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CONTENT

EVALUATION OF SERUM HIGH-SENSITIVITY C-REACTIVE PROTEIN LEVELS DURING VARIOUS PERIODS OF PREGNANCY IN WOMAN, INFECTED WITH PARVOVIRUS – B19 INFECTION

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Abstract

The aim of the research was analyze of C-reactive protein levels in serum blood samples during various periods of pregnancy in women, infected with parvovirus B19 and in case of presence of clinical complications.

129 pregnant women, infected with parvovirus B19 infection and 16 women with physiological pregnancy during first, second and third trimesters of pregnancy were examined. Depending on the presence or absence of clinical complications each group of pregnant women (I, II, III) was divided into two subgroups. The concentration of C-reactive protein in blood serum was determined by the method of immunoassay analysis using diagnostic sets of reagents (ELISA kits, USA). Statistical processing of data was carried out using the package of applied programs Microsoft Office Excel 2010 and StatSoft Statistica 6.1.

The mean age of pregnant woman in our study was 26±6 years. In the Iand II groups of infected B19 parvovirus pregnant women were identified a significant increasing of C-reactive protein levels compared to controls by 62.5 % and 50.0 % ($p<0.05$). The largest increasing of C-reactive protein level relative to control values was observed in women with clinical complications in different pregnancy periods ($p<0.05$).

An increase levels of the marker of systemic inflammation the C-reactive protein in the blood testifies to its active participation in the launch of a complex mechanism for the development of labor activity and the occurrence of fetal disorders, which was confirmed in groups of pregnant women with clinical complications in different periods of pregnancy.

Keywords: pregnancy, parvovirus B19 infection, C-reactive protein.

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1. Introduction

Among the factors which have bad influence on pregnancy, virus infections are one of the most important. Fetal viral infection can cause fetal death, undeveloped pregnancy or miscarriage. Usually these pathologies are associated with acute respiratory viral infections, influenza, measles, parotitis, poliomyelitis, acute Coxsackie viral infection. Parvovirus infection in pregnancy – acute viral infection caused by parvovirus B19 (*Primate erythro parvovirus*) and characterized by a variety of clinical manifestations. Nowadays the role of systemic inflammatory process in the physiology andpathology of pregnancy becomes more important. Most of the inflammatory reactions are localized in the area of damage, particularly in tissues of the endo- and myometrium (that is a result of trophoblast invasion), but in some cases systematic inflammatory response can happen. C-protein is diagnostic marker, which indicates the presence of inflammatory processes in the body of pregnant women [1]. An increase of the CRP level above 8 mg/L indicates the risk of pregnancy loss. Systemic inflammatory response during physiological pregnancy is caused by genetic "foreignness" of fetus, but the reason of pathologic pregnancy is the decompensation of systems that regulate systemic inflammatory response [2]. Role of CRP is not only binding the components of microorganisms and toxins, but also binding of cytokines, IL-4, TGFβ, which possess suppressive properties. Therefore, it could facilitate the switching of the immune response from humoral to cellular. When connected to any of the ligands, CPR acquires the ability to activate the complement (both in classical and alternate way). Also, CRP can bind to lymphoid cells, playing with phagocytes

and platelets the role of an immune modulator. By activating the complement and all dependent reactions (adhesion, chemotaxis, phagocytosis) and modulating the activity of immunocompetent cells and platelets, the CRP carries out the connection between the various components of the inflammatory process. Therefore, the main role of CRP is recognising various substances presented on microorganism's cells or human tissues surfaces, activation of the corresponding functional systems and, consequently, elimination of pathogens, as well as "old" and necrotized cells of the body.Nowadays the role of systemic inflammatory process in the physiology andpathology of pregnancy becomes more important. System inflammatory response during a normal pregnancy occurs because of an effect of «extraneous body», which is a result of normal difference between mother and fetus genotypes. In case of decompensation of system controlling the inflammatory response may cause pathological course of pregnancy [3]. C-reactive protein (hs-CRP) is a blood test marker for inflammation in the body of pregnant women [4]. In most of cases inflammatory centers are located in places of damage of endo- and myometrium during trophoblast invasion [5, 6], but sometimes occurs general inflammation. During the normal pregnancy levels of hs-CRP can increase up to 3 mg/l, if hs-CRP increases over 8 mg/l it enhances probability of premature delivery in two times. High levels of hs-CRP from 5 to 19 weeks of pregnancy can lead to spontaneous abortion [7, 8].

Increased hs-CRP levels during pregnancy are often associated with poor pregnancyoutcomes, such as preeclampsia [9, 10], fetal growth retardation syndrome andintrauterine infection [11]. Hs-CRP is one of the early components, which can protect from infection [12, 13]. Just 2–4 hours after invasion, the level of hs-CRP increases, that lead to stimulation ofimmune reactions, such as phagocytosis, induction of pro-inflammatory cytokines(IL-1, IL-6, TNF-a) [14], monocyte tissue factor and activation of classicalcomplement pathway. Therefore, hs-CRP is considered as the most sensitive andrapid indicator of tissue damage during inflammatory process and it helps to detecteven minimal inflammation, and make faster diagnostic process for using the optimal treatment strategy [15, 16].

Approximately 30 % of pregnant women have no immunity to parvovirus B19infection. Frequency of infection during the pregnancy is near 0.4–3.7 %, and itmay cause spontaneous abortion (5–8 %) or intrauterine fetal infection leading tomal formations [17]. Pregnant women more often have asymptomatic course ofparvovirus infection (60 % of cases). Subclinical forms of parvovirus disease appear much more often than manifestation forms [18, 19]. Therefore, more often pregnant womenhave retrospective thoughts about acute parvovirus infection, when appears disordercommon for parvovirus B19 – "non-immune fetal hydrops" [20]. It is a reason, why hs-CRP test is needed for diagnostic of subclinical forms of parvovirus infectionbetween patients with premature delivery [21, 22], preterm labor or presence of clinicalcomplications.

2. Aimof research

To analyze of hs-CRP levels in serum blood samples during various periods ofpregnancy in women, infected with parvovirus B19 and in case of presence ofclinical complications.

3. Materials and methods

On the basis of the Perinatal Center of Kiev, pregnant women were tested for the presence of parvovirus infection in the 2012–2017period. 129 pregnant women, infected with parvovirus B19 infection (from 19 to 39years old, mean age – 26±6 years) were examined in the research. Patients weredivided into three groups (groups I, II, III) depending of a period of gestation. Subgroups of pregnant women without clinical complications were named as Ia, IIa and IIIa. Subgroups with complications named as Ib, IIb and IIIb. 16 woman formed the control group with physiological course of pregnancy in different trimesters. The concentration of hs-CRP in blood serum was determined by the method of immunoassay analysis using diagnostic sets of reagents (ELISA kits, USA).

The statistical processing of the obtained results was carried out using standard methods of variation statistics, taking into account the differences in the Student t criterion, which was

estimated using the confidence probability (p) less than 0.05 using Microsoft Office Excel 2010 for Windows and StatSoftStatistica6.1. The results are described as M±m. [23].

4. Results of research

From 129 pregnant women with parvovirus infection 57 (44.2 %) of them had increased levels of hs-CRP (exceeding was 20 % from control level in currenttrimester). Infected patients from I and II groups showed significant increasing of hs-CRP levels comparatively with woman from control group: 62.5 % and 50.0 %,respectively (**Table 1**). Levels of hs-CRP of pregnant women from group III haddifference from the control group just on 29.8 % ($p<0.05$).

Table 1

Levels of hs-CRP in blood serum of pregnant B19-infected women in different trimesters

Trimester groups	Level of hs-CRP, mg/l (n=145), M±m					
	Control group, n	Healthy	Main Groups	n	Infected	Δ, %
I trimester (n=50)	5	5.6±0.8	I	45	9.1±1.4*	+62.5
II trimester (n=54)	6	6 5.4±0.5	II	48	8.1±1.0*	+50.0
III trimester (n=36)	5	5 4.7±0.9	III	36	6.1±1.0	+29.8

*Note: * – $p<0.05$ compared to a group of healthy pregnant women; Δ – difference compared with data from healthy pregnant women*

Pregnant women from the main group according to the trimester of pregnancy were divided into subgroups: without clinical complications are grouped into Ia, IIa, IIIa subgroups and withclinical complications during pregnancy – in the Ib, IIb, and IIIb subgroups.

Comparing subgroups of pregnant infected women with or without complications showed significant increasing of hs-CRP in groups Ib, IIb, IIIb (**Table 2**).

Table 2

Levels of hs-CRP in blood serum of pregnant B19-infected women with (groups «b») or without (groups «a») clinical complications

Trimester groups	Level of hs-CRP, mg/l (n=145), M±m					
	n	Healthy	Groups	n	Infected	Δ, %
I trimester (n=50)	5	5.6±0.8	Ia Ib	6 39	7.21.3 9.41.6±*	+28.6 +67.8
II trimester (n=54)	6	5.4±0.5	IIa IIb	19 29	6.7±1.2 9.1±0.9*	+24.1 +68.5
III trimester (n=41)		5 4.7±0.9	IIIa IIIb	30 6	5.8±1.2 7.9±0.6*	+23.4 +68.1

*Note: * – $p<0.05$ compared to a group of healthy pregnant women; Δ – difference compared with data from healthy pregnant women*

Levels of hs-CRP from all groups of B19-infected pregnant women without complications showed almost the same difference with the control group, this difference in groups Ia–IIIa was from 23.4 % to 28.6 %. But in groups with complications (Ib–IIIb) this difference was between 67.8 % and 68.1 % (**Fig. 1**).

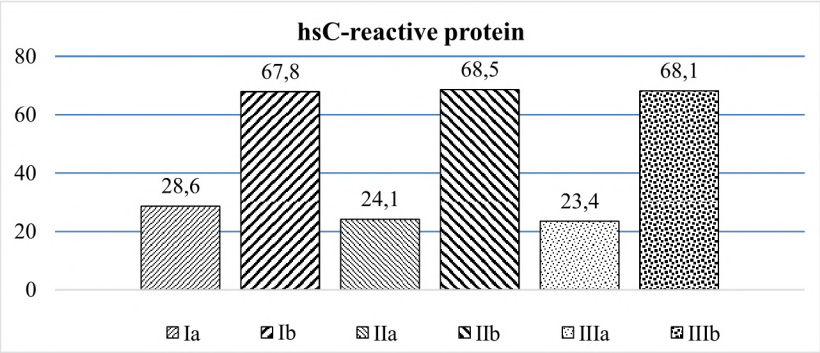

Fig. 1. Comparison content of levels of hs-CRP in groups of B19 infected pregnant women Ia–IIIa (without complications) with groups Ib–IIIb(with clinical complications)

5. Discussion

The present research of levels of hs-CRP has established a moderate increasing its concentration in the blood serum of pregnant women with parvovirus B19 infection, which is connected with slight damage of tissues. It is well-known, that hs-CRP can stimulate inflammatory response by activating of classical complement pathway, tissue damage and induction of pro-inflammatory cytokines in monocytes [10]. Those factors can cause adverse pregnancy outcomes. Current study shows, that parvovirus B19 infection during pregnancy is related with increasing of hs-CRP on all gestation periods. Significant increasing of hs-CRP level can activate phagocytes. Absorption of protein by neutrophils leads to hs-CRP proteolysys, it causes releasing of immunoactive low weight peptides that can modulate the functions of neutrophils, monocytes and macrophages (increases chemotaxis of phagocytes, production of superoxide anion and IL-1). In monocytes hs-CRP enhances the generation of active forms of oxygen and tumor necrosis factor-a. Additionally, hs-CRP affects other immune competent cells: hs-CRP can connect with T-helper cells, cytotoxic T-lymphocytes and NK-cells (also, NK-cells, can produce CRP by themselves).

It is known that CPR can exacerbate inflammatory responses through complement activation, tissue damage, and induction of inflammatory cytokines in monocytes thus lead to adverse pregnancy outcomes. Results of current study show that parvovirus infection during pregnancy is connected with increased levels of CRP on all terms of pregnancy. Significant increase in the level of CRP may provoke activation of phagocytic cells. Absorption of protein by neutrophils leads to CRP proteolysys, which results in the isolation of immunoactive low molecular weight peptides that can modulate the functions of neutrophils and monocytes/macrophages (increased chemoacusis of phagocytes, production superoxide anion and interleukin-1). In monocytes, CRP increases the generation of active forms of oxygen, factor TNF-α. In addition, CRP affects other immunocompetent cells: it can bind T-helper cells, cytotoxic T-lymphocytes, NK-cells (also, NK-cells, can produce CRP by themselves). CRP is the most sensitive and fastest indicator of tissue damage during inflammation. Pregnant women with high levels of C-reactive protein (CRP) in the blood are at high risk for preterm delivery. Increasing level of CRP is able to provoke the activation of phagocytic cells in various intensity. Free CRP is absorbed by neutrophils in large amounts and is rapidly internationalized. The result of CRP proteolysys in the phagosomes of neutrophils is the secretion of immunoactive low molecular weight peptides that can modulate the functions of neutrophils and monocytes/macrophages: enhance chemoacusis of phagocytes, their production of superoxide anion and IL-1. Monocytes not only bind CRP, but also synthesize it. In monocytes, CRP enhances the generation of active forms of oxygen, thereby increasing the ability of macrophages to synthesize TNF-α. So, effect of CRP on monocytes has a more pro-inflammatory character than in the case of neutrophils, when the result of influence depends on the stage of inflammation and activity of neutrophils. In addition, CRP affects other immunocompetent cells: it can bind to lymphocytes (CD4 +, CD8 +), NK cells. Synthesis and secretion of CRP is regulated by cytokines: IL-6, IL-1 and TNF-α.

Therefore, the growth of the marker of systemic inflammation response - C-reactive protein, indicates an active participation in the launch of a complex mechanism of the development of fetal and pregnancy disorders, which was confirmed in groups of women with clinical complications in different periods of pregnancy.

6. Conclusions

1. An increased level of the marker of systemic inflammation hs-CRP in the blood serum testifies to its active participation in the launch of a complex mechanism for the development of labor activity and the occurrence of fetal disorders, which was confirmed in groups of pregnant women with clinical complications in different pregnancy periods.

2. Significant growth (in 1.6 and 1.5 times) of the sensible marker of systemic inflammation of C-reactive protein in infected pregnant women in the 1st and 2nd trimesters was found, the most significant in women with clinical complications of pregnancy and to the same extent (in 1.7 times) in different trimesters of pregnancy.

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CHANGES IN THE CELL SQUAD OF ILIAC LYMPH NODES OF WHITE RATS IN CASE OF LONGTERM INFLUENCE OF NALBUFIN

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Abstract

The article presents data on the change in the cellular composition of the lymph nodes of the white rats, males of reproductive age, who received intramuscular opioid analgesics – nalbuphine every day for six weeks. The weekly dose of nalbuphine was gradually increased, creating a model of physical opioid dependence according to the patent of Ukraine No. 76564 U. All experimental animals were divided into 8 groups.

Morphometric method was used to determine the relative number of cells of the lymphoid series – small, medium and large lymphocytes, blasts and plasmocytes in the cloak zone and the embryonic center of the secondary lymphoid nodes and brain strands of the lymph nodes. Morphometric studies were performed using a system of visual analysis of histological preparations.

It was established that nalbuphine in the lymph nodes causes reactive and destructive changes: the number of large lymphocytes increases in all structural components of the lymph node with a maximum after 4 weeks, respectively, the relative number of small lymphocytes decreases in the nucleus centers and brain tracts, the relative number of plasmocytes in the brain strains increases sharply . In all structural components of the lymph nodes hemocapillaries and venules are dilated and full-blooded, around vascular edema and partial damage to the walls of the microvessels.

One week after the discontinuation of nalbuphine, the relative number of lymphoid cells in the structural components of the lymph nodes does not return to the indicators of intact animals, no reversible changes are noted.

Keywords: lymph node, germ center, lymphocyte, plasmocyte, experiment, nalbuphine, white rat.

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1. Introduction

The study of the influence of narcotic analgesics, in particular opioids, on the immune system of the body, is one of the most important problems, since they are widely used in medical practice [1]. Their medical and social significance is primarily associated with a painkilleractivity [2, 3]. The opioid is a substance of synthetic and semi-synthetic origin, which is in its composition,morphochemically, related to opiate receptors and provides a certain psycho-vegetative action. The representative of this group is nalbuphine – a synthetic opioid of an agonist-antagonist group (agonist of k-receptors and an antagonist of mu-receptors) [4, 5]. Unfortunately, uncontrolled use of opioids leads to an increase in drug addiction not only in Ukraine, but also in the world [6]. Therefore, the issue of the influence of narcotic analgesics, in particular opioids, on organs and body systems acutely faced by scientists.

Alterations in various organs and systems of the body have been investigated under the influence of nalbuphine, in particular:

– the administration of nalbuphine during 7–28 days leads to dystrophic and destructive changes in the hemocapillary and epitheliocytes of the iris-corneal angle of the eyeball [7];

– Nalbuphine causes a diffuse defeat of the mitochondrial apparatus in all areas of the myocardium [8];

– Changes in the skin are observed, which are accompanied by a violation of its microstructure and the bloodstream [9], changes in the structure of all the vessels of the lining of the eyeball [10], tongue tissues [11, 12];

– Blood vessels of the hemomicrocirculatory pancreas [13] and kidneys [14];

– It has been established that six-week administration of nalbuphine causes pathological changes of angioarchitectonics and neurocytes structures of all layers of the cerebellum cortex [15], etc.

However, the influence of opioids, in particular, nalbuphine, on the immune organs has not been sufficiently studied. The effects of nalbuphine on the thymus, the primary lymphoid organ, have already been studied. It was established that six-week administration of the drug leads to a structural rearrangement of the parenchyma of the organ, deep irreversible changes at the submicroscopic level [16] and damage to the thymus's vascular bed [17]. In the scientific literature, there is no data on the influence of opioids, in particular nalbuphine, on secondary lymphoid organs, in particular lymph nodes, on which not only humoral, but also cellular immunity depends. That is why we are interested in this study. We have already investigated that long-term administration of nalbuphine leads to a change in the relative areas of structural components of the iliac lymph nodes [18] and to destructive changes in the lymph nodes parenchyma at the microscopic level [19].

2. Aim of the research

To study the changes in the cellular structure of the structural components of the iliac lymph nodes of white rats-males of reproductive age in the dynamics of the six-week administration of opioid nalbuphine and one week after its discontinuation.

3. Materials and methods

This experimental study was performed on 52 non-breeding white male rats of reproductive age (1.5–2 month olds) with an initial weight of 140–150 g. Opioidnalbuphine was injected into test

animals daily for six weeks in the right intramuscular region of the mitochondria. All experimental animals were divided into 8 groups and the weekly dose of the injection preparation was increased in progressive order (**Table 1**) according to the patent number 76564 U "Method for the simulation of physical opioid dependence in rats" [20]. The studies were also carried out 1 week after the discontinuation of nalbuphine (group 8).

The research was conducted on the basis of the Lviv National Medical University named after DanyloHalytsky, in accordance with the agreement dated November 18, 2013 on cooperation between the department of normal anatomy of the Lviv National Medical University named after DanyloHalytsky and the Department of human anatomy and histology of the Faculty of medicine of Uzhgorod National University during 2014–2017.

Table 1

Dose of nalbuphine according to experimental groups of animals

Group of animals	Group 1 intact animals	Group 2 after 1 week of introduction	Group 3 after 2 weeks of introduction	Group 4 after 3 weeks of introduction	Group 5 after 4 weeks of introduction	Group 6 after 5 weeks of introduction	Group 7 after 6 weeks of introduction	Group 8 abolition
Dose, mg/kg	–	8	15	20	25	30	35	–

Control was provided with 12 rats, which, in place of nalbuphine, were similarly injected with a 0.9 % solution of sodium chloride.

Experiments on animals were conducted in accordance with the provisions of "The European convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986), Council Directives 86/609/EEC (1986), Law of Ukraine No. 3447-I "On protection of animals from cruel treatment "," General ethical principles of experiments on animals ", adopted by the National congress of Ukraine on bioethics (2001).

The animals were kept in vivarium in accordance with generally accepted rules, on a standard diet. For fetching of the studied organs – the iliac lymph nodes – the experimental animals were extracted from the experiment by anesthetizing the with intraperitoneal anesthesia using thiopental natrium (25 mg/kg). Fixed material in 10 % neutral formalin, dehydrated in increasing concentrations of ethanol (50˚, 70˚, 90˚, 100˚) and poured in paraffin blocks. Histological preparations with a thickness of 5–7 microns stained with hematoxylin and eosin were morphometrically studied to determine the relative number of cellular elements (small, medium and large lymphocytes, lymphoblasts, plasmocytes) of structural components of the iliac lymph nodes of white male rats at prolonged exposure of opioidnalbuphine and 1 week after discontinuation of the drug. Images from the histological preparations on the computer monitor were taken from a microscope MICROmedSEOSCAN and using a VisionCCDCamera. Morphometric studies were carried out using programs VideoTest-5.0, KARRA ImageBase and MicrosoftExel on a personal computer. Statistical processing of digital data was performed using the software "Exel" and "STATISTICA" 6.0 using the parametric method. The digital values are represented by selective mean and standard error (M±m). The reliability of the mean values (p) was determined by the Student's t-criterion with a probability level of $p<0.05$.

The half-thickness sections 1–2 microns were made on ultramicrotome LKB-3 (Sweden). They were stained with methylene blue.

4. Results

The dynamics of changes in the relative number of lymphoid cells were studied – small, medium and large lymphocytes, lymphoblasts, plasmocytes in the cloak zone and the embryonic center of the secondary lymphoid nodes and brain stretches of the lymph nodes of the white rats – males of reproductive age in normal, in the dynamics of six weeks under an influence of the nalbuphine and one week after its cancellation (**Table 2, 3, Fig. 1**).

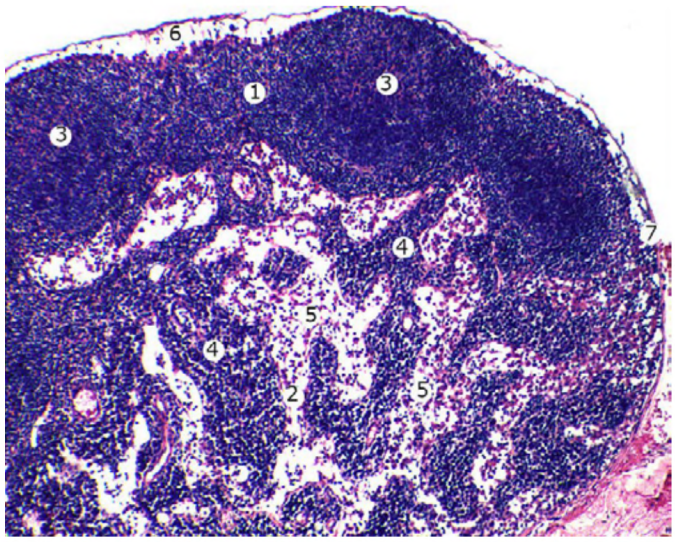


Fig. 1. The structure of the iliac lymph node of the intact white male rat: 1 – cortical substance; 2 – cerebrospinal fluid; 3 – lymphoid knot with a germ center; 4 – brain strain; 5 – the cerebral intermediate lymphatic sinus; 6 – boundary lymphatic sinus; 7 – capsule. Coloring with hematoxylin and eosin. Zoom: op. ×10, oc. ×8

Table 2

Ratio of the cellular composition of secondary lymphoid nodules of the iliac lymph nodes of white male rats at 6 weeks of opioid nalbuphine exposure and after its cancellation

Duration of nalbuphine	The relative number of cells in the lymphoid series, %					
	Cloak zone of the lymphoid nodule			Embryonic center of the lymphoid nodule		
	Small lymphocytes	Medium lymphocytes	Large lymphocytes	Small lymphocytes	Medium lymphocytes	Large lymphocytes
Intact animals	69.47±2.03	27.84±1.17	2.69±0.12	27.93±1.32	63.54±2.54	8.53±0.28
1 week	69.96±3.04	27.11±1.14	2.93±0.14	25.36±1.27	64.42±3.02	10.22±0.37**
2 weeks	70.12±2.95	26.47±1.25	3.41±0.17**	22.20±1.23**	64.75±3.06	13.05±0.32***
3 weeks	70.46±2.14	25.62±1.19	3.92±0.21***	19.92±1.25***	65.17±3.14	14.91±0.42***
4 weeks	71.03±3.21	24.82±1.12	4.15±0.16***	17.70±1.19***	66.51±2.98	15.79±0.44***
5 weeks	71.34±3.33	24.64±1.04*	4.02±0.12***	18.53±1.15***	65.93±3.13	15.54±0.39***
6 weeks	72.03±3.15	24.13±1.05*	3.84±0.22***	19.55±1.12***	65.47±2.98	14.98±0.41***
Cancellation	72.91±3.47	23.72±1.07*	3.37±0.19***	20.73±1.14***	64.97±3.15	14.30±0.38***

Note: * – values that are statistically significantly different from those ones of an intact group of animals: * – p<0.05; ** – p<0.01; *** – p<0.001*

It has been established that in 1–2 weeks of the introduction of nalbuphine changes of the relative number of lymphoid cells in the cloak zone and the embryonic center of the secondary lymphoid nodes and brain strands of the lymph nodes, changes in the cytoarchitectonics of the structural components of the lymph nodes were increasing during the six-week administration of nalbuphine and their cellular composition is not restored, even a week after the drug is cancelled (**Table 2, 3, Fig. 2–6**).

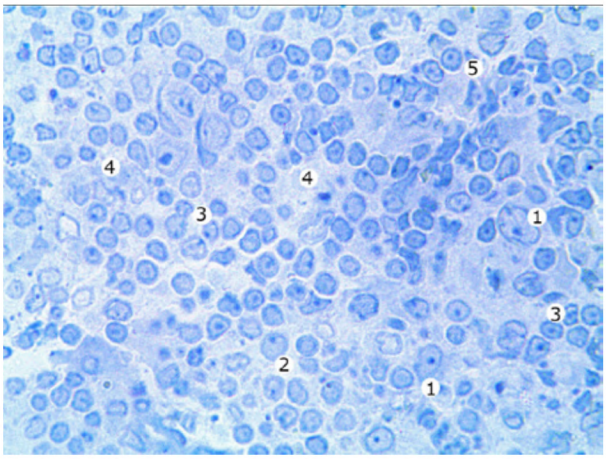
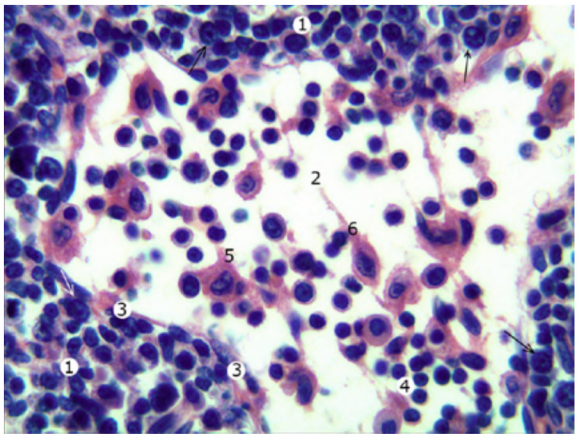
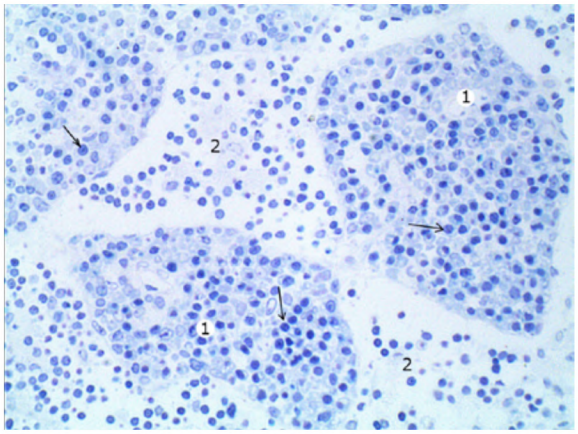


Fig. 2. Fragment of the embryonic center of the lymphoid node of the iliac lymph node of a white male rat after two weeks of nalbuphine administration: 1 – lymphoblast; 2 – middle lymphocyte; 3 – small lymphocytes; 4 – moderately expanded intercellular spaces; 5 – lymphoblast in the stage of mitosis. Thin slice. Coloring with methylene blue. Zoom: ×400



a



b

Fig. 3. Fragment of the brain substance of the iliac lymph node of a white male rat in four weeks of the action of nalbuphine: 1 – cerebral strain; 2 – cerebral intermediate lymphatic sinus; 3 – reticuloendotheliocytus; 4 – small lymphocytes; 5 – macrophage; 6 – reticular cell; plasmocyte (arrow). A – coloring with hematoxylin and eosin. Zoom: op. ×40, oc. ×15. B is a half-cut slice. Coloring with methylene blue. Zoom: ×200.

Table 3

The ratio of the cellular composition of cerebral brain masses of the iliac lymph nodes of white male rats at six weeks of exposure to nalbuphine and after its cancellation.

Duration of nalbuphine	Relative number of cells of the lymphoid series in the cerebral strains of the cerebrospinal fluid, %			
	Small lymphocytes	Medium lymphocytes	Lymphoblasts	Plasmocytes
Intact animals	28.78±1.34	49.14±1.78	2.67±0.13	19.41±0.92
1 week	21.59±1.27***	51.03±2.15	5.04±0.19***	22.34±1.02*
2 weeks	15.60±0.65***	51.94±2.48	7.33±0.21***	25.13±1.06***
3 weeks	11.00±0.45***	52.24±2.15	9.14±0.24***	27.62±1.11***
4 weeks	7.68±0.24***	53.04±2.69	9.86±0.20***	29.42±1.05***
5 weeks	9.24±0.27***	52.87±2.54	9.74±0.23***	28.15±1.14***
6 weeks	10.56±0.36***	52.25±2.47	9.23±0.18***	27.96±1.08***
Cancellation	12.52±0.41***	51.31±2.38	9.19±0.27***	26.98±1.12***

*Note: * – values that are statistically significantly different from those ones of an intact group of animals: * – p<0.05; ** – p<0.01; *** – p<0.001*

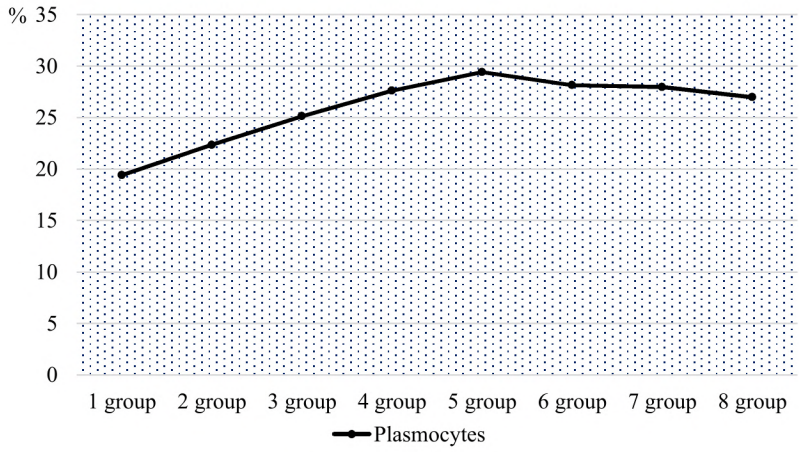


Fig. 4. Changes in the relative number of plasmocytes in the brain strains of the iliac lymph nodes of white male rats in the dynamics of six weeks of opioid nalbuphine exposure:
Group 1 – intact animals; Group 2 – after 1 week; Group 3 – after 2 weeks;
Group 4 – after 3 weeks; Group 5 – after 4 weeks; Group 6 – after 5 weeks;
Group 7 – after 6 weeks; Group 8 – 1 week after cancellation of the drug

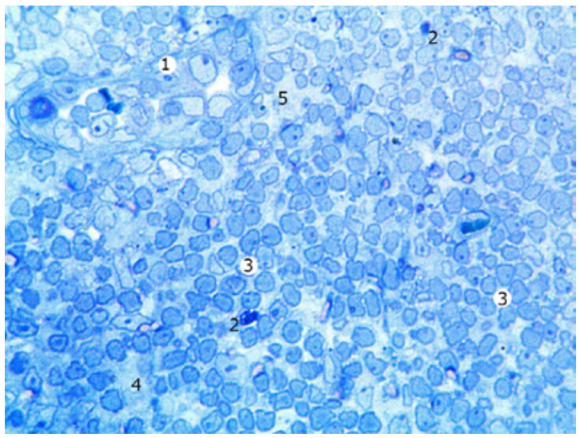
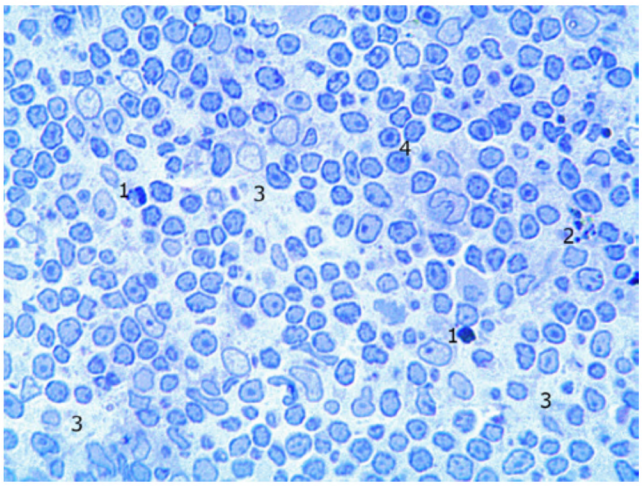


Fig. 5. A fragment of the cloak zone of the iliac lymph nodes male rat after six weeks of nalbuphine administration: 1 – an enlarged vein with a thickened wall; 2 – apoptotic altered lymphocyte; 3 – small lymphocytes; 4 – extended intercellular spaces; 5 – vascular edema. A thin cut. Coloring with methylene blue. Zoom: ×400

Following the cancellation of the nalbuphine, pathological changes in the parenchyma, cellular composition of the iliac lymph nodes and vessels of the hemomicrocirculatory channel are



preserved and not restored (**Fig. 6**).

Fig. 6. Fragment of the embryonic center of the iliac lymph nodes male rat one week after the cancellation of nalbuphine: 1 – apoptotic altered lymphocyte; 2 – macrophage; 3 – extended intercellular spaces; 4 – small lymphocytes. Thin slice. Coloring with methylene blue. Zoom: ×400

5. Discussion

The study showed that in normal (intact animals), the germinal centers of the secondary lymphoid nodes of the iliac lymph nodes of white rats-males of reproductive age are predominantly medium lymphocytes and lymphoblasts, and their cloak zone is small lymphocytes (**Table 2**). There are many plasmocytes in the brain lobes, and middle lymphocytes are also prevalent (**Table 3**).

At short-term action (within one and two weeks), the reactive changes in the cellular composition of the structural components of the iliac lymph nodes were noted on the body of the experimental animals of nalbuphine. Already after 1 week of experiment in the embryonic centers of secondary lymphoid nodes, the relative number of lymphoblasts ($p<0.001$) from 8.53 % (intact animals) to 10.22 % is significantly increased. This indicator increased throughout the experiment with a maximum after 4 weeks of nalbuphine action at 7.26 %. Then the amount of lymphoblasts decreases somewhat until the end of the experimental study, but even after 1 week after the cancellation of nalbuphine, the relative number of these cells is significantly higher by 5.77 % compared to intact animals. The relative number of small lymphocytes decreases accordingly, after 4 weeks they are the least – 17.7 %, at 27.93 % in animals of the intact group. The relative number of middle lymphocytes is almost unchanged (**Table 2**).

In the cloak zone of lymphoid nodules of the iliac lymph nodes of intact animals, small lymphocytes prevail – 69.47 %. Throughout the experiment, the number of small lymphocytes slightly changes, in contrast to large lymphocytes, which in the cloak zone of intact animals is only 2.69 %, but after 4 weeks of action of nalbuphine their number maximally increases to 4.15 %. Subsequently, the number of large lymphocytes is gradually decreasing, but even after 1 week after the cancellation of administration of nalbuphine, this indicator does not normalize, remaining significantly ($p<0,001$) increased and is 3.37 % (**Table 2**).

Small and medium lymphocytes predominate in brain stretches, the relative number of the latter during the experiment almost does not change (**Table 3**). Plasmocytes in this structure of the lymph nodes of intact animals are the most – 19.41 %. Already after 1 week of action of nalbuphine, the amount of plasmocytes increased to 22.34 %. Subsequent daily administration of nalbuphine leads to their maximum increase – 29.42 % after 4 weeks of the experiment. Subsequently, the amount of plasmocytes is somewhat reduced, but even one week after discontinuation of the

drug, this indicator is significantly ($p<0.001$) higher compared to intact animals and is 26.98 % (**Table 3, Fig. 4**). Also, in the brain strains the relative number of large lymphocytes is significantly increased: after 1 week of the experiment, the relative number of them increases by 2.37 %. Subsequently, the number of these cells increases with a maximum of 4 weeks of experiment up to 9.86 %. Then the number of large lymphocytes decreases somewhat, but even after 1 week after the abolition of nalbuphine, this figure is greater by 6.52 % compared with intact animals (**Table 3**). The relative number of small lymphocytes in brain strains under the influence of nalbuphine decreases: after one week of action of the drug – by 7.19 %, and after 4 weeks, these cells are the least as much as 21.1 %. Subsequently, the number of small lymphocytes increases slightly, but after 6 weeks of experiment they are only 10.56 %, and a week after the discontinuation of the drug – 12.52±0.41 at 28.78 % in animals of intact group (**Table 3**).

Comparing the results of the study with the primary lymphoid organ – thymus – where the long-term administration (six weeks) nalbuphine reduces to a slight increase in the density of lymphocytes [21], an increase in the number of lymphoblasts in the embryonic centers and plasmocytes in the brain strands of the lymph nodes suggests the compensatory and adaptive response of this organ to the action of nalbuphine.

6. Conclusions

Long-term six-week administration of nalbuphine causes reactive changes in the cellular composition of structural components of the iliac lymph nodes:

1) in the cloak zone of the secondary lymphoid nodes, the relative number of large lymphocytes is increased to 3.84 % ($p<0.001$); the relative number of secondary lymphocytes is reduced by 3.71 %; the relative number of small lymphocytes does not change substantially;

2) in the embryonic center, the relative number of lymphoblasts increases by 6.45 %, and the relative number of small lymphocytes decreases by 8.38 %; the relative number of middle lymphocytes varies slightly;

3) in the brains of the cerebral substance significantly increases the relative amount of plasmocytes – by 8.55 %; the relative number of large lymphocytes increases 3.4 times – from 2.67 % to 9.23 %, and small lymphocytes – decreases 2.7 times and makes 10.56 % in compare to 28.78 % in intact animals.

One week after the discontinuation of nalbuphine, the relative number of cells of the lymphoid series of structural components of the iliac lymph nodes did not change in comparison with the previous group of experimental animals and did not normalize.

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CORRECTION OF IODINE DEFICIENCY STATES AND DYNAMIC MODELING OF POSITIVE DYNAMICS OF INDICATORS OF THYROID FUNCTIONS BY SUPPLEMENTATION

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Abstract

The problem of iodine deficiency diseases (IDD) is recognized as relevant due to the significant prevalence of iodine deficiency among the population of many countries of the world, an increase in the incidence of diseases with a wide range of clinical manifestations and a marked tendency to increase the frequency and severity of IDD among children of all age groups

We carried out a clinical-anamnestic examination of the child's contingent (187 persons) aged 13–17 years living in an ecologically dependent biogeochemical endemic zone of iodine deficiency, the mountain region of the Zakarpattya region during the period from 2014 to 2015. To identify the pathology of the thyroid gland, a palpatory method of examination was used according to the methodology of the WHO/MRKIDZ, 2001. According to our data 80 pupils (42.8 %) had increased thyroid gland of 1 degree, they were allocated for further and detailed examination and identification of environmentally caused somatic effects. Preventive measures included taking the dietary supplement Yosen, the manufacturer of TOV "OmniFarm", TU U 10.8-35758392-004: 2014 for 6 months. A statistical model for forecasting the dynamics of TSH with supplements with iodine and selenium has been developed. According to our data, the degree of positive changes (decrease of TSH, increase of T4) with supplements with iodine and selenium depends on the starting content of the microelement of iodine in plasma and/or urine: the lower is the initial level of iodine – the more pronounced is the effect of supplements.

Keywords: children, ecologically dependent biogeochemical zone, functional parameters of the thyroid gland.

DOI: 10.21303/2504-5679.2018.00591

1. Introduction

Beginning with perinatal development and throughout life, a person is under constant influence of geochemical factors of the environment [1, 2]. The problem of iodine deficiency diseases (IDD) is recognized as relevant due to the significant prevalence of iodine deficiency among the population of many countries in the world, an increase in the incidence of a large range of clinical manifestations and a marked tendency to increase the frequency and severity of IDD among children of all age groups, which causes the problem not only medical, but also socially significant [3, 4].

Disorders of thyroid function occur due to microelemental insufficiency, which can be prevented and eliminated by supplements with the corresponding microelements – iodine and selenium [5]. In the case of endemic causes of iodine deficiency, supplements should cover a significant part of the inhabitants of the respective settlements. Moreover, the greatest need for preventive measures applies to persons of child and adolescent age, as well as pregnant and lactating women [6]. When developing supplementation programs, it is important to take into account individual characteristics and to select the most optimal dose or mode of use of the microelement preparations [7, 8]. To do this, one should be able to identify the most important factors that can influence the dynamics of the main indicators of the thyroid function, and also predict the expected outcome of long-term supplementation [9, 10].

2. Aim of the research

To carry out a clinical and anamnestic examination of the child's contingent of the mountain region for the detection of physical development disorders, visual-palpation examination of the thyroid gland, followed by the development of a model of multifactorial dependence of thyroid status disorders.

3. Materials and methods

In order to meet the set goals, a preventive survey was carried out for children (187 children) aged 13 to 17 years old living in an ecologically dependent biogeochemical endemic zone of iodine deficiency in the mountainous region of Transcarpathian region during the period of 2014 through 2015 in order to detect violations in the functioning of the thyroid gland with the following somatic effects and taking into account its morphological functional changes. The study group consisted of children aged 13 to 17 years old (mean age 15.90526±1.36), among them 96 boys (63.0±4.18 %) and 81 girls (37.0±5.06 %.) Clinical and anamnestic examination of the child's contingent was carried out. To identify the pathology of the thyroid gland, a visual-palpation method of examination was used according to the WHO/MRKIDZ methodology, 2001 [11]. The research was conducted on the basis of the city children's clinical hospital in Uzhhorod, Transcarpathian region. According to our data, 80 pupils (42.8 %) had an increased thyroid gland of 1 degree, they were allocated for further and detailed examination and identification of ecologically determined somatic effects. The average height of adolescents was 166.22±1.26 from 196 to 158 (boys 175.96±0.37 from 196 to 159 cm and 161.55±0.72 in girls from 166 to 158 cm), which corresponds to 50 centiles, regardless of sex, body weight – 57.78±1.19 from 88 to 46 (62.89±1.37 kg from 88 to 47 in boys and 52.49± ±1.13 kg from 56 to 46 in girls) corresponding to 25 centiles in children, boys and girls. A cohort of 59 adolescents living in an area with endemic iodine deficiency was investigated. 30 of them had hypertrophic changes in thyroid parenchyma, whereas in 29 adolescents parenchyma of the thyroid gland was under reference. In the dynamics, a number of indicators of serum blood and urine were determined [(free thyroxine (T4) and triiodothyronine (T3), thyrotropin (TSH), cortisol, titre of antibodies to thyroid peroxidase (AT-TPO) and to thyroglobulin (AT-TG), thyroxine-binding globulins level (TBG), tyrosine, microelements of iodine and selenium, ferritin, IgM, IgG, cholesterol, high density lipoprotein (HDL) and low (LDL), triglycerides, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), haemoglobin and the erythrocyte sedimentation rate (ESR)] [12]. All listed indicators, as well as the age and condition of the parenchyma (diffuse changes in the thyroid gland or unchanged pelvic syndrome) were studied as possible predictors of the TSH and T4 dynamics as a result of supplements. Preventive measures included the administration of the dietary supplement Yosen manufactured by "OmniFarm" LLC, TU U 10.8-35758392-004: 2014 according to generally accepted recommendations for supplements [13]. Doses were prescribed according to the instructions and age requirements of 1 tablet once a day during meals, drinking water for 6 months. Materials of scientific and practical work were considered at a meeting of the Bioethics Commission. The safety rules of the investigated contingent and the patient's rights were observed. During conducting of scientific and practical investigations of violations of moral and ethical norms in relation to patients was not revealed. The question was formulated as follows: "Is it possible to predict with a satisfactory accuracy the dynamics of the indicator of the thyroid function

under iodine and selenium supplementation, using biochemical parameters?". The accuracy of the forecast, which corresponds to the determination coefficient $R^2 \geq 0.5$, was considered satisfactory, which is twice (or more than twice) less than the mean square error than with pure guessing. Statistical simulation was carried out in the software environment for statistical calculations R 3.4.3. A flexible and modern algorithm for multivariate adaptive regression splines (MultivariateAdaptiveRegressionSplines, MARS) [14, 15] was used to construct regression prediction models.

The MARS algorithm creates an additive nonlinear model, which is described by the following equation.

$$\hat{y} = a_0 + \sum_{m=1}^{M} a_m B_m(x), \tag{1}$$

where $\hat{y}$ – the predicted value of the dynamics, a_0 – the intercept, M is the number of basic functions, B_m and a_m are the m's basic function and its coefficient. Each basic function, in turn, is a power function, which is described by the following equations:

$$\begin{aligned} [+(x-t)]_+^q &= \begin{cases} (x-t)^q, & if\ x \geq t, \\ 0, & \text{in other case,} \end{cases} \\ [-(x-t)]_+^q &= \begin{cases} (t-x)^q, & if\ x < t, \\ 0, & \text{in other case,} \end{cases} \end{aligned} \tag{2}$$

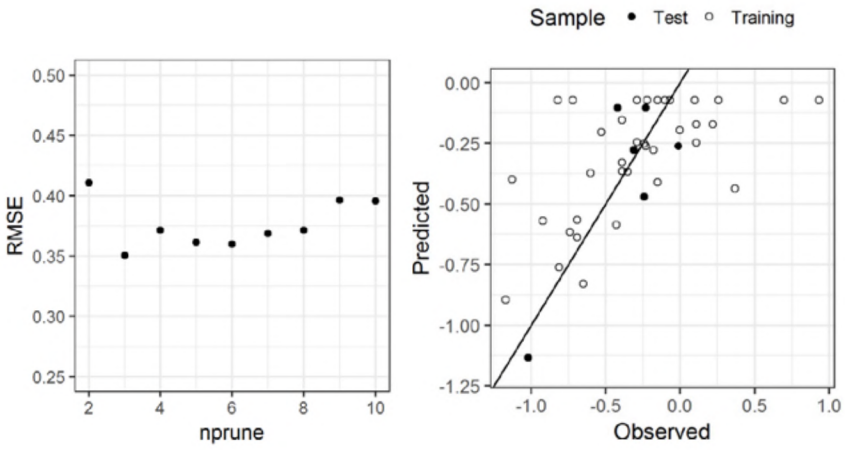
where t is the coordinate of the node, and q (≥ 0) is the spline power [16]. The construction of MARS models goes in two stages: direct and reverse. In the direct phase, the factors are involved in the model for the "greedy" principle, so that an overfitted model is formed. In the next, this model is simplified and improved during the reverse phase. The MARS algorithm is configured with the nprune hyperparameters, which corresponds to the maximal number of components (predictors + free member) in the model after simplification, and q, which is a spline power. In this study, an algorithm with hyperparameters *nprune*={2:10} and q={1; 2} was tested by an exhaustive analysis of all pairwise combinations of hyperparameters. The criterion for choosing the best combination of hyperparameters was the mean-square error (in fact, its square root) of leave-one-out crossvalidation. To evaluate the predictive power of the models, a separate test sample was used, which was constructed by randomization method before simulation. In this study, the size of the test sample was set at the level of 6 people, the training sample – 53 people. To determine the probabilistic nature of the received models, the response permutation test was carried out. During this test, the value of the dependent variable was randomly mixed, and thereafter, a complete cycle of constructing the model was carried out, which included the selection of hyperparameters and fixation of the obtained random variable determination. After 100 iterations of such a procedure, the set of determination coefficients for random models was compared with the determination coefficient of the original model. The test was considered successful if there were less than 5 cases of exceeding the value of the determination coefficient of the original model by the indices of 100 random models. This corresponds to the established $p<0.05$.

4. Results

1. Model of the dynamics of TSH with supplementation of iodine and selenium

Among the studied adolescents, the initial level of TSH in the thyroid gland hyperplasia group was 2.87±0.67 mMol/l, whereas in the group with normal thyroid parenchyma the TSH mean was lower (1.89±0.43 mMol/l). The values of TSH levels according to the authors [17, 18] in children with thyroid hyperplasia corresponded to the level of "normally high", when in patients with normal thyroid parenchyma - it corresponded to the "optimal" level. After 6 months of supplements with iodine and selenium, the TSH level decreased in both groups, the dynamic in the group with diffuse thyroid changes was more pronounced (mean –0.54±0.37 mMol/l compared to –0.13± ±0.42 mMol/l in the group with normal thyroid parenchyma).

Optimization of root mean square error (RMSE) over the field of hyperparameters values allowed to find the minimum error model with spline power q= 1 and the number of components in the model *nprune*=3 (**Fig. 1, *a***). For this model, the determination coefficient R^2=0.45, the results of crossvalidation correspond to Q^2_{LOO}=0.35, the predictive power determined on the test sample is R^2_{ext}=0.58. Comparison of predicted and actual values of TSH dynamics showed that the model does not predict an increase in TSH in the process of supplementation, whereas in some adolescents some increase in TSH has been observed (**Fig. 1, *b***). The response permutation test revealed 2 cases of exceeding the determination coefficient of the original model by its random counterparts. Taking into account that 100 random replicas were constructed during the test, the p-value of the original model is at the level of p=0.02.



a *b*

Fig. 1. Visualization of model errors for predicting the dynamics of TSH under supplementation with iodine and selenium: *a* – error of crossvalidation with different values of nprune hyperparameter; *b* – comparison of predicted and observed values of TSH dynamics

As a result of the modeling with MARS algorithm, the level of AT-TPO and urinary iodine content were found as necessary and sufficient predictors. That is, the introduction of any additional predictors from the array of the biochemical parameters we investigated adds nothing to the predictive value. In addition, after taking into account the levels of AT-TPO and iodine in urine, the differentiation of adolescents in the state of thyroid parenchyma (hyperplasia, norm) has no predictive value for determining the dynamics of TSH. The formula for prediction is as follows:

$$\Delta(\text{TSH}) = -0{,}0728 - 0{,}0734 \times h(10{,}21 - [\text{AT-TPO}]) - 0{,}0173 \times h(41{,}40 - [\text{Iodine in urine}]), \quad (3)$$

where *h* denotes the so-called “hinge” function. The essence of its work on the example of AT-TPO is as follows: if the level of AT-TPO is less than 10.21 mMol/ml, then the difference in the argument of the “hinge” function will be a positive number, and the value of the function will be this difference. If the AT-TPO level is greater than 10.21 mMol/ml, then the difference will be a negative number and the function value will be zero.

As a result, the characteristic of the influence of AT-TPO on Δ(TSH) is as follows: at low levels of AT-TPO (<10.21 mMol/ml), the decrease in TSH due to supplements will be more pronounced, and the lower the level of AT-TPO, the better will be the decrease of the level TSH; if the AT-TPO level is higher than 10.21 mMol/ml, then this component will not have an effect on the dependent variable, which means that the TSH dynamics will be projected at a displacement level corresponding to a slight decrease of –0,0728 mMol/l. Thus, this component regression model takes into account the following feature: a marked decrease in TSH in response to supplements with iodine and selenium is possible only with sufficiently low values of AT-TPO, which means the absence of autoimmune causes of thyroid homeostasis. At high levels of AT-TPO, significant

changes in TSH due to supplementation are not meaningful. The quantitative interpretation of this feature was embodied in the regression model due to automatic analysis by the MARS algorithm of the collected data.

A similar pattern of influence was also demonstrated by a predictor such as the concentration of iodine in the urine. So, with an excess of this indicator of 41.40 μmol/l, the decrease in TSH level due to supplementation is practically non-existent. However, with values of iodine concentration in urine less than 41.40 μmol/l, the positive effect of supplementation increases, and the less the iodine content in the urine will be, the stronger will be the lowering of TSH in the background of iodine and selenium-containing drug administration. This effect has a natural explanation: at a sufficient level of iodine intake, its concentration in the urine will be relatively high, and the effect of supplements with iodine will be absent. Iodine deficiency will primarily be manifested in reducing the elimination of iodine in urine – and in such cases, the additional intake of this micronutrient will contribute to the normalization of thyroid status. The influence of identified predictors on the dynamics of TSH in the training sample is visualized in **Fig. 2**

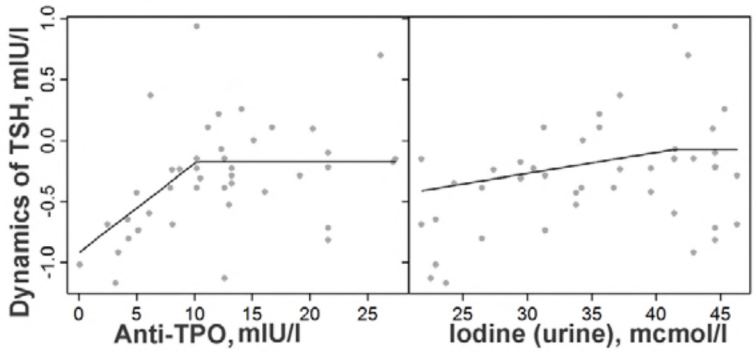


Fig. 2. Graphic representation of the effect of identifiable predictors in the developed regression model for forecasting the dynamics of TSH

The predictive value of the model on the test sample is in the acceptable range (R^2_{ext}=0.58), statistics on the accuracy of the model R^2=0.45 and Q^2_{LOO}=0.35 are in the range of averages.

5. Discussion

The state of the child organism in the endemic zone of iodine deficiency undergoes constant ecologically unfavourable influence. Target body is thyroid gland. Thyroid hormones affect the level of many parts of the homeostasis. The compensatory mechanism of the child's body in conditions of iodine deficiency is an increase in the size of the thyroid gland, which is aimed at supporting the hormonal exchange of the gland and as a consequence – the development of minimal thyroid deficiency with the further development of subclinical hypothyroidism. This condition is increasingly identified by scientists and requires additional research to prevent its development. The increase in the size of the thyroid gland causes a scientific discussion on the subject of the presence of a medical problem – when the onset of minimal thyroid failure [19, 20]. The scientific literature gives a definition of this pathological condition that coincides with the initial stage of subclinical hypothyroidism and is represented by the reference values of thyroid hormones at the level of TSH in the range of 2.5–4.0 mMol/l [8, 16]. We determined the levels of TSH, T3, T4 hormones and calculated the TSH / FT4 indices, the integral thyroid index of ITI (FT3+FT4)/TSH), which give the opportunity to correctly assess the thyroid status of the child. The minimum thyroid dysfunction according to our data was observed in hyperplasia of the thyroid gland, and with unchanged parenchyma observed – euthyroid state. The values of TSH levels according to the authors [17, 18] in children with thyroid hyperplasia corresponded to the level of "normally high", when in patients with normal thyroid parenchyma – it corresponded to the "optimal" level. Therefore, in predicting using this model, one should also take into account a clinical decision that characterizes the patient's condition in association with additional accompanying features in conjunction with the

ultrasonographic examination of the thyroid gland and the use of the TSH/FT4 indices, the integral thyroid index ITI (FT3+FT4)/TTSH)

6. Conclusions

1. Changes in the level of TSH when supplemented with iodine and selenium are dependent on the initial values of the titre of antibodies to thyroid peroxidase and the content of iodine in the urine.

2. The degree of positive changes (decrease of TSH, increase of T4) with supplements with iodine and selenium depends on the starting content of micronutrients of iodine in plasma of blood and/or urine: the lower the initial level of iodine availability, the more pronounced is the effect of supplements.

3. The developed statistical model for forecasting the dynamics of TSH and T4 (free) when supplemented with iodine and selenium provides prediction of T4 and dynamics is characterized by proper accuracy and prognostic ability and is recommended for further use.

4. The predictive value of the model on the test sample is in the acceptable range (R^2_{ext}=0.58), the statistics on the accuracy of the model R^2=0.45 and Q^2_{LOO}=0.35 are in the range of averages. Therefore, when predicting using this model, one must also take into account a clinical decision that characterizes the patient's state of the association with additional accompanying features in conjunction with the data of ultrasonographic examination of the thyroid gland [21].

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RESEARCH OF CERTAIN PATHOGENIC CHARACTERISTICS OF CLINICAL ISOLATES OF STAPHYLOCOCCI OF SKIN BIOME

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Abstract

A serious problem in patients with atopic dermatitis (AD) is the frequent attachment of a secondary skin infection. Among the microbes colonizing the skin of patients suffering from AD, S. aureus takes the lead. According to different authors, from the skin of 80–95 % of patients are sown Staphylococcus aureus. The survival of bacteria in a biotope is promoted by the persistent properties of microorganisms.

Aim of the research: to determine the adhesive properties and antilysozyme activity of clinical strains of staphylococci isolated from the skin of patients with allergic dermatosis.

The study included 50 patients with atopic dermatitis and 20 practically healthy individuals, from which 140 laboratory strains of staphylococci were isolated: 101 strains from patients with AD and 39 control strains. Bacteriological studies to isolate microorganisms and determine a number of pathogenic characteristics were carried out using the methods of classical bacteriology.

The severity of antilysozyme activity (ALA) and adhesive properties of strains isolated from affected areas of the skin was significantly higher than in cultures isolated from intact skin areas, both qualitatively and quantitatively. The obtained data made it possible to assume a certain complicating role of these factors on the course of AD.

Keywords: clinical strains of staphylococci, antilysozyme activity, adhesive properties.

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1. Introduction

Microorganisms of the *Staphylococcus* genus act as the etiological factor of various inflammatory diseases of man, characterized by a variety of course – from the lightest to the heaviest generalized forms [1, 2]. According to different authors, from the skin of 80–95 % of patients are sown *Staphylococcus aureus* [3, 4]. The survival of bacteria in a biotope is promoted by the persistent properties of microorganisms. [5]. Long-term preservation of the population of microorganisms in the host organism is facilitated by the mobility of biological properties, considered as a mechanism for the persistence of microbes [6, 7]. It is known that one of the most important factors for any microorganism is its adhesion to competent host cells, which serves as the first step for the development of the infectious process [3, 8]. It ensures the colonization of tissues by both pathogenic and non-pathogenic microorganisms and is a necessary condition for the natural lifestyle of most bacteria. Adhesion is a trigger mechanism for the realization of pathogenic properties of pathogens, which allows microorganisms to be fixed in a certain biotope and reach the required population level. Most researchers believe that microorganisms that possess high adhesiveness become the causative agents of infections [1, 8]. Of all the varieties of staphylococcus species, one of the most significant super bacteria is *S. aureus*, which is considered to be a highly-adherent microorganism [9, 10]. This property is determined by three regulatory genes: the agr gene (accessory gene regulator), expr (exstracellular protein regulator), sar (staphylococcus accessory regulator) [1, 11]. Staphylococci show a pronounced adhesive activity to the skin, are capable of intense invasion of tissues, significantly inhibit phagocytosis, are able to persist for a long time inside phagocytes [6, 12]. The condition for the survival of bacteria in a biotope is the ability to withstand the existing mechanisms of anti-infective resistance of the host organism due to a number of persistent characteristics, which include the property of bacteria specifically to inactivate the lysozyme of the host, which is defined as antilysozyme activity. Lysozyme or N-acetylmuramidase is an enzyme that ruptures the 1–4 β-glycosidic bond between N-acetyl-glucosamine and N-acetylmuramic acid in the peptidoglycan molecule of the bacterial cell wall, resulting in bacterial lysis [13, 14]. Antilysozyme activity is one of the factors that increase the tolerance of bacteria to the action of serum lysozyme in humans and animals

[15]. The role of lysozyme in protecting the macroorganism from infection is well known. Some authors believe that antilysozyme activity contributes to the long-term survival of bacteria in the macroorganism [16, 17].

Thus, the detection of high levels of adhesiveness and antilysozyme activity in bacteria can be used in bacteriological laboratories to assess the etiological role of isolated cultures of microorganisms and was chosen by us as an initial stage in the study of the pathogenic characteristics of laboratory strains.

2. Aim of the research

Determination of adhesive properties and antilysozyme activity of clinical strains of staphylococci isolated from the skin of patients with allergic dermatosis.

3. Materials and methods

The material for the research was laboratory strains of staphylococci, isolated from the lesions and healthy areas of the skin of patients with allergic dermatosis and practically healthy individuals.

Sowing of biological material from lesions on the skin, identification of isolated bacteria was carried out using methods of classical bacteriology. Interpretation of the results was carried out according to international protocols and normative documents of the Ministry of Health of Ukraine [18, 19].

Determination of adhesion-colonization properties of microorganisms was carried out according to the method of V. I. Brilis [20]. As cellular substrate was formalized human erythrocytes 0 (I) Rh (+). The average index of adhesion (AIA) and the index of adhesion of microorganism (IAM) were determined. AIA – the average number of microbes adhered on one erythrocyte, when counting at least 25 erythrocytes. Adhesiveness was considered zero at an AIA of 0 to 1.0; low – from 1.1 to 2. 0; average – from 2.1 to 4.0 and high – >4.0. IAM is the average number of microorganisms on one erythrocyte participating in an adhesive process. Non-adhesive are microorganisms with IAM≤1.75; low-adhesive – with IAM from 1.76 to 2.5; medium-adhesive – from 2.51 to 3.99 and high-adhesive ≥ 4.0 bacteria / erythrocyte.

Antilysozyme activity of the isolated microorganisms was determined by the method of detached antagonism by the method of O. V. Bukharin [21]. *M. luteus* was used as an indicator strain (strain 2665 of the GISK named after L. A. Tarasevich). The activity of the strains was evaluated in μg/ml. The isolated strains were divided into 3 groups: 1 – strains isolated from affected areas of the skin of patients with AD; 2 – strains isolated from intact sections of the skin of patients with AD; 3 – strains isolated from the skin of healthy individuals.

4. Results of the research

As a result of bacteriological studies, comparative data were obtained on the staphylococcal component of the skin microbiocenosis of 50 patients with atopic dermatitis and 20 practically healthy individuals. During the research 140 laboratory strains of staphylococci were isolated: 101 strains from patients with AD and 39 control strains. In **Fig. 1** is shown the percentage of the most common types of staphylococci.

To study the pathogenic characteristics of staphylococci strains, the initial stage of the research was the investigation of the adhesive activity of isolated microorganisms. In **Fig. 2** is shown the results of the obtained adhesion-colonization indices of *S. aureus* (AIA – the average index of adhesion, IAM – the index of adhesion of microorganisms)

Further, the obtained data were analyzed to determine the percentage distribution of high-, moderate-, low-adhesive isolates. In **Fig. 3** is shown the percentage of strains depending on the degree of adhesion.

In **Fig. 4** is illustrated the difference in the percentage of strains with ALA isolated from different parts of their vegetation.

Fig. 1. Species composition of skin biotopes

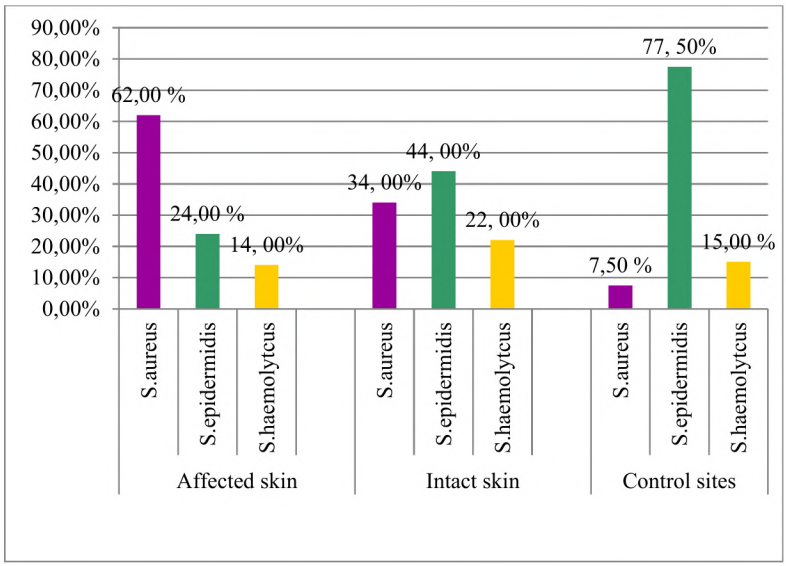


Fig. 2. The results of the adhesion-colonization indices of *S. aureus* ($p<0.05$)

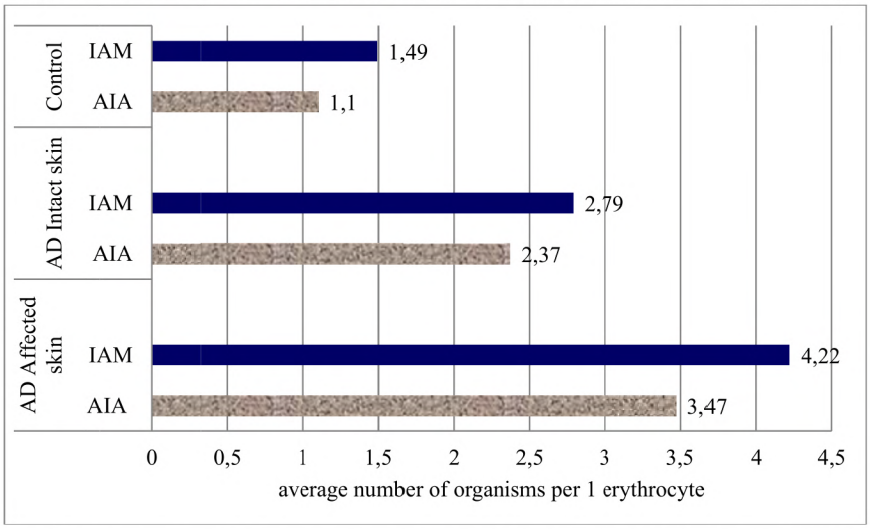


Fig. 3. Distribution of *S. aureus* strains according to the degree of adhesiveness

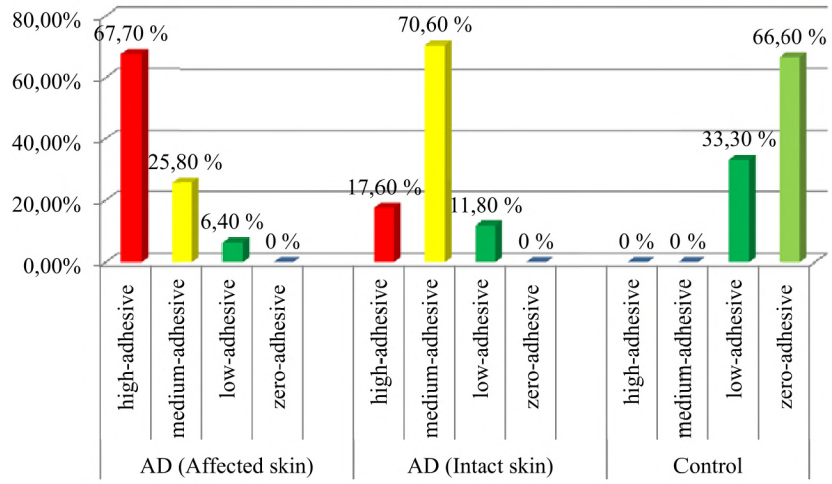
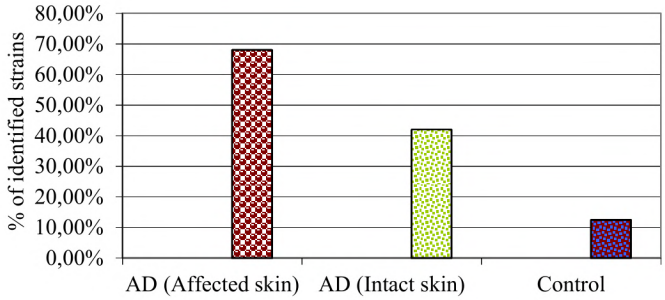
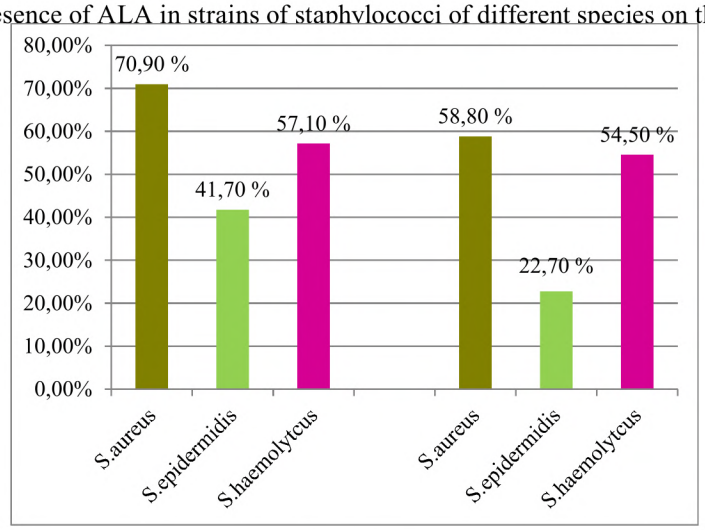


Fig. 4. Frequency of detection of strains of staphylococci with antilysozyme activity



The next step was a comparative analysis to identify this property (ALA) among the dominant species of staphylococci. This group included *S. aureus*, *S. epidermidis* and *S. haemolyticus*. The obtained data are shown in **Fig. 5.**

Fig. 5. The presence of ALA in strains of staphylococci of different species on the affected and



intact sites

Further, a determination of the absolute indices of ALA was made, the values of which are not illustrated by the diagrams, but are given in the discussion.

5. Discussion

As a result of bacteriological studies, a picture of the microbial landscape of the skin biome was obtained. The study of the microbial constituents of the biotope showed the dominance of microorganisms of the Staphylococcus genus on the skin of both patients and healthy individuals. The difference was observed in the species composition of staphylococci and the degree of contamination of lesions and normal skin areas. The emergence of non-resident species of staphylococci with a higher pathogenic potential in the affected and intact skin areas was a distinctive feature for most patients. Staphylococcus strains belonging to the species were selected for the research, the percentage of which was highest. They include *S. aureus, S. epidermidis and S. haemolyticus*, the percentage of which is shown in **Fig. 1**.

After obtaining strains of staphylococci with stable properties, the process of studying their pathogenic characteristics was launched. The initial stage of the study was the study of the adhesive activity of isolated microorganisms. First of all, our attention was paid to the study of the adhesive activity of the pathogen of the genus *S .aureus*. Figure 2 shows the results of the obtained adhesion-colonization indices of *S. aureus* – AIA and IAM. The diagram in **Fig. 2** illustrates that high adhesion-colonization indices were detected in a group of strains isolated from affected areas of the skin of patients with atopic dermatitis: the mean adhesion value of the AIA – (3.47±0.21) bacteria/red blood cell and the adhesion index microorganism IAM – (4.22±0.32) bacteria/erythrocyte,

which is almost 1.5 times higher than in the group of strains isolated from intact areas of the skin of patients with AD and 4 times higher than in the control group.

The distribution of *S. aureus* strains according to the degree of adhesiveness presented in **Fig. 3** shows that in the first group of strains isolated from the affected skin areas of patients, highly adhesive strains dominated, a lower percentage of trait detection was observed among isolates with a moderate degree of adhesion, and low-adhesive microorganism cultures amounted to less than 10 %. Among the strains isolated from the same patients, but from intact skin areas, the picture was somewhat different. The predominance of moderately adhesive strains was noted, while high and low-adhesive strains were found in small amounts. Strains isolated from the skin of practically healthy individuals showed up to a third of low-adhesive, and the others did not demonstrate the presence of the test feature.

As already noted, the development of diseases of microbial etiology largely depends on the persistent properties of microorganisms aimed at inactivating the factors of human natural resistance. Studies of bacterial antilysozyme activity (ALA), which inactivates the lysozyme of the macroorganism, have revealed a wide prevalence of this feature.

In **Fig. 4** illustrated the difference in the percentage of strains with ALA isolated from different parts of their vegetation. As a result of our study, it was found that 68.0 % of the strains isolated from affected skin areas of patients with AD had this feature, while among the strains isolated from intact skin areas and from healthy people this indicator was 42.0 % and 12.5 %, respectively. Thus, when comparing the number of strains possessing this property, it is noticeable that the presence of antilysozyme activity was a more characteristic feature for staphylococci, the site of vegetation of which was parts of the affected skin.

As can be seen in the diagram in **Fig. 5**, the number of strains of *S. aureus* and *S. epidermidis* that possessed this property gradually decreased from 70.9 % to 58.8 % in the series from lesions to intact sites and from 41.7 % to 22.7 %, respectively. For *S. haemolyticus* strains, the number of isolates with this feature was not significantly different between the affected and intact skin areas (57.1 % and 54.5 %). The diagrams do not include data on control strains, since the absolute figures appeared insignificant, and the percentage did not give a proper illustration of the presence of this property. It is understood that, for example, one of the 3 strains of *S. aureus* had one feature, and out of 31 isolates of *S. epidermidis* ALA had been established in 2. For a strain of *S. haemolyticus* species, isolated in healthy people, this property was not revealed.

In determining the absolute indices of ALA, the following results were obtained: in the areas of the affected skin, the antilysozyme activity of *S. aureus* strains reached (2.95±0.2) μg/ml, *S. haemolyticus* – (2.23±0.1) μg/ml , *S. epidermidis* – (1.32±0.08) μg/ml, i. e. the results indicate a higher pathogenic potential of Staphylococcus aureus. The study of intact skin areas showed lower values. But at the same time, it should be emphasized that comparing the results of studying strains with ALA isolated from patients and control areas of the skin of healthy people showed significant differences in antilysozyme activity. First, there were a significantly smaller number of strains with this trait, isolated from the studied topodemes of healthy skin. Thus, out of the 3 *S. aureus* strains obtained, only one was characterized by the presence of ALA, while its quantitative expression was significantly lower. Among the strains of *S. haemolyticus* this trait is not revealed. Finally, only 6.5 % of strains of *S. epidermidis* had this feature, but again the quantitative indices were minimal. The results of the study of individual factors of persistence of different types of staphylococci emphasize the importance of *S. aureus* from the position of potentiating the complications of the course of the underlying disease.

In a comparative analysis of the obtained data with the results of similar studies, it was found that the adhesion rates of our strains were somewhat lower, and the antilysozyme activity was slightly higher than other authors had. In a percentage ratio, the prevalence indicators obtained were comparable to each other [22].

Thus, complex set of virulent properties of *S. aureus* associated with confronting host defence mechanisms, on the one hand, and high adhesive potential, on the other hand, promote active colonization of both affected and intact skin areas, which provides conditions for prolonged persistence.

6. Conclusions

1. Adhesiveness of strains of *S. aureus* isolated from affected skin is almost 1.5 times higher than in strains with intact skin and 3.5 times higher than in strains from control sites. A greater number of strains with high and moderate adhesion rates were detected on the affected skin (67.7 % and 25.8 %), if they were absent in the control areas.

2. The severity of antilysozyme activity in strains isolated from affected areas of the skin was significantly higher than in cultures isolated from intact skin areas, both qualitatively and quantitatively. Determination of high and moderate values of antilysozyme activity of strains of *S. aureus* in lesions in comparison with healthy skin allows assuming a certain complicating role of this factor on the course of blood pressure.

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CLINICAL-LABORATORY MARKERS OF FIBRILOGENESIS DISORDERS IN THE SEVERITY OF PYELONEPHRITIS IN CHILDREN

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Abstract

Aim of the research: to establish the role of undifferentiated connective tissue dysplasia, as a manifestation of violation of fibrillogenesis, in the severity of the course of pyelonephritis in children.

148 children with pyelonephritis from 3 to 18 years were examined. As a result of catamnestic surveillance, they were divided into 2 groups: I – 92 persons, children with chronic pyelonephritis in which were diagnosed 3 or more episodes of relapse of pyelone-

phritis during the year, and II – 56 children with acute pyelonephritis, in which during the year no relapses were noted. The control group were 65 somatically healthy children of the same age (III – health-control). All children had a routine comprehensive clinical and laboratory examination and clinical and laboratory markers of a fibrillogenic disorder were established.

In children with chronic pyelonephritis, the frequency of all analyzed complaints was significantly higher than in children with acute pyelonephritis without relapses: frequent headaches – 56.52 % versus 25.0 %, appetite loss – 28.26 % vs. 19.64 %, frequent abdominal pain – 52.17 % vr. 32.14 %, increased fatigue – 41.30 % vr. 28.57 %.

In children with chronic pyelonephritis, phenotypic signs of undifferentiated connective tissue dysplasia (UCTD) were significantly more marked, such as joint hypermobility (in 52.0 % of children versus 5.4 %), asthenic body structure (59.0 % vs. 26.78 %), visual disturbance (84.8 % vs. 32.14 %), chest deformity (42.4 % vs. 8.9 %), scoliosis (52.17 % vs 10.7 %), arachnodactyly and predisposition to bleeding were observed only in children of the 1st group (22.5 % and 4.34 % respectively).

In practically all children with chronic pyelonephritis, the values of free and bound oxyproline fractions in blood plasma were significantly increased (47.14±0.03 μmol/l and 40.08±0.03 μmol/l, respectively), according to arithmetic meanings, reliably differing from the data of children with acute pyelonephritis (17.65±0.01 μmol/l and 17.22±0.02 μmol/l), in which these oxyproline fractions were elevated only in 12.0 % and 16.0 % of the subjects.

In 97.0 % of children with chronic pyelonephritis, the level of oxyproline in urine was elevated and significantly exceeded the level of excretion of oxyproline in urine in children with acute pyelonephritis.

The presence of UCTD in a child plays an important role in the process of chronic pyelonephritis, and children with its manifestations have a heavier course of disease with frequent relapses, therefore, the presence of signs UCTD is prognostically unsuccessful, which dictates the need for the appointment of metabolic therapy in the first episodes of the disease in children, if they have clinical and laboratory manifestations of UCTD.

Keywords: children, dysplasia of connective tissue, violations of fibrillogenesis, clinical and laboratory markers, oxyproline, pyelonephritis, kidneys.

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1. Introduction

Diseases of the organs of the urinary system take one of the leading places in the structure of childhood diseases. According to statistics, their prevalence has increased over the years. The frequency of their low-symptomatic atypical prograding is increasing [1].

In recent years, not only in Ukraine, but also in the world, attention is focused on the increase in the frequency of diseases of the organs of the urinary system. At the same time, the structure of nephropathy in children is dominated by diseases of the congenital and hereditary genesis, as well as diseases associated with hereditary predisposition, with a latent onset and torpid flow [2]. Particular attention deserves children with manifestations of connective tissue dysplasia (CTD) [3].

Data from studies conducted in the European post-soviet area show the prevalence of undifferentiated connective tissue dysplasia (UCTD) from 9.0 % to 80.0 %, depending on the age, sex, ethnic and clinical groups of the study. At the same time, scientists are concerned about the progressive growth in the population of individuals with a displastic phenotype, which is considered as the result of the impact of new mutagenic factors of social, technological and environmental nature, which form an increase in the so-called "genetic cargo" [4, 5]. The idea of "syndrome violation of gene homeostasis" were formulated, which may be the result of a mutation of one (monogenic theory), and several (polygenic theory) of genes [6, 7]. The uniqueness of the structure and functions of the connective tissue creates conditions for the emergence of a large number of its anomalies and diseases caused by chromosomal and gene defects that have a certain type of inheritance or arise as a result of external mutagenic effects in the fetal period [8]. In addition to a large number of diseases, most often based on gene defects, birth defects of the connective tissue (CT) of multifactorial nature are often present today [9, 10].

CTD are linked both to a violation of the synthesis of collagen and fibrilogenesis, as well as with changes in its biodegradation, fermentopathies, defects of fibronectin, elastin, glycoproteins, proteoglycans, and also with a deficiency of various cofactors of enzymes (magnesium, zinc, copper), ascorbic acid, oxygen and etc., which are involved in the formation of covalent bonds necessary to stabilize collagen structures, which are based on the mutations of genes encoding the synthesis and spatial organization of CT elements [6, 11].

In practice, it is more often encountered with undifferentiated forms of CTD - a genetically heterogeneous group of diseases of multifactorial nature with progressive flow, which are based on the violation of the synthesis, degradation or morphogenesis of the components of the extracellular matrix that occurs during the period of early embryogenesis or postnatal one under the influence of unfavorable environmental factors and can be manifested in different periods of life [12, 13].

Kidneys take part in any pathological processes, since they are one of the main organs that support the homeostasis of the body at any age. Clinical manifestations of many diseases in children to a large extent depend on the degree of maturity of urinary organs and urination [14, 15].

Taking into account the above remains an open question about the role of dysplasia of connective tissue (DCT) in pyelonephritis in children, the signs of which are increasingly encountered among patients nephrology and significantly affect the nature and course of the disease that causes the occurrence, chronicity and its resistance to therapy.

2. Aim of the research

To establish the role of undifferentiated connective tissue dysplasia, as a manifestation of violations of fibrilogenesis, in the severity of the course of pyelonephritis in children.

3. Materials and methods

148 children aged 3 to 18 years old underwent inpatient treatment with a diagnosis of pyelonephritis in the I pediatric department of the KZ LOR LODCL "OKHMATDYT" in 2016–2017. According to the results of the observation of patients in the catamnesis of the 1st and 3rd years, they were divided into 2 groups according to the severity of the disease and the frequency of episodes of exacerbation of the inflammatory process of the kidneys: group I (I-CP – 92 persons) – children with chronic pyelonephritis, in which there was a more severe course of the disease both in the debut and in the catamnesis, they were diagnosed with 3 or more episodes of pyelonephritis relapse throughout the year, the second group (II-AP – 56 children) who had been diagnosed with acute pyelonephritis, was noticed a mild course of pyelonephritis in the debut of the disease during the year of catamnestic observation no relapse of the disease was noted.

The results of the study of the children of the main groups were compared with the results of a survey of healthy children – 65 somatically healthy children of the same age (III-health-control), which were surveyed during the trips of a group of researchers in ecologically clean areas of Lviv region within the framework of the planned research work. All children were given a comprehensive clinical and laboratory examination in accordance with standard, commonly used in pediatric nephrology and pediatrics by clinical, laboratory and instrumental examinations [16].

When the children received next steps were done:

– a thorough survey of children and their parents by a specially designed questionnaire to specify the necessary anamnestic data;

– clinical examination of children and anthropometric measurements to determine the phenotypic signs of undifferentiated connective tissue dysplasia;

– routine clinical, laboratory parameters (general blood test, general urine analysis, biochemical analysis of blood) and instrumental methods of examination (renal ultrasound, cystic cystography, excretory urography) were determined;

– Brighton and modified criteria of Milkovskaya-Dimitrova and Karkasheva [17] determined the phenotypic signs of undifferentiated connective tissue dysplasia.

– levels of excretion of oxyproline in urine were determined based on the method by E. O. Yuryeva, V. V. Ilgov in modification O. O. Dobryk, S. L. Nyankovsky, M. Yu. Iskiv [1, 18] and free and bound fraction of oxyproline in blood serum using N. P. Sharaeva method [19].

The statistical processing of the research results was carried out using the Microsoft Excel program and the application package Statistica 5.0 for Windows. For the processing of the results that fell under the normal distribution, used the statistical method with the deduction of the arithmetic mean (M), standard deviation (SD). Calculations of the main statistical quantities were carried out according to generally accepted formulas [20].

4. Results of the research

In children of all observed groups, clinical and paraclinical manifestations of undifferentiated connective tissue dysplasia and violations of fibrillogenesis were investigated.

In children of the I group, complaints of frequent abdominal pain were noted in 52.17 % of cases, while in children of the II group only 32.14 % of the subjects (in control – 7.5 %). Complaints on frequent headaches were noted in 56.52 % of children in group I, whereas in children of the group II only 25.0 % of children (in control – 4.2 %). The complaints about fast fatigability were noted in 41.3 % of children in group I, whereas in children of group II fatigue was noted only in 28.57 % of children (in control – 5.6 %). Dysuricular manifestations in children of the I group were noted in 63.04 % of children, while in II group - only in 55.35 % of children (in control – 2.04 %). In addition, complaints of frequent nosebleeds were noted in 4.34 % of children in group I, which was not observed in children of the II group (in control – 0.02 %).

In patients of all the observation groups, a history of life was collected, with an emphasis on the study of the frequency of antenatal and postnatal risk factors for the formation of the urinary system pathology and violations of fibrillogenesis (**Table 1**).

Table 1

The frequency of ante- and postnatal non-specific risk factors for the formation of pathology

Non-specific risk factors:	Groups of children:					
	I-CP, n=92		II-AP, n=56		III-health-control, n=65	
	n	%	n	%	n	%
The threat of miscarriage	17	18.48*	9	16.07*	6	0.09
Gestosis of the first half of pregnancy	58	63.04*.**	16	28.57*	10	0.15
Gestosis of the second half of pregnancy	26	28.26*	12	21.43*	5	0.08
Mother's anemia during pregnancy	57	61.96*.**	12	21.43*	5	0.08
Early artificial feeding	48	52.17*.**	13	23.21*	9	0.14
Frequent ARD	56	60.87*.**	17	30.36*	8	0.12
Atopic diathesis in the past	8	8.7*.**	5	8.93*	1	0.02

Note: – the probable difference between the data of children with pyelonephritis and healthy control group p <0.01; ** – the probable difference in the indicator between two groups of children with pyelonephritis; p <0.01*

After children with pyelonephritis came, clinical manifestations and results of ultrasound examination of patients' kidneys were analyzed (**Table 2**).

Clinical manifestations of general non-specific intoxication were registered in most children of the I group: pallor of the skin was noted in 1.5–2 times more often than in children of the II group (52.0 % vs. 32.0 % of the children in the comparison group), periorbital cyanosis was noted 2 times more frequently in patients of the I group (43.0 % vs. 23.0 % of the children in the comparison group); signs of dysmetabolic nephropathy according to ultrasound data were also diagnosed 2.5 times more often in children of the main group (57.0 % against 20.0 % of children in the comparison group), ultrasound signs of inflammatory process of the kidneys met in 93.0 % of children of both group. Reliable difference in performance of muffled tones of the heart, dental enamel hypoplasia in children of observation group was not noted (**Table 2**).

In order to study the possible role of undifferentiated connective tissue dysplasia as the causes of the more severe course of pyelonephritis in children and the propensity to chronize the process, as well as in order to predict frequent relapses of pyelonephritis in children, the nature and frequency of phenotypic manifestations of undifferentiated connective tissue dysplasia were analyzed (**Table 3**).

Table 2

Status of children with pyelonephritis according to clinical and ultrasound examination

Clinical manifestations:	Groups of children: I-CP, n=92		II-AP, n=56	
	n	q	n	q
Pale skin	48	0.52*	18	0.32
Periorbital cyanosis	39	0.43*	13	0.23
Hypertrophy of the tonsils	37	0.40	19	0.34
Micropolyadenitis	13	0.14	9	0.16
Hypoplastics of enamel of teeth, I degree	19	0.21	12	0.21
Hypoplasia of enamel of teeth, II-III degree	17	0.18	10	0.18
The presence of tooth decay	31	0.34	14	0.15
The muffled tones of the heart	14	0.15	8	0.14
Nausea and pain during epigastric palpation	24	0.26*	6	0.11
Pain during palpation of the abdomen	54	0.58*	23	0.41
Ultrasound signs of dysmetabolic nephropathy	49	0.53*	24	0.43
Ultrasound signs of inflammation of the kidneys	86	0.93	52	0.93

*Note: * – the probable difference between the data of two groups of children p<0.01*

Table 3

The nature and frequency of phenotypic manifestations of the examined children

Detected phenotypic manifestations of UCTD:	Groups of children: I-CP, n=92		II-AP, n=56		III-health-control, n=65	
	n	%	n	%	n	%
Hypermobility of the joints	48	52.0**	3	5.4	2	3.07
Asthenic constitution	54	59.0**	15	26.78	8	12.3
Vision disorders	78	84.8**	18	32.14	5	7.69
Arachnodactyly	21	22.8**	–	–	–	–
Deformation of the chest	39	42.4**	5	8.9	3	4.62
Flatfoot	24	26.09**	–	–	–	–
Scoliostic posture	48	52.17**	6	10.7	3	4.62
Tendency to bleeding	4	4.34**	–	–	–	–
Emotional lability	38	41.3**	14	25.0	9	13.85
Umbilical hernia	9	9.78**	3	3.3	–	–

Note: – the probable difference between the data of children with pyelonephritis and healthy control groups p<0.01; ** – the probable difference between two groups of children with pyelonephritis p<0.01*

In order to establish the possible role of undifferentiated dysplasia of connective tissue, as causes of more severe pyelonephritis in children and the propensity to chronize the process, as well as to predict frequent relapses of pyelonephritis in children, all children with pyelonephritis have been evaluated for the levels of oxyproline in plasma and urine as an indicator of increased collagen metabolism and a violation of fibrillogenesis. The obtained data were compared with the data of healthy children in the control group (**Table 4**).

In order to study the processes of collagen degradation in children of both groups of observation, the level of oxyproline in urine as a measure of collagen metabolism and violation of fibrillogenesis was determined by the method of qualitative reaction (by degree of opacity) for all children. The results of excretion of oxyproline with daily urine in children of both groups of surveillance with pyelonephritis compared with the data of healthy children are presented in **Table 5.**

Table 4

Indices of collagen collapse in children with pyelonephritis, (M±m)

Indices	Groups of children: I-CP, n=92		II-AP, n=56		III-health-control, n=65	
	M±m	q	M±m	q	M±m	q
Free oxyproline, μmol/l	47.14±0.03*.**	0.87*.**	17.65± 0.01*	0.12*	12.64±0.38	0.08*
Bound oxyproline, μmol/l	40.08±0.03*.**	0.78*.**	17.22±0.02*	0.16*	8.3±0.29	0.06*

Note: – the probable difference between the data of children with pyelonephritis and healthy control groups p<0.01; ** – the probable difference between two groups of children with pyelonephritis p<0.01*

Table 5

The content of oxyproline in the urine of children with pyelonephritis

Indices:	Groups of children: I-CP, n=92		II-AP, n=56		III-health-control, n=65	
	M±m	q	M±m	q	M±m	q
Oxyproline in urine (+), units of measurement	0.640.02±	0.97*	0.120.01±	0.10	0.060.01±	0.08

Note: – the probable difference between the data of children with pyelonephritis and healthy control groups p<0.01; ** – the probable difference between two groups of children with pyelonephritis p<0.01*

5. Discussion

Comparison of the frequencies of ante- and postnatal non-specific risk factors for the formation of pathology showed that children with chronic pyelonephritis were significantly more likely to be on early artificial feeding in the first year of life (in the I group 52.17 % of children, in the comparison group – 23.21 %, in control – 0.14 %), were more likely to have acute respiratory diseases (in the I group – 60.87 %, against 30.36 % of the children in the comparison group, in the control – 0.12 %), the children of both groups were prone to atopy (8.1 % of children in the It group against 8.93 % of the children in the comparison group, in control – 0.02 %). Mothers of children of the I group were significantly more likely to have anemia during pregnancy (almost 3 times: 61.96 % vs. 21.43 %, 0.08 % control) and 2 times more likely to have clinical manifestations of gestosis in the first half of pregnancy (table 1).

The obtained data allow us to conclude that the frequency of nonspecific ante- and postnatal risk factors for the formation of pathology plays an important role in the formation of nephrological diseases and affects the severity and frequency of relapses of pyelonephritis in the future.

At admission to children of the I group, complaints of frequent abdominal pain were noted in 52.17 % of cases, while in children of the II group only 32.14 % of the subjects (in control – 7.5 %). Complaints on frequent headaches were noted in 56.52 % of children in group I, whereas in children of the group II only 25.0 % (in control – 4.2 %). Fatigue complaints were noted in 41.3 % of children in group I, whereas in children of group II fatigue was noted only in 28.57 % of children (in control – 5.6 %). Dysuria in children of the I group was noted in 63.04 % of children, while in II group – only in 55.35 % of children (in control – 2.04 %). In addition, complaints of frequent nasal haemorrhage were noted in 4.34 % of children in group I, which was not observed in children of the group II (in control – 0.02 %).

Clinical manifestations of general non-specific intoxication were registered in most children of the I group: pallor of the skin was noted in 1.5–2 times more often than in children of the II group (52.0 % vs. 32.0 % of the children in the comparison group), periorbital cyanosis was noted 2 times more frequently in patients of the I group (43.0 % vs. 23.0 % of the children in the comparison group), signs of dysmetabolic nephropathy according to ultrasound data were also diagnosed 2.5 times more often in children of the main group (57.0 % vs. 20.0 % of children in the comparison group), ultrasound signs of inflammatory renal disease met in 93.0 % of the both group. Reliable difference in performance of muffled heart tones, dental enamel hypoplasia in children of observation groups were not noted (**Table 2**).

The analysis of the frequency of phenotypic manifestations of UCTD in the examined children indicates that in children of group I phenothylic signs of undifferentiated connective tissue

dysplasia, such as hypermobility of joints (52.0 % of children versus 5.4 % in the comparison group, in control – 3.07 %), asthenic body structure (59.0 % vs. 26.78 %, in control – 12.3 %), visual impairment (84.8 % vs. 32.14 %, control 7.69 %), deformation of the chest (42.4 % vs. 8.9 %, in control – 4.62 %), scoliosis posture (52.17 % vs. 10.7 %, in control – 4.62 %), arachnodactyly and predisposition to bleeding noticed only in children of the I group (22.5 % and 4.34 % respectively, in the control – 0.0 %), which was not observed in children of the II group. The analysis of phenotypic manifestations of violations of fibrilogenesis points to the role of undifferentiated connective tissue dysplasia, as the causes of the more severe course of pyelonephritis in children and the propensity to chronize the process and predicts frequent relapses of pyelonephritis in children.

According to the literature in older children of the reference group, the level of free and bound oxyproline in the blood plasma is 12.2±0.49 μmol/l and 8.6±0.34 μmol/l, respectively. The higher the figure, the greater is collagen exchange [5].

In almost all of the examined children of both groups, the rates of increased collagen metabolism were significantly higher than healthy children (**Table 4**). However, in children of I group, the increased rates of free and bound oxyproline fractions in blood plasma were diagnosed in a larger number of children (78.0 % and 48.0 % respectively), with mean arithmetic data significantly differing from those in the children of the second group (12.0 % and 16.0 % respectively), indicating an increased collagen exchange in children with chronic and frequent relapses of pyelonephritis.

Determination of the level of oxyproline in the urine of children with pyelonephritis testifies to increased decay and excretion of collagen metabolism products in the child's body in 97.0 % of the examined children of the I group, which significantly exceeds the level of excreted oxyproline in the urine in children of the II group (10 %), which indicates a violation of the connective tissue catabolism in virtually all children with chronic pyelonephritis.

Consequently, after analyzing the results of the clinical and paraclinical examination of children with different course of pyelonephritis, one can conclude that undifferentiated connective tissue dysplasia plays an important role in the process of chronic pyelonephritis in children, and children with UCTD manifestations have a more severe course of the disease with frequent relapses, so the presence of signs of UCTD is prognostically unsuccessful, which makes you think over the necessity of appointing nephroprotective and metabolic therapy in the first episodes of the onset of the disease in children in the presence of they have clinical and laboratory manifestations of UCTD.

6. Conclusions

1. The presence of nonspecific ante- and postnatal risk factors for the formation of pathology plays an important role in the formation of nephrological pathology in children.

2. In children with chronic pyelonephritis with frequent relapses, the frequency of almost all analyzed complaints was significantly and reliable higher than in children with acute pyelonephritis without relapses: frequent headaches – 56.52 % vs. 25.0 % respectively), appetite loss – 28.26 % vs. 19.64 %, frequent abdominal pain – 52.17 % versus 32.14 %, increased fatigue – 41.30 % versus 28.57 %.

3. In children with chronic pyelonephritis and inclination to frequent relapse, phenotypic signs of undifferentiated connective dysplasia such as joint hypermobility (52.0 % of children versus 5.4 % in the comparison group), asthenic body structure of 59.0 % vs. 26.78 %), visual disturbance (84.8 % vs. 32.14 %), chest deformity (42.4 % vs. 8.9 %), scoliosis posture (52.17 % vs 10.7 %), Arachnodactilus and predisposition to bleeding were noted only in children of I group (22.5 % and 4.34 % respectively).

4. In almost all examined children with chronic pyelonephritis, the values of free and bound fractions of oxyproline in blood plasma (47.14±0.03 μmol/l and 40.08±0.03 μmol/l respectively) were significantly increased, for the mean arithmetic data significantly differ from the data of children with acute pyelonephritis (17.65±0.01 μmol/l and 17.22±0.02 μmol/l), in which these oxyproline fractions were elevated only in 12.0 % and 16.0 % examined, respectively, indicating an enhanced collagen exchange in children with chronic pyelonephritis.

5. Determination of the level of oxyproline in urine in children with chronic pyelonephritis testifies to increased decay and excretion of collagen metabolism products in a child's body in

97.0 % of the subjects, which significantly exceeds the level of excretion of oxyproline in urine in children with acute pyelonephritis, indicating the expressed disturbance of collagen catabolism in children prone to recurrence of pyelonephritis.

6. The presence of undifferentiated connective tissue dysplasia in the child plays an important role in the process of chronic pyelonephritis, and children with UCTD manifestations have a heavier course of disease with frequent relapses, therefore, the presence of signs of UCTD is prognostically unsuccessful, which dictates the need for appointment of metabolic therapy in the first episodes of the disease in children, if they have clinical and laboratory manifestations of UCTD.

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EFFICIENCY AND SAFETY OF LEFLUNOMIDE TREATMENT IN PATIENTS WITH PULMONARY SARCOIDOSIS

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Abstract

Patients who have contraindications to the prescription of GCs (glucocorticosteroids), or have developed serious side effects during treatment with GCs, as well as patients with resistance to GCs therapy, are prescribed immunosuppressants.

The aim of the research - to study the efficacy of leflunomide monotherapy in patients with pulmonary sarcoidosis with contraindications to prescription or serious side effects of glucocorticosteroids.

Fourteen patients with sarcoidosis of the respiratory system of stage II were examined – 12 women and 2 men aged 30 to 69 years. In 10 patients there were contraindications to the appointment of GCs (diabetes mellitus – 5, hypertension – 3, obesity – 1, exacerbation of gastric ulcer – 1), which caused the appointment of immunosuppressive therapy as a starting. In 4 cases, serious side effects of SCs were noted, requiring the drug to be abolished (osteoporosis – 3, steroid diabetes – 1). Leflunomide was administered at a dose of 20 mg per day, daily for 3 months. The evaluation of efficacy was carried out using computed tomography of the thoracic cavity organs, body plethysmography, spirometry and determination of the diffusivity of the lungs.

Monotherapy with leflunomide in patients with contraindications to prescription or serious side effects of GCs was successful in 7 out of 13 patients, in 2 patients there was a stabilization of the process, in 4 patients with leflunomide therapy progression of the disease was noted and in 1 case the treatment was discontinued due to serious side effects of preparation.

The results obtained make it possible to recommend the use of leflunomide as monotherapy in patients with pulmonary sarcoidosis with contraindications to the prescription and/or poor tolerability of GCs and methotrexate. It is necessary to continue studying the possibilities of combined use of leflunomide with other drugs of the first line.

Keywords: pulmonary sarcoidosis, treatment of sarcoidosis, leflunomide, side effects.

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1. Introduction

The main drugs for the treatment of sarcoidosis are glucocorticosteroids, their effectiveness has been proven in several randomized researches [1, 2]. Despite the fact that corticosteroids remain the first-line drugs for most patients, cytostatics have been recognized as alternative drugs for the treatment of sarcoidosis. Methotrexate is the most commonly used cytotoxic drug,

however, in a few studies it has been shown that azathioprine and leflunomide are also effective in treating sarcoidosis [3, 4]. In refractory sarcoidosis it is proposed to use infliximab, a drug that is a monoclonal antibody to the tumor necrosis factor (TNF-ά) [5, 6]. In connection with this, a strategy for the treatment of sarcoidosis has been developed. Patients who have contraindications to the appointment of the GCs, or have developed serious side effects during treatment with GCs, as well as patients with resistance to GCs therapy, are prescribed second-line drugs. The main place among which is occupied by immunosuppressants – methotrexate, azathioprine and leflunomide [7, 8].

The work on the study of immunosuppressants in the treatment of patients with respiratory sarcoidosis is few, only one randomized study of the effectiveness of methotrexate in a small group of patients (24 patients) was conducted [9, 10]. Experts of the World Association of Sarcoidosis and other Granulomatous Lesions (WASOG) analyzed literature data on the effectiveness of methotrexate in limited series of observations and developed general recommendations for its use [11, 12].

Leflunomide is an immunomodulating agent of the isoxazole series. It blocks the synthesis of pyrimidine by reversible inhibition of the enzyme dihydroorotate dehydrogenase, which has an antiproliferative effect on activated lymphocytes. The leflunomide efficacy was studied in small groups of patients, and the drug was administered to patients resistant to previous therapy or with poor methotrexate tolerance [13, 14]. Part of the patients received leflunomide in combination with GCs, which did not allow to establish its true efficacy [15, 16].

2. Aim of the research

To study the effectiveness of leflunomide monotherapy in patients with pulmonary sarcoidosis with contraindications to administration or serious side effects of glucocorticosteroids.

3. Materials and methods

The study was carried out on the basis of the clinico-functional department of the State Institution "National Institute of Phthisiology and Pulmonology named after. F. G. Yanovskogo NAMS Ukraine" during the 2017. Fourteen patients with sarcoidosis of the respiratory system of II stage were examined – 12 women and 2 men aged 30 to 69 years. In 10 patients there were contraindications to the appointment of GCs (diabetes mellitus – 5, hypertension – 3, obesity – 1, exacerbation of gastric ulcer – 1), which led to the appointment of immunosuppressive therapy as a starting. In 4 cases, serious side effects of GCs were noted, requiring the drug to be canceled (osteoporosis – 3, steroid diabetes – 1).

The diagnosis of sarcoidosis was verified by computed tomography (CT) scan of the thoracic cavity organs [17, 18]. The function of external respiration was also studied by spirometry body plethysmography, evaluation of lung diffusivity [19, 20]. Leflunomide was administered at a dose of 20 mg per day, daily for 3 months. Before the start of therapy and monthly during the therapy, a general blood test was carried out to determine the number of platelets, the concentration of AST, ALT, bilirubin and creatinine were determined. The results of treatment were evaluated after 3 months, taking into account clinical, functional data and CT results.

4. Results

In one patient during treatment with leflunomide serious side effects developed (pronounced pyrogenic reaction, increase in ALT, which is more than 3 times higher than normal), in connection with which the drug was canceled, the patient was prescribed methotrexate treatment.

In 7 patients out of 13 (53.8 %) after 3 months of treatment with leflunomide, regression of sarcoidosis was observed: a decrease in the density of nodular dissemination of the lung parenchyma, in 2 (15.4 %) – stabilization of the process (no changes in CT data), and 4 (30.8 %) of patients had progression.

Fig. 1, 2 show cases of regression and progression of sarcoidosis after a 3-month course of treatment with leflunomide.

a *b*

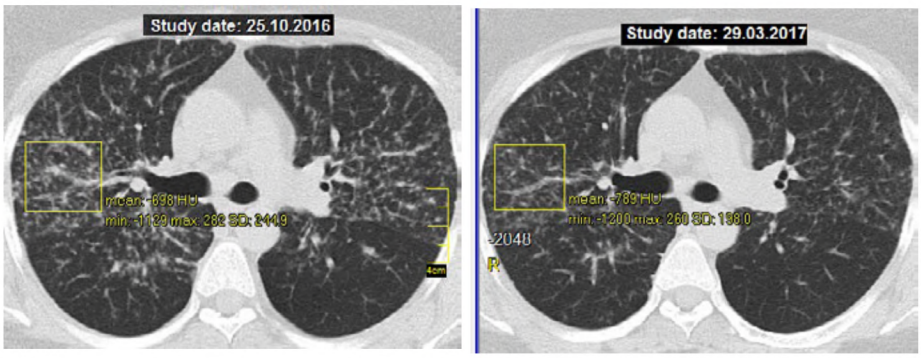

Fig. 1. CT of patient K., sarcoidosis of lungs, II stage: *a* – before leflunomide administration, parenchyma densitometry before treatment – (–698 HU); *b* – in 3 months of leflunomide treatment: a decrease in the density of nodular dissemination of the lungs (densitometry after 3 months of therapy – (–789 HU))

a *b*

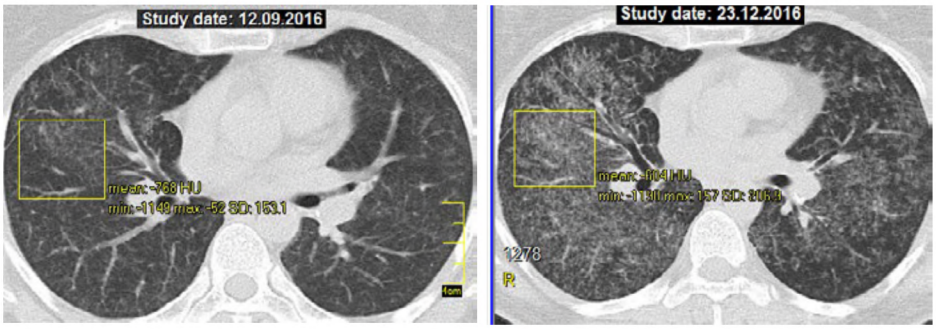

Fig. 2. CT of the patient G., sarcoidosis of the lung, stage II: *a* – before the appointment of leflunomide, (densitometry of the parenchyma before treatment – (–768 HU)); *b* – after 3 months of treatment with leflunomide: significant increase in the density of nodular dissemination of the lungs (parenchyma densitometry after 3 months of therapy – (-604 HU))

The parameters of the function of external respiration are presented in **Table 1, 2**.

Table 1

VC and DLCO indices in the course of treatment with leflunomide

No.	Patient	Result of the treatment	VC (% of normal)		DLCO (% of normal)	
			V1	V2	V1	V2
1	P. I. V.	Progression	110.8	113.2	80.6	85.9
2	K. S. V.	Regression	100.5	106.7	66.9	72.5
3	Ya. L. K.	Progression	92.0	88.9	75.1	64.5
4	Sh. E. A.	Regression	71.0	92.0	65.4	80.5
5	S. L. M.	Stabilization	91.0	92.4	85.0	86.2
6	P. V. M.	Progression	65.0	46.4	68.4	52.3
7	G. A. M.	Progression	101.9	87.1	60.6	60.3
8	Sh. L. F.	Regression	85.0	95.6	46.7	57.0
9	G. G. P.	Regression	125.0	120.7	65.9	65.1
10	S. A. O.	Cancel	101.8	–	52.5	–
11	S. O. M.	Stabilization	88.0	89.4	82.0	83.2
12	G. P. V.	Regression	86.4	90.4	73.4	63.7
13	K. A. V.	Regression	108.7	109.2	76.2	78.3
14	V. O. V.	Regression	93.8	–	82.8	–

Note: V1 – visit before treatment; V2 – visit after 3 months of therapy

In table 1 it is shown that the integral exponent of the external respiration function DLCO was reduced (≤70 %) in 7 of 13 patients. In patients with regression in 3 cases out of 7, the diffusive capacity of the lungs increased. Progression of the process in 2 out of 4 patients was accompanied by a further decrease in DLCO.

Table 2

Indicators of bronchial patency during leflunomide treatment

No.	Patient	Result of the treatment	FVC (% of normal)		FEV_1 (% of normal)		FEV_1/FVC (%)	
			V1	V2	V1	V2	V1	V2
1	P. I. V.	Progression	111.7	113.7	102.5	106.0	79.4	81.0
2	K. S. V.	Regression	97.8	101.6	88.4	96.2	77.4	81.1
3	Ya. L. K.	Progression	92.7	91.8	62.3	75.0	72.9	69.5
4	Sh. E. A.	Regression	65.6	84.8	58.0	75.7	76.8	77.4
5	S. L. M.	Stabilization	90.4	92.0	71.5	72.1	69.4	66.5
6	P. V. M.	Progression	66.0	46.2	58.0	36.3	87.0	67.3
7	G. A. M.	Progression	95.6	84.3	96.5	84.7	86.7	86.3
8	Sh. L. F.	Regression	78.4	84.6	80.5	86.8	84.2	84.1
9	G. G. P.	Regression	129.0	120.8	124.5	116.3	81.7	81.5
10	S. A. O.	Cancel	99.9	–	102.1	–	86.5	–
11	S. O. M.	Stabilization	90.1	91.7	71.2	71.8	69.1	66.2
12	G. P. V.	Regression	90.4	86.3	98.3	91.2	91.3	88.8
13	K. A. V.	Regression	105.6	106.5	98.7	99.7	78.2	79.6
14	V. O. V.	Regression	94.9	–	97.2	–	82.8	–

Note: V1 – visit before treatment; V2 – visit after 3 months of therapy

From table 2 that in 2 patients at the first visit a restrictive type of pulmonary ventilation disorder (FVC≤80 %) was observed, in 2 – obstructive disorders (FEV_1/FVC≤70 %), in other cases spirometry parameters were within the normal range.

Side effects of leflunomide in a dose of 20 mg per day were observed in 6 of 14 patients (42.9 %).

One patient had serious side effects: a pronounced pyrogenic reaction, an increase in ALT above the norm by more than 3 times, which led to the withdrawal of the drug.

In 2 patients (14.3 %) at the beginning of the treatment period, gastrointestinal disorders were observed: nausea, abdominal pain. Patients were recommended to distribute the dose of the drug for two doses – 10 mg twice a day, as a result of these gastrointestinal disorders were not repeated. Two patients (14.3 %) reported frequent respiratory viral infections during leflunomide treatment. One patient (7.1 %) had acute oral herpes. After reducing the dose of leflunomide to 10 mg per day and carrying out antiviral therapy, the patient noted a regression of herpetic infection and sarcoidosis of the lungs (according to CT data).

5. Discussion

Currently, the main drugs for the treatment of sarcoidosis are glucocorticoids [15, 17]. When appointing GCs, a large number of side effects of these drugs should be taken into account, the frequency of which increases with prolonged use [15]. With the development of serious side effects, such as osteoporosis, diabetes mellitus, and also in the presence of contraindications to GCs therapy, second-line drugs - cytostatics are used. The most studied of them is methotrexate [15]. In our clinic we also use it as the most preferable option of the second line. Other drugs of the second line include azathioprine, leflunomide, mycophenolate and antimalarial drugs. However, there are very few scientific data on their use in case of sarcoidosis [11].

Baughman and Lower [13] described the experience of using leflunomide in a small group of patients. According to their data, in 78 % there was a regression of the disease. It should be noted that patients who did not tolerate methotrexate were usually successfully treated with leflunomide. Similar data are presented in the work of D.H. Sahoo with co-authors [16]: of the 33 patients who

took leflunomide due to toxicity from other immunomodulating medications, 20 tolerated leflunomide well (67 %). In our work, we administered leflunomide to patients who had not previously taken cytotoxic drugs. In 10 patients out of 14 there were contraindications to the appointment of GCs, in 4 there were serious side effects of GCs, requiring withdrawal of the drug (osteoporosis – 3, steroid diabetes – 1). The effectiveness of leflunomide therapy in our group of patients was 53.8 %. Perhaps, the lower efficacy of therapy in our group of patients is due to the fact that all patients had stage 2 of pulmonary sarcoidosis. In previous studies, patients had 1 to 3 stages of pulmonary sarcoidosis. As is known, in the first stage of sarcoidosis, spontaneous regression is possible in 90 % of cases.

In our work, as well as in other studies on the efficacy of leflunomide, there was no statistical difference in the parameters of the function of external respiration before and after treatment.

According to a study by D. H. Sahoo with co-authors, side effects were observed in 34 % of cases, similar data were obtained from us – 42.9 %. According to a study by D.H. Sahoo with co-authors, the discontinuation of leflunomide intake due to its toxicity was observed in 18 % of cases. In our work – only 7.1 %. The dosage regimen was the same: 90 % of patients received 20 mg daily. Perhaps a higher level of side effects in the work of D. H. Sahoo and co-authors is associated with a longer period of follow-up.

The disadvantages of our study are a small group of patients and a short observation period. On the positive side of the work was the homogeneity of the group: all patients were with stage 2 of pulmonary sarcoidosis, did not take another therapy before the study.

6. Conclusion

1. Monotherapy with leflunomide in patients with contraindications to prescription or serious side effects of GCs was successful in 7 of 13 patients (53.8 %), in 2 patients there was a stabilization of the process (15.4 %), 4 patients with leflunomide therapy showed progression of the disease (30.8 %).

2. Leflunomide is characterized by satisfactory tolerability: the incidence of side effects from the use of the drug was 35.7 %. At the same time, the drug was withdrawn only in one case (7.1 %).

3. The results obtained make it possible to recommend the use of leflunomide as monotherapy in patients with pulmonary sarcoidosis with contraindications to the appointment and/or poor tolerability of GCs and methotrexate.

4. In the future, attention should be paid to the combined use of leflunomide with other drugs for the treatment of sarcoidosis.

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INFLUENCE OF CONCOMITANT ARTERIAL HYPERTENSION ON ACTIVITY OF INFLAMMATORY PROCESS IN PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA

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Abstract

The aim of the work was to determine the diagnostic value of the markers of surfactant protein D (SP-D) and C-reactive protein (C-RP) in patients with community-acquired pneumonia (CAP) with concomitant arterial hypertension (AH) and its effects on the activity of the inflammatory process.

The study included 79 people. Among them, 63 patients with CAP and 16 healthy individuals who were a control group. Depending on the presence of hypertension, the patients were divided into two groups. The first group included 26 patients with CAP with AH, the second – 37 patients with CAP without AH. All patients were given general-clinical methods of examination, radiography of the chest organs in two projections. Plasma levels of SP-D and C-RP were determined.

Reliable connection ($p<0.05$) was determined between the presence of AH and the probability of occurrence of CAP (OR – odds ratio 2.27 [95 % CI 1.05–4.94]). The level of SP-D and C-RP in patients with AH on the first day was significantly higher than in healthy subjects ($p<0.05$). In patients in the first group, SP-D levels were significantly higher ($p<0.05$) for the first, third and ninth day relative to the second group. The existence of a direct tie of average strength between the presence of AH and SP-D ($R=0.41$, $p<0.05$) has been determined. The presence of a direct correlation link of mean strength ($R=0.38$; $p<0.05$) between the AH and the level of C-RP indicates that arterial hypertension in patients with CAP increases the activity of the systemic inflammatory response.

Keywords: community-acquired pneumonia, arterial hypertension, surfactant protein D, C-reactive protein.

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1. Introduction

Community-acquired pneumonia (CAP), as a disease, remains one of the most important unresolved issues in modern pulmonology [1]. Cardiovascular system dysfunction is almost a constant companion of the CAP and develops from the first hours, with blood circulation disturbances often determine the prognosis and the result of the CAP itself. The spectrum of these violations is varied and depends on the severity of the process [2].

The freaquency of arterial hypertension (AH) can reach 63.9 % with the damage to target organs in patients with CAP. Along with this, the development of anemia is accompanied by violations of microcirculation, which occurs in the mechanism of formation of hypertension, factors of non-specific inflammation characterized by an increase in C-reactive protein (C-RP) [3, 4]. Reducing the surfactant protein D (SP-D) may be due to the absorption and destruction of SP-D by the alveolar macrophages, which, due to damage to the pulmonary epithelium and infiltration of capillaries, causes SP-D to leak into the systemic circulation [5]. The presence of SP-D in the systemic circulation can contribute to the development of cardiovascular disease and mortality in patients with CAP, as its role in the development of endothelial dysfunction is known [6]. It is also important that in the vascular wall SP-D can perform the same anti-inflammatory function as in the lungs or vice versa, leading to a pro-inflammatory reaction [7].

Recent studies have revealed several mechanisms for violation or SP-D modifications that contribute to its systemic leakage from the lungs, while the circulatory level SP-D is a promising biomarker to determine the degree of damage to the lung tissue [8, 9]. The work performed on mice showed a connection between a decrease in alveolar SP-D levels and an increase in oxidative mechanisms [10, 11]. The SP-D concentration gradient thus allows leakage into the bloodstream SP-D with acute and chronic lung injury, as confirmed in experimental studies in mice [11]. Inter-

pretation of the quantitative evaluation of SP-D in alveolar fluid and serum in various pulmonary diseases may be a hindrance because anti-SP-D antibodies may have different susceptibilities in the oxidation process [7, 8]. In recent years, it has been shown that SP-D has different antimicrobial and anti-inflammatory effects, affects lipid metabolism and shows pro-inflammatory effects in the walls of vessels, which increases the risk of atherosclerosis. The common polymorphism of SP-D is associated with atherosclerosis and diabetes, as well as metabolic disorders due to its effect on vascular endothelium [6, 12]. This review summarizes the importance of studying the circulating level of SP-D, the ability to interact in a diagnostic program with already well-known markers of systemic inflammation and the effect or dependence on the presence of comorbidity in patients with CAP in a hospital.

2. Aim of research

Determination of the diagnostic value of markers SP-D and C-RP in patients with CAP with concomitant arterial hypertension and its effect on the activity of the inflammatory process.

3. Materials and methods

The study included 79 people who were on the treatment in the pulmonologic department of the Mechnikov Regional Clinical Hospital and in the therapeutic department of the City Clinical Hospital No. 6 for the period from 2013 to 2016. Among them, 63 patients with CAP, who were diagnosed with CAP of 2 and 3 clinical groups and 16 healthy individuals, who were the control group.

In order to study the effect of comorbid hypertension on the severity of the inflammatory process, the patients were divided into two groups. To the first group included 26 patients with CAP with AH, the second -- 37 patients with CAP without AH. Duration of hypertension in the first group in 35 % of cases was more than 10 years, in 45 % of cases - from 5 to 10 years and in 20 % of cases – less than 5 years. The average systolic blood pressure level was 145±2.4 mm Hg.

Compared to the clinical course, it should be noted that in the first group, the inflammatory process in the lung tissue according to X-ray examination in the form of infiltration was detected on one side in 64 %, bilateral – 36 %, whereas in the second group, the one-side process was found in 84 %, and bilateral at 16 %. The course of the disease in patients with concomitant hypertension was characterized by a longer period of the disease.

Criteria for inclusion of patients in the study: age 18 and over; the presence of clinical and radiological signs of the inflammatory process in the lungs in patients with CAP; the presence of concomitant arterial hypertension in patients with CAP. Criteria for exclusion of patients from the study: refusal of the patient to participate in the study; the duration of antibiotic therapy for more than 24 hours; use in the therapeutic program for any nosology over the past three months of antibacterial treatment; the presence of a disease for tuberculosis, malignant neoplasms, AIDS, alcohol or drug addiction; the presence of any other severe pulmonologic pathology.

The average age of patients in the first group was 52±2.6 years, among them 52 % of men and 48 % of women. The average age of patients in the second group was 45±3.1 years, among them 46 % men and 54 % women. In patients with the first group in 62 % of cases the body temperature reached low-grade markers, 38 % – febrile ones, whereas in patients of the second group the distribution of subfebrile and febrile levels in the percentage ratio was 49 to 51, respectively. The average level of leukocytes in the first group was 14.7±4.9×109/ml, in the second – 13.8± ±4.9109/ml. The average level of erythrocyte sedimentation rate was 32.6±3.6 mm/g and 27.4± ±4.4 mm/g respectively in the first and second groups.

At the research stage, evaluation of the general condition and clinical symptoms in patients with infectious neuropathy was conducted according to a single scheme: for 1–2 days, 3–4 and 9–10 days from the moment of admission to the department. All patients were given general-clinical methods of examination, radiography of the chest organs in two projections.

The plasma level of SP-D was determined by immuno-enzymatic analysis using Hycult Biotech (the Netherlands) reagents according to the manufacturer's protocol. The plasma level of C-RP was determined using the IT-CRP 2* 1S reagents (Lachema, Slovakia) by immunoturbimetric method using the Microlab-200 photometer according to the protocols of the manufacturer.

The obtained results were analyzed using the Microsoft Office Excel program and the STATISTICA 6.1 software (StatSoftInc., Serial number AGAR909E415822FA) using descriptive methods (the description of the central tendency of quantitative attributes was performed with normal distribution in the form of an average arithmetic and standard error M±m; in the opposite case in the form of median and interquat-wise Me [25–75 %], 95 % confidence interval (CI) was calculated to determine the range of indicators in the population, and analytical statistics methods were used References [13].

4. Results of the research

In the course of the study, the results of simple logistic regression analysis revealed a reliable connection ($p<0.05$) between the presence of hypertension and the probability of the occurrence of CAP (OR – odds ratio 2.27 [95 % CI 1.05 – 4.94]). There was no statistically significant relationship between the presence of hypertension and the severity of CAP ($p>0.05$).

According to the rank correlation analysis, the existence of a direct correlation between the level of SP-D and the presence of hypertension in patients in the first group was determined ($R=0.41$, $p<0.05$).

It was also confirmed that there is a direct correlation connection of average strength ($R=0.38$; $p<0.05$) between the comorbid AH and the C-RP level, based on the Spirman rank correlation coefficient.

In the conditions of the study, the correlation analysis of the results did not reveal a probable relationship between the plasma levels of C-RP and SP-D in patients in the first group ($R=0.16$, $p>0.05$) and the second group ($R=0.24$, $p> 0.05$).

Results of measurements of plasma level of C-reactive protein in patients of the first and second groups are presented in **Table 1**.

The level of C-RP was significantly higher in patients with both groups in healthy subjects ($p<0.001$) and on average increased to 73.22–94.70 mg/l, while the maximum values reached 405 mg/l.

As a result of the analysis, it was determined that the level of C-RP in patients with CAP on the first day was significantly higher than that of healthy subjects ($p<0.05$), and subsequently to the third day it gradually decreased in both groups ($p<0.05$), compared with the previous indicators and reached the normal values for the ninth day and did not differ from the control group ($p>0.05$).

In the process of comparing the data obtained from the first and second groups, it was determined that the level of C-RP in patients with concomitant hypertension on the first day was characterized by the presence of statistical tendency to higher values ($p=0.067$) in patients in the first group by 23 %, the third day, the level C-RP decreased and was significantly higher ($p<0.05$) in patients in the first group by 44 %. There was a significant difference between the C-RP indexes in the patients in the first group and the control group ($p<0.05$) in the ninth day, while the second group's statistical significance was not statistically significant in relation to the control group. The C-RP indexes of the first and second groups had a difference of 43 % for the ninth day.

Table 1

Change in the average levels of C-RP in the course of the inflammatory process in patients with CAP in comparison with the control group

Groups	C-RP, the first day Me [25–75 %] mg/l	C-RP, the third day Me [25–75 %] mg/l	C-RP, the ninth day Me [25–75 %] mg/l
First group (CAP with AH), n=26	94.70 [58.44–171.6]°#	48.42 [15.3–132.95]*°#	8.97 [6.31–9.35]*°#
Second group (CAP without AH), n=37	73.22 [34.81–199.52]°	27.04 [16.07–102.61]°*	5.12 [4.1–6.25]*
Control group		4.47 [3.28–5.82]	

*Note: * – $p<0.05$ according to the Wilcoxon criterion relative to the foreground value in each group separately; ° – $p<0.05$ according to the Mann-Whitney criterion relative to the control group; # – $p<0.05$ according to the Mann-Whitney criterion between the groups depending on the presence of hypertension*

Changes in SP-D levels during the course of the process obtained in patients in our study in the process of assessing the severity of inflammation are presented in **Table 2**.

The level of SP-D in patients with the first and second groups for the first day of the statistical data was significantly higher than the control group ($p<0.002$). On average, the values of SP-D in both groups were determined within the range 429.119-608.708 ng / ml for the first day and were significantly higher in the first group ($p<0.05$). The maximum values of SP-D in patients reached 731.261–897.405 ng/ml and were determined mainly for bilateral lung injury.

Table 2

Change in the average levels of SP-D in the course of the inflammatory process in patients with CAP in comparison with the control group

Groups	SP-D, the first day Me [25–75 %] ng/ml	SP-D, the third day Me [25–75 %] ng/ml	SP-D, the ninth day Me [25–75 %] ng/ml
First group (CAP with AH), n=26	608.708 [532.000–663.51]°ˆ	788.821 [695.617–807.681]*°ˆ	373.494 [250.329–491.36]*°ˆ
Second group (CAP without AH), n=37	429.119 [326.851–471.083]°ˆ	582.144 [473.602–622.831]*°ˆ	279.362 [194.268–354.423]*ˆ
Control group		238.050 [144.736–372.132]	

*Note: * – $p<0.05$ according to the Wilcoxon criterion relative to the foreground value in each group separately; ° – $p<0.05$ according to the Mann-Whitney criterion relative to the control group; ˆ – $p<0.05$ according to the Mann-Whitney criterion between the groups depending on the presence of hypertension*

In the period from the first to the third day, SP-D indices increased, they were significantly higher ($p<0.05$) relative to the SP-D index of the first day of both groups and the control group ($p<0.005$). From the third to the ninth day marker values gradually decreased, statistically the data were significantly lower than the previous values ($p<0.001$).

However, the data obtained in the first group were significantly higher ($p<0.05$) for the control group and SP-D indicators of the second group.

Based on the results obtained, the average SP-D values for the first group with concomitant hypertension were 608.708 ng/ml and were significantly higher ($p<0.05$) for the mean of patients in the second group – 429.119 ng/ml. For the third day, there was no statistic difference between the mean values of SP-D in both groups, however, there was a tendency ($p=0.059$) to higher values in patients with the first (788.821 ng/ml) and the second group (582.144 ng/ml), respectively. On the ninth day, the indicators gradually approached the values of the control group, but the level of SP-D was significantly higher ($p<0.05$) in patients in the first group (373.494 ng / ml) relative to the second group (279.362 ng/ml) and statistically different from the control group ($p<0.05$). There was no statistically significant difference between the level of SP-D in patients with the second group and the control group ($p<0.05$).

5. Discussion of the results

Specific pulmonary biomarkers that would be available and could be used for diagnosis, assessment of the course of the infectious process, and the effectiveness of the proposed therapeutic strategies are the aims of many studies [14]. To solve the problems, an analysis of the effectiveness of the use of markers in patients with CAP was performed, depending on the presence of hypertension in terms of comparison with the control group. In the problems of modern medicine, concomitant hypertension in patients with CAP increases the frequency of complications 2.5 times, more than three times the rate of development of pleurisy, lengthens the duration of pulmonary infiltration [15].

For patients with CAP is characteristic an increase in C-RP and its subsequent reduction in the treatment [16], as well as the marker helps in diagnostic determination of the sharpening of the chronic process and the direct presence of the acute inflammatory process of the lungs [17]. However, in both cases, the C-RP level after the therapeutic program is not statistically different

from that of the control group, which corresponds to the data obtained in our study. The presence of concomitant hypertension increases the activity of the systemic inflammatory response. This is confirmed by the results of the correlation analysis, according to which, there is a direct average strength of the relationship between the presence of hypertension and the level of C-RP. Patients in the first group were characterized by significantly higher levels of C-RP ($p<0.05$). Increased levels of C-RP in patients with cardiac pathology are associated with changes in vascular stiffness, atherosclerosis and the development of damage to target organs and increased cardiovascular manifestations [18].

To determine the effect of hypertension on the activity of the inflammatory process and damage to the lung tissue, SP-D levels were studied in patients of both groups at the first, third and ninth day. Thus, in patients with the first group in all three observation points, the data obtained were higher relative to the levels of SP-D of the second group, which is related to the influence of hypertension as a factor that characterizes the higher severity of the systemic and local inflammatory response during acute processes, defining injuries pulmonary tissue [19]. The study by Grith L Sorensen and co-authors showed the presence of SP-D in endothelial cells and its involvement in modulating the local inflammatory response [20], which in turn indicates the feedback of the effect of the development of the process of inflammation in the lungs caused by bacterial agents [21] on the possible complications of concomitant arterial hypertension.

The presence of comorbid hypertension in patients with CAP is accompanied by an increase in the level of SP-D in the bloodstream, which for the ninth day is characterized by significantly higher levels of SP-D ($p<0.05$) in patients with CAP. Higher SP-D rates were also determined in patients with multiple lesion of the lung on the one hand, indicating a marked increase in the area of lung tissue damage and bacterial infiltration and the relationship between pathological changes in the body in which the violation occurs as a consequence of primary changes in their own turn, reinforce each other [22].

Thus, the obtained data indicate that SP-D is capable of displaying the activity of inflammation and the severity of the disease [23], to increase its circulating level in the bloodstream during the period of the inflammatory process of the lung and at the same time increase the risk of complication of the course of hypertension that in its queue in the future requires the search for additional possibilities for a rapid decrease in the level of circulating SP-D in the treatment of infectious processes of the lung.

When discussing reports of SP-D genetic associations with a significant disease list [23, 24] and the clinical utility of circulating SP-D for prediction of respiratory diseases [24], it should be noted that the current study has a further direction for its development. Prospective studies consider the development of SP-D therapy [24], and the open question remains about the possibility of influencing the pathogenetic component of the inflammatory process in the treatment program [25].

6. Conclusions

1. Relative connection ($p<0.05$) between the presence of arterial hypertension and the probability of occurrence of community-acquired pneumonia (OR – odds ratio 2.27 [95 % CI 1.05 – 4.94]) was determined based on the results of simple logistic regression analysis.

2. Concomitant arterial hypertension in patients with community-acquired pneumonia increases the activity of the systemic inflammatory response, which is confirmed by the results of the correlation analysis of the C-RP (Spearman rank correlation coefficient $R=0.38$; $p<0.05$).

3. The existence of a direct link of average strength between the presence of arterial hypertension and the level of surfactant protein D ($R=0.41$, $p<0.05$), which characterizes a higher expressiveness of the local inflammatory response during acute processes, reflecting damage to the pulmonary tissue.

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STUDY OF ACUTE TOXICITY OF A NEW VETERINARY DRUG FOR INTTRAMMARY INTRODUCTION

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Abstract

Preclinical studies of veterinary medicinal products are important and compulsory studies in the development of new dosage forms. The aim of preclinical research is to determine the toxic effect and therapeutic efficacy of the test substance-the future dosage form, its effect on the body's basic systems, as well as the identification of possible side effects.

This work is part of the research on the development of the composition and technology of the veterinary drug – a solution for intramammary application, conventionally called "Argocide", intended for the treatment of mastitis in cattle.

A study of the acute toxicity of the intramammary veterinary drug was carried out in experiments on white rats of both sexes, according to the requirements for potential medicines. The establishment of the value of the average lethal dose (LD50) of the veterinary drug "Argocide" with intramuscular single administration to white mature rats is impossible due to the absence of animal death even when the drug is administered at doses exceeding 5.0 ml/kg. This experiment allows the veterinary preparation "Argocide" to be classified as practically non-toxic compounds (V class).

The analysis of the results of the conducted studies indicates the relative harmlessness of the potential drug for veterinary medicine and allows us to foresee that the "Argocide" preparation can be classified as low-risk substances, which justifies the expediency of its further study and introduction into practice.

Keywords: preclinical studies, acute toxicity, intramammary veterinary drug, "Argocide".

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1. Introduction

Preclinical studies of veterinary medicinal products are important and compulsory studies in the development of new dosage forms, especially in the context of the implementation of the legislation on the control of chemical compounds adopted by member countries of the Organization for Economic Co-operation and Development. First of all, this concerns the implementation of a scrupulous assessment of the potential hazard in conditions of guaranteed quality [1]. The aim of preclinical research is to determine the toxic effect and therapeutic efficacy of the test substance – the future dosage form, its effect on the body's basic systems, as well as the identification of possible side effects. The implementation of the rules of GLP system fully guarantees the quality of innovative medicinal veterinary drugs, their high therapeutic effectiveness [2, 3].

At the initial stage, primary toxicological studies and pharmacological evaluation of new active pharmaceutical ingredients, their separate components, various forms of new veterinary preparations are carried out, which not only determine the successful development of further experimental, clinical research and practical developments, but also has a decisive influence on the possibility of creating a highly efficient competitive and a low-toxic drug. Meeting the requirements of GLP provides a rationale for the safety of pharmaceutical development of a veterinary drug [4].

This work is a continuation of the research on the development of the composition and technology of a veterinary drug – a solution for intramammary administration under the conventional name "Argocide" [5, 6]. The developed combined medicinal product is intended for the treatment of subclinical inflammation of the mammary gland (mastitis) in agricultural animals, in particular in cattle [7].

Mastitis is an inflammation of the mammary gland that occurs in response to the influence of unfavourable environmental factors, in conditions of a decrease in the resistance of the organism and the complication of the infection. Inflammation of the mammary gland leads to a decrease in milk production, changes in the chemical composition, physical and biochemical properties of milk, as a result of which it loses nutritional value, technological properties, which affects its quality and safety [8]. The course and consequences of mastitis depend not only on the localization of the process and the virulence of the pathogen, but also on the immunobiological status of the whole animal's organism and the reactivity of the breast tissue [9, 10]. The development of the inflammatory process in the mammary gland occurs as a result of the action of mechanical, physical, chemical and biological factors. In particular, the biological factor accounts for 85 % of all cases of mastitis [11].

The spread of mastitis is explained by physiological loads on the organism of highly productive cows, inaccuracies in keeping and feeding, as well as non-observance of veterinary-sanitary requirements. To date, preparations based on antibiotics remain the main group for treating patients with mastitis of animals [12, 13]. However, the massive use of antibiotics for the treatment of mastitis has revealed a number of negative factors: the emergence of antibiotic-resistant strains of microorganisms; development of dysbacteriosis and decreased immune status; the presence of antibiotics in milk after the termination of the course of antibiotic therapy and as a consequence, the emergence of unwanted reactions in people who consume such milk [14]. One solution to the problem of dairy cattle disease is the pharmaceutical development of innovative veterinary drugs with antimicrobial substances that do not cause resistance.

2. Aim of the research

Study of acute toxicity of test specimens of a combined veterinary preparation under the conventional name "Argocide", a solution for intracisternal administration, with intramuscular injection of mature white rats.

3. Materials and methods

The research was carried out at the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine. Experiments in which animals were used was conducted in accordance with the international requirements for the humane treatment of animals and compliance with the requirements of Directive 86/609/EEC regarding the protection of animals [15, 16]. All experimental animals were kept in standard sanitary conditions. During the experiment, the animals were kept in a vivarium at 20–24 °C, humidity 30–70 % natural light mode "day-night", standard cells, with a balanced diet.

Investigation of acute toxicity of intramammary veterinary preparation was carried out in experiments on white non adult rats of both sexes (24–12 each sex; 2 animals at each of the investigated doses; 6 control animals (3 of each sex), according to the requirements of potential drug [1, 17]. The drug in the dosage form of solution for intracisternal administration was administered at varying doses – from 2.0 to 6.3 ml/kg at intervals – once daily [18] The volume of liquid which is injected animals. did not exceed volumes defined by the rules of pre-clinical research of drugs [2] The separate group of animals (control) they were treated with solvent (vehicle) preparation – water for injection, in a volume of 1.5 ml intramuscularly.

The duration of observation of the state of the animals after a single administration of "Argocide" in a drug form for intracisternal administration was 14 days. Observation of clinical signs of toxicity and death of the animals was carried out on the first day after administration of the veterinary drug continuously for one hour, and then at 2, 3, 5 hours after

administration. Over the next 14 days, each animal was observed daily, twice a day in 10^{00} and in 17^{00}. We registered cases of appearance and/or disappearance of clinical signs of toxicity, including – animal deaths.

The individual body weight of the animals was recorded before 10^{00} before the administration of the drug, and then 3, 7 and 14 days after the administration of the drug "Argocide". Changes in body weight were calculated in comparison with the weight on the day of administration and the body weight of the animals in the control groups.

Macroscopy. 14 days after the administration of the drug, all animals were withdrawn from the experiment by euthanasia by cervical dislocation under light ether anesthesia. All animals were subject to complete external examination, autopsy taking into account registered clinical disorders and detailed examination of internal organs. Before euthanasia at the end of the experiment, all animals were starving for 3 hours. It was planned to conduct a macroscopic study of animals that may die, immediately after the fact of death was established or the next morning (in 11^{00}), if the animal died at night.

Weight of organs. The following organs were weighed: liver, kidneys, spleen, lungs, and heart. Paired organs were weighed together. The relative mass of organs was calculated in g per 100 g of body weight.

Statistical processing of data. The data are given as mean ± mean error (M±m). Statistical processing of data was performed using of the Student t-test. The changes were considered statistically significant at $p<0.05$. For the statistical processing of data, the MS Excel program was used [19, 20].

5. Results of the research

The degree of acute toxicity of the drug is characterized by a dose value, one-time (or multiple for 1 day) administration of which causes the death of 50 % of animals (LD_{50}).

The main criterion for the toxic effect of the drug was the death of animals. At the same time, data on possible dose-dependent effects of the drug on various body functions were recorded in animals for 14 days, namely: the individual body weight of animals, general condition, changes in body position, skin condition, color of mucous membranes, body temperature (rectal), presence/lack of miosis/mydriasis, lacrimation, salivation, rhinorrhea, changes in the color of urine and feces and their frequency, drowsiness, tremors, convulsions, piloerection, cardiovascular, central nervous and respiratory activity system.

The results of changes in body mass and body temperature (M±m) in sexually mature white rats after a single intramuscular injection of the "Argocide" preparation are presented in **Table 1**.

Table 1

The dynamics of mass and body temperature (M ± m) in mature white rats after a single intramuscular injection of the drug "Argocide" in a dose of 6.3 ml/kg.

Group	Time of observation, (days)			
	Initial data	3 days	7 days	14 days
Control, weight, g (n=6)	182.6±1.08	187.3±1.60	192.8±1.95*	197.1±1.13*
Control, temperature, ºC (n=6)	38.1±0.14	37.9±0.16	37.9±0.13	38.0±0.14
Experiment, weight, g (n=24)	181.2±2.2	190.2±2.45	195.1±1.95*	199.8±2.4*
Experiment, temperature, ºC (n=6)	38.1±0.11	38.4±0.12	38.4±0.12	38.2±0.10

*Note: * – p <0.05 relative to the original data*

When administered intramuscularly to white rats, the drug "Argocide" in a dosage form is a solution for intracisternal administration (applied at doses of 3.16 ml/kg body weight of the animal and higher), the rats displayed certain clinical signs of intoxication, namely: cell congestion, decreased motor activity and reactions to external stimuli, which were noted immediately after the administration of the drug, as well as during the day of observation. Slowing down movements and violation of coordination of movements (ataxia) were noted during the first two hours after a single injection of the drug.

More active motor reactions were observed only 3–5 hours after the administration of the drug, ataxia was no longer noted at this time, while extension of the hind limbs during movement in the cage was observed simultaneously within 1 day after the introduction of the "Argocide". By the third day after the administration of the preparation, the animals had an untidy appearance, which was exuded in appearance – a damp coat, but the consumption of food and water was usual. Other signs of intoxication – seizures, tremors, salivation, changes in respiratory rate, cyanosis of the visible mucous membranes, blepharospasm, lateral position, etc. – in animals were not noted.

After 3 days after intramuscular administration of the drug "Argocide", no clinical signs of intoxication were observed: animals willingly consumed food and water, reactions to external stimuli were common, animals actively moved, a neat appearance were even after administration of sufficiently high doses of the drug – 5.0 ml/kg and 6.3 ml/kg of body weight of the animal.

In animals receiving the drug in low doses (2.0 and 2.5 ml/kg), clinical signs of intoxication were not observed at all. No cases of death of rats were noted, as evidenced by the data presented in **Table 2**.

Table 2

Acute toxicity study of "Argocide" preparation in experiments on white mature rats with intramuscular injection

Animal sex	Doses of the Argocide, ml/kg					
	2.0	**2.5**	**3.16**	**3.98**	**5.0**	**6.3**
Female	0/2*	0/2	0/2	0/2	0/2	0/2
Male	0/2	0/2	0/2	0/2	0/2	0/2

*Note: * – the ratio of the number of dead animals to the number of animals in the group by the indicated dose of the drug*

Among the animals of the control group, after intramuscular introduction of "Argocide" death was not recorded, their behavioral and vegetative functions are unchanged. All control animals (n=6) were active, had smooth hair and clean skin.

Weight of internal organs. A study was made of the absolute mass of the internal organs of sexually mature rats, which are euthanized 14 days after a single injection of the study drug, as well as calculations of the relative mass of internal organs in g per 100 g of body weight of the animal. The absolute mass of the internal organs in the mature rats of the experimental groups did not undergo actual changes in relative to the corresponding index in the rats of the control group. The rats of the control group were examined for comparison with the rats of the test group at the corresponding (identical) observation times. This allowed to provide averaged data for all organs in animals receiving different doses of the drug. The results are shown in **Table 3**.

The relative mass of the internal organs in the mature rats of the experiment groups in obtaining the highest studied doses (3.98, 5.0, and 6.3 ml/kg) did not differ significantly from the relative mass of the internal organs of the rats in the control group. The results are shown in **Table 4**.

Macroscopic studies. Pathomorphological examination of organs and tissues of white mature rats of both sexes was conducted, which survived and was euthanized after 14 days of observation. The animals were once administered the drug "Argocide" in a dosage form solution for intracisternal administration in various doses.

Table 3

Absolute mass (M±m, g) of the internal organs of mature white rats subjected to euthanasia 14 days after a single intramuscular injection of the preparation "Argocide" in a dose of 6.3 ml/kg

Organs	Experiment-female (n=6)	Control-female (n=3)	Experiment-male (n=6)	Control-male (n=3)
Heart	0.89±0.01	0.90±0.01	0.87±0.01	0.90±0.01
Lungs	1.31±0.02	1.29±0.2	1.20±0.2	1.24±0.1
Liver	10.54±1.30	10.34±1.1	10.13±1.2	9.86±0.8
Spleen	1.27±0.02	1.19±0.03	1.18±0.03	1.19±0.3
Kidneys	2.29±0.07	2.16±0.1	2.14±0.08	2.15±0.09

Table 4

The relative mass of internal organs (g/100 g of body weight M±m) in white rats (n=6) after a single intramuscular injection of the preparation "Argocide" at a dose of 6.3 ml/kg, and rats of the control group (n=6), who were euthanized

Organs	Experiment-female+male (n=6)	Control-female (n=3)	Control-male (n=3)
Heart	0.46±0.01	0.45±0.01	0.45± 0.01
Lungs	0.66± 0.03	0.66±0.03	0.63±0.02
Liver	5.35±0.15	5.25±0.2	5.01± 0.24
Spleen	0.65±0.01	0.61±0.01	0.62±0.02
Kidneys	1.17±0.03	1.10±0.01	1.10±0.04

According to the external examination of animals, no signs of pathological changes in their condition were found, hair and skin were clean, the subcutaneous layer of fatty tissue was moderately expressed, no mucous membranes and skin lesions were observed. Eyes, nose, lips, mouth, anal opening and external genital organs had a normal structure in all animals of experimental groups.

When the sexually mature animals were autopsied, the serous membranes of the abdominal, pleural and pericardial cavity were smooth, shiny, without signs of damage or inflammation, the amount of free serous fluid in the cavities is not increased. The brain, organs of the abdominal cavity, as well as the small pelvis are located without deviations from the norm, the colour and consistency of them were not changed; organs of the chest cavity (lungs, heart) had the usual colour and blood filling. Myocardium had viscoelasticity consistency in all animals of all groups, homogeneous on the cut, dark red (cherry) colour. Endocardium of the atria and ventricles was smooth, shiny, the trabecular relief of the ventricular cavity is clear. Heart valves were thin, elastic. Aortic wall of usual thickness, elastic, with a brilliant intima of a whitish colour. In animals that were euthanized, lung tissue is not uniform, loose consistency.

The surface of the mucosa of the larynx, trachea and large bronchi was not changed, covered with a small amount of mucus, the lumen of these organs was free.

In the gastrointestinal tract of all rats of the experimental and control groups were observed no alternative changes or ulcers. The mucous membrane of the stomach had pronounced crypts, without swelling, without damage to the erosive or ulcerative nature.

The liver was of ordinary size, its capsule was smooth, shiny, the liver tissue was uniform, dense-elastic, in a section of reddish-red color. The pancreas was located in the mesentery of the duodenum, a lobate structure, its tissue was whitish-yellow, with no signs of damage and inflammation.

Kidneys were of usual shape and size. Their surface was covered with an unaltered dense fibrous capsule, which was easily separated from the parenchyma of the organ. The tissue of the kidneys was of elastic consistency, with no signs of structural changes, the cortical and cerebral layers of the organ were clearly defined on the cut. Bowls are not dilated, their mucous membranes are thin, smooth, shiny, pale pink.

The wall of the bladder is thin, elastic, pale pink in the cavity of a small amount of transparent urine.

The state of endocrine organs of rats – thyroid gland, adrenal glands had no visible deviations from the norm. Adrenals were of usual size and shape, on a cut of yellow colour. Thyroid gland of usual size and shape, lobate structure, on a section of brownish-red colour. The macroscopic structure of the thymus and spleen was the same as that of the control animals, with no signs of alternative changes, hemocirculatory disorders of atrophy and hypertrophy.

6. Discussion

Pharmacological and toxicological results of studies of drugs and compounds of silver are widely represented in the scientific literature. The authors [21] give data on the toxic properties of Argumistin veterinary medicinal product with a mass content of colloidal silver of 10 and 50 μg/ml, in a dosage form a solution for local and internal use. It has been established that Argumistin is a low-risk chemical substance after introduction into the stomach by laboratory animals according to the classification of the average lethal dose. With prolonged enteral administration, preparations at doses of 5 ml/kg did not exert any noticeable inhibitory effect on the overall state of white non-linear mice.

N. N. Shkil and co-authors determined the parameters of subchronic toxicity of the silver-containing drug Argovit on laboratory animals [22]. For white mice, the cumulation coefficient was 24.15, for white rats – 16.1, which made it possible to classify the test drug as non-cumulative. With the prolonged administration of increasing toxic doses of Argovit to white rats, they found no significant changes in the immunobiochemical parameters of blood serum. During the entire study period, no mortality of laboratory animals was detected, as well as violations of the function of their gastrointestinal tract.

N. S. Ponomar with co-authors studied the acute toxicity of a new drug of ionized silver. The obtained results testified to the absence of any influence of even the maximum possible doses of the study drug on behavioral, neuromuscular and vegetative reactions in rats, as well as the lethality of animals in both enteral and parenteral administration. LD_{50} of a silver preparation in this connection was not established, and the authors of the study drug are classified as low-toxic and safe [23].

A dose-dependent assessment of the toxicity of silver nanoparticles in Wistar rats in vivo is presented by D. K. Tiwari, T. Jin, J. Behari. The studies are devoted to the study of the effect of various doses of silver nanoparticles on rats. Four different doses (4, 10, 20 and 40 mg/kg) were administered intravenously. It has been shown that silver nanoparticles in doses (<10 mg/kg) are safe for biomedical applications and have no side effects, but at high doses (>20mg/kg) are toxic [24].

Ukrainian scientists have studied the acute toxicity of silver nanoparticles in a colloidal solution. It was found that LD_{50}, when administered intraperitoneally to male and female BALB mice, was 34.53±3.87 mg/kg and 22.17±2.36 mg/kg, respectively. The authors classify

silver nanoparticles as substances of the third toxicity class "moderately toxic compounds" according to the K. K. Sidorov classification (1973), and LD_{50} when administered intravenously to white non-linear mice for males and females is 83.2±10.93 mg/kg and 99.92±11.71 mg/kg, respectively [25].

Park K., Park E. J., Chun I. K. et al. investigated the bioavailability and toxicokinetics of silver-coated nanoparticles coated with citrate. Male rats were administered orally or intravenously 1 or 10 mg/kg silver nanoparticles. It was found that, after oral administration, the concentration of silver nanoparticles in blood was very low. However, after injection of the tail vein, a high level of silver in the blood was detected. When silver nanoparticles were administered to rats at a dose of 1 mg/kg, the silver concentration in the blood was significantly increased 10 min after injection; the level subsequently decreased. In rats receiving silver nanoparticles at a dose of 10 mg/kg, an elevated level of concentration persisted during the experimental period. It is noted that silver nanoparticles accumulate in the liver, lungs and kidneys [26].

Souza L. R. R., da Silva V. S., Franchi L. P., de Souza T. A. J. pay attention to the need for in-depth study of the mechanism of cytotoxicity of some silver nanoparticles, as well as possible toxic effects [27].

As part of the pre-clinical study of the combined veterinary drug "Argocide" containing a silver compound, a study was conducted of the acute toxicity of the drug form, a solution for intracisternal administration. Doses were calculated for the dosage form, and not for the active pharmaceutical ingredient. It should be noted that such studies are being conducted for the first time.

According to the analysis of the data obtained (**Table 1**), it was determined that such integral indices of vital activity of animals as mass and body temperature did not experience significant individual fluctuations during the 14 days of observation after a single intramuscular injection of the drug "Argocide". At the same time, a physiological increase in body weight in animals was noted. The animals of the experimental and control groups gained weight in accordance with the physiological norm.

A study of the acute toxicity of the preparation "Argocide" in experiments on white mature rats with intramuscular introduction (**Table 2**) showed that among the animals of the control group, when intramuscular "Argocide" was administered, death was not recorded, their behavioural and vegetative functions were unchanged.

With external examination, autopsy and macroscopic examination of rats of both sexes who received Argocid once, the solution for intracisternal administration in different doses in the range from 2.0 ml/kg to 6.31 ml/kg of body weight, after 14 days of the introduction, there were no signs of a violation of hemocirculation in the tissues of the heart, lungs, stomach and small intestine.

In groups of control and experimental animals, no alternative, inflammatory and hemodynamic disorders in various organs and tissues were detected.

Thus, the research on determining the value of the average lethal dose (LD_{50}) of the veterinary drug "Argocide", a solution for intracisternal administration, with intramuscular single administration to white mature rats is impossible because of the absence of animal death even when the drug is administered in doses exceeding 5,0 ml/kg. The introduction of higher doses is considered inexpedient. This circumstance makes it possible to classify the preparation "Argocide", a solution for intracisternal administration, considering the classification of chemical substances according to the degree of danger, to practically non-toxic preparations (V class).

7. Conclusions

1. The acute toxicity of the intramammary veterinary drug under the conventional name "Argocide" was carried out, in the dosage form – a solution for intracisternal administration.

2. Tests were carried out on white non-linear sexually mature rats of both sexes with intramuscular injection in different doses – from 2.0 to 6.3 ml/kg.

3. It has been established that such integral indices of vital activity of animals as mass and body temperature did not experience significant individual fluctuations during the 14 days of observation after a single intramuscular injection of the "Argocide".

4. It was noted that after intramuscular administration of the preparation "Argocide" at doses of 3.16 ml/kg body weight of the animal and above, certain clinical signs of intoxication appeared in the rats. However, 3 days after the intramuscular application of the drug "Argocide", no clinical signs of intoxication were observed even with the administration of sufficiently high doses of the drug – 5.0 ml/kg and 6.3 ml/kg of body weight of the animal.

5. Animals receiving the drug in low doses (2.0 and 2.5 ml/kg) had no clinical signs of intoxication at all. No cases of death of rats were noted.

6. Conducting studies to establish the value of the average lethal dose (LD_{50}) of the veterinary drug "Argocide" with intramuscular single administration to white mature rats is impossible because of the absence of animal death even when the drug is used in doses exceeding 5.0 ml/kg.

7. This experiment allows us to refer veterinary drug "Argocide", a solution for intracisternal administration, considering the classification of chemical substances according to the degree of danger, to practically non-toxic preparations (V class).

8. Analysis of the results of the conducted studies indicates the relative harmlessness of the potential drug for veterinary medicine and allows to foresee that the "Argocide" can be classified as low-risk substances, which justifies the expediency of its further study and introduction into practice.

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