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CONTENT

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ANALYSIS OF THE PROBLEM OF PROVIDING PERSONAL PROTECTION EQUIPMENT FOR SCIENTIFIC EMPLOYEES OF THE ECOLOGICAL AND HYGIENIC LABORATORY

Altai State Medical University, Barnaul

N.P. Karamyshev, B.A. Balandovich, N.Yu. Tulin, A.V. Kurochkina

The article presents the analysis of the issue of providing personal protection equipment for employees of the scientific laboratory engaged in research and assessment of levels of impact of adverse factors of the ambient environment and production environment. This package of issues is of particular relevance due to the new ISO/IEC 17025-2019 interstate standard "General requirements for the competence of test and calibration laboratories" entering into force from September 1, 2019, where the main responsibilities of the accredited laboratory include the prevention of various air pollution of the working area, of the influence of adverse effects on laboratory activities, and minimization of occupational risks.

Key words: *personal protection equipment, laboratory, labor protection, harmful and dangerous factors, occupational risk.*

Providing personal protection equipment (PPE) for laboratory employees is one of the most important industrial issues, as proper organization of labor protection and protection of staff life and health depends on it in the process of exercising labor functions both in the stationary laboratory workplace and in the conduct of research outside it.

The obligation to provide PPE is enshrined in article 221 of the Labor Code of the Russian Federation: "If there is work involving harmful and/or hazardous labor conditions or work to be carried out under particular thermal conditions or those posing contamination, the employees shall be issued certified individual protection means, means for rinsing and rendering harmless according to the regulations approved in line with the procedure set up by the Government of the Russian Federation" [1].

Today, there are a number of regulations that contain basic rules on labor protection and provision of PPE for laboratory workers, but gaps and conflicts in legislation, the lack of necessary clarifying sectoral by-laws lead to improper provision of personal protection equipment for laboratory employees.

The lack of regulatory provision of PPE in the laboratory is a complex problem as employees can be affected not only by harmful and hazardous factors in the stationary workplace, but also by those factors that are present at the offsites where research is conducted. Laboratory workers face different industrial processes which cannot be stopped for some time in certain cases, so the technological process under study can negatively affect the body of the employee.

The purpose of this study is to identify and analyze problems of providing personal protection equipment for employees of the ecological and hygienic laboratory, as well as to determine ways to minimize the emerging occupational risks.

Materials and methods

Statistical and comparative law methods were used during the study.

The study analyzed statistics from the Ministry of Labor of the Russian Federation and Federal State Statistics Service.

Results and discussion

In carrying out labor functions, employees of the ecological and hygienic scientific laboratory are at risk of exposure to harmful and hazardous factors as the main areas of laboratory activities are occupational health and industrial ecology. Laboratory employees study conditions and nature of work, their impact on human health and functional condition, develop measures to prevent harmful and dangerous action of factors of the work environment and labor process on workers. In turn, this is associated with the professional risk of direct impact of these factors on the body of laboratory employees.

In the analysis of official data of the Ministry of Labor of the Russian Federation, it should be noted that in 2018 there is a steady tendency to decrease in the level of industrial injuries. The number of accidents with severe consequences (group, severe and fatal outcomes) decreased: 4,479 accidents with severe consequences occurred for 11 months of 2018, which is 3% less than in the same period in 2017 (4,614 cases).

In 2018, there is also a decrease in the number of deaths in the workplace: for 11 months of 2018, workers died 2% less than for the same period in 2017 (1,158 and 1,186 people respectively). However, the statistics still show quite a large number of accidents and industrial injuries in certain branches of activity of the organizations [2].

Official data of the Federal State Statistics Service of the Russian Federation show that the number of employees interacting with harmful and haz-

ardous factors and exposed to production injuries remains the highest compared to other economic activities in construction, manufacturing, agriculture and forestry, transport, extraction of minerals (Figure 1). By conducting scientific research in the organizations of the above branches, employees of ecological and hygienic laboratories actively interact with various harmful factors [3].

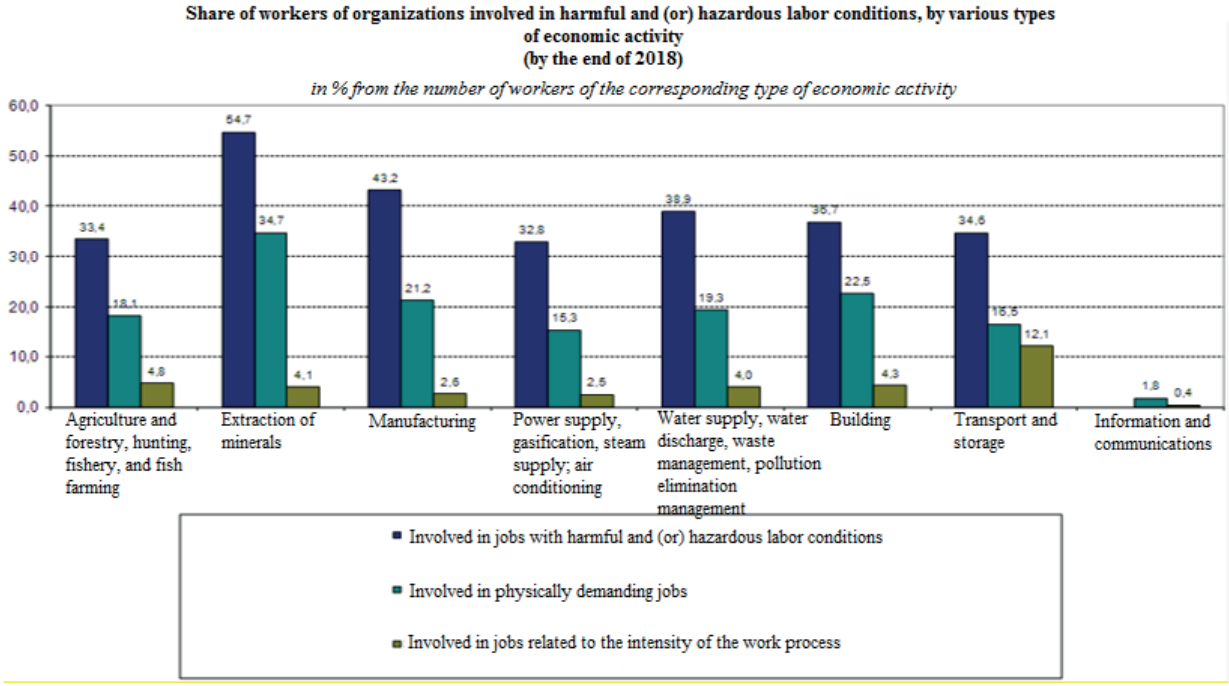

Figure 1.

In carrying out research in laboratories and in the organization of field work, all the requirements of the normative legal acts in the field of labor protection, industrial ecology, sanitary regulations and hygienic regulations, fire safety requirements, electrical safety requirements and instructions for the operation of laboratory and other equipment, including specially designed for clinical and diagnostic, microbiological, virological, chemical and analytical, research laboratories, accredited laboratory of labour conditions hygienic monitoring-https://www.asmu.ru/struktura-agmu/institut-gigieny-truda-i-promyshlenoy-ekologii/, and all necessary personal protection equipment must be obligated.

Depending on the specifics of their activities, laboratory employees are exposed to harmful and hazardous occupational factors, as well as various occupational risks. These include: high neuro-emotional tension, monotony of labor during the sampling of the air of the working zone and atmosphere, harmful chemical substances and biological agents, ionizing radiation under radiation control of X-ray machines and electromagnetic radiation of radio-frequency range in the survey of transmitting radio installations, noise, vibration, moving machines and aggregates, work at heights, and other factors. Appropriate personal protection equipment is required to ensure the labor protection of laboratory employees.

The provision of personal protection equipment for laboratory employees is often not effective enough as the process of PPE delivery is not always risk-oriented and does not always take into account the complexity of the impact of various harmful and hazardous industrial factors. Often enough, the organization of PPE provision is limited only to factors present at the stationary sites in the laboratory, without regard to the conditions affecting employees outside the work places.

Chemical factors are among the most commonly found harmful factors of the labor process for a laboratory employee. According to paragraph 2 of the "Labor Protection Regulations in the Use of Certain Chemicals and Materials", the requirements of these regulations on labor protection and use of PPE are mandatory for employees of any laboratories. They are included in the section "Labor protection requirements in the use of chemicals in laboratories" [4].

It should be noted that the specialized requirements of labor protection and provision of PPE when working in scientific ecological and hygienic laboratories are not fully established, there are no sectoral regulations on labor protection for these institutions. In this regard, it is necessary to develop labor protection instructions and a list of PPE for laboratory workers, guided by a large number of regulations, such as:

- Order of the Ministry of Labor of the Russian Federation of 19.04.2017 No. 371n "On approval of

the Regulations on labor protection in the use of certain chemicals and materials";

- Order of the Ministry of Labor of the Russian Federation of 17.08.2015 No. 552n "On approval of the Regulations on labor protection when working with instruments and devices", POT RO-14000-002-98;

- Order of the Ministry of Labor of the Russian Federation of 9.12.2014 No. 997n "On approval of the Model Rules for free issuance of special clothing, special shoes and other personal protection equipment to employees of cross-industry professions and positions of all economic activities engaged in jobs with harmful and (or) hazardous working conditions, as well as in jobs performed under special temperature conditions or pollution related";

- Order of the Ministry of Labour and Social Protection of the Russian Federation of 28.03.2014 No. 155n "On approval of the Regulations on labor protection at work at heights";

- Order of the Ministry of Labor of the Russian Federation of 24.07.2013 No. 328n (ed. 19.02.2016) "On approval of the Regulations on labor protection in operation of electrical installation";

- Order of the Ministry of Health and Social Development of the Russian Federation of 1.06.2009 No. 290n "On approval of the Intersectoral Rules for providing workers with special clothing, special shoes and other personal protection equipment";

- Regulation "Safety precautions of production equipment" (appr. Ministry of Economy of the Russian Federation, 20.01.1998);

- "POT RO 14000-005-98. Regulation. High hazard jobs. Organization", section 13. Safety requirements for handling hazardous chemical substances; section 14. Safety requirements in chemical laboratories;

- "POT R M-004-97. Intersectoral regulations on labor protection in the use of chemicals";

- local acts of the organization: collective agreement, labor protection regulations;

- operational documentation for equipment, instruments, etc. operated in the laboratory;

- and the type of labor operations and actions performed by laboratory employees.

In accordance with the Order of the Ministry of Labor and Social Protection of the Russian Federation dated December 9, 2014 No. 997n, the minimum list of PPE for laboratory employees include depending on the direction of research:

1. Physical and mechanical tests:

- overall for protection from general industrial pollution and mechanical effects;

- bib apron made of polymer materials;

- goggle;

- filtering respiratory protective equipment [5, par. 62].

2. Chemical analysis:

- overall for protection from general industrial pollution and mechanical effects;

- bib apron made of polymer materials;

- gloves made of rubber or polymer materials;

- goggle [5, par. 66];

- filtering (Figure 2) or insulating respiratory protective equipment.

3. Research in radiometric, dosimetric control:

- suit for protection from general industrial pollution and mechanical effects;

- bib apron made of polymer materials;

- arm sleeves made of polymer materials;

- gloves with polymer coating [5].

It should be noted that these lists of the PPE use in an organization when they are incorporated into local acts may be more extended and combined, for example, if a laboratory employee conducts research in several areas, when they are affected by a greater number of harmful factors, or when conducting research without stopping the production process.

In this case, it is necessary to follow article 221 of the Labor Code of the Russian Federation, which stipulates that "With account taken of the opinion of the elected body of the primary trade union organisation or another representative body of employees and their financial and economic situation, an employer is entitled to establish rated quantities for providing special clothes, special footwear and other means of individual protection to employees, such rated quantities ***improving*** the protection of the employees from the harmful and/or hazardous factors available at the workplace, and also special temperature conditions or pollution as compared with the model rates" [1].

Thus, the employer has the right to expand the list of personal protection equipment that will improve the safety of laboratory employees. For example, if laboratory employees carry out radiation and dosimetric control at an industrial site and work alongside high-risk sources (lifting device, etc.), they will need personal protection equipment for the head, and if they work at height – PPE from falling from height, if it is a noisy factory – personal protection equipment for organs of hearing.

In addition, an important regulatory source enshrining the right to expand the list of PPE is the Order of the Ministry of Health and Social Development of the Russian Federation of June 1, 2009 No. 290n "On approval of the Intersectoral Rules for providing workers with special clothing, special footwear and other personal protection equipment", which stipulates that when expanding the list of PPE, the employer approves this by local regulations on the basis of the results of the Special Assessment of Working Conditions (SAWC) and taking into account the views of the relevant trade union or other body authorized by the employees [6].

Figure 2. The use of personal protection equipment in the utilization of indicator tubes in the accredited Laboratory of Labour Conditions Hygienic Monitoring.

Conclusion

Summing up the study, we can conclude that providing personal protection equipment for laboratory employees is quite a multicomponent issue. The disadvantages of existing legislative acts are the absence of a specific list of PPE for laboratories engaged in comprehensive research, including ecological and hygienic laboratories. These lists would allow for a clear regulation of personal protection equipment required for this type of laboratories.

Another problem is the lack of clear regulatory recommendations on the application of intersectoral legislation and by-laws, regulations on labor protection and PPE. Employers cannot always clearly identify the negative factors that affect employees in the workplace and in conducting research outside the workplace. The results of a special assessment of working conditions do not always determine the whole range of PPE a laboratory employee needs.

When carrying out field studies on the territory of the customer, targeted training on labor protection often is not carried out and the production process is not stopped, which may lead to violations in labor protection and safety.

Thus, it can be concluded that this problem should be solved comprehensively in the following directions:

1. Gaps in the legislation may be solved by the adoption of provisions on labor protection at the level of individual departments that supervise the direction of the work of laboratories or a separate process of production where a clear list of mandatory PPE will be specified;
2. The adoption of local regulations at the level of the organization (collective agreement, labor protection provisions in the organization, list of PPE) will clearly regulate the provision of PPE for employees depending on factors of labor process in the laboratory and outside the laboratory in carrying out research;
3. In carrying out field studies, it is necessary to preliminarily analyze service contracts with customers and include a rule providing for the liability of the customer for non-security of labor protection during the study, for the lack of targeted training and for the failure to ensure the temporary interruption of the production process during the study. This rule will provide labor protection for the laboratory employee when performing a job function outside the workplace;
4. There should be an outreach to laboratory employees that their violation of PPE rules is a disciplinary offence that entails dismissal for a single gross violation of labor discipline, provided for in paragraph d of part 6 of Article 81 of the Labor Code of the Russian Federation: "violation of labor protection regulations by the employee if this violation has leaded to disastrous consequences (industrial accident, damages, catastrophe) or could certainly lead to these consequences" [1].

The set of these measures is aimed at prevention of misuse of PPE in the laboratory, which in turn will prevent cases of industrial injuries and occupational diseases, as well as minimize various occupational risks.

Conflict of interest. The authors declare no conflict of interest.

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ROLE OF MAST CELLS IN LUNG ADAPTATION PROCESSES TO SINGLE AND MULTIPLE DEEP IMMERSION HYPOTHERMIA

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The research objective was to compare the effect of single and multiple daily deep immersion hypothermia on the morphofunctional activity of lung mast cells in Wistar rats in the experiment. The study was conducted on 45 rats. Hypothermia was modeled by placing animals in individual cages in water at a temperature of 5°C with the environment temperature of 7°C. The impact of the cold factor was stopped when animals reached a deep degree of hypothermia, the criterion of which was rectal temperature of 20–25°C. The material for histological examination was taken immediately after hypothermia and after 2, 7, and 14 days. Immediately after the impact of the cold factor, large MC were detected in a state of agranulocytosis in the animal pulmonary tissue. On the 2nd day of the experiment, with multiple hypothermia compared to the single one, the number of MC in the field of view increased by 37.1%; the area of MC increased by 32.2%; the number of degranulating MC increased by 14.4%, and the content of compact forms of MC decreased by 17.9%. On the 7th day, with multiple hypothermia, the number of MC in the field of view increased by 32.3%; the number of degranulating MC was 1.9 times more, and the content of compact forms of MC decreased by 1.4 times. On the 14th day, the number of MC in the field of view increased by 3.1 times; the number of degranulating MC was 1.4 times more, and the content of compact forms of MC decreased by 1.2 times. Thus, MC are an important element in the long-term adaptation of lungs under the impact of multiple deep immersion hypothermia.

Key words: *hypothermia, lungs, mast cells, adaptation.*

In recent years, there has been a clear trend towards increasing respiratory diseases in the Russian Federation, which is most associated with the effects of environmental factors, in particular, cold [1]. In the Siberian part, diseases such as bronchitis, pneumonia, bronchial asthma occupy leading places in the structure of the population morbidity [2]. This is primarily due to the fact that respiratory organs are directly in contact with cold air, they are significantly more difficult to protect against exposure to cold factor, and therefore they most often suffer from the action of low temperatures [3]. Also, the peculiarity of the state of the internal environment of the body leads to a chronic course of respiratory diseases and development of obstructive processes in the climatic conditions of Siberia significantly more often than in the inhabitants of moderate latitudes.

The study of adaptive compensatory reactions of internal organs of animals and humans (lungs, liver, myocard, kidneys, thyroid) when exposed to cold attracts the attention of researchers as diseases of these organs very often take a chronic course precisely in those regions where the body is exposed to low temperatures (Siberia, Far East). The disclosure of adaptation mechanisms to this damaging factor may help in the development of new adaptogens [4].

The study of the activity of mast cells (MC) has important therapeutic aspects [5]. Today, in the clinic of internal diseases, the morphofunctional activity of MC is studied in diseases such as atherosclerosis [6], myocardial infarction [7], dilated cardiomyopathy [8], myocarditis [9]. The important role of MC in the processes of regeneration of different tissues [10, 11] under hypoxic conditions [12], stress [13, 14, 15] has also been identified. They can stimulate carcinogenesis processes [16, 17, 18, 19].

The morphological bases of adaptation processes in organs in hypothermia have been actively investigated recently [20, 21, 22]. MC are important in compensatory adaptive responses of the body to the action of various damaging extreme factors, hypothermia including. The distribution and migration of MC between internal organs and tissues under stress and action of exogenous and endogenous damaging factors can be considered within the framework of adaptation syndrome [23]. All this allowed some authors to express the idea that MC are a single independent regulatory system of the body.

The study of MC in pulmonary tissue is of important clinical significance as they interact with cellular and stromal microenvironment, have direct and inverse relationships with the immune, endocrine and nervous systems of the body [24]. In exocytosis of granules, histamine is released from MC, which stimulates the secretion of mucus by goblet cells and cells of glands of the submucous membrane of trachea and bronchi. Also histamine of MC causes the spasm of small bronchial and bronchiole smooth muscles and increase in the interalveolar septum capillary permeability. MC acti-

vate fibroblasts, macrophages, stimulate migration of neutrophils, lymphocytes into the pulmonary tissue and therefore play a crucial role in immune responses. However, despite the active study of MC in the respiratory system under physiology and pathology, their morphofunctional activity is not sufficiently studied under the impact of endogenous extreme factors, including hypothermia, on the pulmonary tissue.

The research objective was a comparative analysis of morphofunctional activity of lung MC in Wistar rats in single and multiple deep water hypothermia.

Materials and methods

The study was conducted on male Wistar rats weighing 200–240 grams (N=45). The animals were subjected to single (n=20) and multiple (n=20) deep immersion hypothermia (DIH). Hypothermia was modeled as follows: animals in individual cages were placed in water at a temperature of 5°C with the environment temperature being 7°C. The impact of the cold factor was stopped when animals reached a deep degree of hypothermia, the criterion of which was the rectal temperature of 20–25°C. The time of impact of the cold factor averaged 40±5 min. Immediately after the cessation of cooling, after 2, 7, and 14 days, the animals were taken out of the experiment by decapitation, each group had 5 rats. The control group consisted of 5 rats which were placed in individual cages in water at a temperature of 30°C with the environment temperature of 22–25°C. The exposure time in the control group corresponded to the exposure time in the experimental group. The use of rats in the experiment was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and Directives 86/609/EEC.

For histological examination, the pieces of pulmonary tissue were fixed in 10% neutral formalin solution for 24–48 hours, and then treated in the machine TISSUE-TEK VIPTM6 (Sakkura, Nagano Japan) with following paraffin embedding in the corresponding station TISSUE-TEK TEC 5 (Sakkura, Nagano, Japan). Sections 5–7 μm thick were obtained using a rotary microtome Accu-Cut SRM (Sakkura, Japan). The materials were stained with haematoxylin-eosin in a TISSUE-TEK Prisma machine for automatic staining (Sakkura, Nagano, Japan). MC were detected with BiOvitrum toluidine blue (Saint Petersburg). The photomicrography was carried out using the microscope Leica DM 750 E200 (Germany) with a digital video camera Leica EC3 (Germany) with the zoom x400. The density of MC distribution was calculated in the program Image Tool 3.0 in 5 fields of view with the zoom x400. The field of view of the microscope amounted to 0.366 mm^2. We also determined the value of the mast cell degranulation index (the ratio of MC in the state of degranulation to the total number of analyzed cells expressed in percentage). Morphometry of MC was carried out in the morphometric program VideoTest-Morphologia 5.2.

Statistical processing of the obtained data was carried out using the Statistica 10.0. statistical programs package and the statistical analysis package of the MS EXCEL 2010 program. For each of the quantities, we calculated the mean (M) and error of mean (m). The hypothesis about the normality of the data probability distribution was checked using the Shapiro-Wilk test. The reliability of the data was evaluated using the parametric Student's t-test. The critical value of the statistical significance level was 0.05.

Results and discussion

In the lungs of rats of the control group of the study, MC were visible in pleura, in adventitia of bronchi and vessels when staining with toluidine blue. They had a predominantly rounded shape and small size. The MC distribution density ranged from 1 to 4 and averaged 2.0±0.5 in the field of view with the zoom x400. The area of MC was 82.5±3.6 μm^2. There were 87±10.1% of compact forms of MC in which granules were arranged compactly. There were 23±10.1% of degranulating forms of MC when the phenomena of degranulation were detected.

Immediately after exposure to hypothermia. In the pulmonary tissue of animals immediately after the impact of single DIH, MC were found mainly in the peribronchial tissue. They were pale purple in color, had large sizes, were rounded or oval in shape. The MC distribution density ranged from 1 to 6 and averaged 2.7±0.8 in the field of view. The area of MC was 184.5±14.9 μm^2. It should be noted that a significant part of the detected MC was in a state of devastation as a result of secretion of granules and agranulocytosis. The number of degranulating MC was equal to 75±17.1%; compact forms of MC amounted to 25±17.1%.

With multiple DIH, at this time of the experiment, the number of MC in the field of view increased to 2.9±0.8. The area of MC increased to 190.5±10.9 μm^2. The content of degranulating MC was 78±16.1%. There were 22±16.1% of compact forms of MC (Figure 1; Tables 1, 2).

On the second day of the posthypothermic period, after single DIH, MC were located in groups in the walls of thickened interalveolar septums. They were small in size, rounded or oval. The MC distribution density ranged from 4 to 10 and averaged 6.6±0.5 in the field of view. The area of MC averaged 84.2±3.5 μm^2. The number of compact MC was 52.0±8.9%, MC in a state of degranulation amounted to 48.0±8.9%.

With multiple DIH, the number of MC in the field of view increased by 37.1%. The area of MC increased by 32.2%. The content of degranulating MC increased by 14.4%, and the number of compact forms of MC decreased by 17.9% respectively (Figure 2; Tables 1, 2).

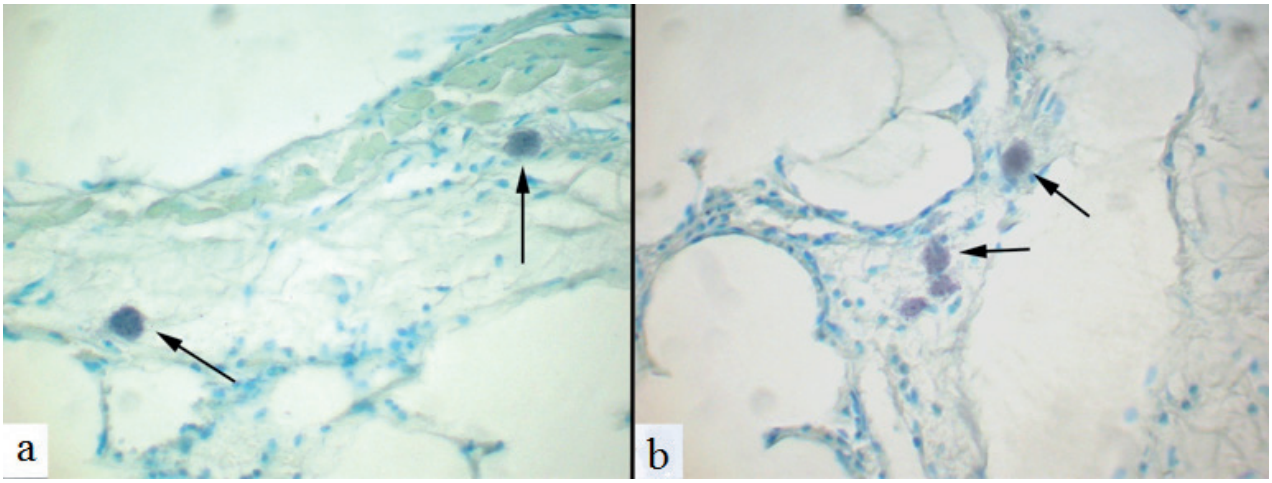

Figure 1. MC (shown by arrows) in a state of desolation and agranulocytosis immediately after hypothermia is performed: a) in single DIH; b) in multiple DIH. Staining with toluidine blue. Zoom x400.

Table 1

Quantitative and morphometric parameters of MC of the pulmonary tissue in rats in single deep water hypothermia

Parameters of mast cells	Day of the experiment			
	Immediately after hypothermia (I)	Day 2 (II)	Day 7 (III)	Day 14 (IV)
Number of MC	2.5±0.8	6.6±0.5	8.0±1.0	4.3±0.3
Number of compact MC (%)	25±17.1	52±8.9	76.7±8.6	70.7±12.2
Number of degranulating MC (%)	75±17.1	48±8.9	23.3±8.6	29.3±12.2
Area of MC (μm²)	184.5±14.9	84.2±3.5	107.9±7.3	90.8±6.2

Note: for the number of MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.01. $P_{II\text{-}III}$, $P_{II\text{-}IV}$ <0.04. $P_{III\text{-}IV}$ <0.001. For the number of compact MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.001. $P_{II\text{-}III}$ <0.01. For the number of degranulating MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.03. $P_{II\text{-}III}$ <0.04. For the area of MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.000005. $P_{II\text{-}III}$ <0.0004.

Table 2

Quantitative and morphometric parameters of MC of the pulmonary tissue in rats in multiple deep water hypothermia

Parameters of mast cells	Day of the experiment			
	Immediately after hypothermia (I)	Day 2 (II)	Day 7 (III)	Day 14 (IV)
Number of MC	2.9±0.8	10.5±0.8	10.3±0.6	13.4±1.9
Number of compact MC (%)	22±16.1	42.7±4.2	54.7±2.9	58.5±4.3
Number of degranulating MC (%)	78±16.1	56.3±4.2	45.3±2.9	42.5±4.3
Area of MC (μm²)	190.5±10.9	124.2±7	100.2±3.0	88.0±6.2

Note: for the number of MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.01. For the number of compact MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.001. $P_{II\text{-}III}$ <0.01. For the number of degranulating MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.03. $P_{II\text{-}III}$ <0.04. For the area of MC: $P_{I\text{-}II}$, $P_{I\text{-}III}$, $P_{I\text{-}IV}$ <0.00004. $P_{II\text{-}III}$ <0.03. $P_{III\text{-}IV}$ <0.04.

On the 7th day of the posthypothermic period, after single GIG, the migration of MC from interalveolar septums to the peribronchial connective tissue was observed. At this time of the experiment, the morphometric parameters of MC differed significantly from the previous term: they had intense purple coloration, elongated or irregular shape, and large sizes. The MC distribution density ranged from 6 to 12 and averaged 8.0±1.0 in the field of view, their area increased to 107.9±7.35 μm². Compact MC amounted to 76.0±8.6%, degranulating cells amounted to 23.3±8.6%.

In multiple DIH, the number of MC in the field of view increased by 32.3%. The area of MC did not change significantly. The number of degranulating MC was 1.9 times more, and the number of compact forms of MC decreased by 1.4 times respectively (Figure 3; Tables 1, 2).

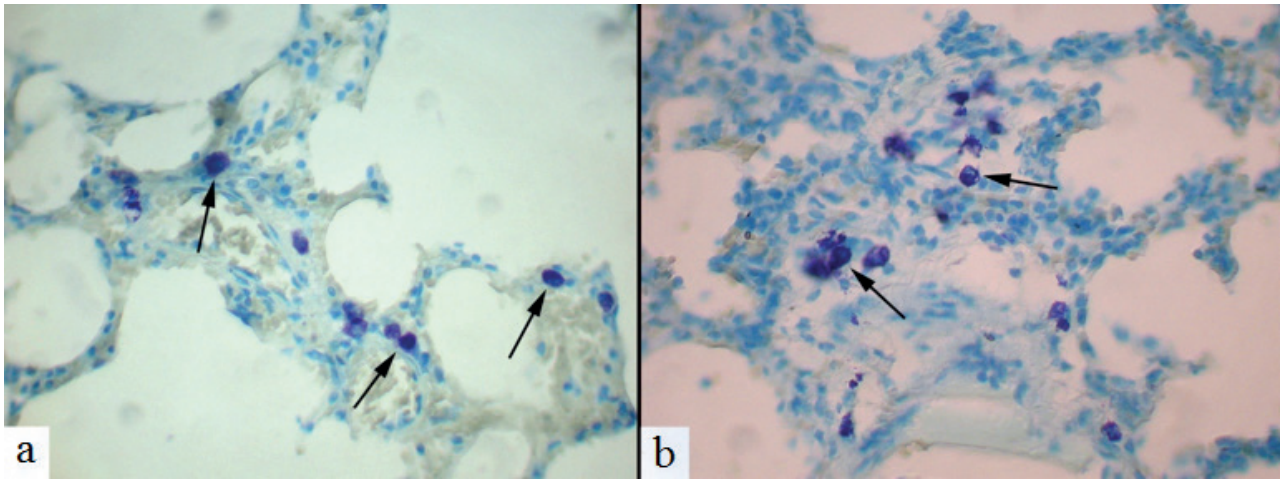

Figure 2. MC (shown by arrows) in the rat lung on the 2nd day of the experiment: a) in single DIH; b) in multiple DIH. Staining with toluidine blue. Zoom x400.

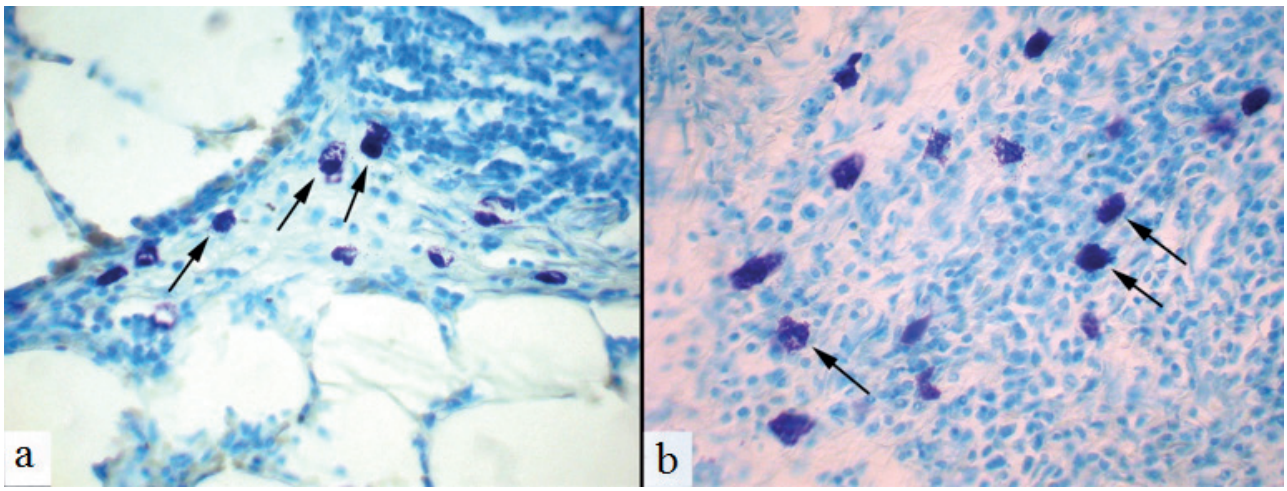

Figure 3. MC (shown by arrows) in the rat lung on the 7th day of the experiment: a) in single DIH; b) in multiple DIH. Staining with toluidine blue. Zoom x400.

On the 14th day of the posthypothermic period, after single GIG, MC were located in the peribronchial tissue predominantly singly or in small groups. MC had small sizes, most of them being rounded in shape. The MC distribution density ranged from 3 to 5 and averaged 4.3±0.3 in the field of view, the cell area averaged 90.8±6.2 μm^2. The number of compact MC was 70.7±13.2%, the number of cells in a state of degranulation was 29.3±12.2%.

In multiple DIH, the number of MC in the field of view increased by 3.1 times. The area of MC did not change significantly. The number of degranulating MC was 1.4 times more, and the number of compact forms of MC decreased by 1.2 times respectively (Figure 4; Tables 1, 2).

In this study, a comparative analysis of morphofunctional activity of MC in single and multiple DIH was carried out. It was revealed that immediately after single DIH the MC system was in a state of deterioration, most mastocytes were in a state of total degranulation and granulolysis. On the 2nd day of the posthypothermic period, the number of MC increased, the cells had small sizes, most of them were in the capillaries of thickened interalveolar septums, which may indicate the migration of young forms of MC into the pulmonary tissue from the blood stream. In our opinion, the migration of MC to the lungs in hypothermia should be considered within the framework of adaptation syndrome. Other authors received similar data. Thus, in experiments on rats subjected to immobilization stress, Artashyan O.S. et al. (2012) also revealed the redistribution of MC between organs: they observed an increase in the content of MC in the adrenal glands, liver, skin, gastric and intestine mucosa, at the same time, the mastocyte content significantly reduced in the bone marrow and thymus. On the 7th day of the posthypothermic period, MC migrated from alveoles to the peribronchial connective tissue, and the number of degranulating forms decreased; on the 14th day of the experiment, the content of MC in a state of degranulation was the smallest, and the number of MC close to normal.

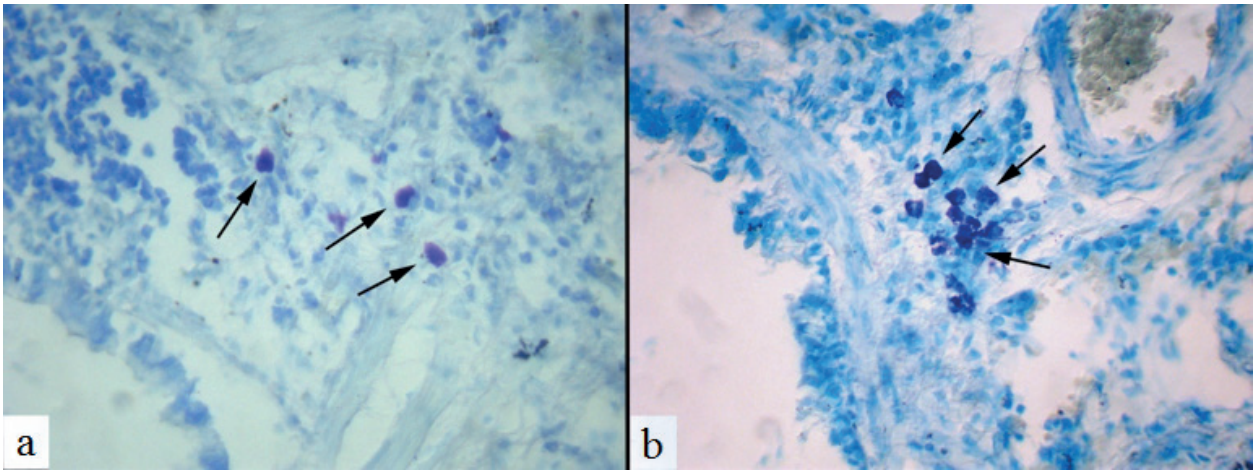

Figure 4. MC (shown by arrows) in the rat lung on the 14th day of the experiment: a) a small number of MC in the peribronchial tissue in single DIH; b) a large number of MC in the peribronchial tissue in multiple DIH. Staining with toluidine blue. Zoom x400.

In multiple daily DIH compared to single, the dynamics of changes in the mast cell population was different. At all terms of the experiment, in pulmonary tissue, there was a smooth increase in the number of MC and a simultaneous increase in the content of degranulating cell forms. Thus, it was revealed that the dynamics of changes in morphofunctional activity of MC in the pulmonary tissue of rats depended on the duration of exposure to cold factor.

Different dynamics of morphofunctional activity of MC discovered in single and multiple DIH, in our opinion, was interconnected with compensatory adaptive reactions. Adaptive reactions are known to divide into two types: 1) urgent but unstable and imperfect adaptation, and 2) long-term, steady adaptation. Urgent adaptive reaction occurs immediately after the beginning of impact of the damaging factor and is implemented by previously formed biological mechanisms. At the same time, the reaction of the body is carried out at the limit of physiological possibilities and the effects of adaptation work not to the full extent. On the contrary, long-term stable adaptation forms during a long multiple exposure of the damaging factor to the body. As a result, there is a gradual accumulation of certain morphological changes that allow organs to acquire new properties and qualities, and thus to turn from unadapted to adapted ones. With that, functional reserves of organs increase, it allows them to withstand higher stress loads.

The results of the study revealed that long-term adaptation of the bronchopulmonary apparatus to cold stress formed in rats in multiple DIH. Long-term adaptation in the pulmonary tissue of rats under the impact of hypothermia, in our opinion, was structurally provided by an increase in the number of effector MC, as well as an increase in their morphofunctional activity. As is known, hypothermia leads to hypoxia and body tissue damage. MC express receptors which react to changes in partial pressure of oxygen and carbon dioxide, so activation of MC under the impact of the hypoxic factor is a natural process [25].

Conclusion

Thus, in daily multiple DIH, in the pulmonary tissue of rats, the phenomena of long-term adaptation form, the structural manifestations of which are the increase in the number of effector MC and increase in their morphofunctional activity.

Conflict of interest. The authors declare no conflict of interest.

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STUDY OF CLAYS OF THE SIBERIAN REGION TO CREATE COMPOSITES BASED ON THEM

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Informative parameters of assessment of Siberian clay mines studied as an example were observed in the work for the clay use in therapeutic practice and cosmetology, creation and storage of biologically active composites based on clay. The results of the study of clays spread in the borders of Kemerovo Oblast, Altai Krai are presented. The X-ray phase analysis and spectral analysis established the main elements of microcrystalline structure, characteristic of kaolin and polymineral clays, their chemical composition. Adsorption capacity, cation exchange capacity important for the use of clays for medicine have been identified along with normalized safety indicators. It is shown that the unified criterion defining the most important physical characteristics of clay prepared for application (plasticity, adsorption, thermal and other) is the content of the smallest (less than 0.01 mm) constituent particles. Enrichment of clays with biologically active additive of carbon acid extract of Siberian fir influenced the biochemical state of the resulting composites, ensured the stability of qualitative indicators in storage dynamics. The therapeutic use of this type of raw material contributes to the realization of resource saving directions.

Key words: *clays, informative parameters, biologically active additives, storage.*

The relevance of scientific search and applied studies to create new medications on the basis of components of natural origin is due to the tasks of improving the level and quality of health of the population including the means of expanding the use of drug-free methods of exposure to the body with clinically effective results. The increasing demand for medical-recreational natural preparations determined the need for research of economically inexpensive and affordable raw materials – kaolin clay (natural sorbent), the efficacy of which as a heat carrier is shown in combined procedures in the treatment of osteoarthrosis, diseases of the female genital sphere, etc. [1, 2, 3]. The expansion of the range of targeted action of these clays is promising by enriching them with biologically active additives for various purposes and creating composites applicable in medicinal preventive institutions and cosmetology.

The complex of positive properties inherent in the clays, crystal-chemical diversity and huge reserves concentrated in the Siberian region offer new opportunities for obtaining active modified products on their basis. In the medicinal use of this type of raw material, not only resource supply of raw materials is significant, but also their quality, environmental safety, and resource saving [4, 5].

The research objective was to determine the most informative parameters of qualitative assessment for the use in medical practice, conditions of pre-procedure preparation, creation and storage of composites based on clays using the Siberian clay mines studied as an example.

Materials and methods

For the use in medicine, we investigated genetically diverse natural clays within the borders of Kemerovo Oblast, Novosibirsk Oblast, Krasnoyarsk Krai, Altai Krai, and the Altai Republic. The mineralogical composition of clays was established by radiographic analysis. Quality for therapeutic use was assessed in accordance with the requirements of methodical instructions of the Ministry of Health of the Russian Federation No. 2000/34 "Classification of mineral waters and medicinal mud for the purpose of their certification", including organoleptic, physical chemical, and sanitary microbiological indicators [6]. In carrying out chemical analyses, we used: weight, spectral, titrimetric, photocolorimetric, extractive-photocolorimetric, potentiometric methods (GOST 26449.1-85, RD 52.24.433-2005, GOST 19723-74, GUEST 11306-2013, GOST 10538-87). Radiological indicators and content of heavy metals were controlled by gamma spectrometric (MVI 15.1.6(2)-14), neutron activation methods. Sanitary microbiological indicators of clays were evaluated according to regulatory criteria of therapeutic mud (coliform bacteria, titer Clostridium perfringens, quantity of mesophilic, mesotrophic aerobes and facultative anaerobes (total microbial count; TMC)), the presence of pathogenic cocci bacteria and Pseudomonas aeruginosa; physiological groups of microorganisms were evaluated by the method of limiting dilution on selective mediums.

Results and discussion

Clays are widespread sedimentary rocks composed mainly of extremely small microcrystals of clay minerals, largely determining their properties. Relative accessibility combined with favorable characteristics inherent in clays (adsorption properties, soft, thermal, etc.) allow them to be considered as a valuable therapeutic raw material.

In Kemerovo Oblast, sedimentary clays of the Barzas group of deposits were the objects of re-

search at the sites: Aprelskoye, Vaganovskoye, Musokhranovskoye, Taldinskoye, Mokhovskoye. In the natural state, these clays are of various coloring (white, red, ochreous, grey, etc.), crumby structure, without smell. Their natural humidity averages no more than 2.0%, rarely reaching 4.0%. The mineral composition, according to X-ray phase analysis, is dominated by associations of thin-scaled kaolinite, to a lesser extent – of hydromica, montmorillonite, minerals of the chlorite group. The chemical composition includes silicon oxides, variations in iron oxide content and other defined elements (Table 1). The obtained values of adsorption capacity (up to 36 mg/g) and cation exchange capacity (10.31 mg-eq/100 g) of clays show the belonging of the studied clays to kaolinic ones.

Table 1

Bulk chemical composition of clays of deposits in Kemerovo Oblast (%)

Deposit	SiO_2	Fe_2O_3	Al_2O_3	TiO_2	MnO	CaO	MgO	SO_3	P_2O_5
Vaganovskoye (red)	30.37	22.03	41.55	0.75	0.01	3.92	0.44	0.78	0.02
Musokhranovskoye (white)	67.30	0.64	26.91	0.10	0.03	3.74	0.10	1.09	0.02
Mokhovskoye (grey)	64.38	6.23	24.85	0.38	0.02	2.44	0.22	1.16	0.02

The results of the granulometric analysis allowed to distinguish areas with the total content of thin dust fractions in the solid phase 50% or more, which provided these clays with satisfactory physical properties (Table 2). Further preparation of the clays consisted of grinding and sifting them through a sieve with 2-3 mm holes, eliminating large inclusions. The main physical-chemical properties, important for therapeutic use, were investigated after their hydration to optimal sticky plastic consistency according to indicators: humidity, bulk weight, ultimate shearing resistance, foreign matter content, ratio of oxidized and reduced forms of iron, adsorption capacity, sanitary microbiological properties. The liquid fraction (pressing of mud) separated after interaction with clay had a variety of ion-salt composition and was characterized by variability in the reaction of the medium: from neutral to alkaline (pH 7.0–8.9), the content of a balneologically valuable component – metasilicic acid (up to 36 mg/dm^3).

Table 2

Granulometric composition of studied clays of deposits in Kemerovo Oblast

Site	Fractions in%, size in mm			
	0.05-0.01 coarse dust	0.01-0.005 medium dust	0.005-0.001 fine dust	<0.001 silt
Aprelskoye	22.53	14.83	15.13	17.52
Mokhovskoye	21.53	7.69	28.44	15.11
Vaganovskoye	15.3	8.81	13.28	28.49
Musokhranovskoye	10.52	4.68	18.70	39.43

The studied clays of the territory of Altai Krai and the Altai Republic are formation outcrops, lie in the form of lenses, have, as a rule, polymineral, mostly illite-chlorite composition. According to the results of granulometric analysis, in the structure of particles that contain clay data, pelitic fractions (less than 0.01 mm) predominate, non-clay particles of composition are represented by hydrogoethite 0.026 μm in size. After moistening to the state optimal for application, the clay data had a weak alkaline reaction (pH 7.5), adsorption capacity up to 4.5 mg/g, satisfactory physical-chemical properties (thermal, shearing resistance, etc.). The content of heavy metals in them, established by spectral analysis, showed compliance with regulatory requirements of therapeutic mud (lead – 0.043 g/t, copper – 0.02 g/t, zinc – 0.027 g/t). The safety of clays by radiological state was assessed by the normative content of natural (K_{40} – 504 Bq/kg, Ra_{226} – 21 Bq/kg, Th_{232} – 30 Bq/kg) and anthropogenic (Cs_{137}, Sr_{90}) radionuclides.

The satisfactory properties of clays of the studied deposits on physical-chemical, sanitary microbiological condition were the basis for the study of the possibility of creating composites based on them. It is known that the phytocomponents added to the therapeutic mud can bring in some characteristics that enhance anti-inflammatory, tonic and

other effects on the body [7]. As a bio-stimulating supplement to natural clays of Vaganovskoye (red) and Mokhovskoye (grey) sites, in the experiment, we used the aqueous fraction of the carbonic acid extract of Siberian fir, the effective dose of which was 0.2% of the mass. The resulting composites were investigated on biochemical indicators: activity of oxidoreductase class enzymes (polyphenol oxidase – PPO, peroxidase – POD), content of organic substances (organic carbon – C_{org}), total lipids and water-soluble vitamin – ascorbic acid, sanitary microbiological state in the dynamics of 1.5 and 3 months of storage (Table 3).

Table 3

Indicators of biological activity of composites based on clays and Siberian fir extract

Indicators	Composites studied					
	Red clay			Grey clay		
	original	1.5 months	3 months	original	1.5 months	3 months
PPO activity, mg 1.4 benzoquinone/30 min per 10 g	<0.1	1.89	5.85	13.94	7.53	6.0
POD activity, mg 1.4 benzoquinone/30 min per 10 g	<0.1	6.50	6.67	<0.1	21.73	37.10
C_{org}, %	0.15	0.06	0.04	0.13	0.12	0.2
Lipids, %	0.18	0.06	<0.01	0.22	0.09	<0.01

The introduction of fir extract at the initial stage increased the C_{org} content in composites (by an average of 30%) on the background of decreased concentration of total lipids and inactivation of defined enzymes. By 1.5 months, the grey clays showed a growth of the PPO enzyme (by 2 times compared to the original content) against the background of stability of the C_{org} content and the lipid level. Oxidative processes were further accompanied by the destruction of lipid structures. The most significant increase in the POD enzyme activity (by 30 times) was observed by the 3-month shelf life of composites.

Microbiological studies on storage of composites with the Siberian fir extract revealed the stability of indicators on the titer Cl. Perfringens (0.1) and Pseudomonas aeruginosa (absence) in all samples during the 3-month shelf life. A significant decrease in mesotrophic, mesophilic aerobes and facultative anaerobes (total microbial count – TMC) was noted, as well as reliable influence of the fir extract on the activity of ammonifying bacteria in dynamics of storage of studied varieties of clays.

Conclusion

The assessment of various natural clays for the purposes of medicine and cosmetology along with established criteria (sanitary microbiological, radiological, and heavy metal content) is the determination of the main forming elements and parts of the mineral composition, the size of its particles. The latter is an essential condition and a unified criterion defining a number of the most important physical characteristics of clay prepared for application: plasticity, adsorption, thermal and other properties, which is confirmed by the testing of deposits of Kemerovo Oblast, Altai Krai, the Altai Republic and other territories of the Siberian region. Enrichment of clay with stimulating biologically active additive of Siberian fir carbonic acid extract influenced the biochemical state of the resulting composites, revealing differences in velocity the oxidation processes of polyphenolic compounds and the transformation of the organic component in them, which ensured the stability of qualitative indicators of composites in storage dynamics. Clays enriched with active additives are more attractive than native (natural) for use in resort activities and medical rehabilitation due to the availability of a range of micro- and macroelements, specific components, etc. This area of research is very promising and requires further study.

Conflict of interest. The authors declare no conflict of interest.

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RESULTS AND PROSPECTS OF PRENATAL DIAGNOSIS AT THE REGIONAL LEVEL

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Prenatal diagnosis of commonly occurring fetal chromosome aberrations is one of the primary methods for predelivery diagnosis of congenital diseases. The objective of this study was to assess the most effective markers of fetal chromosome aberrations and dynamics of prenatal detection of this pathology in the territory of Altai Krai. Based on the analysis of data for the period from 2012 to 2019, prenatal diagnosis of fetal chromosome pathology in Altai Krai was assessed by the results of prenatal screening of the first and second trimester. The effectiveness of prenatal examination of the first and second trimester was assessed for the last 5 years (2015–2019). The obtained results suggest high informativity of early prenatal screening. The combination of ultrasound markers of fetal chromosome aberrations and biochemical screening results showed its maximum efficacy; in this case, prenatal diagnosis was 48.7%.

Key words: *prenatal diagnosis, fetal chromosome aberrations, biochemical screening.*

The problem of diagnosis of fetal chromosome pathology is quite relevant at present [1, 2]. Not only the medical, but also the socioeconomic aspect of this problem is of great importance, which is certainly related to morbidity, mortality, early disablement of children with chromosome pathology [2]. Despite the introduction of modern methods of prenatal diagnosis, risk assessment using modern software, the frequency of prenatal diagnosis in early prenatal screening of the most common chromosome aberrations (trisomy on 21,18,13th chromosomes) is12–60%[3, 4, 5]. As part of early prenatal screening of pregnant women living in the territory of the Russian Federation, an ultrasound examination of the expert level is carried out in the gestation period of 11–13.6 weeks with the simultaneous study of biochemical markers (pregnancy-associated plasma protein-A (PAPP-A) and free β-subunit of human chorionic gonadotropin (β-HCG)), study of history data, the individual risk of the most common fetal chromosome pathology is calculated through the Astraia software installed on the module Fetal Medicine Foundation [5, 6].

Assessment of the most effective markers of chromosome pathology and dynamics of prenatal detection of fetal chromosome aberrations in the territory of Altai Krai was the objective of this study.

Materials and methods

During the analysis of data for the period from 2012 to 2019, prenatal diagnosis of fetal chromosome pathology in Altai Krai was assessed by the results of prenatal screening of the first and second trimester. For comparative analysis of data for the time interval from 2012 to 2014, the data on the surveys conducted in the Altai Interregional Genetic Consultation (Barnaul) were used.

The early prenatal screening program carried out a screening ultrasound in the period of 11–13.6 weeks to identify congenital malformations and commonly accepted markers of fetal chromosome pathology (thickness of the collar space, length of the nose bones, blood flow in the venous duct with measurement of the pulsation index, blood flow through the tricuspid valve). Biochemical markers indicators (PAPP-A, β-HCG) in the 1st trimester were studied, followed by an individual risk assessment based on a threshold (1:100) using the Astraia program.

A second biochemical prenatal screening was carried out during the pregnancy period of 16–18 weeks in the absence of the first screening or on additional indications of medico-genetic counseling, which included the determination of alpha-fetoprotein (AFP), free β-subunit of human chorionic gonadotropin (HCG) and estriol; further, by means of the software, the individual risk was calculated with a threshold cut-off of 1:250 (PRISCA). Medico-genetic counseling was carried out, indications were determined to carry out invasive methods of prenatal diagnostics. Invasive prenatal diagnostics (IPD) involved the collection of the material for the cytogenetic study by biopsy of chorionic villi and cordocentesis, depending on the gestation period.

Statistical data processing was carried out using the Microsoft Office software (Word 2007, Excel 2007), STATISTICA 7.0 application package (StatSoft Inc., USA).

The absolute number and share of these values were used to calculate and analyze qualitative indicators, and the following formula was used to calculate the share: $\hat{p} = \frac{m}{n}$, where n is the total number of patients studied, m of them have the study trait.

Values of share indicators are presented in the form of confidence intervals $\hat{P} \pm S_{\hat{p}} t$, where $\hat{P}$ – the share assessment; $S_{\hat{p}} t$ – 95% limit standard share error. The comparative analysis of qualitative variables involved the construction of 2x2 conjugation tables if the sum of all frequencies in the table is less than 20, and/or in the presence of expected frequencies less than 5, the exact two-way Fisher test was used, with a sum of frequencies more than 20 – the chi-squared test (2) with Yates's correction for continuity.

Results and discussion

According to the results of the study, invasive prenatal diagnosis (IPD) was carried out in 4324 women for the last 8 years since 2012, when in Altai Krai the Regional Center for Prenatal Diagnosis of Child Development Disorders was organized on the basis of the Altai Interregional Genetic Consultation. As a result of cytogenetic studies, chromosome aberrations (CA) were found in 547 fetuses. The dynamics of invasive prenatal diagnosis and ratio with prenatally diagnosed cases of fetal CA for 2012–2019 are shown in Figure 1.

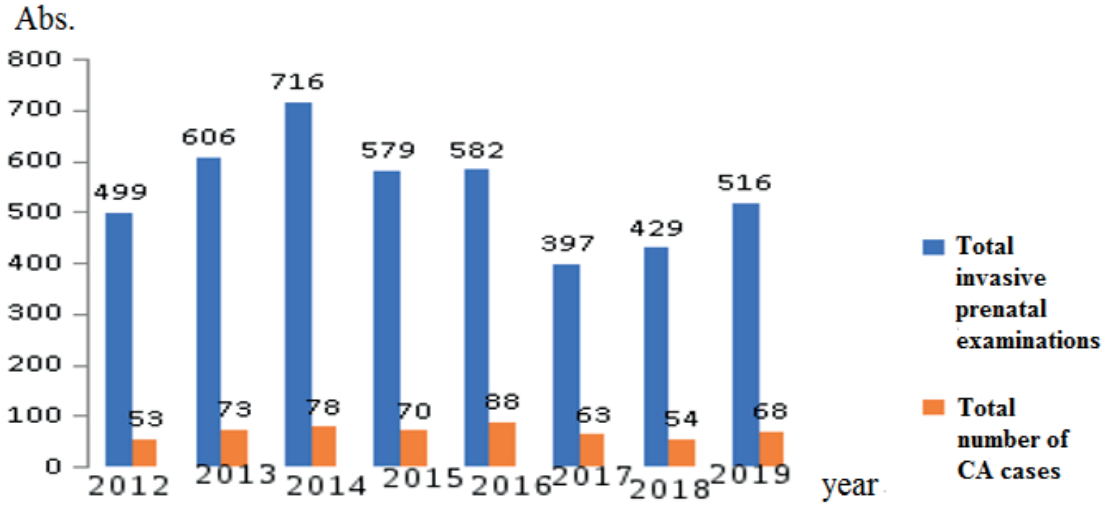

Figure 1. Dynamics of invasive prenatal diagnosis and ratio with prenatally diagnosed cases of fetal chromosome aberrations (absolute value) for 2012–2019.

The percentage ratio of the performed IPD procedures and the number of detected cases of fetal CA during the study period averaged 12.7%±2.5% regardless of the time of performing. The dynamics of prenatal diagnosis of fetal CA in the territory of Altai Krai for the last 8 years is shown in Figure 2.

The main indications for prenatal fetal karyotyping were a combined risk of fetal chromosome pathology 1:100 or more (Astraia), two or more ultrasound markers (expansion of the collar space, hypoplasia, or lack of image of nasal bones and others), presence of congenital malformations (CM), combination of the combined risk (biochemical markers) and sonographic markers of fetal CA. Table 1 shows the proportion of prenatally diagnosed fetal CA cases, depending on the criteria for selection in the "high risk" fetal CA group for early prenatal screening. According to the results of the study, the most informative and effective indications for carrying out invasive prenatal diagnosis from the position of CA detection are high individual risk with the combination of sonographic and biochemical markers in the first trimester of pregnancy, this figure is 48.7±1.0% for the last 5 years; in the second place of significance, there are sonographic markers of CA (23.1%), and biochemical markers have less diagnostic informativity since prenatal diagnosis in this case is only 9.0% (Table 1).

Consequently, in the timely prenatal screening at the gestation period of 11–13.6 weeks, with the combination of sonographic and biochemical markers of fetal CA, the consent of the woman to carry out invasive procedure, almost every second patient confirmed fetal chromosome aberrations in cytogenetic methods.

Based on the analysis of risk factors of the 2nd trimester (sonographic and biochemical) in general and the ratio with prenatally diagnosed cases of fetal CA in the last 5 years, it was revealed that prenatal diagnosis takes place in only 6.5±2.1% of cases (2015 – 7.5%; 2016 – 8.0%; 2017 – 7.3%; 2018 – 4.1%; 2019 – 5.4%). Sonographic and biochemical markers of fetal CA had almost equal importance in determining indications for carrying out invasive prenatal diagnosis in the 2nd trimester, since prenatal diagnosis of CA in sonographic markers of the 2nd trimester was 5.4%, with biochemical markers deviation (PRISCA), the threshold risk was of 1:250 and higher – 5.8% (p=0.157; Table 2). Table 2 shows the dynamics of informativity of fetal CA risk factors over the past 5 years.

Table 1

Dynamics of prenatally detected cases of fetal CA depending on indications (risk factors in the 1st trimester) to prenatal cytogenetic diagnosis for the last 5 years in Altai Krai (2015–2019)

Risk factors for CA in the 1st trimester	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)
	2015		2016		2017		2018		2019		Total	
Two or more CA ultrasound markers, including CM	47	11 (23.4±1.80	21	4 (19.0±3.7)	8	2 (25.0±7.3)	9	3 (33.3±10.3	6	1 (16.7±6.1)	91	21 **(23.1±0.8)**
Biochemical screening results (PAPP-A, β-HCG)	144	7 (4.9±0.3)	173	15 (8.7±0.3)	106	8 (7.5±0.5)	110	10 (9.1±0.5)	137	20 (14.6±0.5)	670	60 **(9.0±0.6)**
Combination of risk factors (CA ultrasound markers and biochemical screening result)	83	29 (34.9±1.1)	83	42 (50.5±1.3)	61	37 (60.7±1.6)	54	28 (51.9±1.8)	56	28 (50.0±1.8)	337	164 (48.7±1.0)

Table 2

Dynamics of prenatally detected cases of fetal CA depending on indications (risk factors in the 2nd trimester) to prenatal cytogenetic diagnosis for the last 5 years in Altai Krai (2015–2019)

Risk factors for CA in the 2nd trimester	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)	Number of IPD	Revealed CA Abs. P± (%)
	2015		2016		2017		2018		2019		Total	
Two or more CA ultrasound markers, including CM	89	6 (6.7±0.5)	63	3 (4.8±0.4)	84	4 (4.8±0.4)	117	3 (2.6±0.3	115	9 (7.8±0.5)	464	25 **(5.4±0.5)**
Biochemical screening results (AFP, β-HCG, estriol)	143	5 (3.5±0.4)	176	11 (6.3±0.5)	98	8 (8.2±0.5)	80	5 (6.25±0.5)	123	7 (5.7±0.5)	620	36 **(5.8±0.5)**

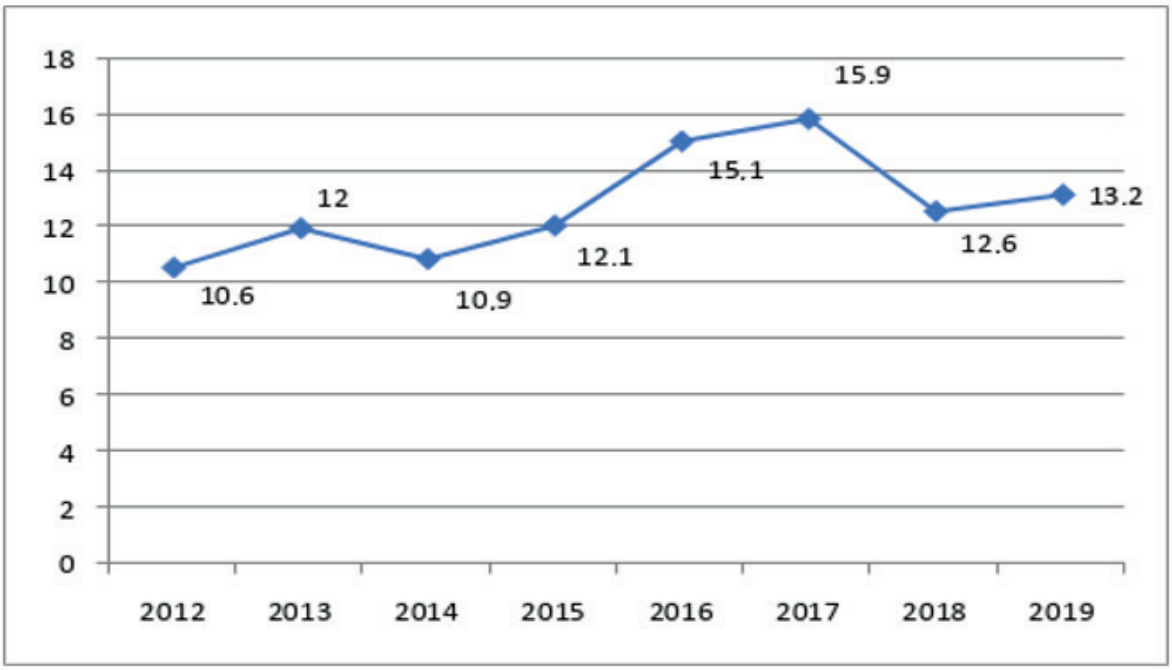

Figure 2. The dynamics of prenatal diagnosis of fetal chromosome aberrations in the territory of Altai Krai (2012–2019).

In total, for the last 5 years (2015–2019) in Altai Krai, out of 343 cases of chromosome aberrations established by prenatal diagnosis methods, 185 (53.9±5.2%) cases of trisomy on the 21st chromosome (Down syndrome) were detected, 55 (16.0±3.9%) cases of trisomy on the 18th chromosome (Edwards syndrome), 16 (4.7±1.2%) cases of trisomy on the 13th chromosome (Patau syndrome); the remaining 87 (25.4±4.6%) cases were represented by other genetic chromosome syndromes (triploidy syndrome, Shereshevsky–Turner syndrome, Klinefelter syndrome, and others; Figure 3).

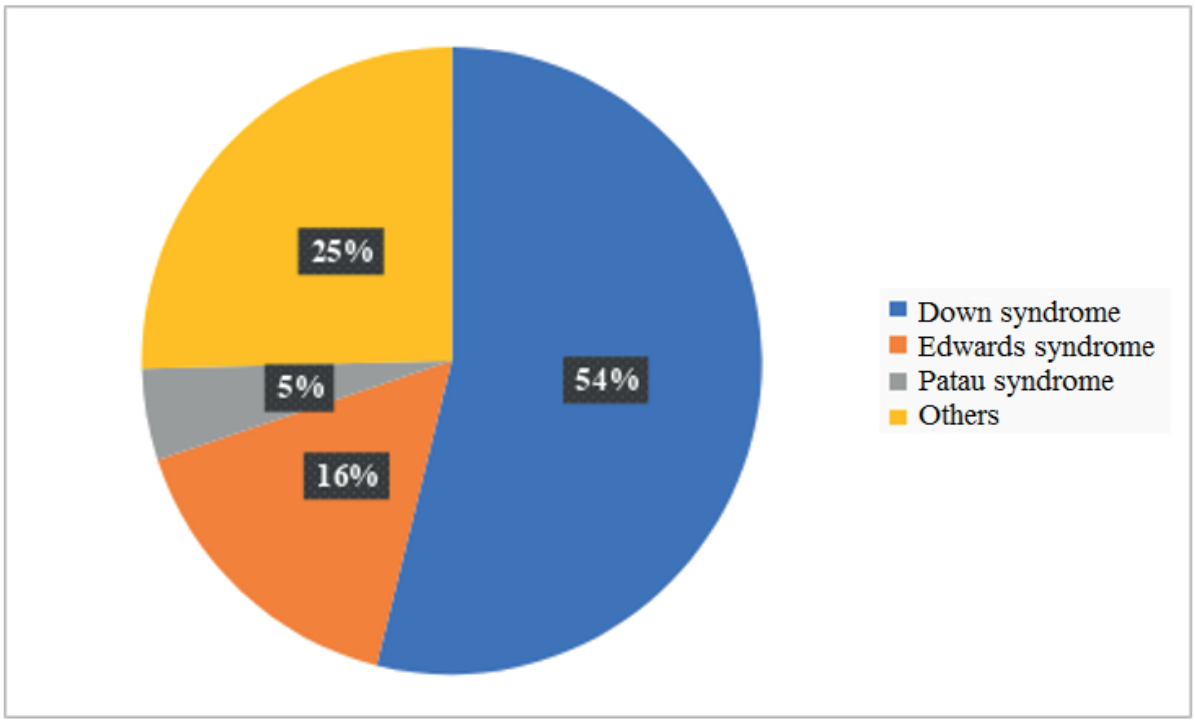

Figure 3. The structure of prenatally revealed fetal CA in Altai Krai for 2015–2019.

Conclusion

Combined early prenatal screening, provided it is timely, allows effective evaluation of sonographic and biochemical markers of fetal chromosome pathology, genetic counseling and determination of indications for invasive prenatal diagnosis methods. The prognostic value of CA markers in the second trimester is sufficiently low, and to a

greater extent they should be considered as a necessary measure in the late visit of a woman to a women's health clinic. Efficacy of prenatal diagnosis determines the possibility of timely execution of ultrasound screening of expert level in the period of pregnancy of 11–13.6 weeks with identification of additional markers of fetal CA, as well as definition of CA biochemical markers that will increase the prognostic significance of non-invasive methods. The determination of fetal DNA in the mother's blood is the promising and non-invasive prenatal test, the introduction of this method in the network of state medical institutions, first of all the third level (perinatal centers, regional medico-genetic consultations), would significantly reduce the number of invasive procedures, better determine indications for invasive methods.

It should be noted that even in the presence of ultrasound markers and exceeding the threshold level of risk calculated by the software, the positive result of a non-invasive prenatal test is not a fact of detecting fetal CA, but only an indication for prenatal cytogenetic study.

Conflict of interest. The authors declare no conflict of interest.

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INFLUENCE OF RHEUMATOID ARTHRITIS ON QUALITY OF LIFE AND PSYCHOLOGICAL STATUS OF PATIENTS IN PRESENCE OF ASSOCIATED RESPIRATORY SYSTEM PATHOLOGY, PROSPECTS OF DRUG-FREE AND DRUG CORRECTION

Altai State Medical University, Barnaul

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The research objective was to comparatively assess the QOL of RA patients and the correlation relationships of its manifestations with the severity of anxiety-depressive disorders in association with respiratory diseases.
Materials and methods. The study included 172 patients, 48 of them were diagnosed with RA (group I), 62 – association of RA and RD (group II), 60 were diagnosed with respiratory diseases (group III). RA was determined in accordance with the classification criterion ACR/EULAR (2010) for RA, with an average score of 8.4±3.11 on 4 positions. The groups are comparable in gender and age. The SF-36 Health Status Survey (SF-36) and HAQ questionnaires were used to assess the QOL of RA patients. The assessment of reactive and personal anxiety was conducted according to the State-Trait Anxiety Inventory.
Results. In group II patients (RA+RD), all QOL indicators were statistically significantly lower than in comparison groups (p<0.001). The greatest changes occurred in physical health indicators (physical functioning (PF), role-physical functioning (RPF), pain (P), general health). Among the QOL indicators characterizing psychological health (V, social functioning (SF), role-emotional functioning (REF), mental health (MH)), REF and V were most reduced: by 52.3% and 36.1% respectively. When combined RA and RD, the level of clinically expressed anxiety was the highest (60.9%; pI-II=0.007; pII-III=0.03). Clinically expressed depression was diagnosed in 56.3% of group II patients (pI-II=0.021; pII-III=0.046); in group III patients, subclinical depression was statistically significantly more likely to be diagnosed (60%, pII-III=0.051). In the majority of RA patients according to the STAI, there was high personal (48%) and situational (56%) anxiety. In regression analysis, the severity of HADS anxiety was directly correlated with age (r=0.44), HAQ index (r=0.69), disease activity according to DAS28 (r=0.53), disease duration (r=0.37), functional class (r=0.38); depression severity was correlated with BMI (r=0.66) and age (r=0.82) of patients.
Conclusion. A comprehensive study of the QOL of RA patients, including association with respiratory system pathology, revealed significant interrelations of QOL indicators with the main clinical characteristics of patients, severity of pain, and the degree of disease activity. The presence of associated lesion of the musculoskeletal, respiratory systems and psychoemotional sphere forms a vicious circle of mutual pressure and dictates the need for a comprehensive examination of the patient for the sake of adequate correction.
Key words: *quality of life, rheumatoid arthritis, respiratory diseases.*

According to the results of epidemiological studies, currently in Russia, the total number of patients with rheumatoid arthritis (RA) is just over 1 million people (almost 1% of the population) [1, 2].

In two-thirds of RA patients during the first two years from the onset of the disease, radiographic signs of articular tissue destruction appear. After three to five years from the onset of the disease, more than 50% of patients become disabled and lose work functions. Disruption of joint mobility, involvement of other organs and systems in the process, adverse side effects of the therapy lead to pronounced psychoemotional disorders, among which anxiety-depressive and social disorders prevail, leading to a significant deterioration in the quality of life (QOL) of patients [3].

According to modern data, rheumatoid arthritis, anxiety-depressive disorders, and respiratory system diseases have not only common risk factors: smoking, chronic psychosocial stress, genetic predisposition, but also combine a number of common pathogenetic mechanisms. Changes in the immune system of a pro-inflammatory nature play the main pathogenetic role, they include disruptions in the production and interaction of T lymphocytes, cytokines, interleukins (IL)-1, 6, 8, tumor necrosis factor TNF-A, as well as oxidative stress, detected in respiratory tract diseases, rheumatological and psychoemotional disorders. Systemic inflammation, like systemic oxidative stress, with an increase in pro-inflammatory cytokines is the main pathophysiological mechanism of the formation and progression of both directly respiratory diseases and the formation of extrapulmonary effects [4, 5].

The research objective was to assess the QOL of RA patients and to identify the relationship of its manifestations with the severity of anxiety-depressive disorders in association with respiratory system diseases (RSD).

Materials and methods

We conducted the direct clinical observation of 172 patients who were on hospital treatment in rheumatological and general therapeutic departments of KSBHI "Regional Clinical Hospital" of Barnaul for the period from 2016 to 2018. All patients were divided into groups:

- group I consisted of 48 RA patients;
- group II included 64 RA patients in combination with RSD;
- group III included 60 patients suffering from RSD.

Exclusion criteria: special, including juvenile, forms of RA, presence of malignant neoplasms, presence of other clinically significant chronic somatic diseases with adverse short-term prognosis, refusal to participate in the study.

Patients were included in the study upon admission. Each patient signed an informed consent form.

Patients were given the complex clinical examination with dynamic observation, which included clinical, biochemical, immunological (RF, CRP) blood tests, general urine test, radiography of joints.

According to the criteria of the American College of Rheumatology (ACR)/EULAR (2010), the diagnosis of RA was determined to be reliable [6]. In the presence of a previously established diagnosis of chronic respiratory system pathology, the analyzed groups of patients II and III were formed. When updating criteria according to GOLD (2020) [7] and GINA (2019) [8], the chronic obstructive pulmonary disease was diagnosed in 63 (50.8%), bronchial asthma in 37 (29.8%), interstitial lung diseases (2018) – in 24 (19.4%), in all in the remission phase without statistically significant differences in groups.

Statistical processing of the material was carried out using the Statistica 8.0 program. The level of statistical significance was taken as $p<0.05$.

Results and discussion

The distribution of patients by sex was assessed. The majority of patients were women, with no statistically significant differences in groups: 87.5% in group I, 82.8% in group II, 81.6% in group III ($p>0.05$). Middle-aged patients prevailed, with the median age of group I patients recorded at 60 [54; 65] years, group II – 57 [51; 64] years, group III – 59 [55; 69] years ($p>0.05$). Thus, the groups were comparable in major demographic determinants.

The duration of RA in group I was 7.8 [6; 12], in group II – 8.3 [6; 11] years ($p=0.56$; U=1260.5 M-W U-test). Extra-articular manifestations of RA were present in 8 patients of group I and 17 of group II ($p=0.334$; Z-test, two-sided). The RA variant seropositive by rheumatoid factor (RF) was diagnosed in 27 patients of group I and 45 of group II ($p=0.031$; Z-test, two-sided). Radiological stage II was most commonly diagnosed (according to Steinbrocker) without statistically significant differences in groups (20 patients of group I and 28 patients of group II, $p=1.00$; Fisher extract p, two-tailed). At the same time, it was found that patients of group II were diagnosed with radiological stage IV more often that patient of group I (15 patients, $p=0.028$; Fisher extract p, two-tailed). High degree of activity (>5.1) on the DAS28 index prevailed in group II patients (in 36, $p=0.000$; Fisher extract p, two-tailed), moderate (3.2£ DAS 28 £ 5.1) and low activity (DAS 28 < 3.2) was more often found in group I patients (in 26, $p=0.120$ and in 12, $p=0.003$; Fisher exact p, two-tailed). The average activity index score (DAS28) was 4.5±1.03 in group I patients and 5.7±1.08 in group II patients ($p<0.05$). More than half of patients had class II functional insufficiency of joints (ACR, 2010) – 23 patients of group I and 31 of group II ($p=1.000$; Fisher extract p, two-tailed); at the same time, FC I was more often diagnosed in group I patients than group II patients (in 20, $p=0.017$), and FC III in group II patients (in 21; $p=0.019$; Fisher extract p, two-tailed).

The basic therapy carried out when including RA patients in the study contained: methotrexate at doses of 7.5–15 mg per week (77% – 84% of groups I and II respectively), sulfasalazine – 10–7%, leflunomide – 14–11%. More than 50% of patients received prednisolone (10.2±7.3 mg/day) with no statistically significant differences in groups. About two-thirds of patients received non-steroid anti-inflammatory drugs.

The QOL of patients was determined by the questionnaire SF-36 (Short Form-36-Item Health Survey) [10]. In order to assess the impact of the main and concomitant diseases on the quality of life of patients on the SF-36 scale, a comparative assessment of the QOL indicators of patients with RA was carried out, with the combination of RA and RSD, as well as indicators of population norms of the corresponding sex and age. The latter are validated by the International Center for Research of the QOL of St. Petersburg with the calculation of 8 main indicators: PF – physical functioning, RPF – role-physical functioning, P – pain, GH – general health, V – viability, SF – social functioning, REF – role-emotional functioning, MH – mental health, and the evaluation of 2 total measurements: physical (PCS) and mental health (MCS), which were compared to population control of the corresponding sex and age. The processing of the SF-36 scales was carried out using a special license package of statistical programs [11, 12].

The obtained results of the QOL parameters of patients of the analyzed groups of the corresponding sex and age, estimated with the help of the SF-36 questionnaire, indicate that all indicators of the QOL of patients were statistically significantly lower compared to population. This was most concerned with group II physical health indicators

(RA and RSD), which were more than 21 points below the average population control values, thus making it possible to conclude that there were significant limitations of group II patients in self-care and physical work. Indicators of the physical health changed most of all (PF, RPF, P, GH). PF was reduced by 45.9%, RPF by 38.4%, P by 34.7% compared to similar indicators in the absence of associated respiratory system pathology.

Among the QOL indicators characterizing psychological health (V, SF, REF, MH), V and SF were most reduced by 37.2% and 33.1% respectively.

Correlation dependencies of some clinical indicators with physical (PCS) and mental health (MCS) SF-36 scale values were analyzed (Table 2).

As can be seen from Table 2, most scales had moderate, statistically significant correlation with age, disease activity, tender joint count, pain by VAS. A high negative correlation between SF-36 scales and the functional status of RA patients by HAQ has been identified. There was no correlation of the QOL with the radiological stage of the disease (r=-0.14; -0.11) and the presence or absence of rheumatoid factor (r=-0.10; -0.09).

Table 1

Distribution of analysed groups of patients according to the SF-36 survey scales

Indicator	RA	RA and RSD	RSD	Kruskal-Wallis	LSD test
PF	58 [40; 75]	44 [29; 61]	68 [44; 83]	H=24.547; p=0.000	P_{I-II} = 0.001; P_{II-III} = 0.000
RPF	44 [28; 59]	39 [25; 59]	58 [41; 69]	H=20.045; p=0.000	P_{I-II} = 0.756; P_{II-III} = 0.000
P	54 [32; 62]	34 [23; 49]	61 [48; 79]	H=36.418; p=0.000	P_{I-II} = 0.001; P_{II-III} = 0.000
GH	50 [30; 59]	37 [29; 55]	55 [39; 62]	H=8.679; p=0.015	P_{I-II} = 0.019; P_{II-III} = 0.008
V	47 [36; 55]	36 [24; 51]	50 [41; 60]	H=24.895; p=0.000	P_{I-II} = 0.001; P_{II-III} = 0.000
SF	55 [40; 64]	48 [26; 59]	65 [57; 75]	H=24.899; p=0.000	P_{I-II} = 0.042; P_{II-III} = 0.000
REF	46 [38; 55]	43 [23; 61]	55 [38; 71]	H=15.423; p=0.000	P_{I-II} = 0.043; P_{II-III} = 0.000
MH	51 [45; 62]	49 [27; 63]	54 [41; 66]	H=4.926; p=0.081	P_{I-II} = 0.025; P_{II-III} = 0.008

Table 2

Correlation dependencies between SF-36 scales and the clinical picture of analysed groups of patients

Indicators	RA		RA + RSD	
	PH	MH	PH	MH
Age, years	-0.35(*)	-0.32(*)	0.44(*)	-0.39(*)
Activity by DAS 28	-0.50	-0.41	-0.59(*)	-0.47(*)
Tender joint count, n	-0.30(*)	-0.33(*)	-0.36(*)	-0.44(*)
Pain by VAS, mm	-0.58(*)	-0.64(*)	-0.68(*)	-0.75(*)
HAQ, points	-0.72(*)	-0.75(*)	-0.82(*)	-0.75(*)
Radiological stage	-0.16	-0.11	-0.15	-0.15
RF, IU/ml	-0.08	-0.11	-0.13	-0.10

Note: the numerical values of the Pearson correlation coefficient, the sign "-" signifies the negative correlation relationship. The correlation statistically significant at p<0.05 is indicated by an asterisk.

A high incidence of anxiety-depressive disorders was identified in RA patients when studying psychoemotional status. According to the Hospital Anxiety and Depression Scale (HADS), clinically expressed anxiety was observed in one in three (35%) RA patients, more than half (56%; p_{I-II}=0.04) had subclinical anxiety. When combined respiratory diseases and rheumatoid arthritis, the level of clinically expressed anxiety was the highest (60.9%; p_{I-II}=0.007; p_{II-III}=0.03). Clinically expressed depression was diagnosed in 56.3% of group II patients (p_{I-II}=0.021; p_{II-III}=0.046); in group III patients, subclinical depression was statistically significantly more likely to be diagnosed (60%, p_{II-III}=0.051). Similar data were obtained when assessing depression on the Beck Depression Inventory.

According to the State-Trait Anxiety Inventory, the majority of RA patients showed high personal (48%) and situational (56%) anxiety with the burdened clinical picture in the presence of associated pathology.

In regression analysis, the severity of HADS anxiety was directly correlated with age (r=0.44), HAQ index (r=0.69), disease activity according to DAS28 (r=0.53), disease duration (r=0.37), functional class (r=0.38); depression severity was correlated with BMI (r=0.66) and age (r=0.82) of patients.

Psychotherapy included individual, group, and therapeutic programs. Most patients participated in supportive individual and cognitive-behavioral group therapy. About half of the patients, mostly women, complained of lack of help and warm support from loved ones, as well as of dependence on their help. Psychological disorders have been found to have the direct correlation with the physical condition of the patient. Based on this, the expected result of psychotherapy in the rehabilitation of RA patients was not only an improvement in the mental state, but also an improvement in the physical well-being, and possibly a modification of the current somatic disease (by reducing the risk of recurrence). We noted positive dynamics based on the results of psychological examination.

At the same time, according to studies conducted by A. Hirata et al. [13], S. Peterson et al. [14], A. R. Ruhaila et al. [15], the use of antidepressants in patients with the association of RA and COPD not only reduces the severity of systemic inflammation by blocking neuronal reuptake of neurotransmitters in the CNS, but also has indirect antioxidant effects by increasing the synthesis of glutathione, peroxidases, catalases, and other components of the antioxidant system. The efficacy of antidepressants and atypical neuroleptics in RA patients has been confirmed by the results of numerous studies.

Conclusion

A comprehensive study of the QOL of RA patients, including association with respiratory system pathology, revealed significant interrelations of QOL indicators with the main clinical characteristics of patients, severity of pain, and the degree of disease activity. Anxiety-depressive disorders are revealed in ¾ of RA patients, the severity of which correlates with the main indicators of activity of the inflammatory process. The presence of associated lesion of the musculoskeletal, respiratory systems and psychoemotional sphere forms a vicious circle of mutual pressure and dictates the need for a comprehensive examination of the patient for the sake of adequate correction.

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CLINICAL AND LABORATORY CHARACTERISTICS OF PATIENTS WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME DEPENDING ON THE DISEASE COURSE SEVERITY

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On the basis of a comprehensive clinical and laboratory examination of 284 patients with hemorrhagic fever with renal syndrome, the characteristics of the course depending on the period and the severity of the disease was given. Correlations between the duration and severity of clinical manifestations of the initial disease period and the severity of the course in the oliguric period have not been revealed. The difficulty of diagnosing hemorrhagic fever with renal syndrome in the initial disease period was shown, which requires differential diagnosis with many infectious genesis diseases. The hemorrhagic syndrome was found only in patients with a severe course of the disease. In most cases, patients with a severe course of the disease required hemodialysis.
Key words: *hemorrhagic fever with renal syndrome, clinical symptoms.*

Hemorrhagic fever with renal syndrome (HFRS) is an acute viral zoonotic natural-focal disease characterized by systemic lesion of small blood vessels, hemorrhagic diathesis, hemodynamic disorders, and kidney lesion with the development of AKI [1].

Over the past decades, hantavirus diseases have been included in the range of urgent and priority issues around the world, so-called "new and returning" infections that threaten complex epidemical situations. This is due to the variability of the genome of hantaviruses, the emergence of new types and genetic variants with high virulence for humans, spread in territories where the disease cases were not registered before [2, 3].

The causative agent of HFRS belongs to the Bunyavirus family (Bunyaviridae) and to the Hantavirus genus. Its replication is carried out in the cytoplasm of infected cells. Hantaviruses are polytropic: monocytes, cells of the lungs, kidneys, liver, salivary glands are infected [6].

More than 30 serologically and genetically distinct hantaviruses are known to date. Two clinical forms of hantavirus infection in humans have been described:

- haemorrhagic fever with renal syndrome, whose pathogens are viruses Hantaan, Seoul, Puumala, Dobrava/Belgrade, Amur;
- hantavirus pulmonary syndrome, whose pathogens are hantaviruses Sin-Nombre, Black Creek, New York, Bayou, Andes, Laguna Negra [10].

Only the first clinical form of the disease (HFRS) is registered on the territory of Russia and seven hantavirus types have been established, four of which are pathogenic for humans [4, 11].

In European foci, the pathogenic agent is Puumala type in the majority of cases. The possibility of circulation of Hantaan, Seoul, and Dobrava is also shown. Viruses Hantaan, Seoul, and Amur circulate in the natural foci of the Russian Far East, South Korea, DPRK, China, Japan. In recent years, there have been reports of occurrence of the Seoul virus in the European part as well, which is able to infect house rats [6, 12].

As many observations show, the viruses Hantaan, Seoul, and Dobrava cause the most severe forms of the disease, whereas the Puumala virus causes relatively lighter forms of the disease that can have atypical manifestation, rarely found hemorrhagic syndrome, patients can tolerate the disease "out of bed". Mortality in HFRS caused by the Puumala virus does not exceed 1% and in other pathogens it is about 5% and more depending on the season.

The infection of humans is mainly air-dust (when aspirated from dried defecations of infected rodents), as well as contact (when rodent bite) and alimentary. A sick person is not contagious. People's natural susceptibility is high. The majority of patients are men (70–90%), mainly agricultural workers, tractor operators, drivers. The suffered infection leaves persistent lifelong type-specific immunity [3, 8, 14].

The infection of HFRS can occur at any time of the year, but there is rising incidence from May to December. In recent years, there has been a shift of seasonal peak from summer-autumn to autumn-winter period [3]. In winter, the infection of people occurs more often in the conditions of rooms (vegetable storehouses, living accommodations, etc.) due to the seasonal migration of rodents to human housing.

The research objective was to study and compare clinical and laboratory changes in HFRS depending on the disease severity.

Materials and methods

For the period of 2014–2016, we examined 284 cases of HFRS (243 (85.8%) men, 41 (14.2%) women). The mean age of the studied was 36.19±0.68 years, the vast majority of patients – 91.2% – were persons of working age (18–50 years) who were in hospital treatment in the Infectious Department of SBHI Infectious Clinical Hospital No. 2 of the Moscow Healthcare Department and SHI City Hospital No. 2 named after E.G. Lazarev in Tula.

Depending on the disease course severity, all patients were divided into 3 groups. The first group included 48 people with the mild course of the disease, the second group included 162 people with the moderate course, and the third group – 74 people with the severe course. The diagnosis of HFRS was established on the basis of generally accepted clinical, epidemiological, laboratory-instrumental data.

The diagnosis of HFRS was confirmed by the indirect immunofluorescence test (IIFT) in paired serums, the polymerase chain reaction (PCR) method to detect viral RNA fragments in the blood.

The research results were processed with the help of parametric methods on the PC using Microsoft Excel. The data are given as arithmetic mean values and error of mean (M±m). The Student's t-test ($p<0.05$) was used to assess the reliability of differences in the comparable means (relative values).

Results and discussion

The analysis of clinical manifestations of HFRS showed that the disease was characterized by a cyclical course with a successive change of disease periods – initial (febrile) period – average duration of 4.24±0.3 days, oliguric period – average duration of 8.01±0.2 days, polyuric period – average duration of 10.5±0.8 days – and convalescence period. The average period of admission of patients to hospital from the onset of the disease was 5.1±0.13 days of disease.

In all HFRS patients during the feverish period, there was the intoxication syndrome in the form of temperature increase, headache, eyeball pain, myalgia, lack of appetite (52.2%), vomiting (37%). The respiratory syndrome in the initial period developed only in patients with mild form of the disease (26%). The leading signs of the respiratory syndrome were complaints of patients about nasal congestion, dry cough, throat irritation. Patients of moderate severity were reliably more likely to have the abdominal syndrome (37%), which was manifested by abdominal pain and diarrhea. Visual impairment (reduced visual acuity, appearance of "fog, mesh" before eyes) was observed in 84% of patients, the pronouncement also depended on the severity of the disease with the greatest frequency at severe form of the disease.

The retrospective analysis of referral diagnoses showed that in 24.0% of cases there was a misdiagnosis. Of the total number of misdiagnoses, there was a referral diagnosis of infectious disease in 18% of cases (leptospirosis, influenza, ARVI, bacterial food poisoning, meningitis). In 6%, there was a therapeutical/surgical disease (pyelonephritis, glomerulonephritis, cholecystopancreatitis, pneumonia, intestinal obstruction).

The asthenovegetative syndrome, manifested by weakness, malaise, reduced working capacity and increasing body temperature to febrile digits, was observed in all patients in the initial period. Clinical signs of the hemorrhagic syndrome were found in 10.2% of patients.

The oliguric period in all patients was accompanied by a decrease in body temperature to normal (14%) and subfebrile (86%) digits with the preservation of the intoxication syndrome. The pronouncement of the intoxication symptoms correlated with the disease course severity. At the same time, the renal syndrome was formed in the form of pain in the lumbar region and oligoanuria. 100% of patients with the moderate, severe forms and 81.3% with the mild form noted lumbar pain. The duration of this syndrome averaged 12.4±0.4 days and correlated with the severity of the infection process. Oliguria was observed in all patients with the severe form of the disease and in 56.2% of patients with the mild form. Anuria was only found in patients with the severe form of the disease. Patients with the severe form of HFRS required hemodialysis in 91% of cases. With the severe course of HFRS, the oliguric period was accompanied by abdominal pain and peripheral edema.

It should be noted that the clinical signs of the hemorrhagic syndrome were found in only 10.2% – 30 patients with the severe form of HFRS, which was accompanied by petechial skin rash in all patients, scleral hemorrhage (24 people), nasal bleeding (17 people), gastrointestinal bleeding (1 person).

The onset of polyuria was accompanied by improvement of the well-being of the patients. Clinical manifestations of hemorrhagic and abdominal syndromes were relieved, appetite improved, edema disappeared, strong thirst appeared. An increase in daily urine volume to 4 to 5 liters was the most important sign of this period. Asthenia persisted, which on average lasted to 23.3±0.5 days of the disease. The severe form was accompanied by longer asthenization and lower back pain.

The following results were obtained in the study of laboratory data.

The comparative analysis of the main hematological indicators revealed that the level of blood leukocytes in the feverish period was reliably higher in patients with the moderate and severe course of HFRS (7.8 x109/L – 13.1±0.4 x109/L). In the mild form, the number of leukocytes did not

increase (4.9±0.06 x109/L). During the oliguric period, the number of leukocytes increased reliably in all three groups compared. Thrombocytopenia was revealed in almost all the groups compared in the febrile period already (159.1±10.8 x109/L, 114.3±8.8 x109/L and 69.2±6.9 x109/L respectively).

Table 1

Results of complete blood count in the oliguric period

Parameters	Mild course	Moderate course	Severe course
WBC	9.63±3.83	11.05±2.39	14.13±3.32
RBC	4.73±0.47	4.7±0.43	5.18±0.37
HGB, g/L	138.93±11.63	137.37±11.36	154.97±12.28
HCT, %	36.88±3.51	36.67±4.18	40.49±4.01
MCV, fL	79.60±4.75	77.35±4.86	79.34±4.80
MCH, Pg	29.80±2.12	28.87±1.34	29.82±1.16
MCH C, g/L	346.38±51.88	375.13±18.38	377.88±17.56
PLT	159.56±58.05	114.37±65.78	69.14±68.23
LY, %	22.89±9.87	29.22±9.54	25.95±6.22
MO, %	8.26±3.46	8.22 ± 2.80	9.58±2.92
GP, %	68.08±13.39	58.97±9.58	62.7±7.82

Table 2

Results of biochemical blood test in the oliguric period

Indicators (min-max)	Mild course (min-max)	Moderate course (min-max)	Severe course (min-max)
AST, IU/L	31.65 – 23.63	39.35 – 13.15	47.61 – 19.46
ALT, IU/L	38.56 – 36.86	44.98 – 23.57	54.67 – 25.60
GGT, IU/L	22.2 – 9.70	43.3 – 19.19	48.72 – 26.06
AP, IU/L	84.25 – 14.25	133.18 – 39.02	93.06 – 21.01
Albumin, g/L	43.05 – 7.55	39.78 – 3.98	37.75 – 4.42
Creatinine, μmol/L	67.93 – 111.31	236.58 – 65.53	543.13 – 65.59
Urea, mmol/L	6.5 – 5.9	18.12 – 14.5	32.76 – 27.9
Total protein, g/L	68.76 – 3.01	53.21 – 5.51	44.38 – 6.85
Cholesterol, mmol/L	5.14 – 0.21	4.06 – 0.77	3.93 – 0.69
Total bilirubin, μmol/L	13.52 – 4.71	10.16 – 3.29	10.52 – 3.51

Among biochemical studies, urea and creatinine levels have traditionally been one of the main criteria for the severity of HFRS and the development of acute kidney failure. In our study, urea and creatinine levels were almost unchanged in the mild form, staying within the normal range throughout the disease. In the moderate form, the average level of urea in the blood reached 18.12±0.7 mmol/L and creatinine was 236.6±20.4 μmol/L. In the severe course of HFRS, urea and creatinine levels were the highest with 32.76±0.9 mmol/L and 543.1±20.8 μmol/L respectively. Normalization of urea and creatinine indicators occurred during the polyuria period at an average of 14.5±0.5 and 18.8±0.5 days of the disease respectively, it correlated with the disease severity.

Table 3

Results of the clinical analysis of urine in the oliguric period

Indicators	Mild course	Moderate course	Severe course
GLU, mmol/L	0	0	0.13 – 0.24
PRO, g/L	0.61 – 0.70	0.86 – 1.13	1.92 – 2.76
PH	5.45 – 0.75	5.1 – 0.6	5.32 – 0.66
Spec. gravity	1015 – 1010	1010 – 1005	1005 – 1001
WBC	0–4	6–14	16–24
RBC	2–6	10–18	28–46
Columnar epithelium	2–8	12–20	22–34

In the oliguric period, hypoisosthenuria, proteinuria, cylindruria, hematuria (micro- and macrohematuria) were determined in the urine in connection with the development of the renal syndrome. The most pronounced changes in the urine were in patients with the severe form. During the polyuria period, hypoisosthenuria persisted.

Conclusion

The analysis of the obtained results makes it possible to conclude that HFRS is currently characterized by a high level of morbidity with the formation of moderate and severe forms. The detection of the following clinical symptoms can help with the proper diagnosis in the initial period: high fever, headache, sore throat and throat irritation, thirst, lower back pain, abdominal pain, decreased visual acuity, presence of hyperemia of the face, neck, collar area, injection of scleral vessels. The severity of the disease can be indicated by the presence of hypotension, tachycardia, pronounced abdominal and lumbar pain, nausea, vomiting, decreased visual acuity. According to the results of our studies, the peripheral edema and hemorrhagic syndrome (petechial skin rash, scleral hemorrhages, nasal and intestinal bleeding) were found only in patients with the severe form of HFRS. From laboratory indicators, the presence of a combination of thrombocytopenia, increased ESR, proteinuria, cylindruria, leukocyturia, microhematuria helps to suspect HFRS. Indirect signs of developing the severe form of the disease in the initial (febrile) period of HFRS already may be pronounced leukocytosis, thrombocytopenia, pronounced proteinuria, leukocyturia, macrohematuria, cylindruria. Changes in the indicators of urea, creatinine are informative for diagnosis only in the oliguric period. Early diagnosis of HFRS (at the level of reception ward) allows to provide the correct tactics of the regime and treatment of patients, alertness on the threat of the development of complications.

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ROLE OF PRESEPSIN IN SEVERE PNEUMONIA AND PNEUMOGENIC SEPSIS IN CHRONIC HEMODIALYSIS PATIENTS

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Research objective. To study presepsin as an inflammation marker to improve diagnosis of severe pneumonia and sepsis in hemodialysis patients.
Materials and methods. 65 patients with severe pneumonia, sepsis, chronic glomerulonephritis, and nephropathy aged from 17 to 77 years were examined. Among them, 22 patients received hemodialysis. The presepsin level was quantified on immunochemiluminiscent analyzer Pathfast (Mitsubishi Chemical Medience Corporation, Japan).
Results. The presepsin level in hemodialysis patients with severe pneumonia was 6587.9±2011.09 pg/ml (n=7). In hemodialysis patients with pneumogenic sepsis, the presepsin level was 6931.1±820.46 pg/ml (n=7). Presepsin in hemodialysis patients with chronic glomerulonephritis, nephropathy was 1693,0±248,24 pg/ml (n=5). The presepsin levels in patients with severe pneumonia, pneumogenic sepsis who received hemodialysis did not differ. Presepsin in hemodialysis patients with severe pneumonia, pneumogenic sepsis was higher than in hemodialysis patients with chronic glomerulonephritis and nephropathy.
Conclusion. The high presepsin level is an indication of an active infection process and an effect of hemodialysis.
Key words: *presepsin, pneumonia, sepsis, hemodialysis.*

The biological marker of bacterial infection presepsin (PSP) is used as an effective indicator of early diagnosis of severe pneumonia, sepsis [1, 2]. It is known that PSP is secreted by kidneys and increases when their functions are impaired [2, 3].

At the same time, the diagnosis of pneumonia and sepsis in hemodialysis patients causes difficulties in general and depending on the identification of biomarkers. Further research is needed to determine the role of PSP in the diagnosis of pneumonia, sepsis in hemodialysis patients.

The research objective was to study PSP to improve the effectiveness of diagnosis of severe pneumonia and sepsis in hemodialysis patients. For this purpose, the PSP level was determined in groups: 1) patients with pneumonia, sepsis; 2) patients receiving hemodialysis without infectious complications; 3) patients with pneumonia, sepsis, who receive hemodialysis.

Materials and methods

65 patients were examined. The patients were treated in the Department of Pulmonology, Department of Nephrology, Department of Resuscitation and Intensive Care of the Regional Clinical Hospital in the period from 2014 to 2019. The age of patients varied from 17 to 77 years, the mean age was 55.8±5.28 years ($\overline{X}$ ±m). There were 37 men (56.9%) and 28 women (43.1%). All patients were divided into 6 groups. The first group included patients with severe pneumonia (n=23, 35.4%), the second group – with pneumogenic sepsis (n=12, 18.5%). The third group included patients with chronic glomerulonephritis (CGN), nephropathy receiving hemodialysis (n=5, 7.7%). The fourth and fifth groups consisted of patients with severe pneumonia and pneumogenic sepsis who received hemodialysis (n=10, 15.3% and n=7, 10.8%). Patients with pneumogenic sepsis from the sixth group (n=8, 12.3%) were treated with hemodialysis on urgent indications due to the acute renal damage. In the construction of the statistical model, we used a group of patients with severe pneumonia and pneumogenic sepsis receiving hemodialysis (n=17, 26.1%) and patients with CGN, nephropathy receiving hemodialysis (n=5).

The PSP level was quantified with the use of the immunochemiluminiscent analyzer Pathfast (Mitsubishi Chemical Medience Corporation, Japan). The measurement results are presented in picograms per milliliter, pg/ml. PSP was determined at admission. Patients were managed in accordance with existing clinical recommendations and standards. Diagnosis of sepsis and septic shock was carried out according to the criteria of the Community of Resuscitation Medicine/European Society of Intensive Care Medicine (2016) with additions [4].

Statistical processing of the data was carried out using the Statistica V. 10.0 software package. To determine statistically significant differences, the Mann–Whitney U-test was used. The differences were considered statistically significant at $p<0.05$. The logistic regression analysis was used to predict the probability of developing severe pneumonia and pneumogenic sepsis by the level of PSP (if $p<0.05$, the null hypothesis is rejected and an alternative hypothesis is accepted that PSP is associated with the development of pneumonia and pneumogenic sepsis).

Results and discussion

The PSP level in patients with severe pneumonia and chronic hemodialysis patients with severe pneumonia is presented in Table 1.

In patients with severe pneumonia, the PSP level was lower than in chronic hemodialysis patients with severe pneumonia.

The PSP level in patients with pneumogenic sepsis and chronic hemodialysis patients with pneumogenic sepsis is presented in Table 2.

In patients with pneumogenic sepsis, the PSP level was lower than in chronic hemodialysis patients with pneumogenic sepsis.

PSP in chronic hemodialysis patients with severe pneumonia, pneumogenic sepsis, CGN, and nephropathy is presented in Table 3.

There was no difference in the PSP level in patients with severe pneumonia and chronic hemodialysis patients with pneumogenic sepsis. PSP in chronic hemodialysis patients with severe pneumonia was higher than in chronic hemodialysis patients with CGN and nephropathy. PSP in chronic hemodialysis patients with pneumogenic sepsis was higher than in chronic hemodialysis patients with CGN and nephropathy. Data on the PSP level can indicate an increase in the PSP level in chronic hemodialysis patients with severe pneumonia, pneumogenic sepsis compared to chronic hemodialysis patients with CGN and nephropathy.

Table 1

PSP in patients with severe pneumonia and chronic hemodialysis patients with severe pneumonia

Indicator	Patients with severe pneumonia (1)	Chronic hemodialysis patients with severe pneumonia (2)	p
	$\overline{X}$ ±m	$\overline{X}$ ±m	1-2
PSP at admission	419.5±56.01 n=23	6587.9±2011.09 n=7	p=0.0001

Note: PSP – presepsin.

Table 2

PSP in patients with pneumogenic sepsis and chronic hemodialysis patients with pneumogenic sepsis

Indicator	Patients with pneumogenic sepsis (1)	Chronic hemodialysis patients with pneumogenic sepsis (2)	p
	$\overline{X}$ ±m	$\overline{X}$ ±m	1-2
PSP at admission	2300.0±843.71 n=12	6931.0±820.46 n=7	p=0.0035

Table 3

PSP in chronic hemodialysis patients with severe pneumonia and pneumogenic sepsis compared to patients with CGN and nephropathy

Indicator	Chronic hemodialysis patients with severe pneumonia (1)	Chronic hemodialysis patients with pneumogenic sepsis (2)	Chronic hemodialysis patients with CGN and nephropathy (3)	p		
	$\overline{X}$ ±m	$\overline{X}$ ±m	$\overline{X}$ ±m	1-2	1-3	2-3
PSP at admission	6587.9±2011.09 n=7	6931.0±820.46 n=7	1693.0±248.24 n=5	p=0.609	p=0.022	p=0.0057

The PSP level in chronic hemodialysis patients with pneumogenic sepsis did not differ from the PSP level in patients with pneumogenic sepsis receiving hemodialysis on urgent indications: 6416.3±1746.81 (n=8, p=0.2715).

The results of the logistic regression analysis showed that PSP has a statistically significant effect (χ^2=6.16, p=0.013) on the development of severe pneumonia and pneumogenic sepsis. Regression equation: y=0.191-0.00049×PSP. Next, using log-

it-transformation, the probability of developing severe pneumonia and pneumogenic sepsis in a certain patient is calculated:

$$P = \frac{1}{1 + e^{-y}}$$

Example: if PSP=2145 pg/ml, then y= -0.86; P=0.299.

A priori probability of developing severe pneumonia and pneumogenic sepsis is 77.3%. The percentage of correct predictions is 72.7%. 77.3%>72.7%. A priori probability exceeds the percentage of correct predictions. Consequently, other factors need to be taken into account.

We obtained data on the higher PSP level in chronic hemodialysis patients with severe pneumonia and pneumogenic sepsis compared to patients with severe pneumonia and pneumogenic sepsis. These data may indicate the effect of chronic hemodialysis on the PSP level in severe pneumonia and pneumogenic sepsis.

The literature data on changes in the PSP level in chronic hemodialysis patients with pneumonia, pneumogenic sepsis are limited.

An increase in the PSP level in sepsis and acute renal damage (ARD) to 1523 (293-16764) pg/ml is known [2].

Nakamura Y. et al. studied PSP in patients with and without ARD, with and without sepsis [5]. Authors determined that the PSP median value increases with an increase in the severity of ARD in the groups of patients with and without sepsis. The researches concluded that the PSP level can be used to determine sepsis in patients with less severe forms of ARD. However, with a significant decrease in renal functioning, PSP can be unreliable.

In their study, Takahashi G. et al. determined the PSP border level to diagnose sepsis accompanying ARD, which was detected by various indicators [4]. Determination of presepsin is useful for diagnosing bacterial infection in ARD.

Our study provides data that indicate an increase in the PSP level in chronic hemodialysis patients with severe pneumonia, pneumogenic sepsis compared to patients receiving hemodialysis but having no infectious complications (1693.0±248.24 pg/ml, n=5).

Nagata T. et al. in their study determined the PSP levels in patients with reduced glomerular filtration rate (GFR) depending on the stage of chronic kidney disease (CKD, KDIGO, 2012) and in chronic hemodialysis patients with anuria (excluding patients with an infection, cancer, liver diseases, autoimmune disorders, using steroids and immunodepressants) [7]. With reduced GFR and no infection, the PSP levels increased depending on the stage of CKD, to a maximum of 251.0 (213–297.5) pg/ml at stage 5 of CKD. The PSP median value in hemodialysis patients made 1160.0 (1070.0–1400.0) pg/ml. After obtaining these results, the PSP level was measured in hemodialysis patients before, immediately after and in 2 days after hemodialysis. After hemodialysis, the PSP level decreased from 1510 (1280–1670) pg/ml to 753 (542–1210) pg/ml. The results obtained by the authors suggested that PSP is filtered in hemodialysis.

The increase in PSP in hemodialysis patients is due to reduced clearance and increased PSP production during the infection development.

Conclusions

1. The presepsin levels in severe pneumonia, pneumogenic sepsis in chronic hemodialysis patients were 6587.9±2011.09 pg/ml and 6931,1±820.46 pg/ml respectively, which is higher than that in hemodialysis patients with chronic glomerulonephritis, nephropathy – 1693.0±248.24 pg/ml.

2. The presepsin levels in patients with severe pneumonia, pneumogenic sepsis who received hemodialysis did not differ.

3. Determination of presepsin allows the assessment of the severity of pneumonia and the sepsis development in hemodialysis patients. Further research is required.

Conflict of interest. The authors declare no conflict of interest.

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POSSIBILITY OF PREDICTING HABITUAL MISCARRIAGE IN THE PREGRAVID PERIOD

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Research objective: to develop and pathogenetically substantiate a new diagnostic complex involving combinations of gene mutations of hemostasis system and folate cycle with autoimmune disorders that increase the risk of habitual miscarriage.
Materials and methods. The frequency of combinations of PAI-1, FV, FII, MTHFR (PCR method) gene mutations and increased levels of antibodies to Fc region of immunoglobulin and/or thyroglobulin (EIA method), their association with miscarriage were studied in 113 women with RCH on the prospective stage. In retrospect, the association of studied combinations with habitual miscarriage was studied in 309 women.
Results. Women with RCH have a combination of genetic and autoimmune changes in 35.4% (40/113) that are more likely to occur in pregnancy loss (p=0.0001). The habitual miscarriage is associated with the combinations studied in 50.0%. The prognostic value of the positive result of certain combinations was 33.3–100%.
Conclusion. Identification of combinations of PAI-1, FV, FII, MTHFR gene mutations and increased levels of antibodies to Fc region of immunoglobulin and/or thyroglobulin in women with burdened obstetric gynecological history in the pregravid period allows to form a risk group for the development of habitual miscarriage.
Key words: *habitual miscarriage, autoantibodies, gene mutations of hemostasis system and folate cycle.*

Habitual miscarriage is a topical issue in modern healthcare. It is customary to consider two or more pregnancy losses in a row as habitual miscarriage. Each repeated miscarriage increases the risk of subsequent pregnancy loss: two miscarriages – up to 29%, three – up to 33% [1, 2]. Habitual miscarriage is associated with genetic, hemostasiological, autoimmune and other factors. Domestic and foreign publications reflect the ongoing search for early predictors, methods of prevention, and treatment of miscarriage [3, 4, 5, 6].

Attention of obstetrician-gynecologists is drawn to the fact of formation of retrochorial hematoma (RCH), at which the miscarriage incidence reaches 40.0% [7]. RCH is associated with habitual miscarriage [8], hereditary thrombophilia [9].

During the past decade, the concept of "body thrombotic readiness" has been widely discussed, according to which conditions are created in the interactions between hemostasis system gene mutations and autoimmune processes that increase the risk of thrombotic complications during pregnancy or surgical treatment [10].

At the pregravid stage, determination of combinations of genetic (mutations of genes of hemostasis system, folate cycle) and autoimmune (autoantibody level increase) factors associated with habitual miscarriage will allow to form high-risk groups, optimize preventive and therapeutic measures.

Research objective: to develop and pathogenetically substantiate a new diagnostic complex involving combinations of gene mutations of hemostasis system and folate cycle with autoimmune disorders that increase the risk of habitual miscarriage.

Materials and methods

At the first (prospective) stage of the study, 113 women of reproductive age with the RCH formation in the gestation period of 6–12 weeks according to the sonography were examined. Women with RCH were divided into 2 groups. The main group included 84 women with threatened miscarriage, the experimental group – 29 women with missed miscarriage.

At the second (retrospective) stage, the primary documentation of 309 women of reproductive age with different outcomes of pregnancies was studied. The main group included 82 women with habitual miscarriage, the experimental group – 136 women with sporadic miscarriage, the control group – 91 women with normal reproductive history, i.e. the presence of at least one case of live birth in the absence of reproductive losses.

The examination included determining the presence of polymorphic gene variants of hemostasis system, folate cycle, deviation of embryotropic antibody level. Polymerase chain reaction determined polymorphic variants of 20210 G->A prothrombin gene (FII), Leiden mutation 1691 G->A of coagulation factor V (FV), 675 4G/5G (5G->4G) plasminogen activator inhibitor gene (PAI-1), A222V thermolabile variant (677 C->T) of methylene tetrahydrofolate reductase (MTHFR). The enzyme immunoassay method determined the level of deviation of autoantibodies to human chorionic gonadotropin (HCG), insulin, thyroglobulin, double-helical DNA, beta-2-glycoprotein I, Fc region

of immunoglobulin, collagen, S100 protein, membrane sperm antigen (Spr-06), kidney cell specific antigen (KiM-05), antigen of vascular endothelium (ANCA) and platelet membranes (TrM-03).

The results of the study were analyzed using the statistical program IBM SPSS Statistics 17.0. Qualitative indicators were expressed in absolute and relative values, the Pearson's χ^2 test was used to calculate the statistical significance of differences (p). The relative risk (RR) with confidence interval (95% CI) was calculated to determine the influence of the studied factor on the outcome. The effectiveness of the diagnostic method of the study was evaluated using sensitivity (Se) and specificity (Sp). The values were considered statistically significant at $p<0.05$. To determine the predictive value of diagnostic tests, the calculation of the predictive value of positive and negative results was applied.

Results and discussion

In order to determine the factors that have the most negative influence on pregnancy development, the frequency of genetic and autoimmune changes in women with RCH was studied at the prospective stage.

The prospective study revealed that the history of women with RCH was burdened with gynecological pathology in 50.4% (57/113), medical abortions in 31.0% (35/113), and miscarriages in 42.5% (48/113) of cases.

92.0% (104/113) of women have the PAI-1, FV, FII, MTHFR gene mutations, women with threatened miscarriage in 97.6% (82/84) of cases, missed miscarriage in 75.9% (22/29) (p=0.001).

Deviation of autoimmunity level in the form of hyperreactivity or hyporeactivity was diagnosed in all types of autoantibodies. The most statistically significant differences between clinical groups were found by the marker of the inflammatory process (antibodies to Fc region of immunoglobulin) and the marker of thyroiditis (antibodies to thyroglobulin), which prevailed in the group with missed miscarriage (p=0.00001). Hyporeactivity was observed in isolated cases, so further analysis of the increase in the level of autoantibodies only was carried out. The frequency of increase in the antibody level to Fc region of immunoglobulin in women with threatened miscarriage was 6.0% (5/84), women with missed miscarriage – 41.4% (12/29) (p=0.0001), the frequency of increase in the antibody level to thyroglobulin was 21.4% (18/84), 72.4% (21/29) (p=0.0001) respectively.

Thrombotic and non-thrombotic effects of thrombogenic alleles are known to interact with autoimmune processes of different nature, potentiate pathological action of each other from early dates pregnancy. Endothelial dysfunction, haemorrhages, thrombosis, and necrosis developing on their background determine the disturbance of invasion and vascularization of chorion, can contribute to the disruption of the pregnancy course [10, 11].

Combinations of PAI-1, FV, FII, MTHFR gene mutations with the increased level of antibodies to Fc region of immunoglobulin and/or thyroglobulin were found in 35.4% (40/113) of women with RCH, more often in the group with missed miscarriage than with threatened (65.5% (19/29) and 25.0% (21/84), p=0.0001 respectively). The presence of studied combinations of genetic and autoimmune changes increases the risk of pregnancy loss by 2.8 times (RR 2.808, 95% CI 1.569–5.026, Se 0.606, Sp 0.750). Combinations of mutations in MTHFR and/or FII genes with the increased level of antibodies to Fc region of immunoglobulin are most important (RR 2.089, 95% CI 1.024–4.259, Se 0.121, Sp 0.963; RR 3.500, 95% CI 2.612–4.691, Se 0.030, Sp 1.000 respectively).

In the analysis of the primary documentation of 309 women, combinations of PAI-1, FV, FII, MTHFR gene mutations and increased levels of antibodies to Fc region of immunoglobulin and/or thyroglobulin were diagnosed in 34.3% (106/309) of cases. In the group of women with habitual miscarriage, combinations were found in 50.0% (41/82) of cases. In groups of women with sporadic miscarriage and normal reproduction, combinations of genetic and autoimmune changes were less frequent (27.9% (38/136), p=0.002 and 29.7% (27/91), p=0.007 respectively).

The studied combinations of genetic and autoimmune factors are associated with an increased risk of habitual miscarriage as opposed to sporadic miscarriage and normal reproductive function (RR 1.759, 95% CI 1.261–2.456, Se 0.500, Sp 0.721; RR 1.544, 95% CI 1.136–2.099, Se 0.500, Se 0.703 respectively).

The risk of developing habitual miscarriage is most increased relative to sporadic one in the presence of combinations of mutations in MTHFR and/or FII genes with increased levels of antibodies to Fc region of immunoglobulin (RR 1.705, 95% CI 1.069–2.719, Se 0.098, Sp 0.963 and RR 2.679, 95% CI 2.255–3.183, Se 0.012, Se 1.0000 respectively). Previously noted combinations increase the risk of habitual miscarriage relative to the normal course of pregnancy (RR 1.592, 95% CI 1.068–2.373, Se 0.098, Sp 0.967 and RR 2.123, 95% CI 1.812–2.488, Se 0.012, Sp 1.000 respectively), as well as a combination of mutation in the FII gene with the increased antibody level to thyroglobulin (RR 2.123, 95% CI 1.812–2.488, Se 0.012, Sp 1.000).

To evaluate the proposed new diagnostic complex consisting of interacting genetic and autoimmune factors, the predictive value of positive and negative results was calculated. In assessing the risk of habitual miscarriage, the most important is the calculation of the predictive value of the combination of the MTHFR gene mutation with the increased level of antibodies to Fc region of immu-

noglobulin (predictive value of the positive result was 50%; predictive value of the negative result – 74.4%), the FII gene mutation with the increased level of antibodies to Fc region of immunoglobulin (100% and 73.7% respectively), the FII gene mutation with the increased level of antibodies to thyroglobulin (33.3% and 73.5% respectively).

The study demonstrates that genetic and autoimmune changes create a negative background at the pregravid stage and are realized during pregnancy in the form of a miscarriage, which is consistent with available literary data [10, 11]. This work identifies for the first time the combinations of PAI-1, FV, FII, MTHFR gene mutations and the increased level of antibodies to Fc region of immunoglobulin and/or thyroglobulin, determining the increased risk of developing habitual miscarriage. The results of the study allow to form risk groups for the development of habitual miscarriage, to carry out complex pathogenetic therapy in a timely manner.

Conclusion

Thus, identification of combinations of genetic (PAI-1, FV, FII, MTHFR gene mutations) and autoimmune (increased level of antibodies to Fc region of immunoglobulin and/or thyroglobulin) factors in women with burdened obstetric gynecological history in the pregravid period allows to form a risk group for the development of habitual miscarriage.

Conflict of interest. The authors declare no conflict of interest.

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CHANGE IN THE STRUCTURE OF VENOUS THROMBOEMBOLIC COMPLICATIONS IN THE POPULATION

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Research objective. To study the structure of VTEC hospitalization and changes in this structure over the last 10 years.
Materials and methods. The retrospective analysis of hospitalization of 2327 patients with VTEC for a 10-year period (2010–2019). Depending on the period of admission, patients were divided into 2 groups: the first group (1104 patients) included people hospitalized in the period of 2010–2014; the second group (1223 patients) – admitted to the hospital in the period of 2015–2019. The structure of hospitalization was analyzed. The dynamics of the number of hospitalizations of VTEC nosological forms was studied.
Results and conclusions. No statistically significant difference was found in the frequency of VTEC hospitalization, but there is a tendency to an increase in the total number of VTEC. According to the data, the annual frequency of VTEC was 65.5 cases per 100 000 population. The significant increase in frequency of hospitalization of PATE patients was found. This fact may be related not only to the improvement of diagnosis, but also to the increase in the intensity of the thrombotic process. The study presented took into account VTEC requiring treatment in a specialized unit, without regard to VTEC recommended for the outpatient stage of treatment.
Key words: *venous thromboembolic complications (VTEC), thrombophlebitis, deep vein thrombosis (DVT), pulmonary artery thromboembolia (PATE).*

Venous thromboembolic complications (VTEC) is a collective concept that includes deep vein thrombosis (DVT), thrombosis (thrombophlebitis) of superficial veins, and pulmonary artery thromboembolia (PATE). This is the third cause in the structure of cardiovascular deaths in the Russian Federation [1]. The frequency of VTEC reaches 180 cases per 100,000 population per year [2, 3] and increases with age to 200 cases per 100,000 population [4]. Data on trends in the VTEC incidence are limited. In the study (Worcester VTE) conducted on the material of hospitalizations of 5,025 patients with VTEC in the period 1985–2009, an increase in the annual frequency of DVT and PATE was found. Authors associate this trend with the improvement of diagnostic methods [5]. At the same time, there is no information on the structure of hospital VTEC and changes in this structure in recent years. Therefore, the research objective was to study the structure of hospitalization of patients with VTEC and changes in this structure over the last 10 years.

Materials and methods

The study was conducted in Barnaul (with the population of 709,372). All cases of hospitalization of patients with VTEC to the Department of Vascular Surgery of the Russian Railway Hospital, providing care to patients with acute vascular events, were taken into account. The department is part of the city vascular center, where all patients with acute vascular diseases are hospitalized every second day. Day and night, lesion diagnostics is performed in the department using laboratory methods (D-dimer, brain natriuretic peptide, and troponin), duplex scanning, X-ray contrast or CT pulmonary angiogram. After verification of the disease – high or intermediate risk PATE, floating or occlusive deep vein thrombosis, saphenofemoral thrombosis, or thrombosis of a large subcutaneous vein on the thigh – patients are hospitalized in an in-patient hospital where surgical or conservative treatment is prescribed.

In a total of 10 years (2010–2019), 2,327 patients with VTEC were hospitalized. Depending on the intensity of the thrombotic process, 6 nosological forms of VTEC are empirically defined (Figure 1). Cases of high and intermediate risk PATE were taken into account. The selection criteria for patients with floating thrombi of the inferior vena cava, iliac or femoral veins were patients with implanted filters or after the thrombectomy followed by the femoral vein ligation. The inclusion criteria for patients with saphenofemoral thrombosis or thrombosis of a large subcutaneous vein on the thigh was the thrombectomy from a saphenofemoral junction followed by ligation of a large subcutaneous vein or isolated crossectomy. Depending on the type of nosology, generally accepted treatments involving antithrombotic or thrombolytic therapy were additionally performed. Depending on the period of admission, patients were divided into 2 groups. The first group included 1,104 patients (47.4%) hospitalized in the first 5 years – 2010–2014, and the second group included 1,223 patients (52.6%) admitted to hospital in the next 5-year period – 2015–2019 (Figure 1).

The structure of hospitalization has been analyzed. The trends in the number of hospitalizations of nosological forms of VTEC were studied, reflecting the intensity of thrombotic process in different time periods. The data are presented as absolute indicators as well as relative indicators in the calculation of the event per 100,000 population per year. The frequency of the trait between groups was compared using tests of fourfold tables.

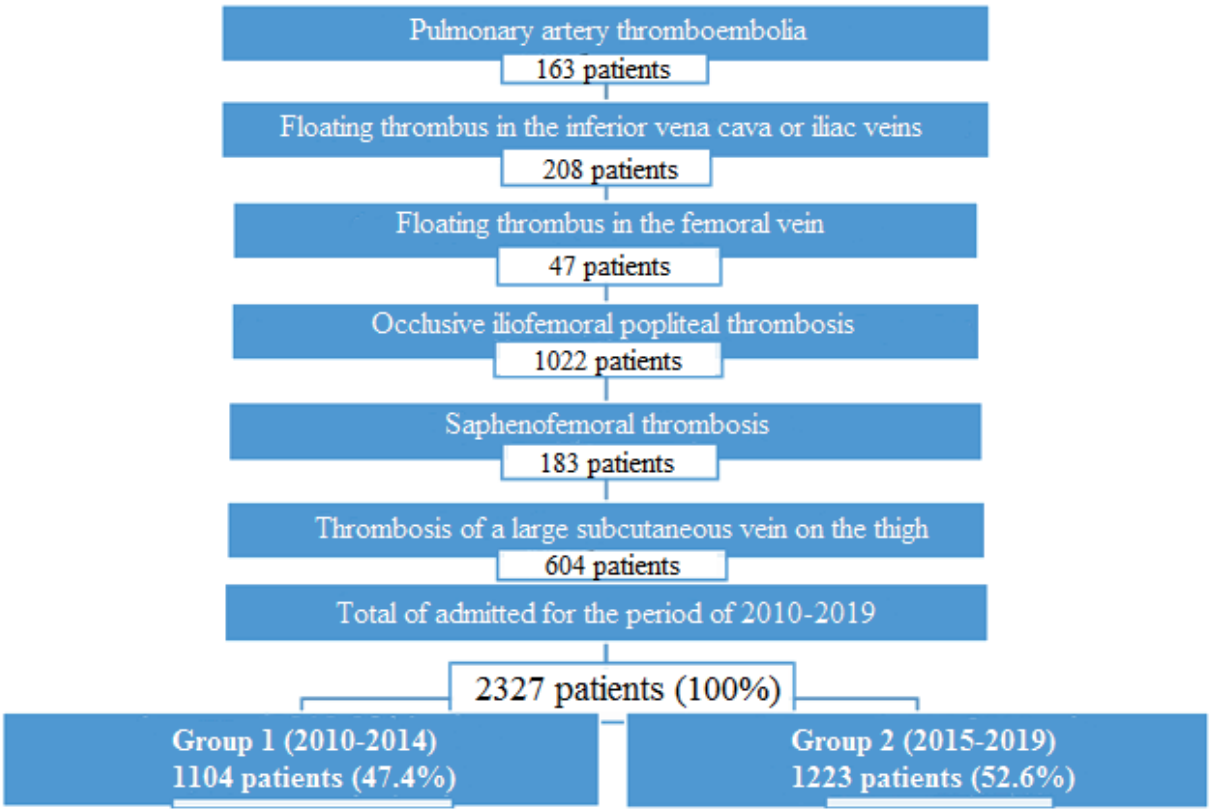

Figure 1. Nosological forms of VTEC, empirically ranked by the intensity of the thrombotic process.

Results and discussion

The annual hospitalization rate over 10 years was 65.5 cases per 100,000 population. No statistically significant difference was found in the frequency of VTEC hospitalization. Only the trend of the increasing number of VTEC continued. It made 62.2 cases per year in the first group (2010–2014) and 69.5 in the second group (2015–2019).

Comparison of the structure of the nosological forms of VTEC in different time periods revealed the following patterns (Table 1). No significant differences were found in the hospitalization rate of patients with floating thrombi in the inferior vena cava, iliac and femoral veins and thrombophlebitis on the thigh. The number of hospitalizations of patients with occlusive iliofemoral popliteal phlebothrombosis decreased statistically insignificant. At the same time, there was an increase in the frequency of hospitalization of patients with saphenofemoral thrombosis (p=0.023) by 1.5 times, and the number of hospitalized patients with PATE increased by 1.8 times (p=0.002).

In the 2019 American Heart Association (AHA) Summary Report, the prevalence of VTEC in the population varies within 100 cases per 100,000 population per year [6]. One of the first research to study the population dynamics of VTEC was conducted in the last century on a group of 2,218 patients (Minnesota, USA) who had a history of deep vein thrombosis or pulmonary embolism during a 25-year period from 1966 to 1990. 117 cases of VTEC per 100,000 (deep vein thrombosis, 48 per 100,000; PATE, 69 per 100,000) per year were established [7]. In our study, the annual frequency of VTEC was 65.5 cases per 100,000 population. This is due to the recording of only hospital events requiring treatment in a specialized unit. In the given sources, both hospital and outpatient thrombotic events were analyzed.

Data on the annual trends of VTEC are different. In Silverstein et. al., 1998 , the frequency of PATE was lower by approximately 45% over the past 15 years, while the frequency of deep vein thrombosis remained constant for men in all age groups, decreased for women under 55 years of age and increased for women over 60 years of age [7]. Later works found no significant change in the VTEC incidence in 1981–2010 [8]. Similar results were found in our study. When comparing two periods, we found no statistically significant differences in

the total frequency of all the nosological forms of VTEC. Among the most commonly recorded acute venous events in hospitalization – embologenic and non-embologenic deep vein thrombosis and thrombophlebitis – no differences were found in different periods of observation.

Table 1

Nosological forms of subgroups of hospitalized patients in different time periods

	Nosological forms of VTEC	Group, periods				p
		The first one 2010–2014		The second one 2015–2019		
		Abs.	%	Abs.	%	
1.	Pulmonary artery thromboembolia	58	5.3	105	8.6	0.002
2.	Floating thrombus in the inferior vena cava or iliac veins	110	10.0	98	8.1	0.1
3.	Floating thrombus in the femoral vein	23	2.1	24	2.0	0.836
4.	Occlusive iliofemoral popliteal phlebothrombosis	553	50.1	569	46.5	0.086
5.	Saphenofemoral thrombosis	72	6.5	111	9.1	0.023
6.	Thrombosis of a large subcutaneous vein on the thigh without spreading into the femoral vein	288	26.1	316	25.8	0.892
Total		1104	100	1223	100	-

Note: the absolute number of hospitalizations per 100,000 population per year.

At the same time, the number of patients with PATE and saphenofemoral thrombosis increases most significantly in the structure of VTEC. The paper shows an increase in the number of cases of PATE by 1.8 times and saphenofemoral thrombosis by 1.5 times between 2010–2014 and 2015–2019. This pattern has been noted in similar studies by other authors. The PATE incidence in the United States increased from 23 per 100,000 in 1993 to 65 per 100,000 in 2012 [9]. This trend is observed in Europe (Denmark), where, according to the national register, the PATE incidence increased from 45 to 83 per 100,000 from 2004 to 2014. Established patterns can be associated with both improved diagnosis of pulmonary embolism and increased number of this complication of the thrombotic process of the inferior vena cava system [10]. If 1.5 times increase in the number of saphenofemoral thrombosis can be associated with improved ultrasound diagnostics, then the increase in the frequency of PATE by 1.8 times in the absence of a significant increase in other nosological forms of VTEC cannot find an explanation only in diagnostic examination technologies.

Conclusions

1. The annual frequency of hospitalized VTEC is 65.5 cases per 100,000 population.
2. In the structure of hospitalization of patients with VTEC, the number of intermediate and high risk PATE cases and the number of patients with saphenofemoral thrombosis increase.

Conflict of interest. The authors declare no conflict of interest.

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