite and energy expenditure in the body. Dysfunction of MC4R in humans leads to hyperphagia, impaired satiety sensation, and the development of obesity [28-30]. A similar compound is the investigated PubChem-25078192. At this stage of scientific research, the compound PubChem-136510605 is not sufficiently studied in terms of its impact on Burkitt lymphoma cells. Scientific sources about PubChem-25078192 currently indicate that it is analogous in action to the inhibitor of blood clotting factor Xa. Similar novel substituted carboxamidic compounds and pharmaceutical compositions made based on them are mainly used to inhibit the activity of factor Xa in patients for specific indications to treat disorders such as deep vein thrombosis, pulmonary artery embolism, cerebrovascular ischemia, coronary artery disease, and oncological diseases [31].

Table 2. Data on biological activity of selected ligands

White	Ligand	Data on biological activity
CD20	CHEMBL505179	Immunoregulatory, antiproliferative action
	PubChem-44406884	Melanocortin cell receptor antagonist
CD22	PubChem-136510605	Inhibitor of blood coagulation factor Xa
	PubChem-126761430	A blocker of epithelial Na^+ channels of the plasmolemma
PD-L1	PubChem-25078192	Melanocortin membrane receptor agonist

Source: compiled by the authors based on [32-34]

Compound PubChem-126761430 (Table 2) has undergone a limited and insufficient amount of scientific research to understand the full spectrum of its action on normal and tumour cells in the body. It has been established that PubChem-126761430 is structurally similar to the blocker of plasma membrane sodium channels in epithelial cells. One approach to restoring the protective layer of fluid on the mucous membrane surface is the “rebalancing” of the system by blocking sodium channels in the cell membrane and corresponding fluid absorption. The protein in the membranes of epithelial cells that mediates the inhibition of sodium ion movement and fluid absorption is the epithelial sodium channel (ENaC). ENaC is located on the apical surface of epithelial cells lining mucous membranes, at the interface of the mucosal surface and the surrounding environment (or the organ cavity). Under normal conditions, to suppress ENaC-mediated sodium ion and fluid absorption, an ENaC blocker of the amiloride class should be delivered to the mucous membrane surface and maintained in place to achieve maximum therapeutic benefit and effect.

Of all the investigated compounds listed, the most promising is the substance CHEMBL505179, which is a macrocycle from the ellagitannin group. CHEMBL505179 is extracted from plant raw materials of the genera *Oenothera, Epilobium,* and *Eucalyptus*. This substance demonstrates a broad spectrum of immunoregulatory effects, including reducing the production of reactive oxygen species by mouse lymphocytes, inducing neutrophil migration *in vitro* and *in vivo*, activating NF-κB factor in monocyte culture, and more. Additionally, this compound exhibits high anti-proliferative activity in experimental studies on prostate carcinoma cells, hepatocellular carcinoma, and other tumours [28], making it promising for the development of a new drug for targeted BL treatment in patients.

Research on NHL group representatives [35], including follicular lymphomas, marginal cell lymphomas, and BL as the main part of malignant neoplasms of mesenchymal cells, focuses on targeted treatment with courses of monoclonal antibody drugs. Due to their high specificity, these drugs are targeted at key markers of malignant B-cells, enhancing the patient's immune system defence mechanisms, leading to apoptosis of malignant cells.

In the conducted study, obinutuzumab was chosen because it has a Fc fragment with increased sensitivity to FcγRIIIa receptors on the surface of effector cells (specifically neutrophils and macrophages) and enhanced ability for antibody-dependent cellular cytotoxicity in the lysosomal immune response, independent of additional apoptotic pathways. The effectiveness of obinutuzumab in lymphomas of various origins is confirmed by other contemporary studies [24, 27, 36]. Obinutuzumab is a type II anti-CD20 monoclonal antibody belonging to the immunoglobulin G2 class. Published data by A. Prica & M. Crump [36] show that targeted therapy against CD20 activity using the monoclonal antibody rituximab led to significant improvement in overall post-therapeutic survival of patients with B-cell NHL and BL. Despite the identified improvements, some patients were diagnosed with relapse or refractoriness after rituximab treatment. Obinutuzumab, despite its recent discovery, is recognised as a promising humanized anti-CD20 monoclonal antibody currently studied in late-phase clinical trials compared to rituximab. Preclinical safety experiments by A. Prica & M. Crump [36] demonstrate that obinutuzumab is a more effective anti-CD20 monoclonal antibody than rituximab, especially in assessing its role in initiating antibody-dependent cellular cytotoxicity and direct cell apoptosis in BL. Obinutuzumab is safe and effective for CD20-positive NHL, including BL, and requires further investigation. As noted by A. Prica & M. Crump [36] and A. Fekih *et al.* [37], obinutuzumab has been recommended for use in clinical treatment protocols for lymphomas since 2013, specifically in combination with chlorambucil; since 2016, it has been recommended for lymphoma treatment in combination with bendamustine followed by maintenance therapy with obinutuzumab every two months for two years in patients refractory to rituximab. The use of obinutuzumab after completion of clinical studies that justify its safety and efficacy compared to or with the use of other drugs will help improve the tactics of targeted and personalised BL therapy.

Among other potential ligands for targeted BL treatment, the study identified the melanocortin receptor antagonist (PubChem-44406884) to the CD20 receptor. Several studies support the use of the melanocortin receptor antagonist for lymphocytic malignancies, particularly mantle cell lymphoma [37]. Melanocortin receptor 1, investigated as a target in modern clinical studies by M. Li *et al.* [38], is being studied for delivering active components to treat metastatic melanoma and other malignant neoplasms. Increased expression of melanocortin receptor 1 is detected *in situ* in histological samples of diagnosed melanoma. B-Raf proto-oncogene (BRAF) inhibitors and histone deacetylase inhibitors significantly increase the expression of melanocortin receptor 1 through MITF-dependent pathways. This effect was enhanced by ligand conjugation to the cell glycoalkalix. The obtained data indicate that the use of BRAF inhibitors and histone deacetylase inhibitors significantly enhances the effectiveness of melanocortin receptor 1-targeted therapy by activating the delivery of active chemotherapy components through regulated expression of melanocortin receptor 1.

Among all the compounds identified during the study, the substance CHEMBL505179, belonging to the ellagitannin group of macrocycles, is the most promising. CHEMBL505179 is extracted from plant raw materials of the *Oenothera* genus and demonstrates a broad spectrum of immunoregulatory action by inhibiting the production of reactive oxygen species by mouse B-lymphocytes and activating the NF-κB factor in monocyte culture [28]. A study by S. Yoshikawa *et al.* [39] on high-molecular-weight polyphenolic molecules in *Barringtonia racemosa* leaves allowed the identification of approximately five previously unexplored ellagitannins. One such compound is barringtonin M1, which is a hydrolysed monomer of tannin. Barrycyclin D1 has a macrocyclic organization formed from casuarictin and tellimagrandin.

From scientific works on PubChem-25078192, it is known that this compound has a similar effect to the blood clotting factor Xa inhibitor, which is focused on malignant lymphomas [40]. Blood clotting factor Xa is a serine protease that has a central role in the mechanism of activating the physiological blood clotting cascade and is therefore investigated as a promising compound for antithrombotic and antitumor drugs. Inhibitors of blood clotting factor Xa can affect the initiation cascades of thrombotic enzymes and catalytic activity. In contemporary research by T. Schmitz *et al.* [41], peptide inhibitors of tridegin and transglutaminase-inhibiting acceptors of Michael are the most promising and potentially successful candidates for developing clinically effective inhibitors of blood clotting factor Xa due to their specific selectivity for activated blood clotting factor Xa.

Rituximab, a CD20 monoclonal antibody, is historically the first immunotherapeutic agent ever used in oncology, and to this day, it remains a cornerstone of lymphoma therapy. Since then, scientists have witnessed the development of humanized antibodies, next-generation anti-CD20 monoclonal antibodies targeting other tumour markers (CD19 and CD22), their microenvironment (PD-1, CD47), antibody-drug conjugates, and bispecific T-cell engagers [42, 43]. Considering their activity, safety, and specificity, mAbs can remain crucial therapeutic tools for treating NHL and other malignant neoplasms.

The activity of the programmed cell death protein 1 (PD-1) ligand is a key mechanism for preserving the tumour from the patient's own immune defence. In malignant B-cell lymphoma cells and their tumour histological microenvironment, PD1-L conjugates with PD-1, leading to the inhibition of cytotoxic T-cell signalling in the immune response and, as a result, to their exhaustion. According to P. Willard *et al.* [43], complete blocking of PD-1 and PD1-L function provided an innovative approach to lymphoma therapy, as pathways for implementing PD-1 inhibitors into B-cell lymphoma treatment protocols were identified and developed. Experimental studies on classical Hodgkin's lymphoma cells and primary mediastinal B-cell lymphomas revealed the pathogenetic basis for blocking the immune response by increasing PD-1L regulation. Two inhibitors of PD-1 from the PD-1 inhibitor group demonstrated high clinical activity and were approved for two subtypes of lymphoma. These registered drugs include nivolumab and pembrolizumab, which are fully humanized monoclonal antibodies, immunoglobulins of class G4, blocking the binding of PD-1L to malignant lymphoma cells.

CONCLUSIONS

Through virtual screening using *in silico* methods with the MOE software, a search for antibody mimetics to B-cell membrane proteins for the treatment of Burkitt lymphoma was conducted, resulting in the selection of five potential anti-lymphoma agents. From the entire pool of screened chemical compounds suitable for *in vitro* anti-lymphoma activitytesting,onlyCHEMBL505179,PubChem-101933623, PubChem-16135635, PubChem-44249983, PubChem-16209234, PubChem-135854913, PubChem-44256795, MolPort-046-788-300, PubChem-45359413, MCULE-5553462135, and MolPort-046-857-137 were chosen.

Docking with the CD22 protein was performed using the amino acids Ile 145, Ser 211, His 213, Gly 214, Asp 232, Thr 233, Gln 235, Val 267, Asn 271, Tyr 274. Docking with the PD-L1 protein was conducted using the amino acids Ala18, Glu45, Asp49, Ala51, Ala52, Ile54, Tyr56, Glu58, Glu60, Asp61, Asn63, Qln66, Val68, His69, Val111, Arg113, Met115, Ser117, Tyr118, Gly119, Ala121, Tyr123, Arg125. Based on the minimal score (S) values, which characterize the binding affinity, five potential anti-lymphoma agents were selected. The analysis of potential anti-lymphoma agents was conducted using data from the PubChem database, revealing that all five selected compounds have already registered biological activity. Four of them, including PubChem-44406884, PubChem-136510605, PubChem-126761430, and PubChem-25078192, are experimentally proven *in vitro* ligands for proteins different from the chosen biological targets characteristic of B-cells in Burkitt lymphoma. Among the identified potential ligands, the compound CHEMBL505179 targeting the CD20 receptor stood out as the most promising. It is a macrocyclic ellagitannin isolated from plant sources (genera *Oenothera, Epilobium, Eucalyptus*). Other potential ligands include an antagonist of the melanocortin receptor (PubChem-44406884), an inhibitor of blood clotting Factor Xa (PubChem-136510605) targeting the CD22 receptor, and a blocker of epithelial Na$^+$ channels (PubChem-126761430). For the PD-L1 receptor, an agonist of the melanocortin receptor (PubChem-25078192) was identified. Thus, the *in silico* mimetics of antibodies to B-cell membrane proteins that cause BL development obtained as a result of the search are a promising method for the treatment of BL using the virtual screening technique. Further directions of this research will be aimed at identifying additional ligands with anti-lymphoma properties that will have an anti-tumour effect for a wider range of non-Hodgkin lymphoma.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Комп'ютерний скринінг пептидоміметиків та маломолекулярних лігандів мембранних білків в-клітин для терапії лімфоми Беркітта

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Анотація. Можливості молекулярного моделювання та докінгу дозволяють знайти нові потенційні лікарські агенти, які дозволяють покращити лікування хвороб, що є актуальним питанням сьогодення. Метою даного дослідження був пошук *in silico* міметиків антитіл до мембранних білків В-клітин для лікування лімфоми Беркітта методом віртуального скринінгу. В даній роботі було використано стандартний протокол структурно-залежного віртуального скринінгу з тією відмінністю, що фармакофори для скринінгу будували не на основі маломолекулярних лігандів, а на основі обраних амінокислотних залишків антитіл. За літературними даними та на основі наявності механізму прямої цитотоксичної дії та наявності 3D структур комплексу було обрано три моноклональні антитіла: обінутузумаб, епратузумабута та атезолізумабу. Ідентифікація біологічної мішені проводилися шляхом пошуку 3D-структур обраних комплексів з білками-мішенями в базі даних Protein Data Bank. Для проведення віртуального скринінгу обрано веб-сервіс Pharmit. За допомогою програми Molecular Operating Environment було побудовано моделі фармакофорів для трьох комплексів: CD20 і обінутузумабу, CD22 і епратузумабу та PD-L1 і атезолізумабу. Докінг з білком CD20, CD22 і PD-L1 проведено за сайтами зв'язування, який впізнає оригінальне антитіло. Методом віртуального скринінгу *in silico* за допомогою програмного продукту Molecular Operating Environment здійснено пошук міметиків антитіл до мембранних білків В-клітин для лікування лімфоми Беркітта, в результаті чого відібрано 5 потенційних антилімфомних агента: CHEMBL505179 до рецептора CD20, до рецептора CD20 – антагоніст рецептору меланокортину (PubChem-44406884), до рецептора CD22 – інгібітор фактору згортання крові Xa (PubChem-136510605) та блокатор епітеліальних Na+ каналів (PubChem-126761430), до рецептора PD-L1 – агоніст рецептору меланокортину (PubChem-25078192). Отримані результати можуть бути застосовані у фармакологічній промисловості та онкологічній практиці з метою покращення терапевтичних результатів лікування хворих з лімфомою Беркітта

Ключові слова: молекулярний докінг; фармакофори; антитіла; міметики; імуногенність

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Microbiological diagnostics: From traditional to molecular genetic methods: A literature review

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Abstract. The development of new and optimisation of known cultural and molecular genetic methods for accurate species identification of microorganisms is an urgent and practically necessary task that is receiving great attention from researchers. The purpose of this study was to analyse and systematise theoretical scientific data on methods of microbial identification and assess their main advantages and disadvantages. For this purpose, a systematic review of 53 randomised research papers published between 2018 and 2023 was conducted. The search for publications using the keywords "microbiome", "microbiological diagnostics", "identification of microorganisms", "sequencing", and "omics technologies" in the title or text of the research paper was carried out in Web of Science, Scopus, PubMed, and Google Scholar. The study provides generalised information on traditional and modern methods of microbial identification. It is established that the advantages of traditional diagnostic methods include the possibility of preserving the obtained microorganisms for further research, in particular, for determining their antibiotic sensitivity. Modern molecular

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genetic methods open up new possibilities for the accurate identification of microorganisms, including those that are difficult to incubate using traditional cultural methods. The use of these methods allows obtaining detailed data on the genetic structure and diversity of microorganisms, which is important in many fields, including microbiology, medicine, and ecology. However, molecular methods are more sensitive to contamination or errors in the sample collection and processing. Moreover, omics technologies (genomics, transcriptomics, proteomics, and metabolomics) open up new opportunities for studying cells at more complex levels of life organisation. In everyday clinical practice, the microbiological method of isolating microbial cultures remains the main one, as it is the only one that allows determining not only the cause of the infection but also the antibiotic sensitivity. However, cultural methods for studying fastidious microorganisms are quite limited. Thus, the results obtained contribute to the development of fast and accurate methods for identifying, classifying, and systematising microorganisms, which facilitates the processes of diagnosis and development of strategies for the control and treatment of infectious diseases in laboratories and clinical institutions

Keywords: microbiology; diagnostics; nutrient medium; identification; sequencing; omics technologies

INTRODUCTION

Microorganisms can affect a person's life both positively and negatively. People have learned to use microorganisms in various areas of their lives. They are closely related to medicine, biotechnology, nutrition, genetic engineering, etc. As noted by Y. Gao *et al.* [1], the ability of bacteria and various types of fungi to produce antimicrobial substances makes them potentially useful in drug production. From the earliest childhood, the human body is also inhabited by a diverse community of microorganisms, which consists of bacteria, fungi, viruses, archaea, and is called the microbiome [2]. The human microbiome is a collection of microbiocenoses that colonise all surfaces of the human body, including the skin, respiratory system, gastrointestinal tract, and genitourinary system. These microorganisms perform a number of functions necessary for maintaining the homeostasis of the human body [3].

However, certain genetic features of bacteria make them dangerous to human health [4]. Antimicrobial resistance, which develops as a result of uncontrolled use of antibiotics, excessive use in animal husbandry and the food industry, poor infection control in hospitals and clinics, and lack of hygiene [5], are recognised as serious international problems. This problem makes it impossible to successfully treat many common bacterial infections and causes a growing number of deaths. A. Salmanov *et al.* [6] suggested possible ways to develop a multidisciplinary approach based on the principles of the "unified health" concept to solve problems related to antibiotic resistance.

Effective treatment becomes difficult without laboratory diagnostics, as the lack of diagnostic tests makes it difficult to establish any diagnosis. It is the qualitative diagnosis that determines the correct decisions of the doctor regarding the treatment methods used, evaluating their effectiveness, and in many cases preventing the occurrence of the disease due to the detection of pathology at an early stage of development [7]. Rapid identification of microorganisms plays a key role in the fight against infections. This helps to accurately identify the types of microorganisms that cause diseases and determine their sensitivity to antimicrobials. However, without rapid and accurate identification of infectious agents, complications associated with the wrong choice of antibiotic therapy for the patient may occur.

The range of diagnostic approaches used in microbiology is extremely wide and ranges from traditional to molecular genetic methods. Each method has its own strengths and weaknesses. Microbiological methods remain fundamental for diagnosing various types of infections in many laboratories. However, the limitations of these methods are the long time to obtain results, the possibility of obtaining false-negative results due to the use of antibiotics, and limited sensitivity to the detection of fastidious microorganisms [8]. Molecular methods are more accurate and specific for rapid diagnosis of aetiological pathogens of infections. M. Shevchenko *et al.* [9] showed the effectiveness of an optimised polymerase chain reaction protocol for identifying *Staphylococcus* spp bacteria, including the stage of detection in agarose gel and testing on clinical isolates from dogs. R. Symonenko [10] concluded that the use of modern methods of molecular diagnostics in periodontal tissue diseases allows quickly detecting periodontopathogens even in small quantities and identifying clinically significant types of microorganisms. In addition, this diagnostic method provides effective screening of periodontal tissue diseases. The study by I. Trubka *et al.* [11] analysed the microbiome of periodontal pockets of patients with rapidly progressive aggressive periodontitis using a molecular genetic method (Multident-5 test system). This approach identified the deoxyribonucleic acid (DNA) of the most clinically significant periodontopathogens in a single biological sample, providing a qualitative and quantitative assessment of the results. However, it is worth noting that although molecular methods open up new opportunities in microbiological diagnostics, their application in many countries with limited resources is quite difficult due to the high cost of this method.

In order to obtain the most accurate identification, classification, and taxonomy of microorganisms, it is extremely important to choose the appropriate methods and to have a thorough understanding of the mechanisms of their mechanisms of action. V. Motronenko & T. Vlasiuk [12] emphasised the importance of correctly interpreting the results obtained using culture and molecular methods in the context of accurate and rapid diagnosis of resistant forms of tuberculosis, and for ensuring effective treatment and care of patients. Microorganisms play a key role both for the benefit and harm of humans, and their identification is of great importance for modern medicine and biotechnology. Therefore, the purpose of the study was to analyse and systematise theoretical scientific data on methods for identifying microorganisms and evaluating their main advantages and disadvantages.

For this purpose, a systematic review of 53 randomised research papers published between 2018 and 2023 was carried out. The criteria for inclusion in the study included scientific publications in Ukrainian and English. The process of selecting publications included the stages of screening and quality assessment, where each paper was carefully analysed with regard to its scientific content and methodological aspects. Using electronic databases such as Web of Science, Scopus, PubMed, and Google Scholar, the authors searched for publications using the keywords "microbiome", "microbiological diagnostics", "microbial identification", "sequencing", and "omics technologies" in the title or text of the paper. It is important to note that the included papers were analysed according to various criteria, such as the year of publication and the main results. This approach helps to systematically summarise the literature data and provides a deeper look at the selected publications for the study.

TRADITIONAL METHODS OF MICROBIAL IDENTIFICATION

Most methods of isolation and identification of microorganisms remain unchanged and are based on the use of specific nutrient media that allow studying their various properties. Microorganisms differ in their needs, and therefore, there is no single environment or set of conditions that would ensure the growth of all species.

Conventionally, Nutrient agar, a universal medium, is used for the cultivation of microorganisms. By their composition, such nutrient media are designed for the cultivation of a wide range of microorganisms. Preparation of blood agar (BA) by adding 5% sheep blood to Nutrient agar expands the possibilities for studying and culturing those microorganisms that may require specific components or conditions for growth [13]. Thus, by the nature of haemolysis on BA, primary identification of streptococci is carried out. To identify certain infectious agents, it is important to use more specific nutrient media. For example, differential diagnostic nutrient media allow determining differences in the metabolic activity of microorganisms using systems of biochemical indicators. Nutrient media are also divided into selective ones, which are characterised by selective growth of a certain type of microorganism due to the addition of antibiotics to the nutrient medium. Metronidazole is often added to the nutrient medium for selective isolation of anaerobic microorganisms.

Chromogenic media are widely used in microbiological practice to identify specific types of microorganisms. The fluorogenic substrates present in these media are hydrolysed under the influence of specific enzymes produced by each type of microorganism. Hydrolysis of the fluorogenic substrate can lead to a change in the colour of the medium, which allows rapid and efficient identification of species [14].

After isolation of a pure culture, the phenotypic properties of the microorganism under study are determined for further identification. One of the main tests is the Gram stain method, which distinguishes between gram-positive and gram-negative microorganisms. The basic biochemical tests in clinical microbiology are the determination of catalase, oxidase, indole H_2S, etc. Most modern biochemical test kits include a combination of a wide range of biochemical determinations that are combined in a single test panel. This facilitates and speeds up the microbial identification process, providing faster and more accurate results.

Although traditional methods are available and provide information on the diversity of microorganisms in the sample, both quantitatively and qualitatively, the time required for identification based on traditional methods is estimated at a minimum of 2 to 5 days [15]. In addition, traditional methods do not provide an opportunity to identify uncultivated microorganisms. However, the advantages of this method are the ability to preserve the resulting microorganisms and test their sensitivity to antibiotics for effective treatment of the patient.

Immunological methods. Such immunological diagnostic methods in microbiology as agglutination, serology, immunoblotting, enzyme-linked immunosorbent assay (ELISA), etc., are worthy of attention. The basic principle of immunological methods is to investigate the interaction between an antibody and an antigen. These methods help in the identification of various microorganisms [16], but their use is accompanied by a number of limitations and disadvantages. Despite the high specificity of the analysis, this can lead to incorrect results or underestimate the presence of a pathogen. Some immunological methods are expensive, which can be a limiting factor for many laboratories due to the use of expensive equipment and reagents. In addition, the presence of chronic or immunodeficiency conditions can affect the accuracy and reliability of this method [17]. The unfavourable aspects of these methods do not reduce their importance as tools for studying and diagnosing microorganisms. Properly used immunological methods remain key in determining the immune response and identifying pathogens.

Mass spectrometry. Improvements in the identification of microorganisms have arisen from the need to reduce the time, cost, and simplify the procedures required to accurately identify these microorganisms. Therefore, the rapid development of mass spectrometry (MS) has led to its widespread use in microbiology [18]. The high speed, reduced cost, and ease of use of MS greatly facilitated and accelerated the identification of microorganisms compared to traditional cultural methods. The main principle of MS is the analysis of ions based on their mass-to-charge ratio (m/c), which allows studying the molecular structure, mass, and concentration of substances in various types of samples [19]. The combination of MS with gas or liquid chromatography significantly expanded the possibilities of this powerful method [20]. This has contributed to a better understanding of biological systems, allowing the analysis of a wide range of biomolecules.

The basic principle of gas chromatography mass spectrometry (GC-MS) is that first, using gas chromatography, the substances of the test sample are separated according to their properties. Next, the separated components are analysed in a mass spectrometer, where individual components are identified by their chemical properties [19]. This method is usually applied to identify microorganisms based on their lipid structures. Thus, S.P. Putri *et al.* [21] investigated 11 undetectable pathogenic Corynebacteria using GC-MS based on mycolic acid analysis. The effectiveness of the GC-MS method has also been demonstrated on yeast-like fungi [22]. The liquid chromatography mass spectrometry (LC-MS) method is based on the separation of substances in a sample using the liquid phase and the analysis of the mass and charge of ions in a mass spectrometer. At this stage, individual components are identified by their mass and chemical properties [20].

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is an advanced tool for the rapid and accurate identification and classification of bacteria [23], fungi [24], and viruses [25]. The principle of MALDI-TOF MS is to ionise microorganisms using laser radiation, which leads to the formation of ions. These ions are accelerated in a vacuum system by an electric field and move towards the mass spectrometer detector in a certain way. The detector registers their mass, and the obtained data are used to identify microorganisms [26]. Among other applications, MALDI-TOF MS is also used to identify various metabolites. For example, the study by J. Doellinger *et al.* [27], using MALDI-TOF MS, investigated enterotoxins produced by pathogenic strains of *Bacillus cereus*. MALDI-TOF MS is also used to quickly detect antibiotic resistance genes in bacteria [28], which allows improving treatment strategies for infections, especially those caused by polyresistant strains of microorganisms.

Bacterial identification using MALDI-TOF MS mainly focuses on improving methods for isolating and cleaning pathogens from clinical samples, expanding spectral libraries and updating software. With the development of technology, many MALDI-TOF MS-based microbial identification databases and systems have been licensed and put into clinical use. However, there is a need to further improve the antimicrobial resistance analysis based on MALDI-TOF MS to provide comprehensive clinical microbiological characterisation [29]. The application of MALDI-TOF MS for the analysis of small molecules is usually limited by the choice of a suitable matrix. Matrices used for large-molecule analysis are often not suitable for analysing low-molecular-weight compounds (m/c <1,000 Da) due to matrix background noise, ion suppression, and uneven crystallisation by traditional organic matrices.

MOLECULAR GENETIC METHODS FOR IDENTIFYING MICROORGANISMS

With the development of molecular biology, the possibilities for identifying microorganisms have significantly expanded. Many microbiological laboratories still use phenotypic and biochemical methods to identify microorganisms. However, the high specificity of molecular methods helps to accurately and quickly identify various cultivated and uncultivated species of microorganisms in any samples without the need to grow them on nutrient media in laboratories. Notably, the significant development of molecular methods helped to launch a large-scale study of the microbiome and its impact on human health in 2007, called the Human Microbiome project [30].

The polymerase chain reaction (PCR) method has become the gold standard in microbiological laboratories, which is used to identify microorganisms. The standard PCR method is based on the identification of bacterial DNA by increasing certain fragments of nucleic acids, that is, their amplification, followed by sequencing and comparing them with databases [31]. Due to the wide variety of microorganisms in biological samples, their identification by cultural methods becomes difficult due to the inability to isolate and identify uncultivated forms. PCR-based methods not only provide rapid identification, but also help identify microorganisms that are difficult to isolate in the laboratory [32]. However, despite its effectiveness, the PCR method has a number of disadvantages. The specificity of the search is that information can be obtained only about those microorganisms for which specific primers are used. PCR does not provide information about the viability of microorganisms, as it uses DNA or ribonucleic acid (RNA), which may be important in some cases. The lack of the possibility of preserving and further using microorganisms limits its use in some studies. In addition, it is worth noting that the method does not provide information about the sensitivity of microorganisms to antibiotics, which is an important aspect in clinical studies.

DNA microarray. The use of DNA microarrays allows simultaneously identifying and sequencing hundreds or thousands of genes in a single study. A DNA microarray is a DNA probe attached to a microplate. The operation of a DNA microarray is based on the phenomenon of hybridisation, during which labelled samples are analysed with fluorescent probes [33]. Unlike other molecular genetic methods, DNA microarrays have a larger coverage area and faster results. M.A. Campanero-Rhodes *et al.* [34] demonstrated the successful use of microarrays to detect type-specific immunoglobulin G (IgG) antibodies against *Streptococcus pneumoniae* capsule polysaccharide in serum (CPS). The results showed the potential of microarrays for simultaneous analysis of the interaction of multiple CPS using small volumes of serum, which can be useful for studying limited volumes of serum samples. In addition, according to Hu. Arengaowa *at al.* [35], the DNA microarray method has been successfully used in the food industry to detect food pathogens. Despite the successful use of the DNA microarray method, it has its drawbacks, which are expensive and the need for high-quality DNA samples. Contamination or poor sample quality can lead to erroneous results, which complicates the research process.

16S rRNA sequencing is one of the most sensitive methods for detecting microorganisms, which is widely used in clinical settings. It is based on the detection of the 16S rRNA gene, which is specific for each type of microorganism [36]. This method provides accurate identification of genera and species of microorganisms that do not meet any recognised biochemical profiles. A. Szymczak *et al.* [37] compared histological method, PCR, and 16S rRNA gene sequencing for identification of *Helicobacter pylori*. Using gastric antral biopsy, the researchers showed that 16S rRNA gene sequencing is the most sensitive method for detecting *H. pylori*. However, there are certain problems that can be encountered when using this method. One of them is that there are variations in the 16S rRNA gene among strains of the same species [38], which may affect the estimation of the abundance of these microorganisms.

Next-generation sequencing (NGS). Continuous improvement of DNA sequencing technology has significantly improved the capabilities of molecular genetic diagnostic methods. The main idea of using NGS is parallel sequencing of many fragments of DNA or RNA, which allows obtaining significant amounts of genetic information in a short period of time [39]. W. Gu *et al.* [40] developed a next-generation metagenomic sequencing test (mNGS) using cell-free

DNA from body fluids to identify pathogens. The results showed that rapid mNGS testing is an effective tool for diagnosing unknown infections. However, NGS research requires expensive equipment, timely and sustainable maintenance, and training of technical specialists, which can be a problem even for middle-income countries [41].

Whole-genome sequencing (WGS) is the most accurate method for identifying microorganisms and is an important tool in microbiology [42]. This method expands the possibilities for analysing genes associated with antibiotic resistance [43], identifying their pathogenicity [44], and other key aspects, making an important contribution to understanding microbial ecology. Despite these advantages, the method has its drawbacks. It is considered the most expensive and time-consuming; it does not provide information about the viability of microorganisms, since it is based on DNA/RNA analysis. In addition, it provides only a correlation of microorganisms, and identification problems are associated with the lack of complete databases of genetic material.

Despite determining the microbial composition of an individual, it is important to understand the functions that bacteria perform in this community, their relationships, and their impact on the host [45]. The rapid development of molecular biology and information technology methods has contributed to the use of various omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics [46]. Omics technologies open up new opportunities for cell research at more complex levels of life organisation.

Genomics is a set of methods that are based on the investigation of the structure and functions of the genome of the studied objects [47], whereas transcriptomics methods focus on studying the transcriptome of an organism, that is, the sum of all its RNA transcripts [48]. Genomics and transcriptomics are two related but different areas of research that help uncover and understand genetic information and gene activity in the body. Proteomics examines the structure and function of all proteins in the body [49]. It helps to uncover the role of proteins in signalling interaction, vital activity, and detection of diseases such as Alzheimer's disease [50]. Metabolomics represents the latest branch of omics technology and is extremely promising for medicine. The main task of metabolomics is to identify and quantify a wide range of metabolites [51]. Detection of specific metabolic changes can serve as an indicator of the development of various diseases, in particular, in diabetes mellitus [52] or in chronic obstructive pulmonary disease [53].

Investigation of the microbial composition of an organism is a key to understanding the relationship between microorganisms and their impact on the host. The use of omics technologies provides new opportunities for studying life systems at different levels of their organisation. The integration of the data obtained with the help of these technologies helps to solve problems related to various pathological conditions of humans and the environment.

CONCLUSIONS

The study analysed and systematised the available methods for identifying microorganisms in clinical diagnostics. Analysing an overview of up-to-date data, it can be concluded that in everyday clinical practice, the microbiological method of isolating microbial cultures remains the main one. This method not only determines the cause of infection, but also helps to determine sensitivity to antibacterial drugs, contributing to the effective treatment of various infections. However, cultural methods for studying fastidious microorganisms are quite long and complex. Immunological methods based on the interaction of antibodies with foreign antigens are widely used in microbiology to identify individual species and serotypes of microorganisms. Despite their speed and simplicity of research, it is important to consider some of the disadvantages of this method. Immunological tests may be vulnerable to changes in the spectrum of antigens or antibodies, which can lead to false results. In addition, the presence of chronic or immunodeficiency conditions can affect the accuracy and reliability of this method.

Compared to traditional methods of microbiological diagnostics, molecular biology methods are characterised by higher sensitivity and specificity. The high sensitivity of these methods allows detecting microorganisms even with a minimal number of them. In particular, the greater specificity of molecular methods helps to more accurately identify a specific type or strain of microorganism. However, molecular genetic methods for identifying microorganisms also have drawbacks. High cost, expensive equipment, and the need for specially trained personnel limit their availability for many laboratories. In addition, molecular genetic methods do not provide information about whether the microorganism is alive in the test sample.

Omics technologies (genomics, transcriptomics, proteomics, metabolomics) are a group of high-performance methods that study large amounts of biological information at various levels of molecular structure and function, which provides a deep understanding of complex biological systems. The disadvantage of omics technologies and molecular genetic methods is their high cost and the need for complex equipment and interpreting large amounts of data requires specialised skills and bioinformatic knowledge.

The results obtained contribute to the choice of a fast and accurate method for identifying, classifying, and systematising microorganisms in accordance with specific needs. In particular, the ability to quickly identify microorganisms in various infectious conditions can significantly speed up the choice of the optimal antibiotic therapy regimen for doctors and provide more effective treatment for patients. Further research may include the development of new methods that combine the benefits of microbiological, immunological, molecular genetic, and omics technologies to improve the accuracy, speed, and accessibility of infectious disease diagnostics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Мікробіологічна діагностика: від традиційних до молекулярно-генетичних методів: огляд літератури

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Анотація. Розробка нових та оптимізація відомих культуральних та молекулярно-генетичних методів для точної видової ідентифікації мікроорганізмів є актуальним і практично необхідним завданням, якому приділяється велика увага дослідників. Метою даної роботи було проведення аналізу, систематизація теоретичних наукових даних щодо методів ідентифікації мікроорганізмів та оцінка їхніх основних переваг і недоліків. Для цього було здійснено систематичний огляд рандомізованих 53 наукових робіт, опублікованих в період з 2018 по 2023 роки. Пошук публікацій із використанням ключових термінів «мікробіом», «мікробіологічна діагностика», «ідентифікація мікроорганізмів», «секвенування» та «омікс-технології» у назві чи тексті науково-дослідницької роботи здійснювали в системах Web of Science, Scopus, PubMed та Google Scholar. У статті надано узагальнену інформацію щодо традиційних та сучасних методів ідентифікації мікроорганізмів. Встановлено, що до переваг традиційних методів діагностики належить можливість збереження отриманих мікроорганізмів для подальших досліджень, зокрема для визначення їх чутливості до антибіотиків. Сучасні молекулярно-генетичні методи відкривають нові можливості для точної ідентифікації мікроорганізмів, зокрема тих, які складно культивувати за допомогою традиційних культуральних методів. Використання цих технік дозволяє отримати детальні дані про генетичну структуру та різноманіття мікроорганізмів, що є важливим у багатьох галузях, включаючи мікробіологію, медицину та екологію. Проте, молекулярні методи більш чутливі до забруднень чи похибок у процесі збирання та обробки зразків. Варто також відмітити omics-технології (геноміка, транскриптоміка, протеоміка та метаболоміка), які відкривають нові можливості для дослідження клітин на більш складних рівнях організації життя. В повсякденній клінічній практиці основним залишається мікробіологічний метод виділення мікробних культур, оскільки тільки він дозволяє визначити не лише причину інфекції, але й чутливість до антибіотиків. У той же час культуральні методи дослідження вибагливих мікроорганізмів досить обмежені. Таким чином, отримані результати сприяють розробці швидкої та точної методики ідентифікації, класифікації та систематизації мікроорганізмів, що полегшує процеси діагностики та розробки стратегій контролю і лікування інфекційних захворювань у лабораторіях та клінічних установах

Ключові слова: мікробіологія; діагностика; поживне середовище; ідентифікація; секвенування; омікс-технології

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Morphofunctional state of the kidneys of laboratory rats during acute respiratory distress syndrome

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Abstract. One of the most common complications of acute respiratory distress syndrome is acute kidney injury, the mechanisms of development of which remain not completely clarified. The purpose of this study is to examine morphofunctional changes in the kidneys of rats with induced acute respiratory distress syndrome at various time intervals after modelling the pathology. For the study, 56 healthy sexually mature male rats weighing 200-220 g were used, divided into 7 groups: control, 6, 24 hours, 3, 7, 14, and 28 days of the experiment. Respiratory distress in animals was caused by inhalation of lipopolysaccharide (5 mg/kg body weight). The kidneys of intact rats had a typical histological structure without specific features. Histological changes in the renal parenchyma of rats in the study groups included compaction of Malpighian bodies, damage and desquamation of epithelial cells of the nephron tubules, and the appearance of signs of disseminated intravascular coagulation. A month after the start of the experiment, both pathological changes in the nephrons and restored or preserved structural components of the kidney are observed, which indicates activation of intracellular reparative processes. The expression of TGF-β1 fibrosis marker as well as CD68 panmacrophage marker increased on days 3 and 7 of the experiment. The number of macrophages in the kidney samples remained consistently high until the end of the experiment, while the level of TGF-β1 decreased on day 28, indicating the start of the resolution phase. Biochemical analysis of renal markers showed an undulating course of inflammatory processes in the kidneys of experimental rats. The maximum concentration of creatinine, urea, and uric acid in the blood serum was observed at 24 hours of the experiment, which indicated the onset of acute kidney injury as a complication of respiratory distress. Preclinical examination of morphofunctional changes in the kidneys during acute respiratory distress syndrome will help choose an effective method for treating this pathological condition in humans in the future

Keywords: lipopolysaccharide; acute kidney injury; renal tests; histological analysis; immunohistochemistry

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a life-threatening condition characterised by insufficient oxygenation and non-compliant or stiff lungs. The disease is associated with damage to the capillary endothelium and diffuse alveolar damage. Studies on the mechanisms and consequences of the development of acute respiratory infections have become particularly relevant during the COVID-19 pandemic. Critically ill patients with an unfavourable prognosis were diagnosed with ARDS, which led to high mortality. However, today there are no effective therapeutic methods to treat this serious condition [1].

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Among the many complications of ARDS, acute kidney injury (AKI) is one of the most common. It is known to cause a rapid deterioration of renal function within a few hours or days [2, 3]. AKI, also known as acute renal failure, is a common clinical disorder that occurs due to certain conditions, such as ischemia/reperfusion injury of the kidney with an acute loss of organ function and a decrease of the renal filtration fraction [4-6].

M. Malek *et al.* [4] note that ARDS provokes an increase in circulating cytokines, chemokines, and activated immunocytes, initiating a pathological cascade that, in turn, leads to the development of AKI. R. Fenoglio *et al.* [5] indicate that AKI is caused by a damage of the renal vascular system and a decrease in glomerular filtration rate due to the release of endothelin factor 1. In more severe cases of nephrotic syndrome, renal ischemia occurs, which reduces the afferent arteriolar blood supply to the glomerulus. Such microvascular lesions, together with inflammatory interstitial fibrosis and epithelial damage, impair the viability of the nephron tubules.

According to the data provided by A. Hosszu *et al.* [6], in humans, the ischemia/reperfusion injury of the kidney leads to the loss of the brush border, the appearance of focal expansion zones in the proximal tubules, and the accumulation of necrotic casts in the distal tubules. Most often, epithelial cells undergo apoptosis or necrosis in very susceptible external medullary areas.

There are various data on the incidence of AKI as a complication of ARDS. According to S.M. Villacrés *et al.* [7], 35% of adult patients with ARDS develop AKI. Therewith, M. Malek *et al* [4] indicated substantially lower rates of AKI in hospitalised patients with ARDS – 7-10%. According to the latest data from the ARDSnet trail meta-analysis, the incidence of AKI as a complication of ARDS was ≈ 24% [8]. Severe renal damage leads to death in almost half of cases, and other devastating long-term consequences of this condition include end-stage renal failure and dialysis dependence [9, 10].

AKI, which complicates ARDS, often indicates a negative prognosis. In the ARDSnet study, the 180-day mortality rate was much higher in those who had AKI compared to those who did not develop this complication (58% and 28%, respectively) [8]. One retrospective study demonstrated an increase in the number of days of artificial lung ventilation (ILV) (10 vs. 7 days) and the duration of switching to independent breathing (41 vs. 21 hours) in patients with ARDS complicated by AKI compared to ARDS alone [11]. A. Panitchote *et al*. [12] concluded that two-thirds of patients with ARDS developed AKI during intensive care, and almost half of these patients progressed to stage III of AKI according to the Kidney Disease: Improving Global Outcomes (KDIGO) classification.

The exact underlying mechanisms of AKI development in patients with ARDS are still unclear and are, therefore, the subject of increased scientific attention. The authors of this study planned a preclinical study of histological changes in the kidneys of rats with ARDS induced in different time periods after modelling the pathology to elucidate the subtle mechanisms of kidney damage and select effective methods for treating AKI during respiratory distress syndrome.

MATERIALS AND METHODS

56 healthy sexually mature male Wistar rats were used for experiments. At the beginning of the experiment, the average body weight of animals was 200-220 g. Rats were kept under standard vivarium conditions (12-hour day/night cycle; t = 20-25 °C; humidity 40-45%) [13], with free access to water and food. Experiments were conducted in the summer. The work with animals was organised in compliance with the principles of the "European Convention for the protection of vertebrates used for experimental and scientific purposes" [14] and the definition of the first National Congress on Bioethics [15].

ARDS was modelled by intranasal administration of lipopolysaccharide (LPS) (SIGMA-ALDRICH), previously diluted with an isotonic sodium chloride solution (1 mg of LPS in 4 mL of NaCl). Using a nebuliser, animals were given LPS at a dose of 5 mg/kg of body weight for 30 minutes. During inhalation, rats were anaesthetised by a peritoneal injection of ketamine at a dose of 50 mg/kg. The animals were randomly divided into 7 groups (8 animals each). Morphological changes in the kidneys were analysed after 6 hours, 24 hours, 3 days, 7 days, 14 days, and 28 days of the experiment. Animals were removed from the experiment by terminal anaesthesia with sodium thiopental at a dose of 150 mg/kg. ARDS modelling was done in the vivarium of I. Horbachevsky Ternopil National Medical University (TNMU).

For histological analysis, pieces of the left kidney were fixed in 10% neutral buffered formalin. Then the tissues were processed in a LOGOS One histoprocessor (Milestone Medical, USA) and embedded into paraffin blocks. 5 microns thick sections obtained on the AMR400 rotary microtome (Amos scientific, Australia) were stained with hematoxylin and eosin [16].

Immunohistochemical evaluation of the expression of transforming growth factor beta 1 (TGF-β1) and CD 68 panmacrophage marker was performed using recombinant rabbit monoclonal primary antibodies (Cat. No. ab215715; No. ab125212, Abcam, USA, respectively) and Mouse/Rabbit PolyVue™ HRP/DAB detection systems (Diagnostic BioSystems, USA). The tissue sections were deparaffinised and rehydrated. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Additionally, the sections were counter-stained with Mayer hematoxylin. Immunohistochemical changes in the kidneys were analysed at 24 hours, 3 days, 7 days, and 28 days of the experiment. These time periods correspond to different phases of ARDS development (acute (24 hours – 3 days), subacute (7 days), and chronic (28 days)) [17]. Histological and immunohistochemical samples were examined under an Eclipse Ci-E light microscope and documented using an M3CMOS 14000 camera (Sigeta, Ukraine) at the laboratory of immunohistochemical and immunocytochemical studies of the experimental sector of the interdepartmental training and research laboratory (ITRL) of TNMU.

Rat blood was taken through a cardiac puncture. The serum was obtained through blood centrifugation and stored at – 80°C until analysis. Serum creatinine, uric acid, and urea levels were determined according to the manufacturer's instructions using Spinreact (Spain) kits. The analyses were conducted on the basis of MNL TNMU.

The study was conducted within the framework of the interdepartmental state-funded comprehensive research project of I. Horbachevsky Ternopil National Medical University "Investigation of the regenerative potential of cellular therapy agents in acute respiratory distress syndrome" (state registration number 0121U100159), 2021-2023.

RESULTS

Histological examination of the kidneys of intact rats did not reveal any pathological changes. Light microscopy examination of the renal cortical substance in intact rats revealed numerous renal (Malpighian) bodies, in which the urinary lumen of the Bowman capsule was clearly visualised. The proximal convoluted tubules were lined with a single-layer prismatic epithelium with a brush border and basal striation. In the distal convoluted tubules, the epithelium was simple cuboidal without a border with deep basal invaginations of the cell membrane. In the interstitium between the tubules of the nephrons, the lumen of the peritubular capillaries was visible (Fig. 1).

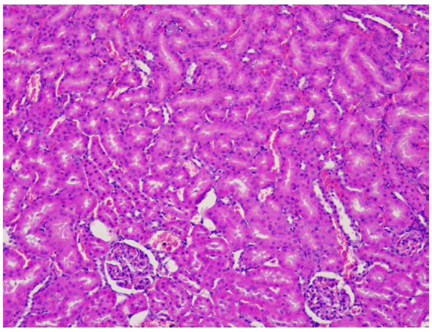

Figure 1. Microscopic structure of the cortical substance of the kidney of an intact rat. H&E staining (x200 magnification)
Source: photographed by the authors

Moderately blood-filled hemocapillaries of the peritubular network were visible in rats with simulated ARDS after 6 hours of the experiment. The epithelium of the proximal tubules was relatively preserved. There was no oedema. Renal corpuscles were deformed with partially blood-filled capillaries. The lumen of the Bowman capsule was narrowed, compaction of the vascular glomerulus is observed. Damage to the apical surface of distal epithelial cells and an enlarged lumen of the tubules were identified. In the proximal tubules, in some places, desquamation of the epithelium was registered. Dilated and blood-filled venous vessels with clotted lumen, oedematous intima, and spasmodic arteries were observed (Fig. 2).

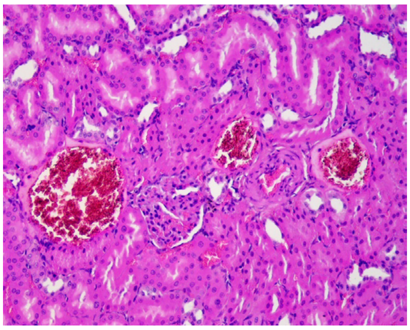

Figure 2. Microscopic structure of the rat kidney at 6 hours of the experiment. H&E staining (x200 magnification)
Source: photographed by the authors

One day after the start of the experiment, signs of stasis were observed in small calibre vessels. The urinary space of the Bowman capsule was practically not visible, the vascular glomeruli were compacted, the lumen of the nephron tubules was not visible due to the accumulation of cellular debris in the proximal region and oedema of the lining epithelium in the distal region (Fig. 3).

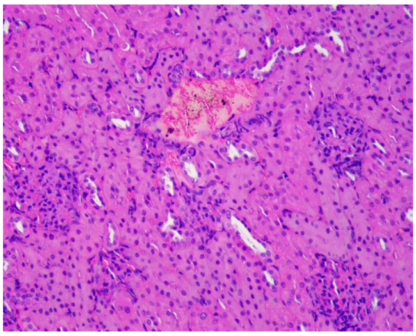

Figure 3. Histological structure of the rat kidney on day 1 of the experiment. H&E staining (x200 magnification)
Source: photographed by the authors

3 days after LPS administration, dilated blood-filled veins with a damaged wall and signs of disseminated intravascular coagulation (DIC) syndrome were identified. Arteries were also spasmodic. Oedema and homogenisation of epithelial cell cytoplasm were observed in the nephron tubules (Fig. 4). The lumen of the Bowman capsule was not visible, which was obviously an indicator of the impaired functional ability of the glomeruli to filter blood plasma.

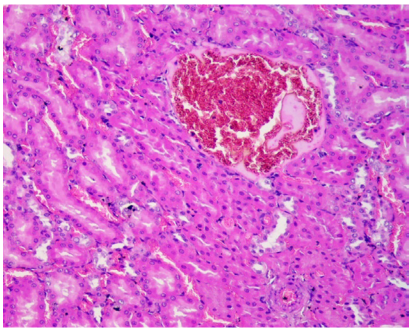

Figure 4. Microscopic structure of the rat kidney on the 3rd day of the experiment. H&E staining (x200 magnification)
Source: photographed by the authors

On the 7th day of the experiment, blood-filled arterial and venous vessels of the renal vascular system with substantially dilated lumen were observed (Fig. 5A). Renal corpuscles contained compacted vascular glomeruli; desquamated cells were visible in the proximal tubules as a result of a disruption of intercellular contacts in the epithelial lining of the tubule (Fig. 5B). 2 weeks after the start of the experiment, signs of DIC continued to be recorded in the vessels of the cortex and medulla of the organ, and accumulation of degeneratively altered epithelial cells in the lumen of the nephron tubules was detected (Fig. 6).

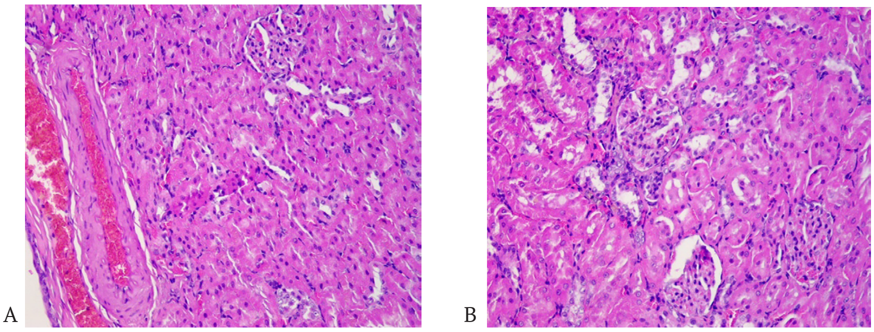

Figure 5. Microscopic structure of the rat kidney on the 7th day of the experiment. H&E staining (x200 magnification)
Notes: A – signs of blood stasis in the vessels of the renal cortex, B – disruption of contacts between epithelial cells of the proximal tubules
Source: photographed by the authors

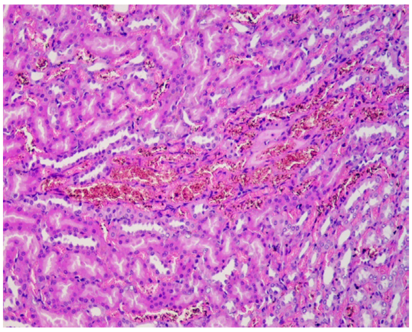

Figure 6. Tubulointerstitial lesions of the rat kidney on the 14th day of the experiment. H&E staining (x200 magnification)
Source: photographed by the authors

On the 28th day of the experiment, less substantial lesions of the vascular bed were observed, which in some places were manifested by blood filling and stasis. The appearance of oedema of the renal stroma and the dilatation of perivascular spaces were registered. The renal corpuscles were of different sizes. Some of the Malpighian bodies had well-defined vascular glomeruli and the urinary lumen of the Bowman capsule, while irregularly shaped Malpighian bodies with damaged and compacted glomeruli were seen nearby. A number of the proximal tubules of the nephrons contained desquamated epithelium (Fig. 7A, 7B).

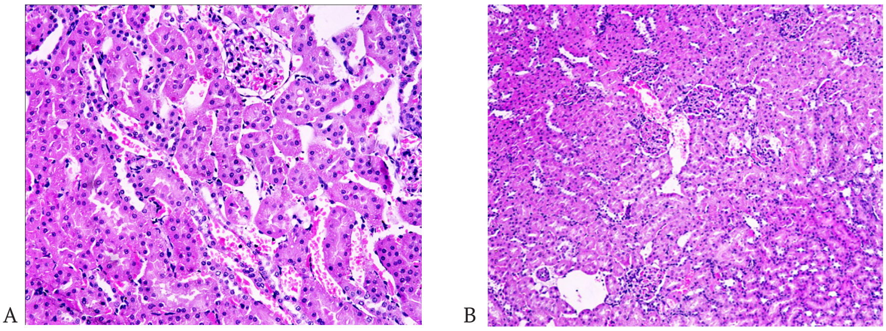

Figure 7. Microscopic structure of the rat kidney on the 28th day of the experiment H&E staining
Notes: A – x200 magnification; B – x100 magnification
Source: photographed by the authors

The development of pathological changes in the renal parenchyma of rats with simulated ARDS occurred gradually. At 6 hours of the experiment, the structure of the nephrons remained relatively unchanged with mild damage to the renal bodies, while at 24 hours, narrowing of the urinary space of the Bowman capsule in the renal bodies appeared, and on the 3rd day and later compaction of the Malpighian bodies indicated impaired plasma filtration. The degree of desquamation of epithelial cells of the nephron tubules also increased with the time of the experiment, and this, in turn, indicated the impairment of the reabsorption of organic components and electrolytes from the primary urine. Therewith, changes in the vascular bed were also documented in the kidneys of experimental animals. In the initial stages (6-24 hours), small vessels of the kidneys were dilated and filled with blood, while on the 3rd day, signs of DIC appeared in the microcirculatory bed. On day 7 and later, stasis in the renal vessels of various calibres was still observed. One month after the start of the experiment, both degenerated renal corpuscles and desquamated epithelium in the nephron tubules as well as the restored structural components of the kidney were observed, indicating activation of intracellular reparative processes in the resolution phase.

Immunohistochemical detection of TGF-β1 in the kidneys of control rats showed a weak response, mainly in the mesangium of Malpighian bodies (Fig. 8A). After staining the material with CD68 macrophage marker, a similar pattern was observed. In addition, an intense positive staining was identified in the area of large vessels (veins) as well as a slight staining of the interstitial space (Fig. 8B)

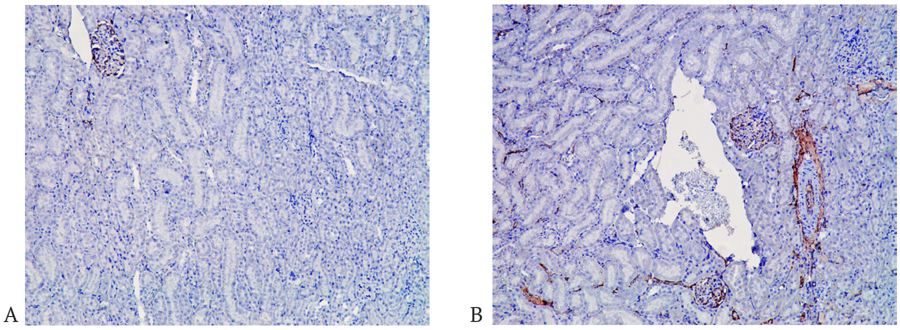

Figure 8. Immunohistochemical staining of intact rat kidneys. x100 magnification
Notes: A – TGF-β1 staining; B – CD68 staining
Source: photographed by the authors

TGF-β1- and CD68-positively stained areas around small calibre vessels are detected in kidney sections of experimental animals 24 hours after ARDS modelling, largely, when stained with TGF-β1. Immunoprecipitation is mostly visible around large vessels (veins). An immunopositive response to TGF-β1 is also observed in the interstitial space (Fig. 9A, 9B).

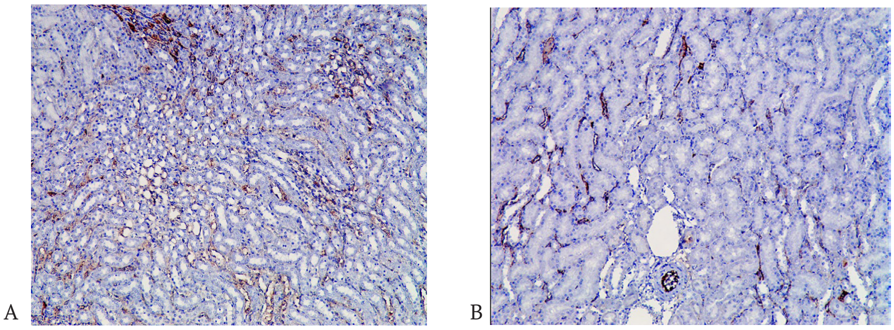

Figure 9. Immunohistochemical staining of kidneys on day 1 of the experiment. x100 magnification
Notes: A – TGF-β1 staining; B – CD68 staining
Source: photographed by the authors

In the kidneys of animals on day 3 of ARDS there was the maximum number of intensely TGF-β and CD68 immunostained perivascular spaces both around the nephron tubules and in Malpighian bodies compared to the control group (Fig. 10A, 10B).

A similar pattern was observed in the kidneys of rats 7 days after the simulated pathology (Fig. 11A). In the case of CD68 staining, a more intense positive staining of the walls of small vessels and intercellular space was also detected at this time point comparing to day 3 of the experiment (Fig. 11B).

On the 28th day of the experiment, mesangium of Malpighian bodies was intensively TGF-β1 immunostained. Immunoprecipitate was also visible although to the lesser extent in the intercellular space of the interstitium (Fig. 12A). CD68 staining revealed a pronounced immunopositive reaction in the vascular areas of the microcirculatory vascular bed and perivascular space (Fig. 12B).

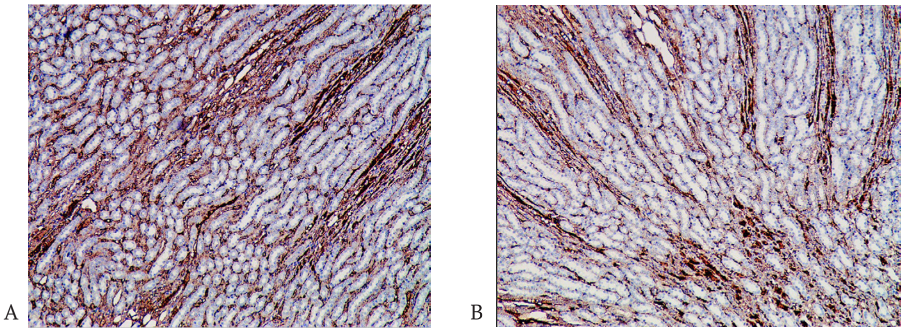

Figure 10. Immunohistochemical staining of kidneys on day 3 of pathology. x100 magnification
Notes: A – TGF-β1 staining; B – CD68 staining
Source: photographed by the authors

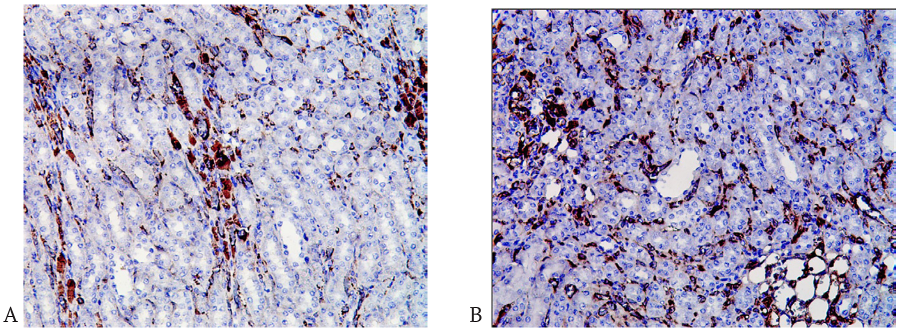

Figure 11. Immunohistochemical staining of kidneys on day 7 of ARDS. x200 magnification
Notes: A – TGF-β1 staining; B – CD68 staining
Source: photographed by the authors

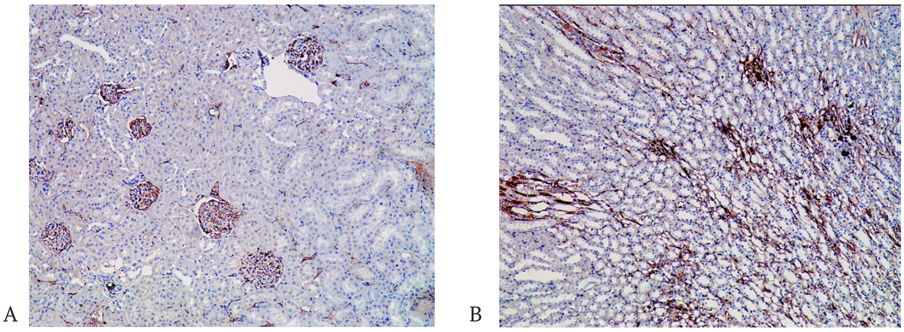

Figure 12. Immunohistochemical staining of kidneys on day 28 of ARDS. x100 magnification
Notes: A – TGF-β1 staining; B – CD68 staining
Source: photographed by the authors

Thus, immunohistochemical analysis of the fibrosis marker TGF-β1 showed an increase in the intensity of immunoprecipitation in kidneys. The most intense expression of TGF-β1 was observed in the subacute stage of ARDS (3-7 days). In the chronic stage (28 days), the intensity of interstitial immunostaining decreased but remained pronounced in the renal corpuscles. This may indicate the development of fibrosis in the mesangia between the capillary loops of the vascular glomerulus. Immunohistochemical detection of the CD68 panmacrophage marker showed an increase in the number of macrophages on day 3 after ARDS modelling, after which the number of these cells remained elevated in the kidney until the end of the experiment.

A biochemical blood test of experimental animals allowed assessing changes in the concentrations of renal markers. The obtained results are shown in the diagrams (Fig. 13A, 13B, 13C).

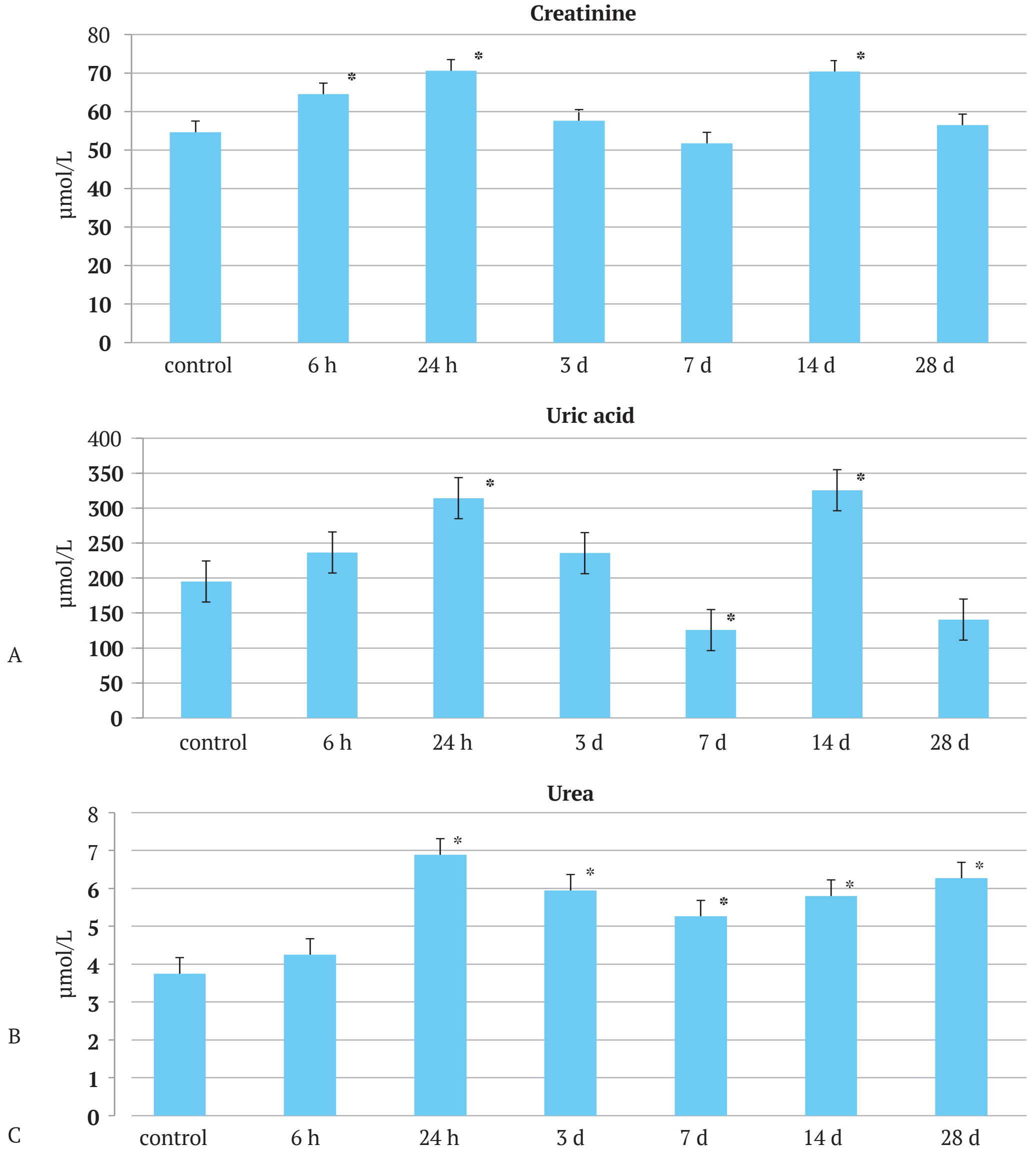

Figure 13. Changes in serum renal marker concentrations after ARDS modelling

Notes: A – change in creatinine concentration; B – change in uric acid concentration; C – change in urea concentration; * – statistically significant difference compared to the control (P⩽0.05)

Source: compiled by the authors

Analysis of serum creatinine, uric acid, and urea levels identified their increase in groups of rats with simulated ARDS. In particular, creatinine increased by 1.2 times in the 6th hour compared to the control, reached the first peak at the 24th hour (1.3 times more than the control value), decreased to the control level on the 3rd day, and remained reduced on the 7th day of the experiment. The second peak of creatinine growth was observed on the 14th day (1.29 times higher than the control), and normalisation of the indicator was recorded a month after the onset of pathology development.

An increase in uric acid concentration was observed starting from the 6th hour, with the first peak on the 24th hour (1.6 times higher than the control) and the second peak on the 14th day (1.7 times higher than the control). A statistically significant decrease (1.6 times) in uric acid concentration occurred on the 7th day of the experiment, while on the 28th day of the experiment, the uric acid level was close to the control value. A pathological decrease in uric acid levels 7 days after ARDS modelling may indicate a malfunction of the liver, which leads to its inability to convert amino acids and ammonia into urea.

The level of urea in the blood serum of rats increased slightly at the 6th hour, rapidly peaked at the 24th hour (1.8 times higher than the control value), slightly decreased at the 3rd and 7th days, but remained statistically substantially higher than in intact animals. After 2 weeks and a month from the start of the experiment, the urea concentration remained substantially higher than the baseline level (1.5 and 1.7 times higher, respectively), which indicates the development of chronic kidney damage in laboratory animals.

The results of a biochemical blood test confirm the occurrence of functional disorders of the kidneys caused by the development of simulated ARDS. Undulating changes in creatinine, urea, and uric acid concentrations indicate that the development of kidney damage was non-linear, with several peaks in the activation of inflammatory processes. The maximum increase in renal marker values in rat blood serum 1 day after ARDS modelling indicates the development of acute kidney injury, which gradually turned into chronic damage.

DISCUSSION

It is known that ARDS occurs in 3 stages: the first phase is acute, or exudation, the second is subacute or fibroplasia, and the third stage is chronic, fibrosis, or resolution [17, 18]. As shown by the results of histological examination of the kidney and biochemical blood analysis in rats, the acute stage is observed from 6 hours to 3 days of the experiment. The subacute stage corresponds to 7-14 days of experiment, while the third phase occurs a month after the onset of pathology. According to the data obtained, the most pronounced signs of AKI appear on 1-3 days of the experiment.

According to R. Vashisht & A. Duggal [19], AKI is observed in ≈ 45% of patients with ARDS, and on average, its signs start to appear 2 days after the initial diagnosis. ARDS causes impaired gas exchange in the lungs, which leads to the development of hypoxemia, hypercapnia and systemic acidosis, and this, in turn, affects vascular resistance, altering renal perfusion pressure and leading to damage to kidney components [20]. Systemic inflammation, hypoxemia, and ILV are considered to be the three main possible mechanisms of renal damage development in ARDS in humans [18, 21]. High alveolar pressure has been shown to alter the hemodynamics of the heart, leading to hypoperfusion in all organs of the body, especially in the kidneys, leading to a decrease in glomerular filtration rate and the development of AKI [19, 21, 22].

According to the report of the pathomorphological study by M. Shao *et al*. [23], acute renal tubule damage was observed in six cases of patients who died from COVID-19. According to H. Su *et al.* [24], in 26 patients with ARDS developed in severe COVID-19, noticeable acute damage to the proximal tubules of the nephrons was identified on the autopsy, which manifested itself in the form of loss of the brush border, vacuolar degeneration, dilation of the lumen of the tubules with cellular debris, and in some cases outright necrosis and detachment of the epithelium with the exposed basement membrane of the tubules (in 4 patients). Similar morphological signs of kidney parenchyma damage were observed in this study.

In the preclinical study by W. Lv *et al.* [25], AKI was induced by peritoneal administration of LPS. As shown in the histological analysis of changes in the kidneys of rats after modelling the pathology, the structure of the organ in the experimental groups of animals became damaged, oedema of epithelial cells of the renal tubules appeared, as well as the narrowing of the lumen of the tubules, renal tissue was infiltrated by a large number of inflammatory cells in contrast to the kidneys of intact rats. Such pathological changes gradually worsened with the duration of the experiment (8 hours, 12 hours, 24 hours, and 48 hours), and the rate of damage to the renal tubules correspondingly increased. H. Gao *et al.* [26], analysing histological sections of rat kidneys in LPS-induced AKI, identified vacuolar degeneration and damage to the brush border in epithelial cells of the renal tubules. In addition, researchers also observed necrosis of epithelial cells of the nephron tubules.

Examinations of AKI in severe cases of COVID-19 have shown that the main mechanism of this complication is similar to severe sepsis. Acute tubular necrosis in the renal parenchyma was reported in 66% of cases [27]. Cytokine storm caused by ARDS causes hypotension, which leads to decrease of renal perfusion [28]. In addition, focal renal fibrin blood clots were located in histological samples of the kidneys, which appear as a result of systemic coagulopathy. Regardless of the root cause, after the development of acute kidney injury, the intrarenal inflammatory cascade is activated, which, if left unchecked, leads to additional damage and irreversible fibrosis.

According to a number of authors, TGF-β is the main regulator of renal inflammation and fibrosis [29, 30]. TGF-β has a multifunctional effect on cell proliferation, apoptosis, migration, and differentiation. Under the influence of this growth factor, mesenchymal transformation of epithelial cells of the tubules and glomeruli of the kidney is observed, which leads to the development of fibrosis [30]. According to Y. Isaka [31], TGF-β1 is highly expressed in the kidneys during a wide range of fibrosis-related diseases. According to the results of immunohistochemical analysis, TGF-β1 was most strongly detected in the kidneys on the 3rd day of ARDS development. At a later stage of the experiment, a decrease in the expression of this marker indicated the resolution of AKI without the development of pronounced fibrosis. This means that the rats' bodies gradually coped with the inflammatory cascade and managed to start the recovery processes in the damaged kidneys. O. Redko *et al*. [32], in their studies on the effect of ARDS on the morphofunctional state of rat liver, showed that in the early stages of pathology, an increased number of M1 macrophages is observed, which indicates the activation of a pro-inflammatory response in the body, while in the later stages of the disease, M2 macrophages with anti-inflammatory function, and the ability to promote tissue repair begin to elevate. These data are consistent with the results of this study showing that the third stage of development of pathology in the kidneys in ARDS follows the path of resolution, and not fibrosis.

In addition to the fibrosis marker, the expression of CD68 panmacrophage marker, which indicates the total number of macrophages in kidney tissue, was analysed. The obtained data indicate a steady increase in phagocytes in the studied organ, starting from the 3rd day of the experiment. The increased number of macrophages in the late stages of ARDS may be due to the appearance of a large number of anti-inflammatory M2 cells. H. Akdam *et al*. [33] also reported an increase in macrophage count during necrotising glomerulonephritis, which positively correlated with an increase in serum creatinine levels.

Biochemical parameters of creatinine, urea, and uric acid in the blood are important markers of kidney injury [34]. Elevated levels of these renal markers were identified, which increased in two peaks (at 24 hours and on 14 days, respectively). This indicates that LPS successfully induced AKI in rats. Changes in these indicators indicate the development of functional disorders in the kidneys. In particular, elevated serum creatinine levels indicate a disruption of glomerular filtration rate [35-37].

W. Lv *et al*. [25] established a substantial increase in serum creatinine and urea levels 8 hours after toxin injection and a peak value 12 hours later, which was three times higher than the baseline level of the control group. However, levels of these renal markers gradually decreased 24 hours, 48 hours, and 72 hours after LPS administration and returned to baseline levels after 7 days. Interestingly, creatinine increased slightly on day 14 after LPS administration, which corresponds to the stage of transition of AKI to CKD. Such observations are consistent with the results obtained in this study.

F.Z. Khamissi *et al.* [38] exposed mice of the C57BL/6 line to bilateral ischemia/reperfusion kidney injury and analysed signs of AKI. Kidney damage caused a substantial increase in blood urea nitrogen levels on day 1 after the development of AKI, indicating severe renal failure, with a slow improvement of about 50% over 3-5 days. Accordingly, serum creatinine levels were substantially elevated on the first day and remained elevated at a lower level for 3-5 days.

A study by S. Liu *et al.* [39] showed that the development of AKI in sepsis and ARDS is also accompanied by an increase in uric acid levels in the blood of patients. Hyperuricemia promotes damage and apoptosis of vascular endothelial cells by increasing oxidative stress, which increases the formation and elimination of reactive oxygen species, causes endoplasmic reticulum dysfunction, and inhibits the activity of endothelial nitric oxide synthase. In addition, elevated uric acid levels promote the formation of sodium urate crystals in vascular intima, enhance the expression of leukocyte adhesion molecules, and stimulate the production of a large number of associated inflammatory factors, further enhancing endothelial cell dysfunction [39, 40]. Thus, based on the results of histological and histochemical analysis of the kidneys and biochemical blood analysis, it was established that ARDS is accompanied by morphofunctional changes in the kidneys, and this fact is an acute medical problem that needs to be solved by developing an effective treatment method.

CONCLUSIONS

The simulated ARDS causes substantial changes in rat kidney tissue and its effect manifests itself in different ways, depending on the duration of the experiment. In the initial stages of pathology, damage to the renal bodies with narrowing of the urinary space of the Bowman capsule was noted. Subsequently, signs of stasis developed in the microcirculatory bed and larger vessels, as well as the destruction of epithelial cells of the proximal and distal tubules. At a later stage of the study, both degenerated renal corpuscles and nephron tubules and restored structural components of the kidney were observed.

According to the results of an immunohistochemical examination of TGF-β1 fibrosis marker, it was revealed that the change of immunostaining intensity depended on the duration of the experiment. The most intense expression of TGF-β1 was observed on 3-7 days (subacute phase of ARDS – stage of fibroplasia), afterwards the intensity of immunoprecipitation in the interstitium decreased, which indicated a transition to the resolution stage (28 days). However, fibrotic changes remained pronounced in the renal corpuscles, which indicated the deposition of collagen in the mesangia between the capillaries of the vascular glomerulus. The result of immunohistochemical detection of CD68 total macrophage marker showed an increase in the number of macrophages on the day 3 of the experiment, after which their level remained elevated in the kidney until the end of the experiment.

Biochemical analysis of renal markers in rat blood serum confirmed the presence of functional renal disorders caused by the development of simulated ARDS. The recorded maximum increase in creatinine, urea, and uric acid values at 24 hours from the start of the experiment indicates the development of an acute phase of kidney damage, which gradually turned into chronic damage. Undulating changes in the levels of renal markers indicate a nonlinear course of inflammatory processes in the parenchyma and stroma of the kidneys.

Histological, immunohistochemical, and biochemical analyses of rat kidney damage form an overall picture of morphofunctional changes in this organ during simulated ARDS. The obtained data will contribute to assessing the nephroprotective effectiveness of the latest treatment agent, the therapeutic potential of which is planned to be studied at the next research stage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Морфофункціональний стан нирок лабораторних щурів за умов гострого респіраторного дистрес-синдрому

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Анотація. Одним з найчастіших ускладнень гострого респіраторного дистрес-синдрому є гостре ураження нирок, механізми розвитку якого залишаються до кінця нез’ясованими. Метою даної роботи було вивчення морфофункціональних змін у нирках щурів з індукованим гострим респіраторним дистрес-синдромом у різні відтинки часу після моделювання патології. Для дослідження використано 56 здорових статевозрілих щурів-самців, масою 200-220 г, яких розподілили на 7 груп: контрольна, 6 та 24 год, 3, 7, 14 та 28 діб експерименту. Респіраторний дистрес у тварин викликали за допомогою інгаляторного введення ліпополісахариду (5 мг/кг маси тіла). Нирки інтактних щурів мали типову гістологічну будову без видових особливостей. Гістологічні зміни паренхіми нирок щурів дослідних груп включали ущільнення мальпігієвих тілець, пошкодження та десквамацію епітеліоцитів канальців нефронів та появу ознак дисемінованого внутрішньосудинного згортання крові. Через місяць від початку експерименту спостерігалися як патологічні зміни в нефронах, так і відновлені чи збережені структурні компоненти нирки, що вказувало на активацію внутрішньоклітинних репаративних процесів. Імуногістохімічно встановлено зростання експресії маркера фіброзу TGF-β1 та підвищення інтенсивності імунозабарвлення препаратів за панмакрофагічним маркером CD68 на 3 і 7 доби досліду. Кількість макрофагів у зразках нирки залишалась стабільно високою до кінця експерименту, в той час як рівень TGF-β1 знижувався на 28 добу, вказуючи на розвиток фази розрішення патології. Біохімічний аналіз ниркових маркерів показав хвилеподібний перебіг запальних процесів у нирках дослідних щурів. Максимальна концентрація креатиніну, сечовини і сечової кислоти в сироватці крові спостерігалась на 24 год експерименту, що свідчило про настання гострого ураження нирки як ускладнення респіраторного дистресу. Доклінічне вивчення морфофункціональних змін нирок за умов гострого респіраторного дистрес-синдрому допоможе в майбутньому підібрати ефективний метод корекції даного патологічного стану у людей

Ключові слова: ліпополісахарид; гостре ураження нирок; ниркові проби; гістологічний аналіз; імуногістохімія

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Inactivation of microorganisms by high hydrostatic pressure: A literature review

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Abstract. The use of high hydrostatic pressure is intended to perform non-thermal inactivation of microorganisms in food products, to ensure their freshness and to prevent foodborne infections. These infections impact the healthcare system, the food industry, and consumers directly. This study aims to analyse the literature on the effectiveness of high hydrostatic pressure against pathogenic and opportunistic microorganisms transmitted through the consumption of contaminated food. Scientific publications for 2011-2023 were selected for the review. A total of 44 scientific publications were selected, the information from which was critically analysed, systematised and presented in the form of a literature review. The mechanisms of high hydrostatic pressure's effect on microbial cells are described. To illustrate the effectiveness of high hydrostatic pressure against microorganisms, data from selected publications regarding efficiency and treatment parameters are presented in tables. The inactivation of such clinically important microorganisms as *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus* and *Toxoplasma gondii* in liquids and food has been demonstrated. High-pressure treatment has been shown to be a non-thermal food processing method, which distinguishes this method from traditional thermal processing methods such as boiling or pasteurization. One of the notable advantages of using high hydrostatic pressure is the non-thermal inactivation of various microorganisms, which preserves the nutritional and flavour properties of the processed product. It is also noted that food products can be processed in the final packaging, which reduces the risk of microbial contamination at the post-processing stages. The main disadvantages are the impossibility of complete inactivation of bacterial spores and the high cost of high-pressure processing equipment. Combining high-pressure treatment with other methods, such as heat treatment, can overcome the limitations of spore inactivation

Keywords: high pressure; pathogens; neutralization; non-thermal processing

INTRODUCTION

Gastrointestinal diseases account for a significant proportion of infections worldwide, and among them, diarrhoeal diseases are the second leading cause of global mortality. Food- and waterborne diseases cause the premature loss of millions of lives annually, most of which are among vulnerable groups such as children [1, 2]. According to S. Chuang *et al.* [3], these diseases not only have devastating health consequences, but also a significant economic impact in many sectors, including healthcare systems, tourism, food industry, and consumer welfare. While setting and enforcing regulatory standards and guidelines is a means of reducing food contamination by pathogens [4], the importance of effective food processing and preservation methods cannot be overstated. These methods have been used since ancient times and have a dual purpose: to contain the spread of pathogens responsible for foodborne infections, as well as to control microorganisms that cause spoilage and degrade food quality. The historical range of food processing and preservation methods includes heating, salting, freezing, drying, freeze-drying, fermentation, canning, and the use of antimicrobial agents. In addition, modern advances have added new processing methods, such as ionization radiation, pulsed electric fields, ohmic processing, ultraviolet light disinfection,

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and high-pressure processing (HPP) [5, 6]. Since the late 1990s, HPP has become a convincing and commercially feasible alternative for food processing [7]. This trend is driven by the growing consumer demand for food products that are not only microbiologically safe, but also retain their freshness and nutritional value [8].

Despite significant efforts to improve food safety, foodborne illness outbreaks associated with pathogenic bacteria reaching dangerous levels in food have been widely documented [9]. Bacteria can contaminate food at any stage during harvesting, processing, storage, and transportation, as well as during food preparation in restaurants or kitchens. According to the Centres for Disease Control and Prevention (USA), which is cited by M. Al-Mamun *et al.* [10], the pathogens that most often cause foodborne infections leading to hospitalization and death are *Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* (most often of the O157:H7 serotype), *Campylobacter* spp., *Clostridium perfringens,* and *Toxoplasma gondii*. These pathogens are pathogenic or opportunistic microorganisms that demonstrate the ability to persist or even multiply in various food substrates, making them an important foodborne hazard. This highlights the need to implement technological measures to effectively mitigate the food safety risks associated with these pathogens. Among the various methods, HPP treatment has been comprehensively studied in research aimed at neutralizing these infectious threats to improve food safety.

In the Ukrainian-language scientific literature up to 2023, high-pressure treatment is mentioned as one of the methods of improving food quality. In particular, T. Lozova [11] mentions ultra-high-pressure treatment to improve the quality and storage of bread. In the work of I. Bernyk *et al.* [12], high-pressure treatment is used to saturate meat raw materials with substances, including table salt. In this publication, high pressure is also mentioned as an alternative to heat treatment, but there are no microbiological data, the authors focus on the consistency and organoleptic properties of the product. The lack of Ukrainian scientific works and the positive results of world scientists on the inactivation of pathogens and microorganisms that cause food spoilage make this area of research interesting and promising. The purpose of this manuscript was to bring together data from various English-language scientific studies that both highlight the potential of HPP as a method of inactivating a wide range of microorganisms and reveal limitations to its use.

Google Scholar was used to search for literature sources. The literature was searched using a combination of keywords: “high-pressure processing”, “high hydrostatic pressure”, “inactivation”, “log reduction”, “bacteria”, “fungi”, “protozoa”. The English-language literature sources for 2011-2023 were reviewed. Preference was given to articles describing high hydrostatic pressure treatment alone without the use of combined treatment with other non-thermal or thermal methods. Data on high-pressure treatment alone were selected from publications with several types of treatment or combined treatment. Articles with a focus on pathogenic and opportunistic microorganisms that cause disease were selected; articles on the inactivation of microorganisms that cause food spoilage were excluded. Book chapters, dissertations, and reports or abstracts from scientific conferences were not considered. Both open-access articles and articles from paid resources were reviewed. The data from the selected publications were systematised by the consistency of high-pressure processed foods and entered into the relevant tables.

EFFECT OF HIGH HYDROSTATIC PRESSURE ON PATHOGENS AND FEATURES OF ITS APPLICATION

HPP is a non-thermal treatment method that involves subjecting food products, beverages, or other materials to elevated pressure in the range of 100 to 1000 MPa, usually at room temperature or slightly higher [13]. This process is also known as ultra-high-pressure processing (UHP). HPP is known to inactivate various microorganisms, including bacteria, viruses, yeast, mould, as well as enzymes, while preserving the nutritional and taste properties of the processed product [1, 14, 15]. It is noted that HPP does not break covalent bonds, so changes in low-molecular-weight compounds such as vitamins, pigments, and flavourings are minimal [16].

The mechanism of microbial inactivation by HPP is not fully understood, but it is considered to be multifactorial. R. Levy *et al.* [6] mention that one influencing factor is the denaturation of proteins and enzymes, leading to the disruption of structures and functions of the affected by pressure cells. Hydrostatic effects induce mechanical stress on cellular structures, causing deformation and damage to cell membranes; the loss of membrane integrity contributes to cell death [17, 18]. Damage to bacterial cell membranes by high pressure induces intracellular oxidative stress [19, 20]. S. Ceuppens *et al.* [21] point out that another important factor is the impact on nucleic acids. Although they are much less sensitive to high-pressure effects compared to flexible lipid membranes, hydrogen bonds that maintain their spiral structure can be disrupted under high pressure [22]. Thus, pressure-induced structural changes in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules may interfere with DNA replication, transcription, and translation.

The mechanisms of impact of HPP are particularly effective against the vegetative cells of microorganisms, but processing without additional thermal treatment cannot completely inactivate bacterial spores, whose dense shells make them resistant to many processing methods [23, 24]. However, research by M.R. Sarker *et al.* [25] has shown that high pressure can trigger the germination of *Bacillus* spp. bacterial spores, making them susceptible to further treatments. J.H. Mok *et al.* [26] also indicated better processing efficiency with multiple pressurization-depressurization cycles compared to a single-cycle treatment of the same duration.

High-pressure processing is considered a non-thermal method for treating food products, despite the adiabatic temperature increase that occurs during pressure application to physical objects. For juices and other beverages, the temperature increase is 3°C for every 100 MPa at the initial product temperature of 25°C [13]. However, this temperature increase is temporary and local, lasting only during the pressure application period. When the pressure is released, the temperature also decreases and may even drop below the initial temperature if heat exchange occurs between the processed product and the elements of the high-pressure chamber. Additionally, as mentioned by M. de Alba *et al.* [15], processing can be carried out with additional cooling of the high-pressure chamber jacket. This distinguishes this method from traditional thermal processing methods, such as boiling or pasteurization, where the entire product is subjected to heating by external heat sources over an extended period, leading to a uniform temperature increase. Although the temperature increase caused by pressure does occur, it is not the primary means of achieving microbial inactivation. This processing method minimizes the impact of heat on sensitive nutrients, flavourings, and other desirable product properties that may be reduced or lost through prolonged exposure to elevated temperatures [15, 16]. Y. Zhou *et al.* [27] report that HPP can be performed in the final packaging, minimizing the risk of microbial contamination of the food product after processing. Inactivation of microorganisms slows down the spoilage of products, thereby extending their shelf life. T. Rode *et al.* [28] reported an increase in the shelf life of fresh fish processed with HPP at 200 and 500 MPa for 120 seconds. K. Aaby *et al.* [29] reported an extension of the shelf life of strawberry purée and juice when using 400-600 MPa for 1.5-3 minutes, and noted that HPP is better suited for products with a lower initial level of enzymes compared to thermal processing.

The practical application of HPP may be constrained by several factors that food industry companies need to consider. Among these factors is the cost of equipment: HPP requires specialised equipment capable of generating and withstanding high pressure. These devices are typically expensive to purchase and maintain. For small food product manufacturers or businesses with limited budgets, the cost of acquiring HPP equipment can be a significant barrier to implementing this technology [14]. Another factor is the potential change in product texture. Some food products may become softer or undergo other structural changes. For certain food products, such as fruits and vegetables, this change may be acceptable or even desirable. However, for others, especially those valued for their specific texture, such as certain cheeses or bakery products, these changes may be undesirable and limit the application of HPP [30]. High-pressure processing is typically conducted in a high-pressure chamber. To withstand the pressure, food products must be packaged in packaging made of robust and flexible materials [31]. As noted by H.W. Huang *et al.* [14], the packaging material used in HPP must have a compressibility of at least 15%, making only plastic packaging materials suitable for such processing. V.M.B. Balasubramaniam *et al.* [32] describe the penetration of propylene glycol into plastic packaging during combined HPP and thermal processing. The same authors report delamination of metal-plastic packaging with the penetration of propylene glycol between the aluminium and plastic layers. Therefore, companies using HPP need to invest not only in equipment but also in appropriate packaging materials.

Due to the direct impact on the structural elements of microbial cells, HPP offers many advantages in the processing of food products. Among them are extended shelf life and improved microbiological safety of food products. However, the practical application of HPP may be limited by a range of factors. Companies must weigh the benefits against financial expenses and potential texture changes to determine whether HPP is suitable for their specific products.

INACTIVATION OF PATHOGENS THAT CAN CAUSE FOOD INFECTIONS

Due to the mechanisms described above, HPP has been effectively applied for the inactivation of microorganisms that can contaminate food products and pose a danger to human health and life. Comparisons of pressure resistance among vegetative cells revealed that, among foodborne pathogens, strains of *Escherichia coli* O157:H7 were the most resistant. The United States Department of Agriculture (USDA) recognizes this strain as an indicator, and the HPP process achieving a 5-log inactivation of this strain is deemed sufficient to ensure the microbial safety of the product [14]. To illustrate the effectiveness of HPP treatment on bacterial suspensions and various liquid food products contaminated with different microorganisms, data from selected publications are compiled in Table 1.

Table 1. Inactivation of microorganisms in liquids and liquid food products by high hydrostatic pressure

Pathogen	Substrate	Pressure (MPa)	Initial temperature (°C)	Processing time (min)	Inactivation (log CFU/mL)	Reference
Bacillus cereus CECT 131 (vegetative cells)	Human milk	379 593,96	10	233 s*	4.62 6.93	[2]
A mixture of strains *Escherichia coli* (NCTC 11601, NCTC 11602, NCTC 11603, NCTC 9706, NCTC 9707)	Raw milk	400	18	1	0,85	[33]
				3	1.1**	
				5	2.2**	
		500		1	0.9**	
				3	1.9**	
				5	2.6**	
		600		1	3.6**	
				3	5.6	
				5	6.8	
Escherichia coli O157:H7 NCTC 12900	Suspension of bacteria in physiological solution	450	Not indicated	5	5.02	[34]

Table 1. Continued

Pathogen	Substrate	Pressure (MPa)	Initial temperature (°C)	Processing time (min)	Inactivation (log CFU/mL)	Reference
A mixture of strains *Listeria monocytogenes* (FMT 1750, NCTC 11994, NCTC 5214, NCTC 10888, NCTC 19118)	Raw milk	400	18	1	1.42	[33]
				3	1.5**	
				5	2.1**	
		500		1	1.6**	
				3	3.1**	
				5	5.48	
		600		1	3.2**	
				3	5.65	
				5	5.91	
A mixture of serovars *Salmonella* (ATCC numbers 13076, 8387, 6962, 9270, 14028)***	Orange juice	103	25	10	0.75	[35]
		241	25	2	0.53	
				4	0.69	
				8	1.44	
				10	1.88	
		380	25	2	2.76	
				4	5.56	
				8	>7	
				10	>7	
Staphylococcus aureus CECT 976	Human milk	379	10	233 s*	2.73	[2]
		593,96			5.81	
Staphylococcus aureus strains	Brain Heart Infusion culture	400	15	10	2.01	[36]
CTC 1008		600	15	10	7.96	
CTC 1019		900	Not indicated	5	>8	
		400	15	10	1.55	
		600	15	10	5.78	
CTC 1021		900	Not indicated	5	>8	
		400	15	10	1.82	
		600	15	10	7.32	
CECT 976		900	Not indicated	5	>8	
		400	15	10	1.81	
		600	15	10	7.79	
CECT 4466		900	Not indicated	5	>8	
		400	15	10	1.94	
		600	15	10	7.90	
		900	Not indicated	5	>8	

Notes: * – units of measurement according to the original publication; ** – data are presented in the form of a graph without specifying exact numbers, the numbers in the table are reproduced approximately; *** – description of the used microorganisms according to the original publication. Strain numbers correspond to *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076, *Salmonella enterica* subsp. *enterica* serovar Montevideo ATCC 8387, *Salmonella enterica* subsp. *enterica* serovar Newport ATCC 6962, *Salmonella enterica* subsp. *enterica* serovar Anatum ATCC 9270, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028

Source: compiled by the author based on data collected during the literature review

When processing bacterial cultures of S. *aureus* with an initial concentration of approximately 9 log CFU/mL at a pressure of 400 MPa, A. Jofré *et al.* [36] demonstrated that, with inactivation of 1.55-2.01 log CFU/mL, a portion of microorganisms (up to 1.41 log CFU/mL) were subjected to sublethal damage and did not grow on a selective medium (tryptic soy agar with yeast extract and 10% NaCl). J. Kameník *et al.* [37], in the HPP inactivation of *E. coli* applied to tartare beefsteak, were able to recover sublethally damaged bacteria by inoculating samples into a liquid enrichment medium, regardless of the applied pressure (400 and 600 MPa for 5 minutes). To illustrate the effectiveness of HPP treatment on semi-solid and solid food products contaminated with different microorganisms, data from selected publications are compiled in Table 2.

Table 2. Inactivation of microorganisms in liquids and liquid food products by high hydrostatic pressure

Pathogen	Substrate	Pressure (MPa)	Initial temperature (°C)	Processing time (min)	Inactivation (log CFU/g)	Reference
Campylobacter jejuni	Ground chicken	200	4	5	0*	[38]
				10	0*	
				15	0*	
		300		5	2.7	
				10	⩾6.87	
				15	⩾6.87	
		400		5	⩾6.87	
				10	⩾6.87	
				15	⩾6.87	
A mixture of vegetative cells of strains *Clostridium perfringens* type F (KCCM12098, KCCM40946, KCCM40947, KCTC5101)	Emulsion-type pork sausage	500	4	4 cycles of 3 min	1. 9	[39]
A mixture of serovars *Escherichia coli* (O26:H11, O45:H2, O103:H2, O111:NM, O121:H19, O145)	Ground beef	250	5-7	5	0.7	[40]
				15**	1.6	
				30**	2.3	
		350		5	2.4	
				15**	3.5	
				30**	4.7	
		450		5	4.4	
				15**	5.6	
A mixture of serovars *Escherichia coli* (O91, O146, O153, O156)	Tatar beefsteak	400	3±1	5 min of processing + 168 h of storage at 4 °C	1.8	[37]
		600			>4.7	
A mixture of strains *Escherichia coli* O157:H7 (ATCC 43888, 43889, 43890, 45756, 11082)	Ground beef	250	5-7	5	0.2	[40]
				15**	0.7	
				30**	1.0	
		350		5	1.0	
				15**	2.1	
				30**	3.2	
		450		5	3.5	
				15**	6.9	
A mixture of strains *Listeria monocytogenes* (Scott A, 43256, 51742)	Melon purée	400	8	5	2.9	[41]
			15		3.1	
		500	8	5	5.6	
			15		⩾7	
A mixture of strains *Salmonella enterica* (*S.* Poona RM 2350, *S.* Newport H1275, *S.* Stanley H0558)	Melon purée	300	8	5	2.7	[41]
			15		3.1	
		400	8	5	4.8	
			15		5.8	
		500	8	5	⩾7	
			15		⩾7	
A mixture of strains *Salmonella Typhimurium* DT 104, *S.* Newport ATCC 6962, *S.* Enteritidis ATCC 13076, *S.* Senftenberg ATCC 8400, *S.* Kentucky FSIS 074	Ground chicken	250	4-6	15	0.5	[9]
		350		15	1.7	
		450		10	>5	
Toxoplasma gondii strain VEG	Raw ham	100-400	6±1	1	Infectious***	[42]
		600		20	Non-infectious	
	cured ham	600		3-10	Infectious***	
				20	Non-infectious	

Notes: * – treatment at 200 MPa did not show statistically significant inactivation; ** – treatment for 15 and 30 minutes consisted of repeated cycles of 5 minutes each; *** – ham samples were tested for infectivity by inoculation of mice with subsequent detection of cysts in their brains

Source: compiled by the author based on data collected during a literature review

The analysed studies indicated that the inactivation of the test strains is proportional to the hydrostatic pressure and treatment time, but it is important to note that the reaction of bacteria to high pressure can vary depending on the species, strain, and physiological state of the bacterial cells. In general, gram-negative bacteria are more sensitive to HPP treatment than gram-positive bacteria. This is due to differences in the structure of the cell wall of these types of bacteria. The outer membrane of gram-negative bacteria contains lipopolysaccharides that are sensitive to high pressure, whereas its absence and a thicker layer of peptidoglycan in the cell walls of gram-positive bacteria provide them with some resistance to damage caused by HPP. However, even within a species, the sensitivity of strains to HPP can vary. For example, Y. Liu *et al.* [19] showed that among 19 strains of *Campylobacter jejuni* inoculated into ground chicken that had been treated with HPP at 300 MPa and 30°C for 3 minutes, the inactivation data varied greatly. The sensitive strains were inactivated within about 3 log CFU/g, while the inactivation of the most resistant C. *jejuni* strain HCJ2316 was only 0.5 log CFU/g. Y. Zhou *et al.* [27] conducted a similar experiment with the laboratory strain of *Escherichia coli* JM109 and six strains of *E. coli* O157:H7 inoculated into ground beef. While high-pressure treatment at 400 MPa and 25°C for 15 minutes inactivated the laboratory strain completely (~8 log CFU/g), the inactivation of the pathogenic strains ranged from 1.57 log CFU/g (strain SEA13B88) to 3.49 log CFU/g (strain WM98A06026). These results emphasize why a single strain should not be used in studies evaluating the effectiveness of the HPP inactivation process.

S.H. Lee *et al.* [39] demonstrated that even with low microbial inactivation by HPP, there was a slowdown in the growth of vegetative cells of *Clostridium perfringens*. After HPP treatment at 500 MPa for 4 cycles of 3 minutes each, a bacterial concentration reduction of 1.9 log CFU/g was achieved. Following this treatment, the bacterial population did not increase for two weeks at 4°C.

Despite the positive results from experiments on the inactivation of various microorganisms by HPP, treated food products are not sterile and should be stored under refrigeration. Sublethally damaged bacterial cells, under appropriate conditions (availability of nutrients, temperature changes, product storage time), may recover and proliferate [43]. In contrast, some researchers have shown that sublethally damaged cells do not recover and die when storage conditions are maintained [44]. For instance, an experiment with a mixture of seven strains of Campylobacter jejuni inoculated in chicken liver demonstrated that HPP at 250 MPa and 4°C for 10 minutes resulted in a 1.3 log CFU inactivation. Storage at 4°C for 1 week led to an additional reduction in the *C. jejuni* population by 1.6 log CFU. Thus, the efficiency of HPP treatment against a wide range of foodborne pathogens has been demonstrated, and HPP technology can be combined with existing processing methods in the food industry to enhance their effects.

CONCLUSIONS

A review of English-language literature demonstrates the effectiveness and wide application of HPP for inactivating pathogenic microorganisms in liquid, semi-solid, solid food products, as well as physiological solutions. The collected data from scientific publications illustrate the potential of this food processing method as a standalone technique and suggest its prospective use in combination with other thermal or non-thermal processing methods. The broad spectrum of high hydrostatic pressure action makes this processing method versatile for controlling vegetative microbial contamination in various food products and beverages, consequently reducing cases of foodborne illnesses or other diseases transmitted through the consumption of contaminated products. Among the processed sources that compared the inactivation of gram-positive and gram-negative microorganisms, results indicated a higher sensitivity of gram-negative microorganisms to HPP treatment, explained by the unique structure of their cell walls containing more pressure-sensitive structures. Inactivation of both types of microorganisms was proportional to the magnitude of hydrostatic pressure and the processing time. In most reviewed sources, HPP was applied without combining it with other processing methods, but there are publications demonstrating its potential enhancement with other methods.

The introduction of high hydrostatic pressure processing technology will reduce the processing time of products, decrease heat-induced changes in products, while preserving natural vitamins, pigments, and flavourings highly valued by consumers. The reduction of microbial contamination, in addition to the improved safety of processed food products, will also extend their shelf life. One drawback of this method is its insufficient efficacy in inactivating bacterial spores, a limitation shared with other non-thermal processing methods and well-known in the scientific community. This limitation can be overcome by combining high hydrostatic pressure processing with other methods. Among other considerations, it is worth mentioning that high-pressure processing is not suitable for all products, but only for those with a homogeneous consistency, ensuring processing uniformity. Products with specific textures or porosity are not suitable for HPP treatment. To ensure quality processing for the packaging of such products, non-toxic flexible material with resistance to compression must be used.

Despite these drawbacks, high hydrostatic pressure processing is a promising technique for enhancing the safety and quality of food products. The results of the review indicate a convincing path for further necessary research and development that meets the evolving demands of both the food industry and consumers. A possible direction for future research is exploring the potential of combining HPP with other thermal and non-thermal methods to inactivate a broader range of pathogens, including spore-forming microorganisms.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Інактивація мікроорганізмів високим гідростатичним тиском: огляд літератури

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Анотація. Використання високого гідростатичного тиску призначене для здійснення нетермічної інактивації мікроорганізмів у харчових продуктах, забезпечення збереження їх свіжості та запобігання харчовим інфекціям. Останні впливають на систему охорони здоров›я, харчову промисловість та, безпосередньо, на споживачів. Метою даної роботи було проведення аналізу літературних даних стосовно ефективності високого гідростатичного тиску проти патогенних та умовно патогенних мікроорганізмів, які передаються через вживання контамінованої їжі. Для огляду обиралися наукові публікації за 2011-2023 роки. Було обрано 44 наукових публікації, інформацію з яких було критично проаналізовано, систематизовано та оформлено у вигляді огляду літератури. Зазначені механізми впливу високого гідростатичного тиску на клітини мікроорганізмів. Для ілюстрації ефективності високого гідростатичного тиску проти мікроорганізмів дані з вибраних публікацій щодо ефективності та параметрів обробки зібрано у таблицях. Продемонстрована інактивація таких клінічно значимих мікроорганізмів як *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus* та *Toxoplasma gondii* у рідинах та продуктах харчування. Показано, що обробка високим тиском є нетермічним методом обробки харчових продуктів, що відрізняє цей метод від традиційних термічних методів обробки, таких як кип’ятіння або пастеризація. Однією з помітних переваг застосування високого гідростатичного тиску є нетермічна інактивація різних мікроорганізмів, при якій зберігаються поживні та смакові властивості обробленого продукту. Також зазначається, що харчові продукти можуть оброблятися в кінцевій упаковці, що зменшує ризик їх мікробної контамінації на етапах після обробки. Основними недоліками є неможливість повної інактивації бактеріальних спор та велика вартість обладнання для обробки високим тиском. Поєднання обробки високим тиском із іншими методами, наприклад, термічною обробкою може вирішити обмеження стосовно інактивації спор

Ключові слова: високий тиск; збудники; знешкодження; нетермічна обробка

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Mathematical modelling of peripheral haemodynamics of the shin in volleyball players of mesomorphic somatotype

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Abstract. Statistical modelling of peripheral haemodynamics indicators, which is conducted based on determining anthropometric and somatotypic features of the organism, can be considered a new way of personalising instrumental diagnostic examination. Therefore, determining the suitable rheovasographic parameters of the shin in highly skilled volleyball players of a particular somatotype is relevant. The purpose of this study was to investigate the total influence of indicators of the external structure of the body on the value of indicators reflecting the specific features of blood circulation in the shin in volleyball players of mesomorphic somatotype. A comprehensive clinical and laboratory study of the state of health and physical development of 108 volleyball players aged 16-20 years was conducted. The method of tetrapolar rheography with the use of a diagnostic computer multifunctional apparatus was used to determine the rheovasographic parameters of the shin, amplitude, time, and indicators of the ratio of amplitude and time rheovasographic parameters were evaluated. An anthropometric study of total and partial body measurements was

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carried out. Somatotyping was performed using the Heath-Carter method. The study selected 28 volleyball players of mesomorphic somatotype. Statistical models were created using direct stepwise multivariate regression analysis. For all amplitude and time rheovasographic parameters of the hip in volleyball players of mesomorphic somatotype, statistical models with high accuracy of description of signs were built. The 10 models included 94 anthropometric dimensions. The key determinants of shin blood circulation parameters are body circumference (most often forearm, neck, and shin circumference), chest and pelvic diameters, thickness of skin and fat folds (most often on the shin, abdomen, flank), and width of the epiphyses of long tubular bones. It was found that the indicators of external body structure within 63.84-99.99% determine the value of indicators of regional blood circulation on the shin in volleyball players of mesomorphic somatotype. Statistical modelling makes it possible to determine the proper values of individual rheovasographic indicators on the shin depending on the anthropometric parameters of individuals of a certain gender, age, somatotype, which is especially significant for athletes of a certain sport, since their cardiovascular system indicators are greatly influenced by the specifics of sports activity

Keywords: stepwise regression; statistical modelling; rheovasography; anthropometric dimensions; mesomorphic somatotype; volleyball

INTRODUCTION

Technical and tactical efficiency in modern sport is determined by the functional state of the cardiovascular system of athletes, the indicators of which form the body's response to exogenous and endogenous influences [1, 2]. D. Berhtraum *et al*. [3] proved that changes in cardiovascular system indicators can signify the body's adaptive capacity, which was also noted in the studies of other scientists [4, 5]. O. Voloshyn *et al*. [6] emphasise that the ability of the human body to adapt will largely be determined by the functional state of its cardiovascular and respiratory systems, emphasising the significance of such studies in adolescence, when the importance of these systems increases for the harmony and sufficiency of physical development of the level of health of a young organism against the background of considerable changes in living conditions. The possibility of prompt diagnosis of changes in adaptive potential is an effective way to prevent overwork and overtraining in athletes. Scientists emphasise the significance of central haemodynamics indicators for sports performance and the specificity of these parameters in individuals engaged in various sports [5, 7, 8]. However, Y. Zhang *et al*. [7] note that the response of central haemodynamics to physical activity is transient, and therefore it is difficult to investigate it to obtain objective results. The importance of peripheral circulation indicators for sports performance was proved in the study by O. Usova *et al*. [9], noting that a decrease in the functional capabilities of the body will be accompanied by a decrease in blood filling of blood vessels, which is expressed in a change in the value of time and amplitude indicators of the rheogram. This is because the condition of the vessel walls, blood flow, and microhaemodynamics of skeletal muscles, especially in the lower extremities, can hinder the successful sports career of any athlete. During physical activity, the greatest changes in blood supply are inherent in skeletal muscle circulation, and therefore the condition of peripheral vessels is crucial in the development of adaptive responses to sports activities. S. Malyuga *et al*. [10] point out that it is necessary to consider the functional state of blood vessels, which can have a considerable impact on the processes of rapid recovery after static physical activity. However, along with sports activities, functional parameters of regional blood circulation can also be affected by the specific features of the athlete's external body structure. Therefore, it is crucial to determine the appropriate values of regional blood circulation parameters of the lower extremities, specifically, rheovasographic parameters of the shin, by statistical modelling. Athletes involved in sports, particularly volleyball, need special attention to the condition of the vessels of the lower limb. According to the findings of the study by O. Khapitska *et al*. [11], it is known that volleyball players can often show signs of venous stasis without substantial symptoms of organic vein pathology.

Thus, the literature analysis shows the significance of the morphological and functional state of the cardiovascular system, specifically peripheral haemodynamics, which may vary depending on the characteristics of sports activity and the constitutional characteristics of the athlete. Therefore, the study aimed at conducting statistical modelling of the rheovasographic parameters of the shin based on the determination of the constitutional characteristics of the organism is of great practical importance, as it will allow comparing the factual values of rheovasographic parameters with the respective values determined by concrete anthropometric features. The purpose of this study was to create statistical models for determining the suitable value of rheovasographic indicators of the shin in volleyball players of mesomorphic somatotype, considering anthropometric dimensions.

MATERIALS AND METHODS

During 2017-2023, a comprehensive clinical and laboratory study of the health and physical development of 108 female volleyball players aged 16-20 (youthful period of ontogeny) of high sports categories (from second adult to masters of sports) was conducted at the Research Centre of National Pirogov Memorial Medical University, Vinnytsia (Pirogov VNMMU). The female volleyball players played in the following teams: “Bilozhar – Medical University”, “Dobrodii – Medical University – SHVSM”, volleyball student teams of Vinnytsia higher education institutions, children's and youth sports schools of Vinnytsia and Kalynivka. Each female athlete who had at least 3 years of sports experience and the appropriate level and age (inclusion criteria) gave informed consent to take part in the study. All examinations were carried out no less than 12 hours after training. Admission to further studies was based on a preliminary electrocardiographic and echocardiographic examination. Exclusion criteria were hypertension, pathological hypotension, arrhythmias, mitral valve prolapse of II-III degree, pathological myocardial hypertrophy.

Rheovasographic parameters of the shin in volleyball players were determined by tetrapolar rheocardiography using a certified computer diagnostic multifunctional complex (developed by scientists of the Pirogov VNMMU) and the amplitude, time, and ratio indicators of amplitude and time rheovasographic parameters (integral) were evaluated. An anthropometric study of the total body and chest, pelvis, limbs, and head was performed according to the recommendations of R. Shaparenko [12], and a total of 50 external body dimensions were determined. Measurements were taken with certified equipment of the following sizes: Martin's anthropometer was used to determine longitudinal dimensions – body height and anthropometric points (cm); centimetre tape – girth dimensions (cm), large spreading caliper – transverse and sagittal body diameters (cm), sliding caliper – width of distal epiphyses of long tubular bones (cm), body fat caliper – thickness of skin and fat folds (mm), and medical scales – body weight (kg). The body surface area was calculated using the DuBois formula [12].

$$S = W^{0.425} \times H^{0.725} \times 0.007184, \qquad (1)$$

where S is the body surface area (m^2); W is the body weight (kg); H is the height (cm).

The somatotypic study was conducted using the Heath-Carter calculation method [13], based on anthropometric measurements, and the size of the somatotype components (ectomorphic, mesomorphic, endomorphic) was determined in points (from 1 to 7). After somatotyping, it was found that 28 female volleyball players belonged to the mesomorphic type. The results were analysed using the licensed software Statistica 5.5.

To determine the variability of peripheral haemodynamics of the shin from the complex total effect of anthropometric and somatotypic parameters, a multivariate stepwise regression analysis was performed in compliance with the following conditions [14]. Firstly, the coefficient of determination R^2 of the regression polynomial should be equal to or greater than 0.60, which would indicate that a particular rheovasographic indicator depended on the total influence of constitutional factors by more than 60%, and this would be the basis for mathematical modelling. Secondly, the value of the Fisher's F-criterion must be at least 2; the calculated value of the Fisher's criterion must be less than its actual value. Thirdly, if the coefficient of determination approached 1,000 and the regression polynomial included many terms, a ridge regression was applied, with the correlation coefficient artificially lowered to allow for more stable beta coefficients.

The Bioethics Commission of the Pirogov VNMMU found that this study does not violate the basic bioethical standards established in the Declaration of Helsinki [15], as confirmed by the decision of the Bioethics Commission of the Pirogov VNMMU (Protocol No. 1 of 31 January 2018).

RESULTS

It was found that the value of the basic impedance (Ohm) in female volleyball players of the mesomorphic somatotype was 90.54% dependent on the complex influence of anthropometric dimensions and somatotype components. Most of the coefficients of this regression polynomial were statistically significant, except for the free term, the width of the distal epiphysis of the upper arm, and the sagittal arch of the head. The regression polynomial of the baseline impedance was significant ($p < 0.001$), and the analysis of variance confirmed this ($p < 0.001$). Since the Fisher's criterion of 14.89 is much higher than its calculated value ($F_{cr.} = 9.14$), it could be argued that the constructed mathematical model is significant:

BASIC IMPEDANCE = 66.12 + 9.323 × width of the distal femoral epiphysis - 7.576 × shin circumference + 0.996 × thickness of the skin and fat fold on the thigh + 1.100 × height of the finger point - 3.314 × width of the distal epiphysis of the shoulder - 3.091 × width of the shoulders + 3.978 × head circumference - 0.861 × thickness of the skin and fat fold on the abdomen - 1.546 × sagittal arch of the head.

The coefficient of determination R^2 of 96.91% substantiated the dependence of the systolic wave amplitude at the shin (Ohm) on the complex effect of somatometric parameters. All coefficients of this regression polynomial were statistically significant. Since the Fisher's criterion of 24.12 is significantly higher than its calculated value ($F_{cr.} = 13.10$), it could be argued that this regression polynomial is highly significant ($p < 0.001$). This is evidenced by the results of the analysis of variance ($p < 0.001$). The statistical equation of the model is presented as follows:

SYSTOLIC WAVE AMPLITUDE = - 0.261 + 0.006 × fold thickness on the back of the shoulder + 0.004 × neck circumference - 0.004 × shin circumference - 0.010 × fold thickness on the forearm + 0.005 × head circumference + 0.003 × thickness of the crease on the shin - 0.001 × thickness of the crease on the abdomen + 0.003 × inter-ridge distance - 0.005 × forearm circumference at the bottom - 0.001 × inter-axial distance + 0.002 × thigh circumference - 0.002 × upper leg circumference.

It was found that the value of the incisor amplitude (Ohm) was 83.34% dependent on the variability of the complex of anthropometric indicators. Most of the coefficients of this regression polynomial were significant, except for the free term. Since the Fisher's criterion of 9.38 is greater than its calculated value ($F_{cr.} = 8.15$), it could be argued that this regression polynomial is significant ($p < 0.001$). This is evidenced by the results of the analysis of variance ($p < 0.001$). The statistical equation of the model is presented as follows:

AMPLITUDE OF INCISURE = 0.015 + 0.004 × relaxed shoulder girth - 0.002 × waist circumference - 0.003 × hand girth + 0.002 × intercristal distance + 0.004 × width of the face + 0.002 × sagittal arch of the head - 0.006 × forearm circumference at the bottom - 0.001 × thickness of the fold at the lower angle of the scapula.

The coefficient of determination R^2 of 99.65% substantiated the variability of the diastolic wave amplitude at the shin (Ohm) from the complex effect of 15 anthropometric dimensions. Most of the independent variables of this polynomial had strong interrelationships with each other, and therefore the so-called multicollinearity arose, which required the additional use of the ridge regression method. In the new regression polynomial, the determination coefficient R^2 was already only 70.83% responsible for the variability of the diastolic wave amplitude. Most of the coefficients of the independent variables included in this regression equation were significant, except for the free term and the width of the lower tibial epiphysis. Since the actual value of Fisher's criterion of 8.74 was higher than the calculated value ($F_{cr.} = 5.18$), it could be argued that this regression polynomial is significant ($p < 0.001$). This is evidenced by the results of the analysis of variance ($p < 0.001$). The statistical equation of the model is presented as follows:

DIASTOLIC WAVE AMPLITUDE = 0.001 - 0.002 × thickness of the fold on the anterior surface of the shoulder + 0.006 × endomorphic component of the somatotype - 0.002 × lower arm circumference + 0.002 × relaxed arm circumference + 0.003 × width of the distal tibial epiphysis.

It was found that the value of the fast blood filling amplitude of the rheovasogram of the shin in mesomorphic volleyball players (Om) was 85.73% dependent on the total complex effect of indicators of the external structure of the body. Most of the coefficients of this regression polynomial were significant, except for the free term of this polynomial and the chest circumference at exhalation. Generally, this linear regression polynomial is significant ($p < 0.001$). Since the Fisher's criterion of 9.31 is higher than the value of its calculated value ($F_{cr.} = 9.14$), it could be argued that the constructed mathematical model is significant:

FAST BLOOD FILLING AMPLITUDE = 0.012 + 0.004 × relaxed shoulder circumference - 0.002 × smallest head width + 0.002 × transverse mid-thoracic diameter - 0.001 × neck circumference + 0.004 × width of the distal tibia epiphysis - 0.003 × shin circumference + 0.002 × sagittal mid-thoracic diameter + 0.001 × height of the finger point - 0.001 × chest circumference at exhalation.

It was found that all time parameters of the rheovasogram of the shin had a rather high dependence on the complex influence of anthropo-somatotypic characteristics of the organism of volleyball players of mesomorphic somatotype. Specifically, the variability of the rheographic wave duration on the tibia (c) depended by 96.11% on the total complex effect of anthropometric and somatotypic indicators. Most of the coefficients of this regression polynomial were statistically significant, with the exception of foot circumference. Generally, this regression linear polynomial was statistically significant ($p < 0.001$), as evidenced by the analysis of variance ($p < 0.001$). Since the Fisher's criterion of 32.46 was three times higher than its calculated value ($F_{cr.} = 10.13$), it was possible to assert the significance of the constructed mathematical model:

RHEOGRAPHIC WAVE DURATION = -0.771 + 0.072 × shin crease thickness + 0.049 × sagittal mid-thoracic diameter - 0.023 × lateral crease thickness + 0.044 × neck circumference - 0.034 × lower forearm circumference + 0.115 × width of the distal epiphysis of the upper arm - 0.012 × width of the shoulders - 0.021 × foot circumference + 0.030 × transverse mid-thoracic diameter - 0.011 × height of the acetabulum.

The coefficient of determination R^2 of 89.61% substantiated the variability of the time value (s) of rise part from the complex influence of somatometric indicators. All coefficients of this regression polynomial were statistically significant. Since the Fisher's criterion (16.09) was twice as high as its calculated value ($F_{cr.} = 8.15$), it could be argued that this regression polynomial was highly significant ($p < 0.001$), and the analysis of variance confirmed this ($p < 0.001$). The statistical equation of the model is presented as follows:

TIME OF THE RISE PART= -0.303 + 0.021 × hand circumference + 0.012 × hip circumference - 0.025 × lower forearm circumference - 0.021 × smallest head width + 0.018 × lower jaw width - 0.004 × waist circumference + 0.008 × relaxed shoulder circumference - 0.004 × external conjugate.

The variability of the time value (s) of the down part of the rheovasogram of the shin by 94.72% in mesomorphic female volleyball players is conditioned by the value of 10 anthropometric body sizes. Most of the coefficients of this regression polynomial were statistically significant, except for forearm circumference in the lower third and the width of the distal tibial epiphysis. Since the Fisher's criterion (23.29) was more than twice as high as its calculated value ($F_{cr.} = 10.13$), this regression linear polynomial was considered reliable ($p < 0.001$), and the analysis of variance confirmed this ($p < 0.001$). The statistical equation of the model is presented as follows:

TIME OF DOWN PART = -0.637 + 0.059 × thickness of the fold on the shin + 0.053 × sagittal mid-thoracic diameter - 0.019 × thickness of the fold on the side + 0.033 × neck circumference - 0.031 × inter-articular distance + 0.041 × width of the distal epiphysis of the upper arm - 0.027 × lower forearm girth + 0.025 × inter-articular distance - 0.039 × face width - 0.042 × width of the distal epiphysis of the shin.

The coefficient of determination R^2 of 95.91% determined the dependence of the time of rapid blood filling (s) on the complex effect of 10 indicators of the external structure of the body. Almost all of the coefficients of this regression polynomial were significant, with the exception of the largest head width. Since the Fisher's criterion (30.35) was three times higher than its calculated value ($F_{cr.}$ = 10.13), the polynomial for determining the time of rapid blood filling was considered highly significant ($p < 0.001$), and the analysis of variance confirmed this ($p < 0.001$). The statistical equation of the model is presented as follows:

RAPID BLOOD FILLING TIME = - 0.124 - 0.020 × smallest head width + 0.015 × transverse mid-thoracic diameter + 0.012 × sagittal mid-thoracic diameter - 0.003 × inspiratory chest circumference + 0.003 × relaxed shoulder circumference + 0.002 × finger point height - 0.004 × intercristal distance - 0.002 × thickness of the side crease + 0.001 × thickness of the abdominal crease + 0.004 × largest head width.

The variability of the value of the slow blood filling time (s) of the shin rheovasogram depended by 99.81% on the total effect of 13 indicators of the external structure of the body. To reduce the multicollinearity of the independent variables of this polynomial, a constant was added to the correlation matrix. However, after applying the method of ridge regression, it turned out that the Fisher's criterion was 5.79, which was lower than its calculated value ($F_{cr.}$ = 6.17), which contradicted the condition of the regression analysis, and therefore the mathematical modelling was carried out without ridge regression. All coefficients of the independent variables of the polynomial were significant. Generally, the regression linear polynomial was significant ($p < 0.001$). Since the Fisher's criterion of 36.78 was three times higher than its calculated value ($F_{cr.}$ = 13.10), the statistical model was statistically significant:

SLOW BLOOD FILLING TIME = - 0.071 + 0.007 × neck circumference - 0.004 × waist circumference - 0.002 × shoulder width + 0.006 × largest head width + 0.002 × thigh crease thickness + 0.004 × smallest head width - 0.018 × distal tibia width + 0.006 × transverse mid-thoracic diameter + 0.003 × chest crease thickness - 0.001 × forearm crease thickness + 0.005 × forearm girth at the bottom - 0.004 × width of the distal epiphysis of the upper arm + 0.001 × width of the lower jaw.

Since the integral rheovasographic parameters of the shin are derived from amplitude and time parameters, it was not advisable to perform statistical modelling to determine the appropriate values of each of them, but the analysis of the constitutional determination of their variability was performed. Specifically, the coefficient of determination R^2 of 88.71% determined the dependence of the dicrotic index on the complex effect of 10 indicators of the external structure of the body. The variability of the diastolic index depended on the complex effect of 22 anthropometric parameters by 99.99%, and after applying the method of ridge regression, the coefficient of determination R^2 decreased to 63.84%. The variability of the average slow blood filling rate of the shin rheovasogram by 71.23% depended on the complex effect of constitutional parameters. Indicators of the tone of all arteries of the shin rheovasogram and the tone of medium and small diameters in mesomorphic volleyball players depended on the complex influence of a considerable number of anthropo-somatotypic parameters by 100%; after applying the method of ridge regression, the coefficient of determination in the first case decreased to 66.23%, in the second – to 53.33%. Other integral rheovasographic parameters of the shin had low accuracy of feature description.

DISCUSSION

Under the influence of constant physical exertion in the athlete's body, the mechanism of adjusting various organs and systems to the demands of a particular sport is launched, which ensures rational or irrational adaptation to training and competitive activity [16]. Systematic volleyball training leads to substantial changes in the cardiovascular system in adolescent female athletes. Thus, Y. Yakusheva [17] found that stroke volume and stroke index, minute blood volume, blood volume velocity, left ventricular power were significantly higher in female volleyball players compared to the control group, while the total peripheral resistance in female volleyball players was several orders of magnitude lower. Furthermore, the author argues that volleyball players with different somatotypes have substantial differences in central haemodynamics. S.L. Popel *et al.* [18] also found changes in response to physical activity in the cardiohemodynamics of volleyball players, specifically, the values of stroke and minute blood volumes, and changes in red blood cells in the peripheral blood were recorded. The peripheral circulation of female athletes in this specialisation also underwent certain changes. I. Stepanenko *et al.* [19] note that female volleyball players, compared to girls who did not engage in sports, had substantial differences in the value of regional blood circulation indicators. Specifically, they registered higher values of amplitude and time indicators on the shin, namely, the amplitudes of fast blood filling, systolic and diastolic rheovasogram waves, rheographic wave duration, time of the rise and down parts of the rheogram, and slow blood filling of the shin rheovasogram.

For female volleyball players to achieve high sporting results, it is crucial to be able to predict possible deviations in regional blood circulation and to predict the appropriate rheovasographic parameters. One of the ways of such mathematical forecasting is statistical modelling of the proper values of peripheral haemodynamics indicators based on the influence of constitutionally determined anthropometric parameters. L.C. Summer *et al.* [20] note that changes in body weight composition during the competitive season in field hockey athletes are accompanied by changes in sports performance. N. Nalyvayko *et al.* [21] conducted a correlation analysis between haemodynamic bioimpedance indices and body composition in women with different types of haemodynamics. O. Vysochanskiy [22] found numerous correlations between anthropometric and somatotypic parameters and hip and tibia rheovasograms in practically healthy boys from Podillia. Therefore, the present study was based on statistical analysis to investigate the relationships and interdependencies between the rheovasographic parameters of the shin and indicators of the external structure of the body in volleyball players of mesomorphic somatotype. The authors of the present study built 10 statistical models for all amplitude and time parameters of the shin rheogram. Notably, the principal factors of variability of the baseline impedance in female mesomorphic volleyball players were mainly anthropometric dimensions of the lower limb and the shape of the head, which can be considered as a somatotypic attribute. The baseline bioelectrical impedance reflects the body's resistance to alternating current, which results from tissue resistance to current and the reactive resistance associated with the capacitive component of the tissue. O. Di Vincenzo *et al*. [23] conducted a retrospective analysis of the significance of determining the bioelectrical impedance in representatives of various sports and noted that changes in its value are directly related to sports performance. Furthermore, the variability of the bioelectrical impedance itself depends on age and gender. J.C. Koury *et al*. [24] found that the values of bioimpedance indices in male athletes of different sports were lower at a younger age, and there were direct correlations with body weight and body mass index. However, despite the interest of scientists in using such a non-invasive and affordable method in the practice of sports medicine, there is no unanimous understanding of the reasons that can lead to changes in performance. Therefore, the use of multivariate regression analysis to determine the causes of variability in the rheovasographic parameters of the shin may provide an answer to this question. Each built statistical model makes it possible to individually determine a separate indicator of peripheral haemodynamics for a female volleyball player of mesomorphic somatotype, considering their anthropo-somatotypic characteristics.

The 10 statistical models built in this study included 94 dimensions of the external structure of the body. Among them, body circumference was the most frequently represented, accounting for 35.11% of all predictors of the regression polynomial and being included in all the models built (100%). Forearm circumference was included in 7 models; neck circumference was included in 5 models; shin circumference was included in 4 out of 5 statistical models of rheovasogram amplitude indices.

Notably, a considerable influence on the value of rheovasographic parameters of the shin in volleyball players of mesomorphic somatotype of the chest and pelvis diameters was established – they accounted for 19.15% of all anthropometric sizes of regression polynomials and were included in 90% of the built models, only the value of the amplitude of incisure was not significantly influenced by body diameters. Of this group of anthropometric measurements, the most commonly included in the models were transverse mid-thoracic (40% of models), anterior-posterior mid-thoracic (40% of models), shoulder width (30% of models), and intercristal distance (30% of models).

Furthermore, the variability of these regional circulation parameters also depended on the width of the epiphyses of the long tubular bones, which accounted for 9.57% of all predictors and were represented in 6 models (60%). The thickness of the skin and fat folds accounted for 17.02% of all predictors of the regression polynomials and was included in 8 models (80%), most often skin and fat folds were present on the shin, abdomen, and flank area.

V. Khavtur *et al*. [25] performed statistical modelling of thigh rheovasographic parameters in female volleyball players of ectomorphic somatotype, for which 16 regression models were built, and they also modelled integral rheovasographic parameters. Notably, in female volleyball players of ectomorphic somatotype, who are distinguished by significant height, predominance of longitudinal body dimensions over transverse ones, low fat deposition, variability of regional blood circulation indicators on the thigh by fewer indicators of external body structure, because the 16 regression models included 94 anthropometric and somatotypic dimensions. To the greatest extent, the variability of rheovasographic parameters of the thigh in female ectomorphic volleyball players was conditioned by the thickness of fat folds, head size, girth and anterior-posterior body dimensions, and somatotype components.

Thus, modelling cardiovascular system parameters using statistical methods is a modern approach that allows individualising test results, considering the unique features of each person's body structure and revealing the biomechanics of physiological processes.

CONCLUSIONS

The obtained findings, which were based on the use of multivariate regression analysis, made it possible to fulfil the purpose of this study. This is because for all amplitude and time indicators of the rheovasogram of the shin in female volleyball players of mesomorphic constitutional type, statistical models with high accuracy of description of signs were built, which make it possible to determine the appropriate values of regional blood circulation indicators on the shin, considering the anthropo-somatotypic characteristics of each athlete.

A reliable predominant influence of indicators of the external structure of the body on the variability of rheovasographic parameters of the shin in volleyball players of mesomorphic somatotype was established, as evidenced by the high values of the coefficients of determination of regional blood circulation in the shin. For the amplitude indicators of the shin rheovasogram, the values of the determination coefficients were within the following limits: $R^2 = 0.833\text{-}0.996$; for time – $R^2 = 0.896\text{-}0.998$, for integral – $R^2 = 0.638\text{-}0.999$.

The 10 statistical models of the amplitude and time indices of the shin rheovasogram included 94 dimensions of the external body structure. According to the results of the stepwise regression analysis, the largest value of the parameters of shin rheovasography was determined by the body circumference, which accounted for 35.11% of all predictors (most often the circumference of the forearm, neck, and shin), and was included in 100% of the models; chest and pelvic diameters (19.15% of all anthropometric dimensions of regression polynomials and included in 90% of the models); thickness of skin and fat folds, which accounted for 17.02% of all predictors and included in 80% of the models (most often folds on the shin, abdomen, and flank); width of the epiphyses of long tubular bones (9.57% of all predictors and included in 60% of the models).

The obtained results make it possible to analyse and determine the appropriate parameters of peripheral haemodynamics in youth volleyball players of mesomorphic somatotype during screening studies of female athletes, considering their personal values of anthropometric dimensions. In the future, it is worth using the identified anthropometric parameters for screening athletes to determine their peripheral haemodynamics and improve the efficiency of the training process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Математичне моделювання показників периферичної гемодинаміки гомілки у волейболісток мезоморфного соматотипу

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Анотація. Статистичне моделювання показників периферичної гемодинаміки, яке проводиться на основі визначення антропометричних і соматотипологічних особливостей організму можна вважати новим способом персоналізації інструментального діагностичного обстеження, тому визначення належних реовазографічних параметрів гомілки у висококваліфікованих волейболісток окремого соматотипу є актуальним. Мета роботи полягала у дослідженні сумарного впливу показників зовнішньої будови тіла на величину показників, які відображають особовості кровообігу на гомілці, у волейболісток мезоморфного соматотипу. Проведено комплексне клініко-лабораторне дослідження стану здоров’я та фізичного розвитку 108 волейболісток віком 16-20 років. Для визначення реовазографічних показників гомілки використовували метод тетраполярної реографії із застосуванням діагностичного комп’ютерного багатофункціонального апарату, оцінювали амплітудні, часові та показники відношень амплітудних і часових реовазографічних параметрів. Здійснено антропометричне дослідження тотальних та парціальних розмірів тіла. Соматотипування проведено за методом Heath-Carter. Відібрано 28 волейболісток мезоморфного соматотипу. Створення статистичних моделей проведено з використанням прямого покрокового багатофакторного регресійного аналізу. Для всіх амплітудних і часових реовазографічних параметрів стегна у волейболісток мезоморфного соматотипу були побудовані статистичні моделі з високою точністю опису ознак. До 10 побудованих моделей увійшло 94 антропометричних розмірів. Найбільше визначають величину параметрів кровообігу гомілки обхватні розміри тіла (найчастіше обхват передпліччя, шиї та гомілки), діаметри грудної клітки та тазу, товщина шкірно-жирових складок (найчастіше на гомілці, животі, боку), ширина епіфізів довгих трубчастих кісток. Встановлено, що показники зовнішньої будови тіла у межах від 63,84 % до 99,99 % детермінують величину показників регіонального кровообігу на гомілці у волейболісток мезоморфного соматотипу. Статистичне моделювання надає можливість визначити належні значення окремих реовазографічних показників на гомілці залежно від антропометричних параметрів осіб певної статі, віку, соматотипу, що особливо важливо для спортсменів певного виду спорту, тому що їхні показники серцево-судинної системи зазнають колосального впливу специфіки спортивної діяльності

Ключові слова: покрокова регресія; статистичне моделювання; реовазографія; антропометричні розміри; мезоморфний соматотип; волейбол

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