Научно-практический журнал для работников здравоохранения

Включён в Перечень ведущих рецензируемых периодических изданий ВАК Минобрнауки РФ

Архивъ внутренней медицины

THE RUSSIAN ARCHIVES OF INTERNAL MEDICINE

www.medarhive.ru

ИЮНЬ 2018 (№ 3(41))

УЧРЕДИТЕЛЬ И ИЗДАТЕЛЬ
Общество с ограниченной ответственностью «Синапс»
109089, Москва, ул. Угрешская, д. 2, стр. 145
Тел.: (495) 777-41-17
E-mail: info@medarhive.ru

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Тираж 3000 экземпляров.

Издание зарегистрировано в Федеральной службе по надзору в сфере связи, информационных технологий и массовых коммуникаций (Роскомнадзор).

Свидетельство о регистрации
ПИ № ФС77-45961 от 26 июля 2011 г.

ISSN 2226-6704 (Print)
ISSN 2411-6564 (Online)

Отпечатано в типографии «Onebook.ru»
ООО «Сам Полиграфист»
г. Москва, Волгоградский проспект, д. 42, корп. 5
www.onebook.ru

Ⓐ авторский материал

Ⓟ публикации на правах рекламы

Журнал включен в Российский индекс научного цитирования (РИНЦ)

Статьи журнала представлены в Российской универсальной научной электронной библиотеке www.elibrary.ru

Подписной индекс в каталоге «Почта России» 87732

DOI: 10.20514/2226-6704-2018-3

Scientific and practical journal for health professionals

Included the List of the Russian reviewed scientific magazines in which the main scientific results of theses on competition of academic degrees of the doctor and candidate of science have to be published.

Архивъ внутренней медицины

THE RUSSIAN ARCHIVES OF INTERNAL MEDICINE
www.medarhive.ru
JUNE 2018 (№ 3(41))

FOUNDER AND PUBLISHER
«SYNAPSE» LLC
109089, Moscow, Ugreshskaya str., 2-145
info@medarhive.ru

CHIEF EXECUTIVE OFFICER
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109089, Moscow, Ugreshskaya str., 2-145
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PAGE-PROOFS
Kotov Vitaly

ADVERTISING
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Circulation 3000 exemplars

It is registered by state committee of the Russian Federation on the press

The certificate on registration of mass media ПИ № ФС77-45961, 26 July 2011

ISSN 2226-6704 (Print)
ISSN 2411-6564 (Online)

Printed «Onebook.ru»
«Sam Poligrafist»
Moscow, Volgograd Prospect, 42-5
www.onebook.ru

The journal is included in Russia Science Citation Index (RSCI)

Journal data are published on website of Russian General Scientific Electronic Library www.elibrary.ru

Subscription index in the catalogue «Russian Post» 87732

DOI: 10.20514/2226-6704-2018-3

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UDC 616.24-073.756.8

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GROUND GLASS OPACITY ON CT OF THE CHEST IN CLINICAL PRACTICE: PATHOGENESIS, CLINICAL RELEVANCE, DIFFERENTIAL DIAGNOSIS

Abstract

Ground glass opacity, mosaic perfusion and air-trapping signs on chest computed tomography are types of one of the most common CT patterns — the increased lung density pattern. It is important to remember that these signs require the differential diagnosis and are not a diagnosis itself. Differential diagnosis ranges widely, since this pattern commonly occurs in diseases that affect small bronchi, pulmonary vessels, alveoli and interstitial tissue. A combination of lesions of various components of the pulmonary parenchyma is often observed thus leading to CT patterns formation. The understanding of this formation helps the doctor find a clue to the correct diagnosis. Another problem in the evaluation of these patterns is the distinction between pathological and "healthy" areas of lung tissue. Thus, in certain diseases, areas of increased lung density may not be pathological. The objective of this lecture is to analyze the reasons of ground glass opacity, mosaic perfusion and air-trapping CT-signs formation normally and in pathology and to identify their distinctive features, which allow to determine lung parenchyma elements underlying the pathological process, and thereby to narrow the differential diagnosis.

Key words: *computed tomography, ground glass opacity, mosaic perfusion, air-trapping, differential diagnosis*

For citation: Karnaushkina M.A., Averyanov A.V., Lesnyak V.N. GROUND GLASS OPACITY ON CT OF THE CHEST IN CLINICAL PRACTICE: PATHOGENESIS, CLINICAL RELEVANCE, DIFFERENTIAL DIAGNOSIS. The Russian Archives of Internal Medicine. 2018; 8(3): 165-175. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-165-175

DOI: 10.20514/2226-6704-2018-8-3-165-175

Computed tomography (CT) of the chest

Introduction

Computed tomography (CT) of the chest is today one of the leading radiographic methods for the diagnosis of lung diseases, which have become widespread in clinical practice.

Many diagnostic centers, hospitals, and outpatient clinics are currently equipped with modern CT scanners. However, clinicians are still underinformed about the capabilities of computed tomography, including CT of the chest, and for them the radiography reports are often the final diagnosis. The reason is that the radiological terms for thoracic imaging are often difficult for clinicians to interpret due to their lack of knowledge of how CT images are formed. This prevents the doctor from fully mastering the approach to making the accurate diagnosis.

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This lecture is aimed to help the clinician to form the correct diagnostic algorithm based on one of the signs of CT pattern of increased lung density: ground-glass opacity (GGO). In the lecture, we outline the principles of the diagnostic imaging and interpretation of CT findings. This information is necessary for resident physicians, primary care doctors, general practitioners, and pulmonologists to understand the diagnostic value of the changes in lung parenchyma that are revealed during chest CT.

The CT Pattern of Increased Lung Density

There are many CT signs of pulmonary diseases, but in order to ease our understanding of them and classify them, four CT patterns can be distinguished:

- Increased lung density pattern.
- Decreased lung density pattern.
- The reticular pattern.
- Focal lung lesions pattern.

CT signs of increased lung density are presented in Table 1 [1, 2].

The main mechanism that creates this pattern is as follows. The healthy person's lung tissue density in CT scans is slightly higher than the density of air. It is determined by three components: the lung tissue itself, pulmonary vasculature, and the amount of air in the alveoli.

Therefore, the reasons for increased lung density are:

- The appearance of additional masses.
- Lung tissue that is more dense and less airy because the intralobular airways and/or interstitial space are filled with pathological inclusions, or atelectasis.
- Increased capillary blood volume.

Any of these mechanisms or a combination of these mechanisms can cause GGO.

Ground-Glass Opacity

Ground-glass opacity (GGO) is an insignificant increase in lung tissue density with preserved bronchial and vascular markings in the area of pathological changes [1, 3]. The GGO areas of pathological changes are clearly distinguished from the healthy lung tissue and look grayish. The bronchial contours appear to be "too black" in comparison with the surrounding lung tissue (Fig. 1).

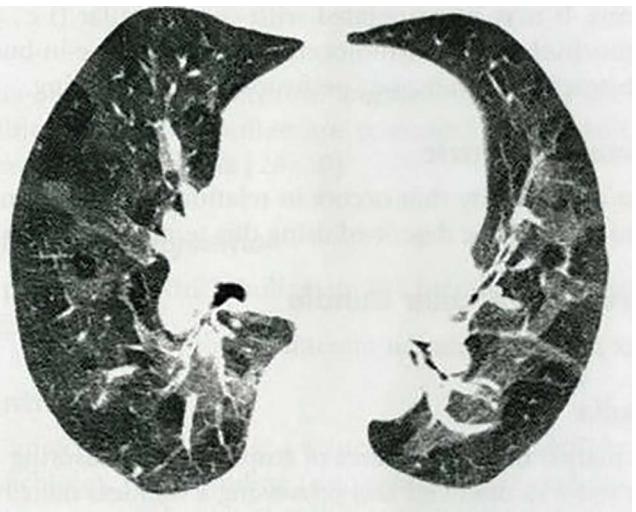


Figure 1. *High-resolution CT shows ground-glass opacity*

Table 1. *CT pattern – Increased Lung Density*

CT-pattern	CT signs of Increased Lung Density Pattern
Increased Lung Density Pattern	• Ground-Glass Opacity • Mosaic perfusion or "Mosaic lung" sign • Lung consolidation • Atelectasis • Soft-tissue density in the lungs ("expansive process")

It should be noted that the GGO is not always a sign of the pathological process in the lungs.

The physiological GGO can be observed in the gravity-dependent (lower) parts of the lungs, due to an increase of their blood filling due to the gravitational force [2]. This phenomenon is often found in patients with obesity. If such a patient undergoes CT scanning in a prone body position, the posterior segments of the lungs in which changes were detected in a supine body position will become translucent again (Fig. 2). However, the areas of infiltrative or fibrotic changes remain unchanged regardless of the position of a patient.

The GGO can be observed when a healthy person expires and when all the pulmonary fields acquire an even grayish color. This phenomenon occurs due to the physiological expiratory decrease of the lung airiness [2]. It can be easily distinguished from pathological changes in the lung tissue. Since the healthy person's expiratory decrease of the lung airiness occurs almost evenly, the X-ray attenuation coefficient (the gray scale) is even for the whole pulmonary field (Fig. 3), which is unlike what is true of the pathological process, which has a mosaic pattern (Fig. 4).

Ground-glass opacity can be found in CT images of patients who have increased lung tissue density

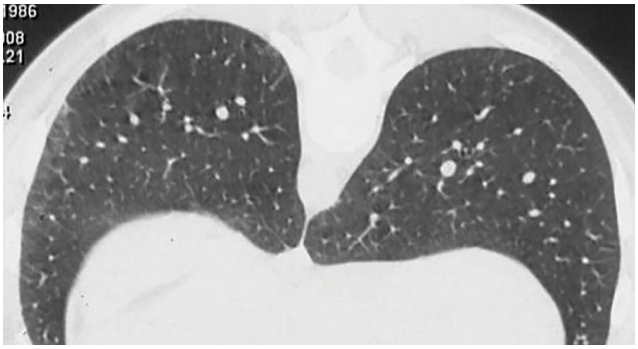


Figure 2b

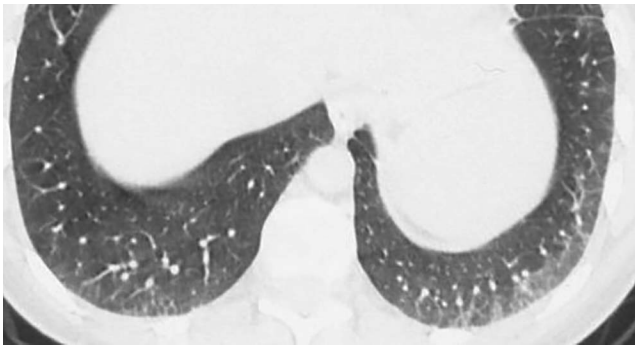
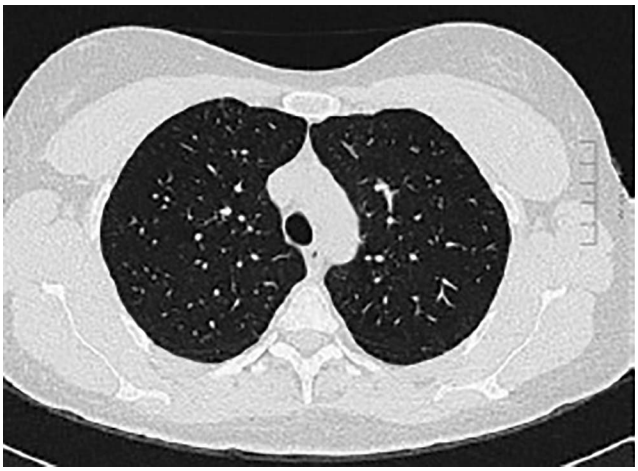


Figure 2a

Figure 2. *High-resolution CT of the normal lung at upper and middle levels in supine (**2a**) and at lower level in prone (**2b**) body positions*



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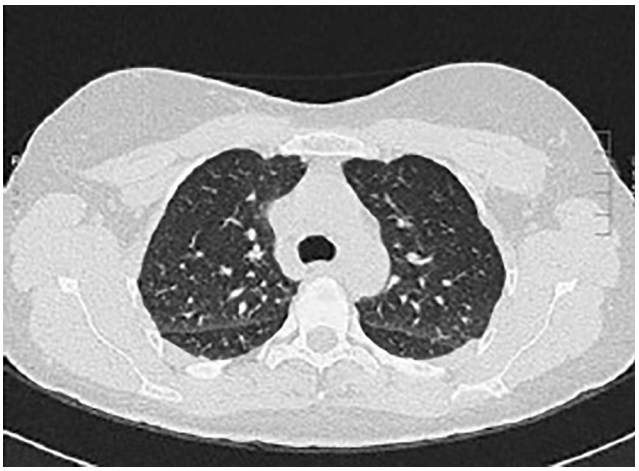

Figure 3b

Figure 3. *High-resolution CT of the normal lung (**3a** – suspended deep inspiration; **3b** – suspended deep expiration) [2]*

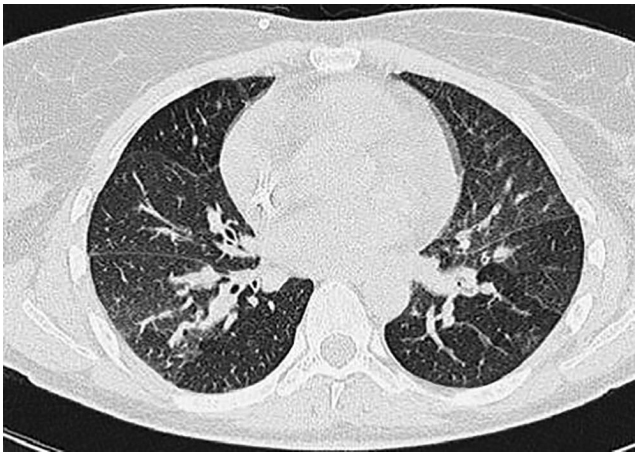

Figure 4a

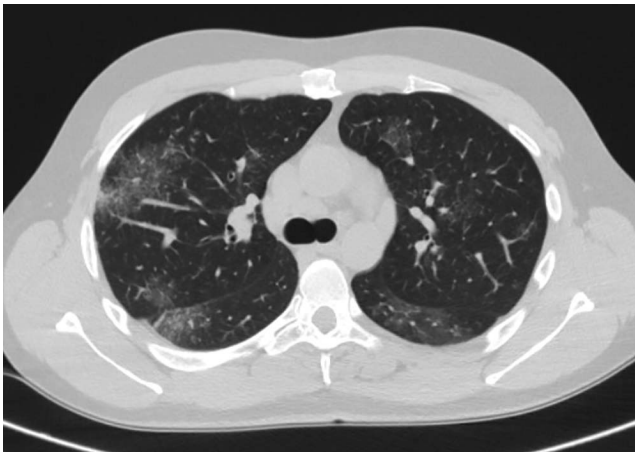

Figure 4b

Figure 4. *High-resolution CT of the lung (**4a** – COPD (areas of air-trapping); **4b** – pneumonia (ground-glass opacity)).*

due to bronchopulmonary disease. This sign can be both true and false in a patient with respiratory pathology.

True Ground-Glass Opacity Sign

Morphological substrate: True GGO develops due to three main mechanisms [1, 2, 4, 5]:

1. Figure 5 demonstrates the mechanism of formation of the pathological substrate in interlobular septa (septal interstitium) and the intralobular interstitium (most often of inflammatory origin). At the same time, the air-containing spaces (alveoli and bronchioles) remain almost completely airy. The diameter of the vessels in the areas with different densities is not changed.
2. Partial filling of alveolar spaces with cellular masses, exudate, transudate, or other pathological inclusions. If the alveoli are completely filled with liquid instead of air, the ground-glass opacity is transformed into a pulmonary consolidation (Fig. 6).

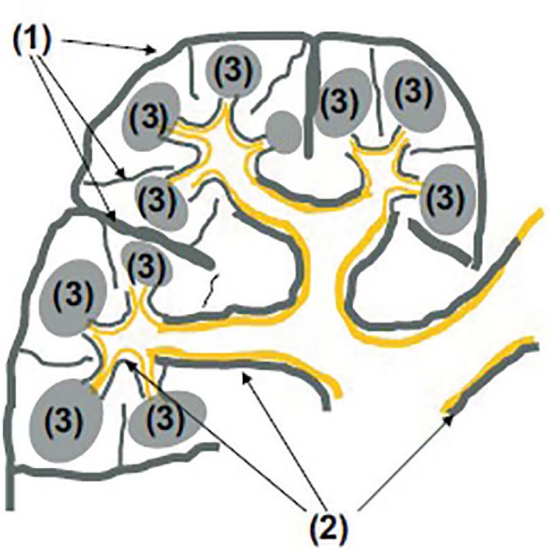

Figure 5. *The pulmonary interstitium can be divided into three component parts that communicate freely: (1) the peripheral connective tissue; (2) the axial connective tissue; (3) the parenchymatous connective tissue [2]*

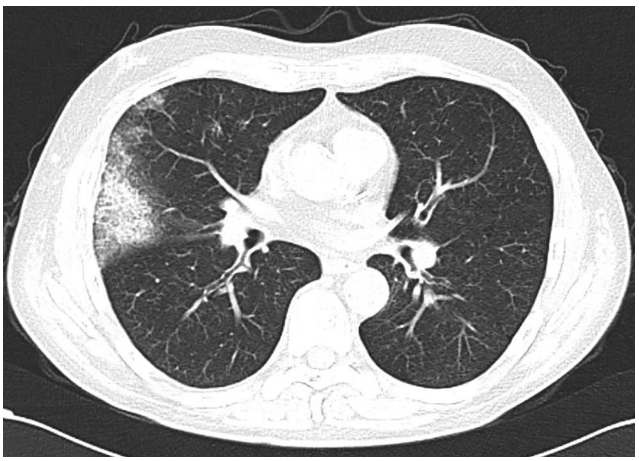

Figure 6. *Pulmonary haemorrhage in the right lobe in a patient with an injury after a car accident. Ground-glass opacity and areas of lung consolidation [2]*

Table 2. *Differential diagnosis of ground-glass opacity [4-9]*

Clinical Course	Disease
Acute disease	Pulmonary edema
	Pulmonary haemorrhage
	Acute respiratory distress syndrome, viral pneumonia, acute interstitial pneumonia
	Pneumonia (bacterial, viral, Mycoplasma pneumonia, Chlamydia pneumonia)
Subacute disease	Pneumocystis jiroveci pneumonia
	Fungal pneumonia
	Hypersensitivity pneumonitis
	Cryptogenic organising pneumonia
	Desquamative interstitial pneumonia
	Eosinophylic pneumonia (chronic)
	Micobacteriosis
Chronic disease	Nonspecific interstitial pneumonia
	Usual interstitial pneumonia (UIP): idiopathic pulmonary fibrosis and disease associated UIP
	Vasculitidis (Churg-Strauss syndrome)
	Sarcoidosis
	Bronchioloalveolar carcinoma (mucinous)
	Alveolar proteinosis

3. The decrease of lung airiness due to pathological processes (hypoventilation, respiratory depression, pulmonary fibrosis).

Type of the lung disease: acute, subacute or chronic clinical course leads to the appearance of additional CT signs, which help to perform a differential diagnosis.

Table 2 shows the main diseases that lead to the formation of the true ground-glass opacity and additional CT signs of corresponding pathology (Table 2).

If the clinical course is acute or subacute, GGO is mostly represented on CT scans as:
1. Pure GGO (Fig. 1).
2. GGO + consolidation (Fig. 7). The common cause of lung tissue consolidation is the

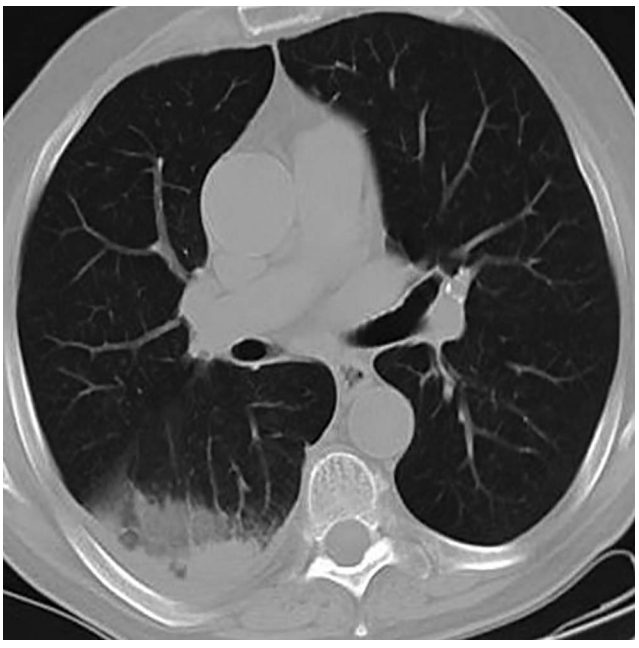

Figure 7. *The combination of ground-glass opacity and lung consolidation*

replacement of air in alveoli with liquids, cells or tissue. However, GGO is found along the consolidation area periphery if lung airiness is partially preserved and the interstitial component is predominant.

1. GGO + bronchiolitis (Fig. 8). Bronchiolitis is characterized by Y-structures with widening at the ends. It resembles the buds on tree branches. This symptom is associated with the filling of intralobular bronchioles with liquid (usually it is an inflammatory liquid), their dilation, and the thickening of their walls.

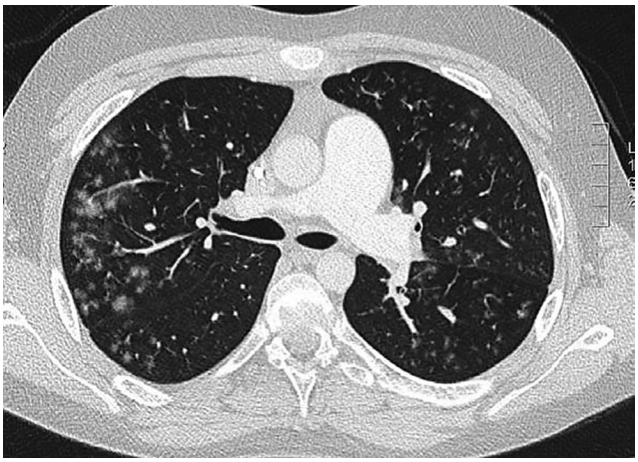

Figure 8. *Infectious bronchiolitis ("tree-in-bud" sign) with ground-glass opacity in a patient with Mycoplasma pneumonia*

The detection of GGO without signs of fibrosis, traction bronchiectasis, and lung tissue structural damages thus means the development of an active, most often reversible process in the lung tissue (Fig. 9).

If the clinical course is subacute or chronic, the GGO is represented on CT scans on the background of reticular (fibrotic) changes [4–9] as:

1. GGO + reticular changes (Fig. 10).
2. "Crazy paving" pattern (Fig. 11). It is a bilateral lung lesion, imaged as GGO, combined with the reticular pattern caused by interlobular and parenchymal interstitium thickening and peripheral lobular air space filling with the pathological inclusions.

The "crazy paving" pattern is specific for pulmonary alveolar proteinosis, but it also occurs in other diseases, including acute diseases (nonspecific interstitial pneumonia, cryptogenic organising pneumonia, vasculitides, eosinophilic pneumonia) (Table 3).

3. GGO + bronchiectasis and/or bronchiolectasis (Fig. 12).
4. GGO + cysts (Fig. 13).

Note that the appearance or expansion of the ground-glass opacity area in subacute and chronic diseases may indicate the degree of the pulmonary pathological process activity [4, 8, 10, 11].

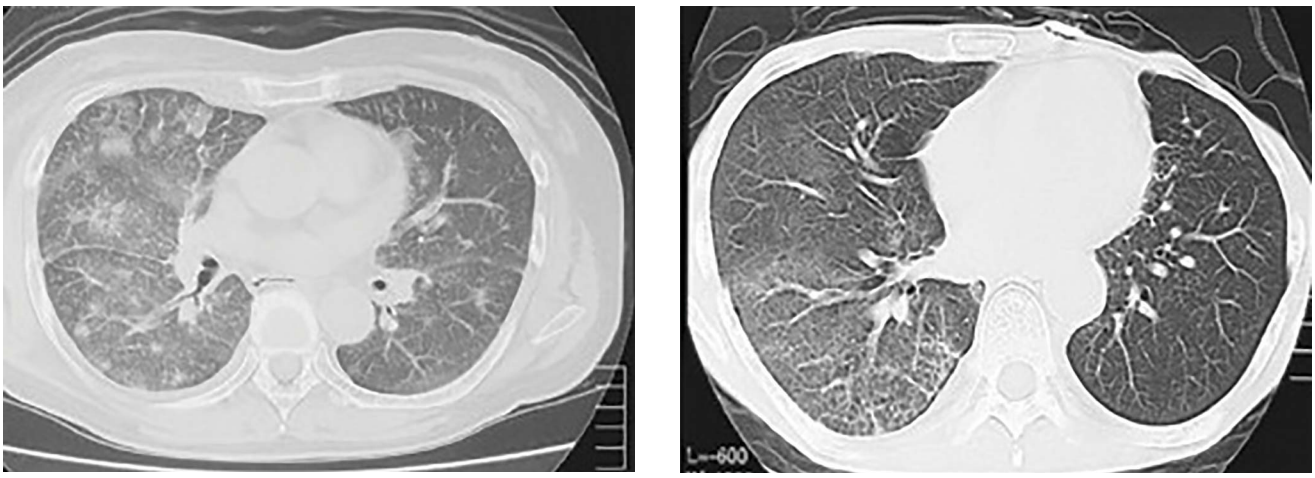

Figure 9. *Dynamics of ground-glass opacity sign in a patient with hypersensitivity pneumonitis before and after corticosteroids therapy (3 months) [1]*

The decrease of lung tissue density on CT images as an insignificant lung tissue density increasing with preserved bronchial and vascular markings in the area of pathological changes may be associated not only with interstitium thickening and partial filling of alveoli with the pathological inclusions ("true GGO"), but also with a perfusion defect, the "air trapping" occurrence. This phenomenon is called "false GGO" or the "mosaic lung" sign [1, 11], since its X-ray pattern on inspiratory CT scan is very similar to true GGO.

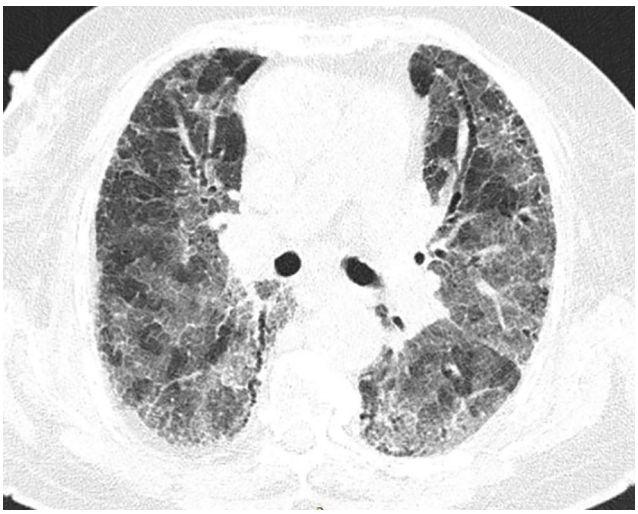

Figure 10. *Diffuse ground-glass opacity and the reticular pattern in a patient with hypersensitivity pneumonitis*

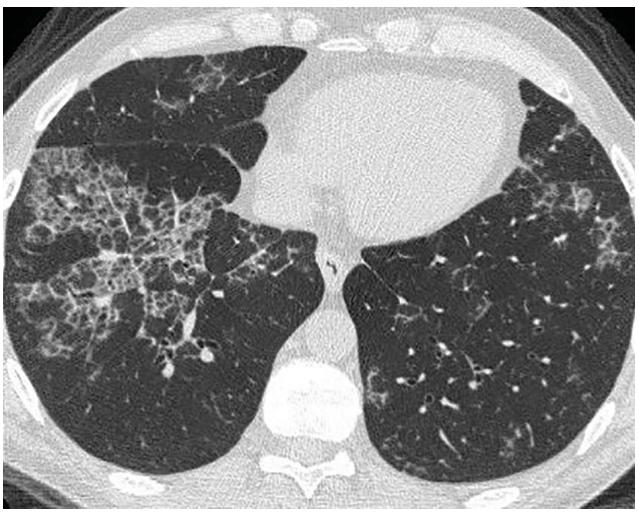

Figure 11. *The combination of ground-glass opacity and intra- and interlobular lines creates the crazy-paving pattern, in a patient with alveolar proteinosis*

Table 3. *Diseases than can cause the crazy-paving pattern formation [2, 4, 9-11]*

Acute disease	Subacute/chronic disease
Pulmonary edema	Alveolar proteinosis
Acute respiratory distress syndrom	Usual interstitial pneumonia (UIP): idiopathic pulmonary fibrosis and disease associated UIP
Pulmonary infection (bacterial, viral, Pneumocystis pneumonia, Mycoplasma pneumonia)	Nonspecific interstitial pneumonia
Pulmonary haemorrhage	Organising pneumonia
Acute interstitial pneumonia	Vasculitis (Churg-Strauss syndrome)
Radiation pneumonitis	Bronchioloalveolar carcinoma
Acute eosinophilic pneumonia	Chronic eosinophilic pneumonia
	Lipoid pneumonia
	Sarcoidosis

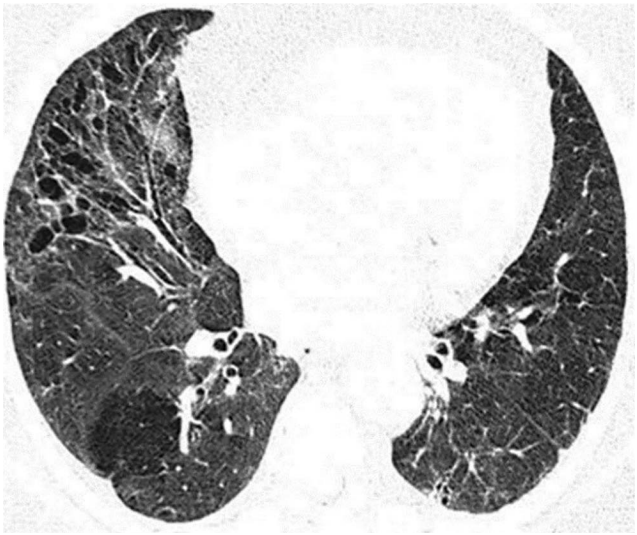

Figure 12a

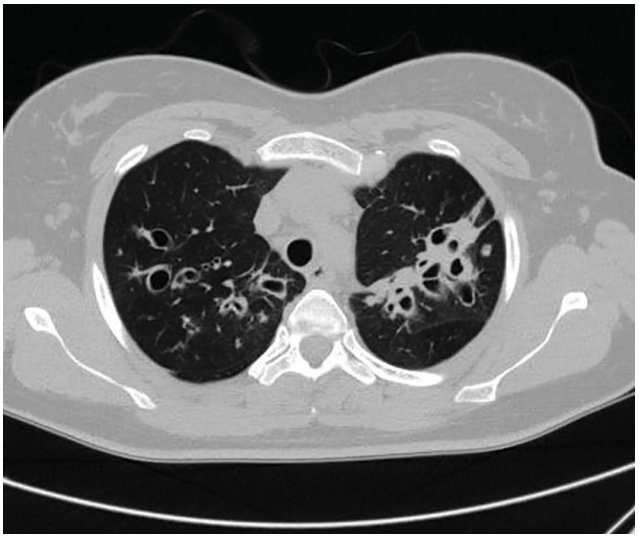

Figure 12b

Figure 12. *Ground-glass opacity in a patient with bronchiectasis (**12a** – diffuse ground-glass opacity and traction bronchiectasis in a patient with chronic hypersensitivity pneumonitis; **12b** – the combination of ground-glass opacity and cysts bronchiectasis)*

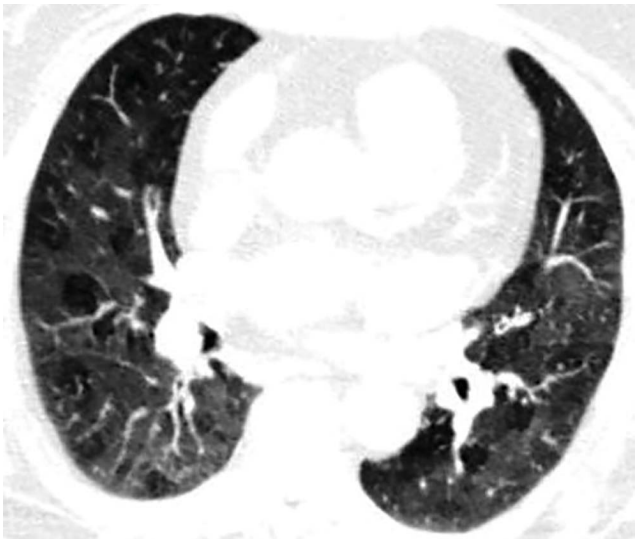

Figure 13. *Lymphocytic interstitial pneumonia. A few thin-walled cysts and GGO are seen in both lower lobes [10]*

«Mosaic Lung» Sign or Mosaic Perfusion (False Ground-Glass Opacity)

The «mosaic lung» sign or mosaic perfusion is a combination of areas with increased and reduced lung tissue density that are not associated with the decreased alveoli airiness or pulmonary interstitium lesions [1, 2, 11] (Fig. 14).

Morphological substrate: It occurs as a result of two mechanisms or a combination of mechanisms [1, 11]:

1. Affected by pathological changes in small bronchi with subsequent reflex vasoconstriction and redistribution of blood to healthy (well-aerated) areas of the lung tissue.
2. Affected by lobular arteriostenosis (obstruction, spasm).

The «mosaic lung» sign is a mosaic decrease of lung tissue density. It is very similar to true GGO in inspiratory CT images. In other words, it is manifested by a combination of increased and decreased density areas (alternating light gray and dark gray regions). However, the areas of true GGO reflect the lung tissue with preserved hemodynamics and reduced airiness, whilst mosaic perfusion occurs as a result of hemodynamic anomalies or pathology of small airways [1, 2, 11, 14].

In order to distinguish between these two CT signs, it is necessary to:

1. Compare the diameters of the vessels in the light gray and dark gray regions of the lung parenchyma during the examination while the patient breathes in. The diameters of the

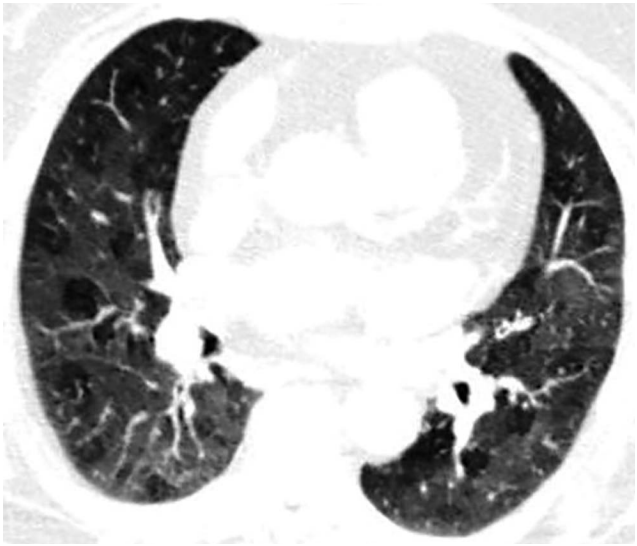

Figure 14. *Mosaic perfusion secondary to chronic pulmonary embolism. The areas of ground-glass opacity are «normal» lung regions with increased perfusion and increased blood volume*

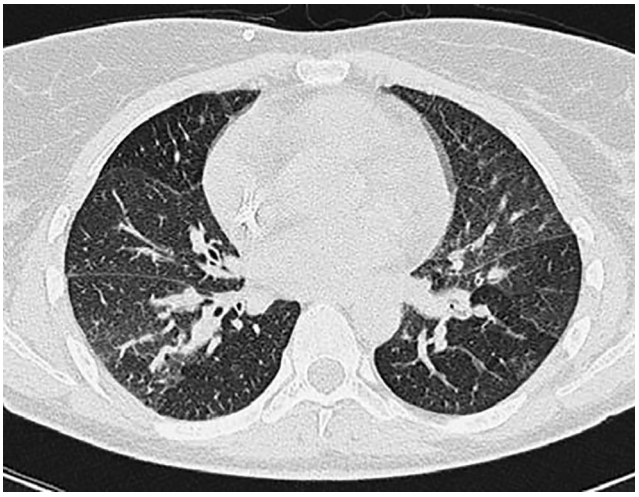

Figure 15. *Mosaic perfusion pattern*

vessels in different density areas are almost the same with true GGO in CT images of the chest. With false GGO, the diameters of the vessels in different density areas are not the same: they are less in decreased density areas (Fig. 15).

2. One of the best methods to distinguish the causes of the mosaic CT sign is the evaluation of the data obtained during inspiration and expiration. If the changes are related to hemodynamic anomalies and are presented as mosaic perfusion and true GGO, the density difference between these areas disappears or decreases: areas with a lower attenuation coefficient (darker regions) become more dense (become grayish like the healthy areas of the lung tissue) (Fig. 16).

If the «mosaic lung» sign is caused by the pathology of the small airways, pathological areas retain

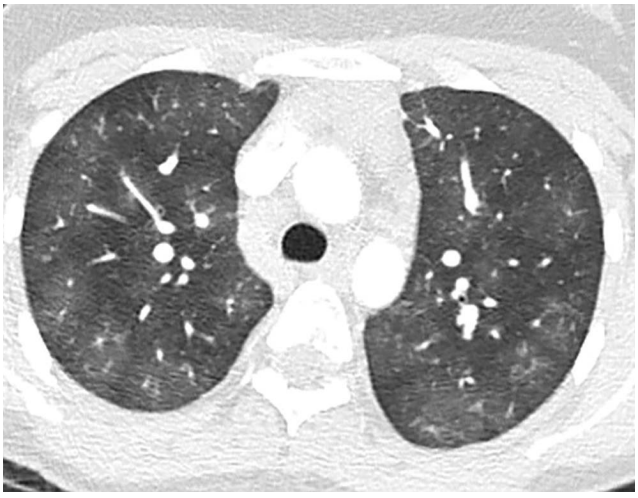

Figure 16a

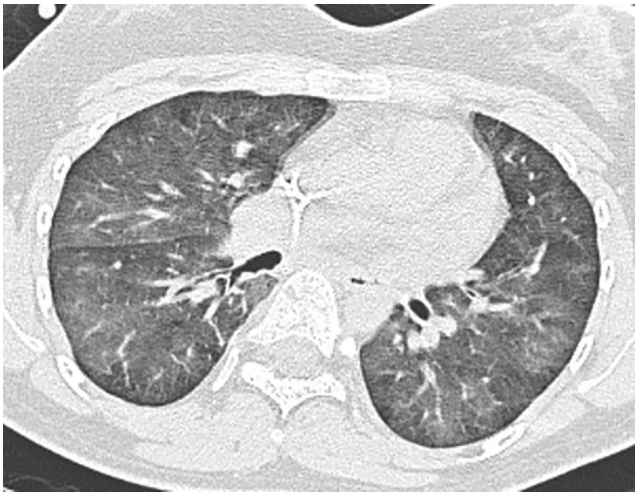

Figure 16b

Figure 16. *Multiple areas of mosaic perfusion in a patient with chronic pulmonary embolism. Differential diagnosis is predominantly based on normal vessel size in the high- and low-density areas: inspiration (16a) and expiration (16b)*

the same density irrespective of the respiratory phases, or the differences in the density of areas increase even more («air trapping») (Fig. 17).

It is necessary to examine the distribution of GGO in order to perform a proper diagnosis. It may be patchy or diffuse (Table 4).

The ground-glass opacity can also exhibit features of regional distribution in the lung parenchyma (Table 5).

Conclusion

The objective of this lecture was to explain to clinicians the pathogenic mechanisms by which the ground-glass opacity develops normally and in pathology. We presented the main approaches that are used to identify and conduct a differential diagnosis of true and false ground-glass opacity CT patterns in patients with lung diseases. We hope that this lecture will help you use the obtained knowledge for proper diagnosis and for

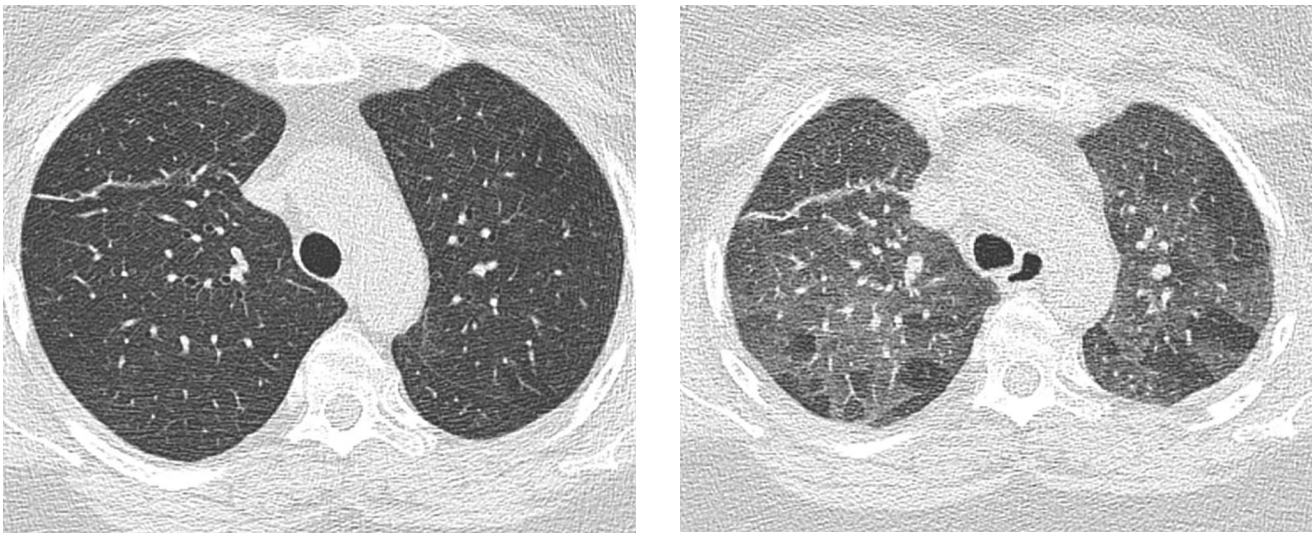

Figure 17. *Inspiration (a) and expiration (b) CT scans. Multiple large areas of air-trapping are seen in both lungs*

Table 4. *GGO distribution [2, 4, 11, 13]*

Patchy distribution	Diffuse distribution
Nonspecific interstitial pneumonia	Hypersensitivity pneumonitis
Desquamative interstitial pneumonia	Smoking related parenchymal lung disease, respiratory bronchiolitis (Respiratory bronchiolitis — interstitial lung disease, Desquamative interstitial pneumonia)
Hypersensitivity pneumonitis	
Alveolar proteinosis	Nonspecific interstitial pneumonia
Pulmonary embolism	Pneumonia (viral, bacterial, fungus)
Vasculitidis	Pulmonary edema
Sarcoidosis	Pulmonary embolism
	Acute respiratory distress syndrome
	Acute interstitial pneumonia
	Alveolar proteinosis

Table 5. *GGO localization [2, 4, 11, 13]*

Upper lung	Lower lung	Diffuse distribution
Sarcoidosis	Pulmonary edema	Hypersensitivity pneumonitis
Туберкулез/Tuberculosis	Usual interstitial pneumonia [UIP]: idiopathic pulmonary fibrosis and disease associated UIP	Pneumonia (viral, bacterial, fungus)
Chronic Eosinophilic pneumonia	Nonspecific interstitial pneumonia	Sarcoidosis
	Desquamative interstitial pneumonia	
	Lymphocytic interstitial pneumonia (Sjögren syndrome, AIDS)	
	Organising pneumonia	
	Pulmonary embolism	

developing the most optimal algorithm for choosing additional clinical and instrumental tests when you perform diagnoses. However, despite the knowledge about the formation mechanisms and radiographic features of CT sign of ground-glass opacity that you have learned, a doctor should primarily take into account the clinical picture of the disease when making a diagnosis.

Conflict of interests

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов / The authors declare no conflict of interests.

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Article received 09.04.2018
Adopted for publication 13.04.2018

UDC 616.74-009.1-02-03-07-08

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IMMUNOPATHOGENESIS OF MYASTHENIA GRAVIS (REVIEW)

Abstract

Myasthenia gravis is a progressive autoimmune disease, which is characterized by the production of antibodies against the structures of the neuromuscular junction. High clinical heterogeneity of autoimmune myasthenia and the initiating course of the disease increase the urgency of its pathogenesis studying, searching for specific methods of marker diagnostics, developing algorithms for predicting the features of the development of the disease. At the present time, there are different approaches to the study of the etiology and pathogenesis of the disease, which include serological, biochemical, genetic, etc., theories of its development. For decades, researches have been carried out to find new pathogenetic links in myasthenia gravis. Today, a number of antibodies were described, such as antibodies against muscle-specific tyrosine kinase (MuSK), ryanodine receptors, titin, lipoprotein bound receptor 4, cortactin, etc. The serological diagnosis of myasthenia gravis has been used as a "gold standard" in clinical practice. The prognostic criteria describing the course of myasthenia gravis and the type of antibodies isolated in the blood serum of the patient are described. Mechanisms of immunological tolerance disorder, which triggers the production of antibodies against their own structures, have already been developed as well, and their genetic bases are also described. Thanks to the development of biotechnological methods, the researchers were able to identify the subtype of lymphocytes involved in the development of myasthenia gravis. Isolation of individual subpopulations of lymphocytes also became available. Researchers continue to search for new targets, allowing to improve diagnostics, to develop new directions in the therapy of the disease. However, despite the active study of various mechanisms for the development of myasthenia gravis, many unresolved problems still remain. The article briefly describes the main investigated mechanisms of complex myasthenia gravis pathogenesis.

Key words: *autoimmune myasthenia gravis, myasthenia gravis pathogenesis, myasthenia gravis markers*

For citation: Gasymly E. D. IMMUNOPATHOGENESIS OF MYASTHENIA GRAVIS (REVIEW). The Russian Archives of Internal Medicine. 2018; 8(3): 176-185. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-176-185

DOI: 10.20514/2226-6704-2018-8-3-176-185

HLA — major histocompatibility complex system, LRP-4 — low-density lipoprotein receptor-related protein 4, MuSK — muscle-specific tyrosine kinase, T_{reg} — regulatory T cells, TCR –T cell receptor, ILs — interleukins, Th — T-helper cells, TNF — tumor necrosis factor

Myasthenia gravis is an autoimmune disease, which is based on mechanisms aimed at the synthesis of antibodies against the neuromuscular junction structures. The causes triggering the breakdown of immunological self-tolerance are not completely defined. It is considered to be a multifactorial disease [9, 10].

The disease has a high clinical heterogeneity, a chronic progressive course, affects mostly young people, and it is debilitating in nature [3, 51, 68, 70, 96]. Autoimmune myasthenia gravis is the most common neuromuscular pathology, accounting for up to 60% of patients [2]. Despite the widespread prevalence [16], the pathogenesis of myasthenia gravis remains poorly understood due to the diversity of antigenic "targets" of the neuromuscular synapse [9].

Active research into autoimmune diseases, including myasthenia gravis, has been going on for decades. The attention of scientists is attracted both by serological and immunological, and genetic features, endogenous and exogenous factors [8]. In addition, myasthenia

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pathogenesis may also involve proteins (for example, survivin), cellular (micro-RNA) structures, synaptic cholinergic receptors, changes in the structure of the ion channels of axon, muscle structures, etc. [10, 44].
Recently, the role of survivin protein in the pathogenesis of autoimmune myasthenia gravis has been studied. It is functionally important for cell division, apoptosis and, possibly, for the biogenesis of micro-RNA. It also takes part in the implementation of adaptive immune reactions, controls the differentiation of memory CD4+ and CD8+ T cells and maturation of B cells. In the literature, there is evidence supporting the possible application of survivin as a diagnostic and prognostic marker for rheumatoid arthritis, psoriasis, pulmonary arterial hypertension, multiple sclerosis, inflammatory bowel disease, and myasthenia gravis [36, 56].
According to a number of scientists, components of the complement system participate in the blocking of neuromuscular transmission, which is clinically manifested by the development of muscular fatigue. Blocking the activity of the complement system in patients brought about relief of the disease symptoms [24, 25, 43, 99].
In the history of the study of the pathogenesis of autoimmune myasthenia gravis, definite success has already been achieved. A number of antibodies against antigenic structures in myasthenia have been identified, which can serve as an additional diagnostic criterion for this disease and provide a source of information that allows for the course of the disease to be predicted [1, 65, 69].
Analysis of the concentration of serum markers is the standard for diagnosis of the disease. In the genetics of the disease, a number of HLA-system genes have been identified whose polymorphisms have an impact on the development of myasthenia gravis.

The authors of the article tried to describe the existing directions in the research of pathogenesis of autoimmune myasthenia.

The Role of Antibodies in the Pathogenesis of Myasthenia Gravis

The method for determining marker antibodies is widely used in the diagnosis of autoimmune diseases [34, 121]. A number of serological markers related to myasthenia gravis have been described in the literature [107]. These data emphasize the diversity of mechanisms of pathogenesis and, perhaps, explain the existence of differences in clinical manifestations [44, 66].
Determination of the antibody titer to the structures of acetylcholine receptors is one of the criteria for the diagnosis of seropositive myasthenia gravis. According to the literature, exceeded reference values are recorded in 80–85% of patients, among whom patients with the generalized form predominate [1, 106].
Epidemiological analysis of patients revealed a bimodal distribution of increased titer of antibodies against acetylcholine receptors. Seropositive patients prevailed among women between the ages of 20 and 40, and men of the elderly age group of 60–80 [44].
In addition to subunits of the nicotinic receptor, a number of other postsynaptic structures (muscle-specific tyrosine kinase, lipoprotein-bound receptor protein 4 — LRP4), and of muscle tissues (titin, ryanodine receptors, agrin) have immunogenic properties [48, 106].
Approximately in 70% of cases of seronegative myasthenia gravis antibodies against muscle-specific tyrosine kinase (MuSK) are detected. Moreover, in many of these cases, there is a tendency for the disease to take a severe course, when the respiratory and bulbar muscles are predominantly affected. Such forms of the disease rarely respond to hormonal therapy and require the prescribing of combined immunosuppressive therapy [1, 27, 72, 73, 82, 121].
Antibodies against LRP4 are detected in 2–27% of cases in double-seronegative patients (antibodies against acetylcholine receptor and MuSK in the blood are not detected). Among LRP4-positive patients, female patients predominate (2:1), muscle weakness is moderate. The distribution in muscle groups is similar to seropositive patients, but in the fifth part is limited only to ocular manifestations. They have shown a positive response to inhibitors of acetylcholinesterase [1, 91, 106, 118].
Another component of the postsynaptic membrane is agrin. Neuronal agrin is a protein of the extracellular matrix used by motoneurons to induce clustering and post-functional differentiation of acetylcholine receptors. Agrin binds to LRP4 to form a tetrameric complex that interacts with MuSK and activates it to initiate subsequent signaling pathways [30].
According to F. Romi and other researchers, the study of antibodies against striated muscle tissue in the serum of seronegative patients should produce promising results. These include antibodies against the components of striated skeletal or cardiac muscle (SH antibodies), antibodies against citric acid extract

of striated muscle (CAE antibodies) and antibodies against titin and ryanodine receptor (RyR), which are used in the diagnosis of myasthenia gravis associated with the presence of thymoma. Among them, the determination of a combination of serological indicators for muscle tissue, especially for titin and ryanodine receptor, is the most sensitive and specific to detecting thymoma [47, 97].

Antibodies that attack potassium channel structures were recorded in 12–28% of patients with myasthenia gravis in Japan. The presence of these antibodies is associated with bulbar disorders, possible myasthenic crises, thymoma, myocarditis and prolonged QT intervals on electrocardiograms, and the detection of neuromyotonia in an electrophysiological study. However, in the European population, these markers are isolated in patients with local forms of myasthenia gravis, mainly the ocular form, in which the signs of neuromyotonia are not detected by EMG [86].

In some patients who were classified as double-seronegative, antibodies against cortactin, a key regulator of actin reorganizations in response to changes in tyrosine kinase signaling, were detected by Gallardo E. in 2014 [57]. It is known that actin chains play an important role in various cellular processes aimed at remodeling of the plasma membrane and the movement of intracellular vesicles and particles. Cortactin is exposed to tyrosine kinases. Its phosphorylation reduces the activity of Src family kinases. Phosphorylation of cortactin provides binding sites for specific signaling proteins in SH2 domains, which can regulate a number of cellular functions. It should also be noted that cortactin is expressed in breast cancer and squamous cell carcinoma of the head and neck [12, 28, 53, 57, 87].

Analysis of serum antibodies against the structural components of neuromuscular junction is an important tool that is used in healthcare practice. A variety of serological markers can be used to predict the character of the clinical course.

The Role of Cytokines in the Pathogenesis of Myasthenia Gravis

Cytokines play an important role in the development of autoimmune diseases, determining the intensity of inflammatory changes in tissues, including neuromuscular structures [101, 103, 115].

As mediators, they take part in the differentiation of immunocompetent and hematopoietic cells and in the formation of mechanisms responsible for the intercellular interaction underlying the immune response. Their main biological activity is the regulation of the immune response at all stages of its development [117].

In general, it should be noted that all this large group of endogenous regulators participates in the division and differentiation of progenitor cells of functionally active immunocompetent cells, in the changing of antigens and various markers expression, in the chemotaxis, in the switching of immunoglobulin synthesis, in the inducing cytotoxicity in macrophages, in the proliferation of antigen-sensitive lymphocytes, in the differentiation of B cells to the producers of immunoglobulins, in the switching of the immunoglobulins synthesis from one isotype to another, in the ensuring the maturation of progenitors of cytotoxic T cells to the mature effectors, in the inducing cytotoxicity in macrophages, and in the formation of the inflammation site [101, 103].

Cytokines play a coordinating role in the pathogenesis of autoimmune diseases. They participate in the interactions between B lymphocytes and T helpers (Th) [15]. So, the cytokine can affect the receptors of the very cell that synthesizes it, and it can also affect the adjacent cells as well as the cells of distant organs. Yilmaz et al., 2015, have shown on the experimental model of autoimmune myasthenia gravis that a decrease in the number of cytokines correlates with a decrease in the level of antibodies against acetylcholine receptors. Scientists have identified a relationship between an increase in the level of TNF-α (tumor necrosis factor α), interleukins 17A and 21 (IL-17A and IL-21) and the severity of the disease with MuSK-positive myasthenia gravis [15, 117].

TNF inhibits the activity of regulatory T cells, which reduce the autoreactivity of immunocompetent cells, while reducing TNF levels results in the restoration of the function of these cells [55].

The ability of IL-10, which is synthesized by regulatory type 1 T cells, to inhibit the activation of effector immune cells during autoimmune responses, underscores their essential role in maintaining immune tolerance. Interleukin-27 (IL-27), a member of the IL-12 family of heterodimeric cytokines, has been identified as an important cytokine that suppresses the Th17 cell effector and promotes the formation of Th1 cells [81].

Th17 cells are involved in the development of autoimmunity. T cells producing IL-17 and IL-10 are functioning in the suppression of inflammatory reactions [3, 38, 59, 69, 72, 77, 83, 88, 108]. IL-17 is

present in sites of tissue inflammation in autoimmune diseases [40]. T helper cells also synthesize IL-23, which contribute mechanisms that upset self-tolerance in the central nervous system [58].

Scientists have used the experimental model of myasthenia to show that IL-12 is the determinative cytokine for the differentiation of Th1 cells that are involved in the development of myasthenia gravis, and IL-10, which is a powerful differentiation factor for B cells, also promotes the development of myasthenia gravis. In contrast, IL-4 has an antagonistic effect. It inhibits the development of symptoms of myasthenia gravis. An increase in the level of IL-10 in the patients with generalized myasthenia gravis is significantly higher than in patients with a local form of the disease [67, 74, 116].

A number of scientists have shown that in patients receiving immunosuppressive therapy, the number of memory cells increases. When stimulating CD40 in patients with myasthenia, significantly lower levels of IL-10 and IL-6 were obtained than in the control group. When stimulating CD40 and B cell receptor in addition to these cytokines, the production of TNF-α also decreased [116, 117].

The works of Akiyuki Uzawa et al. (2016) have demonstrated that in the blood serum of seropositive patients there is an increase in the content of IL-15, IL-19, IL-20, IL-28A, IL-35, which induce ligand proliferation, which is a vascular endothelial growth factor. Changes in the cytokine profile in patients indicate the role of these molecules in the development of myasthenia gravis [101, 102].

Perhaps, the detection of changes in the cytokine profile in myasthenia patients can function as an important prognostic factor in diagnosis, and it can also be used in the development of medicines with a new mechanism of action.

The Role of Immune Cells in the Pathogenesis of Myasthenia Gravis

The protective function of the immune system is provided by its ability to recognize practically the full range of pathogens, the presence of immunological memory, which is designed to produce a rapid response, and immunological tolerance, which makes it possible to avoid damaging own body structures [33, 62]. It is known that the main reason why a large amount of autoantibodies are released in the organism is the positive selection of autoreactive T cells and the selective loss of regulatory T cells. In connection with this, another direction in the research of the characteristics of pathogenesis of myasthenia gravis is represented by the study of self-tolerance mechanisms at the intercellular level [33].

By participating in the formation of immunological memory, in recognizing antigens and inducing immune response, T cells are one of the key links in the pathogenesis of autoimmune diseases. T-lymphocytes also have the ability to recognize antigens on the surface of antigen-presenting cells in combination with their own histocompatibility antigens [7, 26, 32, 62, 74].

There are several types of T cells. Populations of T cells differ both in membrane markers and in the method by which antigens are recognized and functions are performed. A receptor complex with a unique structure, which determines the functioning of T cells, functions on the surface of T cells. In addition to the main receptor complex, a number of auxiliary protein complexes, co-receptors, have a pronounced presence on the surface. CD4 and CD8, CD3, CD28 are the most significant auxiliary receptor complexes. The functionally important co-receptors CD4 and CD8 are associated with tyrosine kinase systems and a costimulatory molecule [99].

Part of the T cells is involved as cytotoxic cells, significantly reducing intense immune response and autoaggression and acting as regulatory T cells (T_{reg}). In barrier tissues, they interact with epithelial cells, stimulating their survival and functions and facilitating the recovery of the epithelium when it is damaged [71, 108, 113].

Natural regulatory T cells prevent other T-lymphocytes from reacting to their own antigens, limiting all forms of immune response. It is these cells that guarantee the suppression of the activity of autoreactive cells, which have avoided negative selection during development. In addition, when regulatory cells are differentiated, other functionally important membrane molecules have a pronounced presence on their surface [71, 112].

Scientists also found a decrease in the suppressive activity of lymphocytes when there is a defect in the structure of T_{reg} [33, 108].

T_{reg} cells are a subpopulation of T cells that inhibit the activation of other immune cells and thereby support

the homeostasis of the immune system. The influence of T_{reg} cells on the pathogenesis of autoimmune diseases, including myasthenia gravis, is actively being studied by a number of researchers from different countries. The researchers suggest that the functional deficiency of T_{reg} cells can lead to the inability to suppress autoreactive T cells [65, 67, 109, 114].

The effect that is produced on these cells, according to some researchers, presents a promising direction in the therapy of autoimmune disorders. According to a number of researchers, regulatory T-lymphocytes represent the most promising area in the study of the pathogenesis of myasthenia [97, 107].

In addition to T cells, B cells also participate in the development of myasthenia gravis by producing autoantibodies. The study of cellular mechanisms that participate in the development of autoimmune disease is a promising direction for targeted therapy [10, 21, 23].

The study of the disrupted functioning of regulatory T cells, which are associated with the severity of the disease, has attracted a great deal of interest from scientists. Approaches are actively being developed to improve and even to correct the functioning of T-lymphocytes, which can be used in the treatment of myasthenia gravis and other diseases [31, 33, 92, 98, 99].

The Role of Receptors and Enzymes in the Pathogenesis of Myasthenia Gravis

The result of all interactions occurring at the cellular level is chemical transformations. Earlier, the study of the cascade of signaling pathways was impossible due to technical reasons, and scientists could only guess about the possible role that certain biological substances played. External factors affecting the receptors of the cell membrane lead to conformational changes in their structure, thereby leading to the activation of enzymatic systems that play a role of secondary intermediaries in signal realization [94, 104].

Another direction in the research of myasthenia gravis pathogenesis and other autoimmune diseases is the study of a cascade of signaling pathways that ensure the functioning of immune cells. At different stages of signal transmission, this functioning is carried out by enzyme molecules (mainly protein kinases that activate proteins at each next stage of signal transmission) as well as adaptor and GTP-binding proteins [6, 113].

The most promising in the study of the pathogenesis of myasthenia are T-cell receptor associated signaling pathways, which are determined by the interaction of the main lymphocyte receptor with coenzyme molecules as well as Toll-like receptor signaling pathways [4, 11, 17, 22, 45, 52, 77].

The end product is the transcription factors that lead to a change in gene activity resulting from the enhancement or suppression of the secretory function of cells in the immune system [93].

T cell receptor (TCR) determines the functional activity of each T-lymphocyte, which is the most important structure on the lymphocyte membrane. The receptor makes it possible to recognize only antigen fragments associated with histocompatibility molecules. Each T cell has its own unique receptor. Each TCR is strongly associated with CD3 as well as with CD4 or CD8 co-receptor molecules [38]. TCR and co-receptors are bound by an enzyme of non-receptor Src family tyrosine kinases (Fyn, Blk, Lyn in B-lymphocytes, Lck and Fyn in T-lymphocytes) [112].

Its dephosphorylation occurs when there is antigenic stimulation with the participation of phosphatase CD45, which causes it to be activated. Activated Src kinase (Lck) phosphorylates ITAMs* that are bound to the receptor, which increases the activity of another kinase, Zap70, which begins to phosphorylate the adaptor proteins: LAT (Linker for Activation of T cells) and SLP-76, BLNK and SLP-65 [109, 114]. Adaptive proteins, binding to enzymes (tyrosine kinases) of the Tec family, increase the activity of one of the most important key enzymes — the phospholipase Cy, which breaks up the phosphatidylinositol biphosphate on the cell membrane for phosphatidylinositol triphosphate and diacylglycerol. These molecules trigger activation of the pathways responsible for the function of transcription factors NF-kB, NFAT and AP-1, initiating the transcription of genes responsible for the differentiation, proliferation, and effector activity of T cells [89].

In the generation of signals transmitted from the polypeptide chains of the TCR-CD3 complex, the presence in the cytoplasmic part of the complex of the ITAM activation sequence associated with ZAP-70 is the key factor in the signal transmission from TCR when it binds to the ligand [76, 109, 111, 114].

* ITAM — (Immunereceptor tyrosine-based activation motif) — tyrosine-containing activation sequences of amino acids in immunoreceptors.

In addition to CD45, another key regulator of the activation cascade of transcription factors affecting the functioning of immunocompetent cells is presented by PTPN22, an immune homeostasis regulator that inhibits T cell receptor signaling and the selective promotion of type I interferons, which affects ZAP-70 activity by Lck kinase after activation of receptors [29, 49].

It should also be noted that the T cell receptor, due to the fact that it binds to other molecules and co-receptors, can transmit both strong and weak signals, which are necessary both to maintain cell survival at the periphery and to create self-tolerance mechanisms [112].

Pathologies in the structure of this complex can thus cause the malfunctioning of T cells and the development of autoimmune diseases such as autoimmune diabetes, systemic lupus erythematosus, and systemic scleroderma [46]. Since most autoimmune diseases are considered antigen specific, the pathology in the T cell receptor structure, or the disruption of its functional activity, play a decisive role in the pathogenesis of diseases [13, 89].

Each receptor complex has connections with the intracellular system of enzymes, and the most important are tyrosine kinases and phosphatases.

The function of tyrosine kinases lies in the substrate phosphorylation of tyrosine groups of the target proteins. These are responsible for their activation and the manifestation of cell functions. The role of PTPN22 and CD45 in the development of several autoimmune diseases has been demonstrated the most conclusively, and there are some reports on their role in the pathogenesis of myasthenia gravis [14, 31, 61, 77, 105].

The main role in the transfer of receptor kinases to the "working" state is performed by the molecule CD45, in which tyrosine phosphatase (double) is active. CD45 deficiency leads to the development of manifestations of severe combined immunodeficiency. CD45 serves as a genetic modifier for autoimmune, infectious, and malignant diseases. Its prominence is limited to all nuclei of hematopoietic cells. In general, it becomes more prominent as the cells mature. There are several isoforms whose functional activity affects the functioning of T cells [105].

Some studies have shown a reduction in the prominence of CD45 in patients with SLE in comparison with the control group. However, information on the possible involvement of CD45 in the pathogenesis of myasthenia gravis is contradictory [54, 84].

Another enzyme that plays the role of a potent inhibitor of activation of T cell signaling is PTPN22, due to dephosphorylation processes. It suppresses the function of Lck and Fyn and activates the Lyp-enzymatic pathway [20, 29, 37, 80, 82].

A number of studies have shown the association of PTPN22 with the development of diseases such as type 1 diabetes, rheumatoid arthritis and SLE, as well as its role in increasing the risk of developing juvenile idiopathic arthritis, thyrotoxicosis, autoimmune thyroiditis, myasthenia gravis, generalized vitiligo, and others belonging to the group of autoimmune diseases [14, 61].

The Role of Genes in the Pathogenesis of Myasthenia Gravis

According to modern data, the mechanisms that disturb tolerance to autoantigens are associated with changes in expression of autoantigens caused by exposure to harmful factors as well as genetic peculiarities. Many genetic factors affect the predisposition and onset of the disease [19, 35]. It is known that the lack of immune response to its own antigens is a consequence of the formation of immunological tolerance at a certain stage of individual development. There are both active and passive mechanisms for the formation of self-tolerance. The passive mechanism is the ignoring of autoantigens by the immune system, which is caused by their low concentration or by the isolation from it. The active ones include elimination of autospecific clones, correction of autoreceptor genes, induction of anergy of autospecific clones, and inhibition of immune response by regulatory cells. Currently, the search for new candidate genes involved in the pathogenesis of the disease is underway [18, 19, 60].

More than thirty years ago, the genes of the HLA-system (major histocompatibility complex (MHC) class II locus)* were identified, which are associated with an increased risk of myasthenia gravis [39, 50]. During studies that were conducted over the last decade, such genes as the gene of the type 22 protein

* MHC (abbr. of English *Major Hystocompatibility Complex*) in humans was later described in the works of J. Dosse. It has been designated as HLA (abbr. of English *Human Leukocyte Antigen*) in humans, as it is associated with leukocytes. There are two main classes of MHC molecules: it is conditionally accepted that MHC class I induces a predominantly cellular immune response while MHC class II produces a humoral response.

tyrosine phosphatase non-receptor (*PTPN22*), *TNFAIP3* — the gene of interacting proteins 1 (TNIP1), the gene of cytotoxic T-lymphocyte protein 4 (*CTLA4*), , and a number of other genes have been identified [19, 24, 64].

An association with these genes has also been revealed in pediatric patients [42]. This fact can become an argument in favor of the theory of unified genetic mechanisms for the formation of neuromuscular transmission disorders.

It is believed that the relationship of myasthenia with a number of other autoimmune diseases has recently become more evident, many patients often have a serious medical history or family medical history with a number of other diseases, the associations of myasthenia with autoimmune thyroiditis, rheumatoid arthritis, and type 1 diabetes are mentioned most often [31, 75].

According to different authors, in the youngest patients the most frequent myasthenia gravis is associated with the genes *PTPN22* (a gene that encodes tyrosine phosphatase*, where defects lead to an increase in autoreactivity), *HLA* and *TNFAIP3* [63]. The key genes involved in the development of myasthenia gravis are *IRF5* (the gene of the interferon-5 regulatory factor), *TNFAIP3* (the gene of predisposition for TNF-α-induced protein 3, also known as A20), and the interleukin-10 gene (*IL10*); the genes *TNFSRF11* and *CTLA4*** are associated with myasthenia gravis in the elderly because of their regulatory function.

Therefore, it is advisable to study the role in the development of myasthenia of those genes, which are related not only to the HLA system [19, 39].

Conclusion

There are currently still many questions regarding the characteristics of pathogenesis of myasthenia gravis. Researchers continue to search for new targets that make it possible to improve diagnosis and to develop new directions in the treatment of disease. In addition to the antibodies that target acetylcholine receptors that were isolated in the 70s and 80s, other antibodies (that target MuSK and ryanodine receptors, titin, lipoprotein-bound receptor 4, etc.) have also been identified. Differences in the clinical manifestations of myasthenia in patients with different serological markers have been described.

With the development of biotechnological methods, researchers were able to identify the subtype of lymphocytes involved in the development of myasthenia gravis. It became possible to separate individual populations of lymphocytes from the patient's blood and to study their function *in vitro*. The development of genetic technologies and decoding of the human genome has made it possible to investigate the role of genes that are related to more than just the HLA system in the pathogenesis of myasthenia gravis. However, despite the active research of various mechanisms by which myasthenia gravis develops, many unresolved problems still remain.

The successes that have been achieved are prerequisites for the search for new therapeutic targets. Every year, the number of studies that seek to compare clinical manifestations depending on the serological and genetic characteristics of patients continues to grow [41, 68, 111].

Conflict of Interests

The authors declare no conflict of interests.

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* Tyrosine phosphatase functions as a key regulator of immune homeostasis. Normally, it inhibits the transmission of T cell receptor signals and selectively stimulates the response of type I interferon in myeloid cells. When it has a defect, it results in an increase in autoreactivity [14, 61, 95].

** Synthesis of the CTLA4 gene is higher in the activated T cells. It increases T cell mobility and reduces contact periods between T cells and antigen-presenting cells, which leads to a decrease in cytokine production and proliferation. The defect of the CTLA4 gene has a significant effect on the T cell component of immune system regardless of age [98].

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Article received on 13.03.2018
Accepted for publication on 15.05.2018

UDC 616.36-002-03-08

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HYPERAMMONEMIA IN PATIENTS AT PRE-CIRRHOTIC STAGE: CLINICAL REALITY?

Abstract

Ammonia belongs to the common neuro- and cytotoxic metabolites in the human body. It is established that ammonia has hepatotoxic properties. Ammonia induces the formation of oxygen active forms, reduces the activity of endothelial NO synthase, dose-dependently decreases the cellular metabolism and proliferation of stellate cells, and promotes fibrogenesis, disturbance of intrahepatic hemodynamics and, accordingly, the formation of portal hypertension. The article describes causes of hyperammonemia in pathological conditions and physiological functions disorder. The increased level of ammonia is associated not only with various neuropsychiatric disorders in patients with liver cirrhosis, but is also shown in patients with chronic liver disease (CLD) at the pre-cirrhotic stage. The sign of minimal hepatic encephalopathy in patients with chronic hepatitis is a cognitive impairment, which manifests as a decrease in concentration, in particular when driving. The effect on hyperammonemia becomes a target for therapy in steatohepatitis of various etiologies. The use of the oral form of L-ornithine-L-aspartate effectively reduces the level of ammonia in the blood, improves cognitive function and positively affects the functional state of the liver in patients with CLD at the pre-cirrhotic stage.

Key words: *steatosis, steatohepatitis, chronic hepatitis C, hepatic encephalopathy; hyperammonemia, L-ornithine-L-aspartate*

For citation: Ilchenko L. Yu., Nikitin I. G. HYPERAMMONEMIA IN PATIENTS AT PRE-CIRRHOTIC STAGE: CLINICAL REALITY? The Russian Archives of Internal Medicine. 2018; 8(3): 186-193. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-186-193

DOI: 10.20514/2226-6704-2018-8-3-186-193

eNOS — endothelial nitric oxide synthase, LOLA — L-ornithine-L-aspartate, NH_3 — ammonia, NH_4^+ — ammonium ion, NO — nitric oxide, ATP — adenosine triphosphate, VEPs — visual evoked potentials, BBB — blood-brain barrier, CFF — critical flicker-fusion frequency, MHE — minimal hepatic encephalopathy, NAD — nicotinamide adenine dinucleotide, NAFLD — non-alcoholic fatty liver disease, NASH — non-alcoholic steatohepatitis, TrR — Traffic Rules, HE — hepatic encephalopathy, NCT — number connection test, CH — chronic hepatitis, CHB — chronic hepatitis B, CHC — chronic hepatitis C, CLD — chronic liver diseases, CNS — central nervous system, HC — hepatic cirrhosis

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a range of neurological or mental (cognitive or behavioral) disorders that occur in patients with hepatic insufficiency and/or portosystemic shunts. The clinical picture of HE varies from minimal and subclinical to pronounced manifestations, which can result in a coma [1, 2].

The pathogenesis of HE has not been completely deciphered. It is complex and involves many factors. In recent years several hypotheses

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have been discussed, but much of the evidence points to ammonia as the main cause of impaired brain function, and researchers have focused on developing therapies for hyperammonemia [3, 4]. However, despite the understanding of the importance of the role of ammonia in the pathogenesis of HE, its elevated level in the blood is not as essential for its development as the amount of ammonia penetrating the blood–brain barrier (BBB) [5].

Ammonia and Its Metabolism

Ammonia is the final product of nitrogen metabolism in the human body. Under normal conditions, the balance of nitrogen and ammonia is constantly maintained. Up to 60% of ammonia is formed in the liver with deamination of glutamine and other amino acids. A small amount is produced by the decay of glutamine in the small intestine and in the muscles during exercise. In addition, the gut microbiota decomposes protein and urea, which as a result also produces a certain amount of ammonia [6].

In the blood of a healthy person, the normal level of ammonia (NH_3) varies from 15 to 60 μg / 100 ml (21–50 μmol/l). In tissues and intercellular fluids normally, ammonia is usually proton-bound and presents in an ionized form — ammonium ion (NH_4^+). The concentration of unionized NH_3 is negligible (about 1%), and it does not penetrate the BBB. Ammonium ion content in fresh plasma is less than 20 μg per 100 ml, which confirms the extraordinary effectiveness of biochemical reactions for the removal of this highly toxic substance.

There are several mechanisms involved in ammonia neutralization, the main one of which undergoes in the periportal hepatocytes. In the Krebs-Henseleit urea cycle, about 30 g of urea is thus formed from 100 g of protein supplied with food, which is excreted by the kidneys. In addition, ammonia is metabolized by the formation of glutamine from glutamate in perivenous hepatocytes, muscles and in the brain (in astrocytes), as well as by the amination of alpha-keto acids in the synthesis of amino acids [3, 6].

Causes of Hyperammonemia

A heightened level of ammonia in the blood is defined as hyperammonemia. Being a toxic compound, ammonia is present in the blood of a healthy person in relatively low concentrations, but even a slight increase has an adverse effect on the body, and, above all, on the central nervous system (CNS). Symptoms of poisoning are manifested when the ammonia level is exceeded by 2–3 times.

In actual clinical practice, there are two main types of hyperammonemia:

1. Acquired hyperammonemia caused by the development of hepatic cirrhosis (HC) and/or portosystemic shunts.
2. Hereditary hyperammonemia resulting from various genetic defects in the enzymes of the urea formation cycle.

Increased level of ammonia in the blood is an indicator of the change of its metabolism in the liver. In previous studies, when a patient is suffering from non-alcoholic fatty liver disease (NAFLD) at the stage of steatosis and at cirrhosis, the activity of the enzymes in the urea synthesis cycle and the synthesis of glutamine in hepatobioptates thus decrease by 20% and 50%, respectively, in comparison with healthy individuals [7, 8]. An increase in the blood ammonia level is associated with various pathological conditions or disorders of physiological functions [1, 2, 9].

Hyperammonemia is typical not only for patients with hepatic insufficiency. It can be observed when there is bleeding from various parts of the gastrointestinal tract in patients without HC as well as in patients suffering from heart failure, pulmonary heart disease, leukemia, certain endocrine disorders (decompensated diabetes mellitus, severe thyrotoxicosis), patients underwent bypass surgery, etc.

In addition, an elevated level of ammonia is noted in patients suffering from Reye's syndrome (acquired deficiency of the enzymes of the ornithine cycle of urea synthesis and, as a consequence, microvesicular steatosis), disorder of liver perfusion, metabolic alkalosis and acidosis,

bacterial overgrowth syndrome, and prolonged constipation.

Hyperammonemia can be observed in any pathological conditions that are accompanied by increased protein catabolism (those suffering from extensive burns, compression or crush syndrome, extensive purulent necrotic processes, gangrene of the extremities, sepsis, etc.). These disorders cause body muscle atrophy, antioxidant defense depletion, as well as significantly weakened and suppressed immunity.

High protein diet, fasting, overeating, intense physical activity (mainly in men and bodybuilders), childbirth can also cause the level of this toxin to increase in the body.

The level of ammonia in the blood will increase when taking a number of medications, namely: salicylates, tetracycline, asparaginase, thiazide diuretics, valproic acid, ethacrynic acid, isoniazid, etc.

Hyperammonemia can develop due to the consumption of a large amount of alcohol together with the use of psychoactive drugs. And smoking even one cigarette increases the level of ammonia in the blood by 10 μmol/l.

Ammonia Is an Endogenous Toxin

Ammonia is one of the main neurotoxic metabolites in the human body. The increased supply of ammonia through the BBB depletes the reserves of glutamate and, on the contrary, promotes the excessive accumulation of glutamine in the brain (in the ammonia neutralization reaction via glutamine synthetase), which causes swelling and edema of astrocytes, inhibition of gamma-aminobutyric acid synthesis (GABA), and impaired transmembrane transport of electrolytes (Na^+ and K^+), thus worsening the chemical neuromediation. In addition, when ammonia is insufficiently neutralized, a decrease in the concentration of α-ketoglutarate (a product of glutamate metabolism), inhibition of transamination, and synthesis of neurotransmitters are noted. These pathological processes, along with the increase in alkalosis with hyperammonemia, increase hypoxia and decrease metabolism in astrocytes, neurons and, ultimately, lead to the development of HE [6].

In addition to its neurotoxicity, ammonia has general cytotoxic, including hepatotoxic, properties, which have been confirmed by new data obtained in the recent years [10, 11].

In a liver with only steatosis, in the absence of clinical manifestations of inflammation and hepatic insufficiency, ammonia thus induces formation of active forms of oxygen, dose-dependently decreases the cellular metabolism and proliferation of stellate cells, reduces the activity of endothelial NO synthase (eNOS), enhances the processes of fibrogenesis, disrupts intrahepatic hemodynamics and, accordingly, contributes to the formation of portal hypertension.

Minimal Hepatic Encephalopathy

In the case of HE, varying degrees of neuropsychiatric symptoms that reflect changes in consciousness, intelligence, behavior and neuromuscular disorders are evaluated. There are 4 stages of HE (ranging from mild to coma). In addition, in patients with chronic liver disease (CLD) minimal hepatic encephalopathy (MHE) is also identified, in which case the detection of neuropsychiatric symptoms requires the performance of various psychometric tests, and no clinical manifestations of HE are found as the result of routine clinical examination. This, first of all, can concern patients with CLD at the precirrhotic stage. Earlier, MHE was defined as latent or subclinical HE.

Evidence of the presence of MHE is the cognitive impairment that is revealed during the course of testing of the speed of psychomotor reaction / executive functions, or neurophysiological changes without clinical signs of mental changes. Such patients have decreased attention span, operative memory, difficulty in making decisions, decreased ability to drive a car, and altered handwriting. In general, the appearance of MHE worsens the quality of life and increases the risk of developing clinically pronounced HE.

A lot of tests have been proposed for the detection of MHE. In routine clinical practice, the number connection test (NCT), the digit-symbol

substitution test, and the line tracing test have become the most widely adopted [1]. They reveal an impairment of visual-spatial orientation, lowered cognitive processing speed, and decreased accuracy of fine motor skills in patients. To reduce the learning effect, the determination of visual evoked potentials (VEPs) of the brain, the critical flicker-fusion frequency (CFF) are also used for the purpose of dynamic estimation of MHE. In addition, the so-called "Repeatable Battery for the Assessment of Neuropsychological Status" (RBANS) is used in scientific research to evaluate neuropsychological status, including a test for the examination of eyesight, memorization of multisense words from the list, text (story) or numbers, association test, pattern copying, counting backwards or with an interval of 3, different scales (anxiety, depression, sleep disorders) [12, 13]. Training models are also developed in the form of either computer games or training programs that recreate real situations, which can be used to diagnose MHE when the following symptoms are manifested: decreased attention, delayed decision-making, and decreased ability to drive a car.

Hepatic Encephalopathy in Chronic Liver Diseases in Pre-Cirrhotic Stage: Clinical Reality?

No one has any doubt about the possibility of developing HE in patients with HC. To identify it, various methods are used, but the clinical manifestations of HE may not be obvious, which makes it difficult to estimate its incidence and prevalence rates. At the time when HC is diagnosed, the prevalence of apparent HE is 10–14% [14]. MHE occurs in 20–80% of patients with HC [15, 16]. However, in routine clinical practice, some patients with CLD in the pre-cirrhotic stage note a decrease in memory and attention, mood change, loss of interest in previously important personal values, etc. These patients also have difficulties making decisions and make frequent mistakes while driving a car. These manifestations require interpretation, and other causes that can lead to these disorders (in particular, vascular pathology, metabolic or electrolyte disorders, mental disorders, etc.) must be excluded.

Studies from recent years have allowed us to accumulate data on the diagnosis of MHE in patients with steatosis, chronic hepatitis C (CHC) and B (CHB), alcoholic and non-alcoholic steatohepatitis (NASH), and they have also allowed us to determine hyperammonemia in patients with initial liver changes [17–20] that cause cognitive impairment, which are manifested in particular in stressful situations that require a decision to be made [17–21].

For example, in our psychometric tests 78% (109/140) of the patients with different stages of fibrosis, including patients with chronic hepatitis, had a significant increase in the NCT time, and 40.7% (57/140) exhibited a drop in the frequency of perceived VEPs flickers (HEPAtonorm™ Analyzer, Germany) [18]. A negative correlation ($r = -0.53$, $p < 0.01$) was obtained between the frequency by which VEPs flickers were perceived and the NCT time, as well as between their changes as HE became more pronounced. Using the spectrophotometric method of ammonia determination, it was possible to record its elevated content in venous blood (up to 89 μg/dl) in 57/78 (78.1%) patients with CLD with pre-cirrhotic stage (CH), which may be the cause of MHE development in this group.

Correction of hyperammonemia in patients with CLD in the pre-cirrhotic stage was performed with L-ornithine-L-aspartate (LOLA) at a dose of 15 g of granulate per day for 2 weeks. The ammonia level normalized, and the obtained results of psychometric tests improved [18].

Evidence of the presence of MHE in patients with CHC includes cognitive impairments, which are manifested by the decreased ability to concentrate when driving a car [21].

In order to establish the connection between the frequency of violations of the Traffic Rules (TrR) and the presence of minimal signs of liver damage, the "Smart Radar" study was performed. Sixty men with symptoms of low activity MHE and CHC were examined. All patients regularly drive a car. NCT was performed, CFF and NH_4^+ content in plasma were determined, and data on the frequency of TrR violations were analyzed. All patients with CHC received LOLA at

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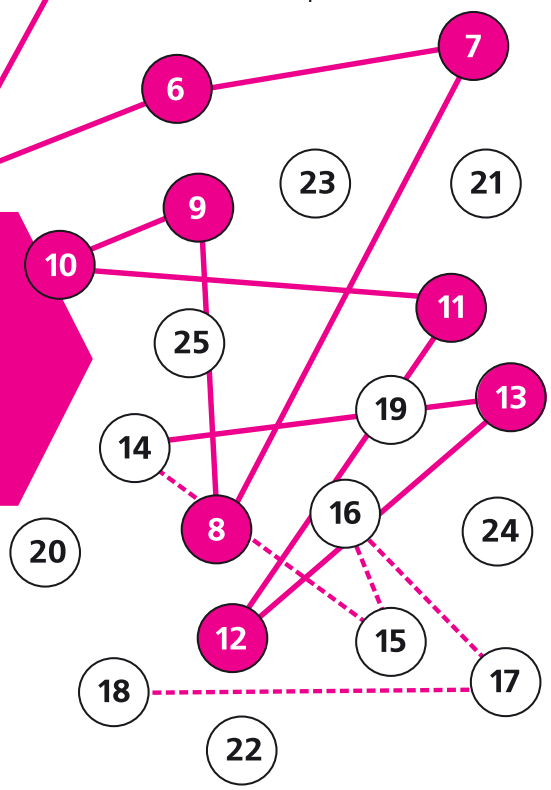

*Е. А. Агеева, С. А. Алексеенко «Опыт применения пероральной формы препарата «L-орнитин-L-аспартат» при гипераммониемии у больных с хроническими заболеваниями печени на доцирротической стадии». **R.Jalan, F.De Chiara et al. J.Hepatology 2016 vol.64 p. 823-833. ***Grungreiff K., Lambert-Baumann J., Die Medizinische Welt, 2001; 52: 219-226. ИНФОРМАЦИЯ ДЛЯ СПЕЦИАЛИСТОВ. РУ в РФ: П № 015093/01 от 22.03.2007

a dose of 12 g/day every 2 months followed by a break of 2 months. The duration of the study was 12 months. As a result of the therapy, patients with CHC were able to improve their concentration while driving vehicles: the frequency of violations of TrR, the time of performance of the NCT were significantly reduced, and there was an increase in CFF in comparison with members of the healthy control group. In addition, the mean concentration of NH_4^+ decreased in plasma (from 141.8 ± 35.8 μM to 91.8 ± 32.6 μM, $p < 0.003$) [21].

When fractional LOLA therapy was performed, a decrease in the ammonia content, improvement of cognitive functions and, as a result, a reduction in the frequency of violations of TrR (one of the leading symptoms of MHE) was thus all observed.

In another study, the effectiveness of different treatment options using the oral LOLA form was evaluated with hyperammonemia in 37 patients with NAFLD and CHC who had stages 1–2 fibrosis [20]. Six months after the first course of therapy (9 g/day for 4 weeks), the level of ammonia in the venous blood that was determined using the enzymatic method was maintained within reference values in 25 patients. Twelve (32.4%) patients with newly detected hyperammonemia underwent a second course of treatment with the same daily dose for 10 days per month for 12 weeks. After the completion of the therapy in this group of patients, the level of ammonia in the blood decreased to 25.4 ± 1.9 μmol/l, which corresponded to the parameters of the control group of healthy individuals [20].

Important conclusions have been drawn that hyperammonemia occurs in patients with CLD (NAFLD and CHC) at the pre-cirrhotic stage; there is a recurring course of hyperammonemia, and the use of the oral form of LOLA effectively reduces the level of ammonia in the blood with different courses of treatment.

The progression of fibrosis is the key mechanism leading to the development of HC and its complications, which contributes to increased mortality in patients with NAFLD. In this regard, a decrease in the level of ammonia, as a hepatotoxin, may become a new target in the treatment of non-alcoholic steatohepatitis [22].

"Hepatoprotective" Properties of LOLA

LOLA has a number of positive properties, which allow us to classify it as one of the medicines that have hepatoprotective properties (complete absorption, presence of the effect of first pass through the liver, suppression of fibrogenesis, natural metabolism in the liver disorder, lack of toxicity, etc.) [23].

In hepatocytes, ornithine-aspartate promotes an increase in the synthesis of NAD (nicotinamide adenine dinucleotide) and prevents the decrease in the content of ATP (adenosine triphosphate) as a result of decreased cytolysis under the influence of alanine, which is synthesized via the metabolism of aspartate. In addition, due to transamination with α-ketoglutarate, ornithine acquires antioxidant properties [24].

Numerous studies have established the hepatoprotective properties of LOLA, which can be effectively prescribed to patients with CLD of different etiologies [25–31].

Data from a non-randomized prospective cohort study performed at multiple centers in Germany in 2001, involving 1,167 patients with CLD, including 648 patients with NASH and 253 with CH, demonstrated that LOLA was highly effective (decreased activity of alanine and aspartate aminotransferases, gamma-glutamyl transferase at 40–50%) and well tolerated [25].

During the course of clinical trials, patients with steatosis and steatohepatitis of various etiologies experienced relief of their asthenic, dyspeptic and pain syndromes and lost excess body weight, which made it possible to expand the set of indications for the use of LOLA [32]. The therapeutic efficacy of the oral form of LOLA was demonstrated, which was manifested in the improvement of the functional state of the liver, the positive effect on lipid metabolism, the reduction of cognitive impairment, and the improvement in the quality of life [27–31].

During the ornithine cycle, LOLA participates in the synthesis of arginine. When it is stimulated, nitric oxide (NO) is produced, which helps promote blood flow in the liver, muscles, brain, etc.

By affecting porto-hepatic hemodynamics, LOLA thus improves intrahepatic blood flow, which was obtained by performing polyhepatography in patients with chronic hepatitis of different etiologies, including NASH [33].

The correction of porto-hepatic blood flow disorders is an important aspect of pathogenetic therapy. Its effectiveness makes it possible to improve the regeneration of liver cells and reduce the progression of CLD.

New Possibilities for Determining Ammonia

Ammonia is one of the most important neurotoxins. However, in routine clinical practice there are no methods for identifying it in the brain. In order to identify its content in the blood, various methods have been developed: ionometric, spectrophotometric, enzymatic, etc. However, most methods of quantitative analysis of ammonia are rather laborious, since it is necessary to observe a cooling procedure and blood sampling technique. Prolonged application of a tourniquet or clenching of a fist can thus lead to an increase in its concentration and a false-positive result. In addition, the analysis should be carried out as soon as possible after blood sampling, as the concentration of ammonia increases in direct proportion to the storage period of the sample. The arteriovenous difference in the content of ammonia in the vessels, whose concentration is lower at rest in venous blood than in the arterial blood, should also be considered. It is caused by the binding of ammonia in the muscle tissue.

Rapid point-of-care testing is ideal for the quantitative analysis of ammonia. For this purpose, a portable PocketChem BA analyzer was developed, which makes it possible to determine the level of ammonia in the entire blood by microdiffusion. Ions of ammonia from a blood sample (20 μl), when applied to a test strip impregnated with a salt of boric acid, pass into the gaseous state and, when they reach the indicator (bromocresol green), change its color. The degree of color change is proportional to the concentration of produced ammonia (measuring range — 8–285 μmol/l, time of the test — 180 s). The device is extremely compact: it fits in the palm of an adult person, it is easy to operate, and it can be used by the patient himself [34].

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 03.05.2018
Accepted for publication on 21.05.2018

UDC 616-097-022:578.826.6

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NOSOLOGY AND AGE-GENDER STRUCTURE OF HIV-INFECTED PATIENTS AT EMERGENCY MEDICAL SERVICE CLINICS

Abstract

We retrospectively studied the age-gender structure and nosology of urgent pathology among HIV-infected persons at N. V. Sklifosovsky Research Institute of Emergency Medicine. For the period from 2008 to 2015, the number of patient's hospitalizations with HIV to the emergency medical service clinics increased by 1.5 times among men ($R^2 = 0.63$, $p = 0.0188$) and women ($R^2 = 0.84$, $p = 0.0013$). The greatest number of HIV-infected persons registered at the emergency departments ($R^2 = 0.57$, $p = 0.03$). The main shares of people with HIV infection were in the age groups of 18–30 and 31–40. At the same time, we revealed multidirectional trends connected with decreasing number of hospitalized patients in the age group of 18–30 ($R^2 = 0.51$, $p = 0.04$) and increasing in groups of 31–40 ($R^2 = 0.71$, $p = 0.008$) and 41–50 ($R^2 = 0.89$, $p = 0.0004$). The share of HIV-infected men decreased from 68.1 to 65.1% while for women it increased from 31.9 to 34.9%. The differentiation of the epidemic process for HIV-infection among patients in different clinical departments was noticed.

Key words: *HIV, HIV-infection, prevalence, hospital, emergency room, retrospective study*

For citation: Solonin S. A., Shakhova O. B., Belova M. V., Godkov M. A. NOSOLOGY AND AGE- GENDER STRUCTURE OF HIV-INFECTED PATIENTS AT EMERGENCY MEDICAL SERVICE CLINICS. The Russian Archives of Internal Medicine. 2018; 8(3): 194-203. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-194-203

DOI: 10.20514/2226-6704-2018-8-3-194-203

UNAIDS — Joint United Nations Programme on HIV/AIDS, MSC AIDS — Moscow City Center for AIDS Prevention and Control, SRIEM — N. V. Sklifosovsky Research Institute of Emergency Medicine, PSs — psychoactive substances, EMSC — emergency medical service clinics

Introduction

According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), Russia as well as Ukraine, Estonia and Moldova lead the world in the growth of the HIV incidence rate. In 2015, of all the member countries of the WHO Regional Office for Europe, our country accounted for 80% of all new HIV infections [1]. The territorial centers for the prevention and control of AIDS have reported 95,475 new cases of infection, and the total number of HIV-infected citizens of the Russian Federation has exceeded 1 million. Currently, an increase in the incidence and prevalence of HIV infection in the population is observed in most regions [2]. The main reason for the high HIV prevalence in the population is associated with the high rate of viral shedding among drug users. According to the Ministry of Internal Affairs of the Russian Federation, the number of people who use psychoactive substances (PSs) at varying degrees of frequency is more than 2.2 million people [3]. Sharply increased migration flows also contribute to the development of the epidemic process [4].

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Previously it was believed that HIV infections are concentrated mainly among socially marginalized groups of people whose way of life is socially stigmatized or is associated with crime [5]. Recent data indicate that the HIV epidemic is spreading from vulnerable groups to the general population [2]. HIV is actively spreading among socially advantaged members of society [6, 7]. The increase in HIV infection rate among women has caused much concern: in 2016 more than 39,000 new cases of infection were revealed; in total there were more than 410,000 such cases [2]. The main reason for this is related to the active sexual transmission of the virus. An important piece of evidence that HIV is being transmitted sexually is the increasing number of cases of infection detected in pregnant women [8].

One of the key priorities for combating the HIV epidemic in Russia is to ensure that a large percentage of the population undergoes testing in order to detect infection and prescribe antiviral therapy in a timely fashion [4]. However, when assessing the incidence, it is extremely important to take into account not only the coverage of testing, but also the demographic characteristics of the surveyed population. In our country, an annual increase in the number of completed HIV tests is associated with a low level of testing of members of vulnerable groups [9]. These groups are traditionally thought to include injecting drug users, men who have sex with men, commercial sex workers, homeless, prisoners, and illegal migrant workers [4]. Representatives of these groups avoid seeking treatment at outpatient clinics or do not indicate their membership in these cohorts for fear of social discrimination and stigmatization [10]. Rules and regulations at institutions of outpatient care prevent these individuals from obtaining necessary medical assistance for the diagnosis, prevention, and treatment of HIV infection [11]. According to the evaluation of experts at the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor), up to a third of infected Russians are not aware that they have HIV [12]. In such a situation, a high percentage of the population that is vulnerable to HIV infection remains outside the existing epidemiological surveillance system.

One way to estimate the prevalence of HIV infection among such cohorts is to analyze the results of a screening survey of patients seeking specialized care at emergency medical service clinics (EMSC). This approach is particularly relevant due to increased rates of physical trauma reported by citizens suffering from drug abuse and socially deviant behavior (alcoholism, drug addiction, etc.), that is, among those most at risk of HIV infection.

Objective: To study the nosology and age-gender structure of HIV-infected patients entering the emergency medical service clinic.

Materials and Methods

A retrospective analysis of the types of requests for treatment of patients with HIV infections at the specialized departments of EMSCs was conducted. Testing for HIV infection was carried out with the informed consent of the patient or his legal representative. HIV testing was performed at the Department of Laboratory Diagnostics of the N. V. Sklifosovsky Research Institute of Emergency Medicine (SRIEM). The enzyme-linked immunosorbent assay and test systems, which have been approved for use in the Russian Federation in accordance with established procedure, were used. Confirmation of the results of the screening study was performed by immunoblotting in the laboratory of the Moscow City Center for AIDS Prevention and Control (MCC AIDS). The incidence of HIV was assessed in absolute terms and in terms of the rate of detection as a percentage. The amount of HIV-positive blood samples was estimated as the amount of HIV-infected patients' requests to EMSCs. Epidemiological evaluation was carried out according to departments specialization and also while taking into account the data on the age-gender structure of the patient population for 2008–2015. Statistical processing of the data was performed using Graph Pad Prism 7 (Graph Pad Software, USA). Pairwise comparison of the distribution of HIV incidence rates in groups was performed using the Fisher's exact test (two-tailed P values). A regression analysis (ordinary least squares) was used to determine trends. In order to assess the informativeness and significance of the regression equation, the coefficient of

determination R^2 was calculated. Differences were assessed as statistically significant at the 95% probability ($p < 0.05$) or higher.

Results

For the period from 2008 to 2015, 191,564 patients were examined for HIV infection at the clinical and intensive care departments of SRIEM. There were 2,946 cases of admission of HIV-infected patients, which amounted to 1.5% of the total number of those who were surveyed. Over the course of seven years, the number of HIV-infected patients applying to the hospital increased by 1.5 times ($p = 0.0068$) from 273 to 410 people, and the detection rate increased by 0.3% (from 1.2 to 1.5%) (Fig. 1). Statistics on the number of identified HIV cases in patients at SRIEM during the analyzed period did not follow any clear trends: linear growth was recorded in 2008–2013 ($R^2 = 0.87$, $p = 0.005$) with peaks in 2012–2013, though that trend was reversed by a gradual decline in 2014–2015. The causes of changes in admission trends of patients with HIV at SRIEM require further thorough study.

It should be noted that the detection of HIV infection in patients at the Institute exceeds the comparable data for other Moscow clinics specialized in non-infectious diseases by double on average [13]. The high incidence of HIV infection in the SRIEM patients can be attributed to the fact that the EMSC has specialized units that provide emergency and primary medical care to victims with injuries and acute illnesses of various origins, including an overdose of PSs. In addition, this is due to the characteristics of the contingents of patients who are in need of emergency and primary medical care. It is shown that among the patients at SRIEM, the share of citizens suffering from various kinds of drug abuse and socially deviant behavior is high. They lead ways of life that are associated with increased rates of various kinds of physical trauma [14].

Fig. 1 presents a very general picture of the HIV situation at the hospital. When assessing the epidemiological situation at SRIEM, it is important to consider not only the trends in admissions and rates of detection, but also the specialization of the units in which they are identified.

A retrospective study of the types of calls for medical care of HIV-infected people at the specialized EMSC departments has revealed a statistically significant trend towards an increase in the number of admissions to the intensive care units at the Institute ($R^2 = 0.57$, $p = 0.03$). During the analyzed period, the number of infected patients admitted to the resuscitation department at SRIEM increased by 1.8 times ($p = 0.0004$) (from 126 in 2008 to 224 in 2015), which was 46 and 55% of the number of all patients admitted to the hospital with HIV, respectively (Tables 1, 2).

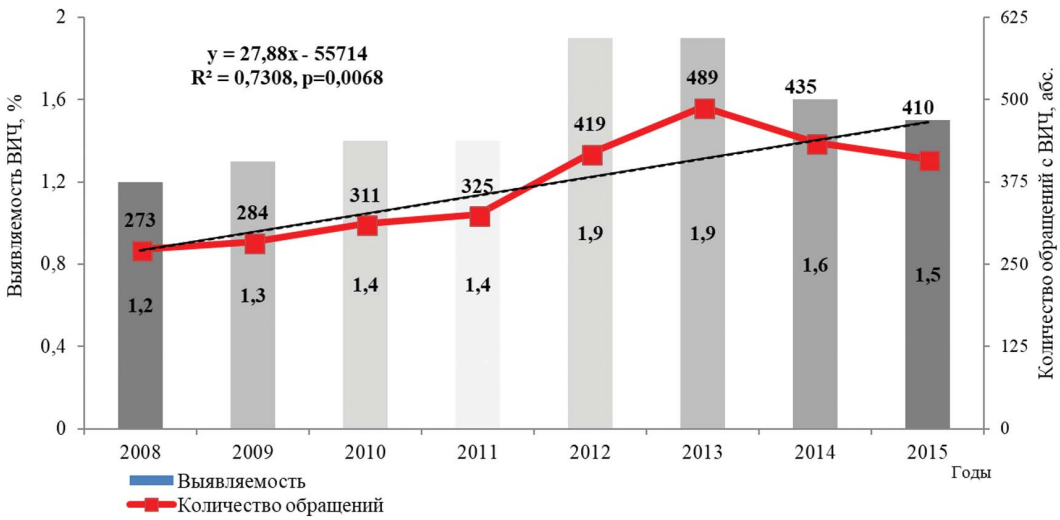

***Fig. 1** — Number of cases and detection of HIV-infection in patients at the N. V. Sklifosovsky Research Institute of Emergency Medicine, 2008–2015*

Table 1 *— Detection and admission profile of HIV-infected patients to N. V. Sklifosovsky Research Institute ofEmergency Medicine, 2008–2011*

Profile/year	2008			2009			2010			2011		
	*NTP	n	%	NTP	n	%	NTP	n	%	NTP	n	%
Toxicological resuscitation	1,903	91	4.8	1,697	100	5.9	1,515	119	7.9	1,618	143	8.8
1st Unit of acute poisoning treatment	1,477	22	1.5	1,385	26	1.9	1,329	32	2.4	1,296	22	1.7
2nd Unit of acute poisoning treatment	858	27	3.1	853	23	2.7	834	28	3.4	788	34	4.3
Mental health unit	777	20	2.6	708	21	3.0	674	23	3.4	627	23	3.7
Neurosurgical intensive care unit	89	0	0.0	72	2	2.8	87	0	0.0	123	0	0.0
Neurosurgery	1,806	16	0.9	1,679	11	0.6	1,608	6	0.4	1,631	9	0.6
Burn unit	459	3	0.7	443	5	1.1	443	1	0.2	375	3	0.8
Burn resuscitation	312	6	1.9	283	6	2.1	269	4	1.7	306	3	1.0
General intensive care unit	1,165	20	1.7	1,050	15	1.4	1,209	13	1.1	1,218	22	1.8
Surgical intensive care unit	595	7	1.2	626	9	1.0	500	3	0.6	442	4	0.9
Traumatology	3,003	19	0.6	2,975	19	0.6	2,814	19	0.7	2,648	11	0.4
Surgery	4,807	32	0.7	4,377	32	0.7	4,314	26	0.6	4,260	22	0.5
Cardiac resuscitation	933	2	0.2	971	10	1.0	804	9	1.1	876	8	0.5
Cardiology	550	0	0.0	575	1	0.2	1,236	8	0.6	1,530	6	0.4
Gynecology	1,891	3	0.2	1,929	2	0.1	1,904	8	0.4	2,135	12	0.6
Other units	2,529	5	0.2	2,372	2	0.1	2,218	12	0.5	2,746	3	0.1
Total	**23,154**	**273**	**1.2**	**21,995**	**284**	**1.3**	**21,919**	**311**	**1.4**	**22,619**	**325**	**1.4**

***** NTP — the number of tested patients.

Table 2 *— Detection and admission profile of HIV-infected patients to N. V. Sklifosovsky Research Institute of Emergency Medicine, 2012–2015*

Profile/year	2012			2013			2014			2015		
	*NTP	n	%	NTP	n	%	NTP	n	%	NTP	n	%
Toxicological resuscitation	1,932	223	11.5	2,253	266	11.8	1,969	183	9.3	1,839	173	9.4
1st Unit of acute poisoning treatment	1,320	31	2.3	1,408	27	1.9	1,471	32	2.2			
2nd Unit of acute poisoning treatment	900	33	3.7	1,004	47	4.7	1,049	50	4.8	1,205**	42**	3.5**
Mental health unit	609	31	5.1	591	16	2.7	622	20	3.2	841	33	3.9
Neurosurgical intensive care unit	134	3	2.2	65	0	0.0	77	0	0.0	129	4	3.1
Neurosurgery	1,544	9	0.6	1,717	9	0.5	1,438	5	0.3	1,572	3	0.2
Burn unit	439	5	1.1	429	5	1.2	413	10	2.4	411	4	1.0
Burn resuscitation	268	0	0.0	305	5	1.6	337	5	1.5	327	5	1.5
General intensive care unit	1,230	15	1.2	1,391	33	2.4	1,283	28	2.2	1,301	20	1.5
Surgical intensive care unit	500	3	0.6	543	8	1.5	503	8	1.6	633	10	1.6
Traumatology	1,730	9	0.5	2,157	12	0.6	2,569	17	0.7	2,928	18	0.6
Surgery	4,086	30	0.7	4,874	31	0.6	5,083	35	0.7	5,865	50	0.9
Cardiac resuscitation	925	4	0.4	1,172	7	0.6	1,197	10	0.8	1,181	8	0.7
Cardiology	1,371	3	0.2	1,627	3	0.2	1,676	4	0.2	1,610	2	0.1
Gynecology	2,093	7	0.3	2,461	5	0.2	2,672	8	0.3	2,829	12	0.4
Other units	4,563	13	0.3	5,484	15	0.3	5,798	20	0.3	5,111	26	0.5
Total	**22,273**	**419**	**1.9**	**25,341**	**489**	**1.9**	**26,481**	**435**	**1.6**	**27,782**	**410**	**1.5**

****** In 2015, the 1st and 2nd Units of acute poisoning were merged into the Department of acute poisoning treatment.

The detection of HIV infection varied significantly, depending on the specialization of the departments, and, consequently, the type of emergency medical care that the patients received. Among the departments in the intensive care unit, the highest rate of detection of HIV infection was recorded in the toxicology (4.8–11.8%), general care (1.1–2.4%), burn (0.0–2.1%) and cardiology (0.2–1.1%) intensive care units. Among the departments with a clinical specialization that reported the highest incidence rate were the 1st (1.5–2.4%) and 2nd departments for the treatment of acute poisonings in mental patients (DTAP) — 2.7–4.8%, the department of crisis states and psychosomatic disorders (further somatopsychiatry) — 2.6–5.1%, the departments of surgery (0.5–0.9%), traumatology (0.4–0.7%), and gynecology (0.1–0.6%) (Tables 1, 2). Particular attention should be paid to the fact that among the departments in the intensive care, the proportion of patients requiring toxicological resuscitation over various years accounted for 70 to 90% of all admissions of HIV-infected patients.

Based on the data on the number of admissions and the rates of detection of HIV infection in patients, the departments at SRIEM can be divided into several subgroups:

- With a high rate of detection of HIV with a statistically significant upward trend — toxicological resuscitation (R^2 = 0.51, ρ = 0.04), 2nd DTAP (R^2 = 0.77, ρ = 0.004).
- With a high rate of detection of HIV, which does not have a statistically significant upward trend — 1st DTAP, somatopsychiatry, burn unit, general, surgical, neurosurgical and burn resuscitation.
- With a low rate of detection of HIV, which has a statistically significant downward trend — department of neurosurgery (R^2 = 0.67, ρ = 0.01).
- With a rate of detection of HIV fluctuating within a relatively narrow range of values — cardiac resuscitation, surgery, traumatology, cardiology, and gynecology.

At SRIEM, there are thus distinct differences in the trends in the frequency of requires of patients with HIV for medical care, depending on the specialization of the EMSC unit. Among the patients of toxicological resuscitation, 2nd DTAP, somatopsychiatry, general and surgical resuscitation, there is an increase in the rate of detection and number of admissions of HIV-infected people. In the group of other specialized departments, on the contrary, there is either a decrease in or stabilization of the number and proportion of infected patients at the previous level. However, in the group of specialized departments with low rates of detection of HIV infection, for example, gynecology, there are also certain observations that, in our opinion, require changes in the approaches to organizing medical care for HIV-positive patients. This, in particular, concerns patients with complications of early pregnancy, especially with progressive uterine pregnancy and HIV infection, which account for more than 40% of HIV-positive patients with pregnancy complications. The prevention of vertical transmission of infection in this group of patients requires a comprehensive approach, aimed at advising patients and encouraging them to get treatment [15].

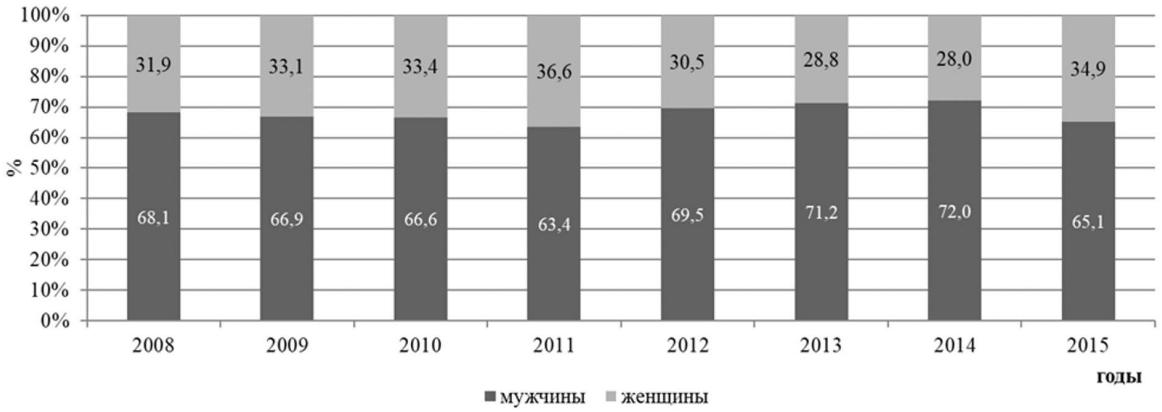

***Fig. 2** — The distribution of HIV-positive patients by gender for the period from 2008 to 2015*

We analyzed the age-gender characteristics of patients who require emergency and primary medical care who were admitted with HIV. Among this group of patients, the incidence of HIV infection in men exceeded the reported rates for women during the entire observation period ($p < 0.05$) (Tables 3, 4). At the same time, statistically significant tendencies of an increasing number of calls to the EMSC among both HIV-infected men ($R^2 = 0.63$, $p = 0.0188$) and women ($R^2 = 0.84$, $p = 0.0013$) were noted. However, over the course of seven years of observations, the proportion of hospitalized men affected by HIV declined from 68.1 to 65.1%, and women, on the contrary, increased from 31.9 to 34.9% (Fig. 2). The increase in the proportion of women was observed in all age groups: 18–30 — from 38.9 to 44.8%, 31–40 — from 24.7 to 29.6%, 41–50 — from 11.8 to 34.8%, 51–60 — from 0.0 to 44.5% and over 61 — from 33.3 to 66.7%, respectively (Table 5).

Table 3 — *The distribution of HIV-positive patients at N. V. Sklifosovsky Research Institute of Emergency Medicine in age groups by gender, 2008–2011, per cent*

Age, gender/ year	2008				2009				2010				2011			
	male		female		male		female		male		female		male		female	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
18–30	96	51.6	61	70.2	75	39.5	57	60.6	64	30.9	53	51.0	56	27.2	55	46.2
31–40	70	37.6	23	26.4	89	46.8	27	28.7	110	53.1	40	38.5	118	57.3	51	42.9
41–50	15	8.1	2	2.3	17	9.0	6	6.4	23	11.1	6	5.8	21	10.2	8	6.7
51–60	3	1.6	0	0.0	9	4.7	3	3.2	10	4.8	3	2.9	8	3.9	3	2.5
> 61	2	1.1	1	1.1	0	0.0	1	1.1	0	0.0	2	1.9	3	1.5	2	1.7
Σ	**186**	**100.0**	**87**	**100.0**	**190**	**100.0**	**94**	**100.0**	**207**	**100.0**	**104**	**100.0**	**206**	**100.0**	**119**	**100.0**

Table 4 — *The distribution of HIV-positive patients at N. V. Sklifosovsky Research Institute of Emergency Medicine in age groups by gender, 2012–2015, per cent*

Age, gender/ year	2012				2013				2014				2015			
	male		female		male		female		male		female		male		female	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
18–30	62	21.3	47	36.7	91	26.1	42	29.8	77	24.6	43	35.2	48	18.0	39	27.3
31–40	189	64.9	74	57.8	204	58.6	86	61.0	176	56.2	60	49.2	162	60.7	68	47.6
41–50	30	10.3	5	3.9	39	11.2	10	7.1	41	13.1	14	11.5	45	16.9	24	16.8
51–60	10	3.4	1	0.8	11	3.2	3	2.1	15	4.8	3	2.5	10	3.7	8	5.6
> 61	0	0.0	1	0.8	3	0.9	0	0.0	4	1.3	2	1.6	2	0.7	4	2.8
Σ	**291**	**100.0**	**128**	**100.0**	**348**	**100.0**	**141**	**100.0**	**313**	**100.0**	**122**	**100.0**	**267**	**100.0**	**143**	**100.0**

Table 5 — *The share of HIV-infected men and women in patients of N. V. Sklifosovsky Research Institute of Emergency Medicine in different age groups, 2008–2015*

Age	2008		2009		2010		2011		2012		2013		2014		2015	
	male	female	male	female	male	female	male	female	male	female	male	female	male	female	male	female
18–30	61.1	38.9	56.8	43.2	54.7	45.3	50.5	49.5	56.9	43.1	68.4	31.6	64.2	35.8	55.2	44.8
31–40	75.3	24.7	76.7	23.3	73.3	26.7	69.8	30.2	71.8	28.2	70.3	29.7	74.6	25.4	70.4	29.6
41–50	88.2	11.8	73.9	26.1	79.3	20.7	72.4	27.6	85.7	14.3	79.6	20.4	74.5	25.5	65.2	34.8
51–60	100.0	0.0	75.0	25.0	76.9	23.1	72.7	27.3	90.9	9.1	78.6	21.4	83.3	16.7	55.5	44.5
> 61	66.7	33.3	0.0	100.0	0.0	100.0	60.0	40.0	0.0	100.0	100.0	0.0	66.7	33.3	33.3	66.7

Table 6 — *The share of HIV-infected patients of N. V. Sklifosovsky Research Institute of Emergency Medicine in different age groups, 2008–2015*

Age/years	2008	2009	2010	2011	2012	2013	2014	2015
18–30	57.5	46.5	37.7	34.2	26.0	27.1	27.6	21.2
31–40	34.1	40.8	48.2	52.0	62.7	59.3	54.3	56.1
41–50	6.2	8.1	9.3	8.9	8.4	10.0	12.6	16.8
51–60	1.1	4.2	4.2	3.4	2.6	3.0	4.1	4.4
> 61	1.1	0.4	0.6	1.5	0.3	0.6	1.4	1.5
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

It should be noted that the maximum increase in the proportion of men among HIV-positive people (2012–2014) almost completely coincides with peaks of increase in the total number of people with HIV at time of admission and the rate of detection of HIV infection in the hospital.

The ranking of patients by age showed that the largest proportion of people affected by HIV infection was in the age groups of 18–30 and 31–40 (Table 6). It should be noted that in the 18–30 age group there was a decrease in the number of visits ($R^2 = 0.51$, $p = 0.04$), whereas in the age group of 31–40 ($R^2 = 0.71$, $p = 0.008$) and 41–50 ($R^2 = 0.89$, $p = 0.0004$), on the contrary, growth was recorded (Table 3, 4). It should be specially emphasized that the significant reason why the rate of detection of HIV infection in the EMSC in the 18–30 age group declined was mainly due to the sharp decrease in the number of infected women ($R^2 = 0.94$, $p < 0.0001$), which was not true of men ($R^2 = 0.17$, $p = 0.3$).

The detection of HIV in patients admitted to the intensive care units during the entire period of observation was higher than in the clinical units. Such differences, apparently, are due to the medical backgrounds and social characteristics of citizens. Most often, these are people who practice socially deviant behavior. The deviations from the accepted norms of behavior are associated in such contingents with an extremely high level of accidents, poisoning and injuries, including those resulting from the use of alcohol and PSs.

Discussion

According to the UNAIDS report, Russia is among the top twenty countries in terms of the number of new HIV infections [1]. The key reasons for the worsening of the HIV epidemic in our country include the high level of drug use, active sexual transmission of the disease, and poor access to antiretroviral therapy within the affected population [16]. At present, in most of the federal subjects of the Russian Federation, the incidence of HIV infection has been increasing. In 2016, in five regions that make up the Ural Federal District, specialists of Rospotrebnadzor recorded that the HIV epidemic had spread from concentrated pockets to the general overall region [17]. At the same time, over the last ten years in Moscow, the incidence of HIV infection has stabilized. In 2014, this indicator was 3.8 times lower than the average for the Russian Federation [9].

The spread of HIV infection is significantly affected by the socio-economic conditions of life. When these conditions deteriorate, rates of drug addiction grow uncontrollably, alcoholism becomes widespread, and the behavioral habits of people and their socio-psychological health worsen. HIV-infected people need specialized psychological help, as they are often in a state of social maladjustment and are prone to suicide [18].

According to the Ministry of Internal Affairs of the Russian Federation, the kinds of PSs that have been seized and confiscated in Russia have changed [3, 19]. "Classic" opioid drugs, such as heroin, are being replaced by "designer" PSs, including synthetic cannabinoids, mephedrone, methylone, methylenedioxymethamphetamine, methylenedioxypyrovalerone, etc. [20]. It is often difficult for doctors to diagnose poisoning by such drugs because of similar clinical symptoms with general-somatic diseases as manifested in the cardiovascular and nervous systems [21]. Synthetic PSs have

a pronounced empathogenic effect. Their use provokes risky sexual behavior and greatly increases the risk of HIV transmission during sexual intercourse [22].

Often, the occurrence of serious injuries is due to the aberrant social behavior of people, which does not comply with officially established or actually established rules and norms in our society. This kind of behavior is regarded by sociologists as deviant (from the Latin *deviatio*) [23]. Persons with deviant behavior tend to engage in asocial (alcoholism, drug addiction, prostitution, vagrancy, disorderly conduct, etc.), and risky (engaging in sports with a high risk of injury, sexual perversions, etc.) behavior. Quite often deviant behavior is observed among people who come from a socially vulnerable part of the population. These features can usually be identified by analyzing the medical and social characteristics of the victims [14, 24]. Often, the injury can be attributed to chronic intoxication with alcohol and PSs as well as the presence of mental disorders [25]. Persons who are intoxicated or who suffer from alcoholism are at increased risk of victimization, i.e., they are more often victims of various crimes and traffic accidents [23].

According to S. F. Bagnenko, one third of the Russian population seeks emergency medical care for emergency situations each year; every tenth inpatient is admitted for emergency indications; more than 60% of those admitted for inpatient care require emergency care [26]. Multi-specialization hospitals that provide general care are increasingly becoming the institutions that provide the "first line" of diagnosis of HIV infection. In some patients, HIV infection is detected accidentally in the course of seeking emergency and primary specialized care [15, 27, 28].

HIV infection has a negative effect on the clinical course of physical illnesses and increases the length of stay in the hospital [29]. In HIV-infected people, complications often occur in the form of hospital-acquired pneumonia, recurrent purulent-inflammatory diseases and sepsis [30]. The frequency by which antibiotic-resistant isolates of opportunistic microorganisms are identified in patients with HIV is also significantly higher than in comparison groups [31].

Data on the rate of detection of HIV infection in patients in the clinical and emergency care departments of SRIEM reflect the changes that are occurring in the contingents at risk of being HIV-infected. On the one hand, we observed a stably high incidence of HIV infection in patients with poisonings with various causes. On the other hand, there have been changes in the kinds of patients who are admitted to SRIEM, mainly due to an increase in emergency and primary surgical pathology. This may be associated with worsening of the overall physical condition of patients compounded with progressive HIV infection.

It is necessary to pay attention to the steady trend towards an increase in the number of infected women entering the EMSC and their share among all admitted HIV-infected patients (feminization of the epidemic), as well as the dynamic spread of the virus among male and female patients in the age groups of 31–40 and 41–50.

The increased incidence of HIV infection in these age groups can occur due to both aging of PSs users and the active sexual transmission of the disease among different segments of the population of the megalopolis, as well as an increase in the life expectancy of HIV-infected citizens due to the effect of antiretroviral therapy.

The high rate of detection of HIV in the EMSC makes it possible to consider patients in need of emergency and primary care as a risk group for the spread of HIV infection related to the provision of medical care [32]. Urgent medical care for such patients is associated with a high risk of occupational exposure. According to the Federal Scientific and Methodological Center for AIDS Prevention and Control, in 2014 151 new cases of HIV infection among medical personnel were registered in the Russian Federation (code 115) [33]. Infection of medical personnel can occur during emergency situations because they are injured by piercing-cutting sharp medical tools as the result of the emergence of a complex clinical situation; a lack of time; long-term exposure to a large volume

of infected biological material; improper use of personal protective equipment by employees; non-observance of sanitary and epidemiological rules in the collection and disposal of medical waste; as well as the delayed performance of post-exposure measures to prevent infection [34, 35].

The treatment of diseases in patients with HIV infection requires a complex interdisciplinary approach. In this situation, it is extremely important to constantly enhance the professional knowledge of specialists who provide emergency and primary care to treat and diagnose patients with HIV while taking into account nosological features.

The study of the admissions trends for HIV-infected patients at the EMSC has broad epidemiological significance. It allows us to provide an objective evaluation of the prevalence of HIV infection and the specific features of emergency pathology among different segments of the population, especially in vulnerable and socially maladaptive population groups. Based on the data that has been obtained, it is possible to develop operational guidelines and technologies for providing emergency medical care to HIV-infected patients in the hospital. The revealed age-gender differences and the incidence of physical trauma of HIV-infected citizens require further study and analysis targeted at the development and implementation of programs for the prevention of the spread of HIV among different segments of the population.

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 26.03.2018
Accepted for publication on 21.05.2018

UDC 616.379-008.64:[616.092:612.217.1]

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RESPIRATORY MUSCLES STRENGTH CHANGE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS COMPLICATED BY THE DIABETIC FOOT SYNDROME

Abstract

The relationship between cardiorespiratory disorders and the diabetic foot syndrome has not been adequately studied. In particular, there is no information on the strength of the respiratory muscles in this category of patients. **The objective of the study**: to reveal respiratory muscles weakness in patients with type 2 diabetes mellitus complicated by the diabetic foot syndrome. **Materials and methods.** 72 patients were examined, 16 of them with type 2 diabetes mellitus complicated by Wagner grade I-IV neuroischemic diabetic foot made up the first (main) group. The second group (comparisons) included 29 patients with type 2 diabetes mellitus not complicated by the diabetic foot syndrome. The third group (comparisons) included 27 patients without diabetes. The groups were randomized by gender and exclusion criteria. RM strength determination was carried out by measuring the maximum static inspiratory and expiratory mouth pressures, which the patient created during the maximum inspiration and maximum expiration with closed airways. **Results**. Respiratory muscles strength on inspiration in patients with type 2 diabetes mellitus complicated by the diabetic foot was reduced by 18.5 cm H_2O ($p<0.01$) compared with diabetic patients without diabetic foot syndrome and by 17.3 cm H_2O ($p<0.01$) compared with patients without diabetes. The expiratory effort showed a decrease in respiratory muscles strength in patients of the first group by 49.4 cm H_2O ($p <0.01$) compared to patients of the second group and by 27.4 cm H_2O ($p <0.05$) compared to patients of the third group. In women with the diabetic foot syndrome, the inspiratory muscles strength was reduced in comparison with patients without diabetic foot and without diabetes by 27.1 ($p <0.01$) and by 23.3 ($p< 0.05$) cm H_2O respectively. In men with the diabetic foot syndrome, the same index was lowered by 13.9 ($p> 0.05$) and 17.7 ($p <0.05$) cm H_2O compared to the second and third groups respectively. The expiratory effort revealed a decrease in respiratory muscles strength in men in all groups in approximately the same range, without a significant difference between the groups. In women with the diabetic foot syndrome, there is a significant decrease in expiratory muscles strength: by 48.4 cm H_2O ($p <0.01$), compared to women of the second group, and by 20.6 cm H_2O ($p <0.05$) patients of the third group. **Conclusions**. Patients with type 2 diabetes mellitus complicated by the diabetic foot syndrome showed a decrease in inspiratory and expiratory muscles strength. In women with the diabetic foot syndrome, there is a more significant decrease in respiratory muscles strength on expiration compared to men with this pathology.

Key words: *diabetes mellitus, diabetic foot syndrome, respiratory muscles strength*

For citation: Sivozhelezova O.K., Ivanov K.M., Miroshnichenko I.V., Kunarbaeva A.K. RESPIRATORY MUSCLES STRENGTH CHANGE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS COMPLICATED BY THE DIABETIC FOOT SYNDROME. The Russian Archives of Internal Medicine. 2018; 8(3): 204-208. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-204-208

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DOI: 10.20514/2226-6704-2018-8-3-204-208

RM — respiratory muscles, DM — diabetes mellitus, DFS — diabetic foot syndrome

Introduction

Diabetes mellitus (DM) is one of the topic medical, social and economic health issues not only in Russia but also in many countries due to the increase of the incidence rate and the frequency of diabetic complications [1]. Diabetic foot syndrome (DFS) is the most common and socially significant late diabetic complication, which worsens the quality of life of patients [2, 3].

There are data on various pulmonary function impairments in patients with DM without respiratory and cardiovascular systems pathology [4, 5]. The decrease of the Tiffeneau index and FEV1 has provided authors with an opportunity to think about obstructive changes of pulmonary function in patients with DM [5, 6]. On the contrary, the data gathered by E. I. Sokolov support a restrictive respiratory pattern in DM [7], especially in patients with severe complications [8]. It remains unclear whether functional changes in the lungs precede the emergence of DM or develop later.

Previous authors have described the correlation between respiratory dysfunction and diabetic complications (retinopathy and nephropathy) [9]. At the same time M. S. Boulbou et al. did not reveal any connection between functional impairments of the respiratory system and other diabetic complications [10]. The correlation between cardiorespiratory disorders and DFS has not been adequately studied. In particular, there is no information on changes in the respiratory muscles (RM) strength in this category of patients. The RM dysfunction is today conventionally subdivided into fatigue and weakness. Respiratory muscle fatigue is a process in which there is a decrease in the strength and speed of contraction of RM as a result of the fact that they work excessively. Muscle fatigue is an inversive process. Functional recovery is possible after rest. RM weakness is the state of their reduced strength at rest. The most common cause of RM weakness is metabolic, inflammatory and degenerative changes leading to RM, nerves or neuromuscular junction dysfunction [11]. It should be assumed that the development of RM fatigue or weakness is possible in patients with DM.

Objective: To reveal RM weakness in patients with type 2 DM complicated by the diabetic foot syndrome on the basis of changes in respiratory muscles strength.

Materials and methods

72 patients were examined, treated at the non-governmental healthcare organization Departmental Clinical Hospital at Orenburg Station of OAO Russian Railways, Surgical Department and Department of Internal Medicine. The patients were divided into 3 groups. The first (main) group consisted of 16 patients with type 2 DM complicated by Wagner grade I-IV neuroischemic DFS (classification of the International Working Group on the Diabetic Foot, 2000). The second group consisted of 29 patients with type 2 DM without DFS. At enrollment, type 2 DM was subcompensated and decompensated in all patients. Inclusion criteria: confirmed type 2 DM; the absence of concomitant bronchopulmonary diseases, other visceral diseases in the decompensation stage; and the ability of patients to perform breathing manoeuvres during the examination of the respiratory system. Exclusion criteria: Wagner grade V DFS, significant organic lesion of CNS, acute disorder of cerebral circulation in past medical history, lower limb amputation in past medical history, third-degree obesity (BMI > 40 kg/m^2), II-III CHF, moderate to severe anemia (hemoglobin < 90 g/l), significant liver and renal dysfunction, non-sinus rhythm, administration of psychotropic agents. The third group consisted of 27 patients without DM.

RM strength determination was carried out by measuring the maximum static inspiratory and expiratory mouth pressures, which the patient

created during the maximum inhalation and maximum exhalation with closed airways For this purpose, MicroRPM for RM testing by Micro Medical Ltd. (UK) was used to determine MIP (maximal inspiratory pressure) and MEP (maximal expiratory pressure) in cm H_2O. No less than 3 manoeuvres were carried out with the rest periods of 1 minute. Only the best result was recorded. Patients were sitting, and a nose clip was used to prevent air leakage. The requirement for recording the maximal inspiratory and expiratory pressures was to maintain them for at least 1 second. The normal values of MIP were: men > 100 cm H_2O, women > 70 cm H_2O. The normal values of MEP were: men > 140 cm H_2O, women > 90 cm H_2O.

The study was approved by the Ethics Committee. All patients signed the informed consent (PIC) to participate in the study.

Statistical analysis was carried out using the Statistica 7.0 software package on the base of nonparametric methods (the Wilcoxon-Mann-Whitney test). Differences between the examined groups were considered significant at $p < 0.05$.

Results and Discussion

Patients were randomized by gender, age, body mass index (BMI), hypertension and coronary heart disease (CHD) (Table 1).

The RM strength was reduced in all the examined patients in comparison with the normal indices. However, the indices varied significantly between groups (Table 2).

The RM strength of inspiratory effort was reduced in both experimental groups, without a significant difference between the indices. In patients belonging to the main group, with DM and DFS, a significant decrease of MIP index by 18.5 cm H_2O (34%) was found compared with data on patients with uncomplicated DM ($p_{I-II} < 0.01$), and by 17.3 cm H_2O (33%) compared with the indices in patients without DM ($p_{I-III} < 0.01$).

The expiratory effort revealed higher RM strength values in DM patients compared to the third group by 22.0 cm H_2O (25%) ($p_{II-III} < 0.05$). In patients with DM complicated by DFS, a significant decrease in the MEP index by 49.4 cm H_2O

Table 1. *Initial characteristics of patients*

Indices	Studied groups		
	Group I n=16	Group II n=29	Group III n=27
Mean age, years old	62,6±0,9	61,5±1,2	60,8±0,7
Men/Women, absolute number	7/9	15/14	13/14
Hypertension, percentage of patients, %	100	100	100
CHD, percentage of patients, %	56	50	65

Chart 2. *The respiratory muscles strength in the studied groups (M ± m)*

Studied groups	Indices of RM strength	
	MIP, cm H_2O	MEP, cm H_2O
Group I, n = 16	53,9±6,9	59,4±7,4
Group II, n = 29	72,4±4,8	108,8±6,4
Group III, n = 27	71,7±7,3	86,8±5,3
p	p_{I-II}<0,01 p_{I-III}<0,01 p_{II-III}>0,05	p_{I-II}<0,01 p_{I-III}<0,05 p_{II-III}<0,05

Note: p — the reliability of the difference between the studied groups

(83%) was found compared with data on patients with uncomplicated DM ($p_{I-II} < 0.01$), and by 27.4 cm H_2O (46%) compared with the indices in patients without DM ($p_{I-III} < 0.05$).

The analysis of the changes in the RM strength revealed various MIP and MEP in the groups of patients depending on gender. MIP and MEP indices in men were significantly higher than in women in all groups. Mean MIP indices were lower in men and women in all groups. The MEP index was determined within normal limits only in women belonging to the second group (97.9 ± 6.4 cm H_2O).

Among women, the smallest MIP index was found in the group of patients with DFS: compared with the second group, it decreased by 27.1 cm H_2O (66%) ($p_{I-II} < 0.01$), and compared with the third, it decreased by 23.3 cm H_2O (55%) ($p_{I-III} < 0.05$). Among men, the strength of inspiratory muscles is reduced by 13.9 cm H_2O (20%) in patients of the main group compared with patients with DM ($p_{I-II} > 0.05$) and by 17.7 cm H_2O (26%) compared with patients without DM ($p_{I-III} < 0.05$).

When assessing the strength of the expiratory muscles in men, there were no significant differences between the groups, unlike what was true of the women. Among women, the MEP index is reduced by 20.6 cm H_2O (41%) in patients with DFS compared with patients without DM ($p_{I-III} < 0.05$) and by 48.4 cm H_2O (98%) compared with patients with DM without DFS ($p_{I-II} < 0.01$).

Figure 1 shows the deviation of the RM strength during inspiration and expiration in the examined groups of patients, depending on gender (Fig. 1).

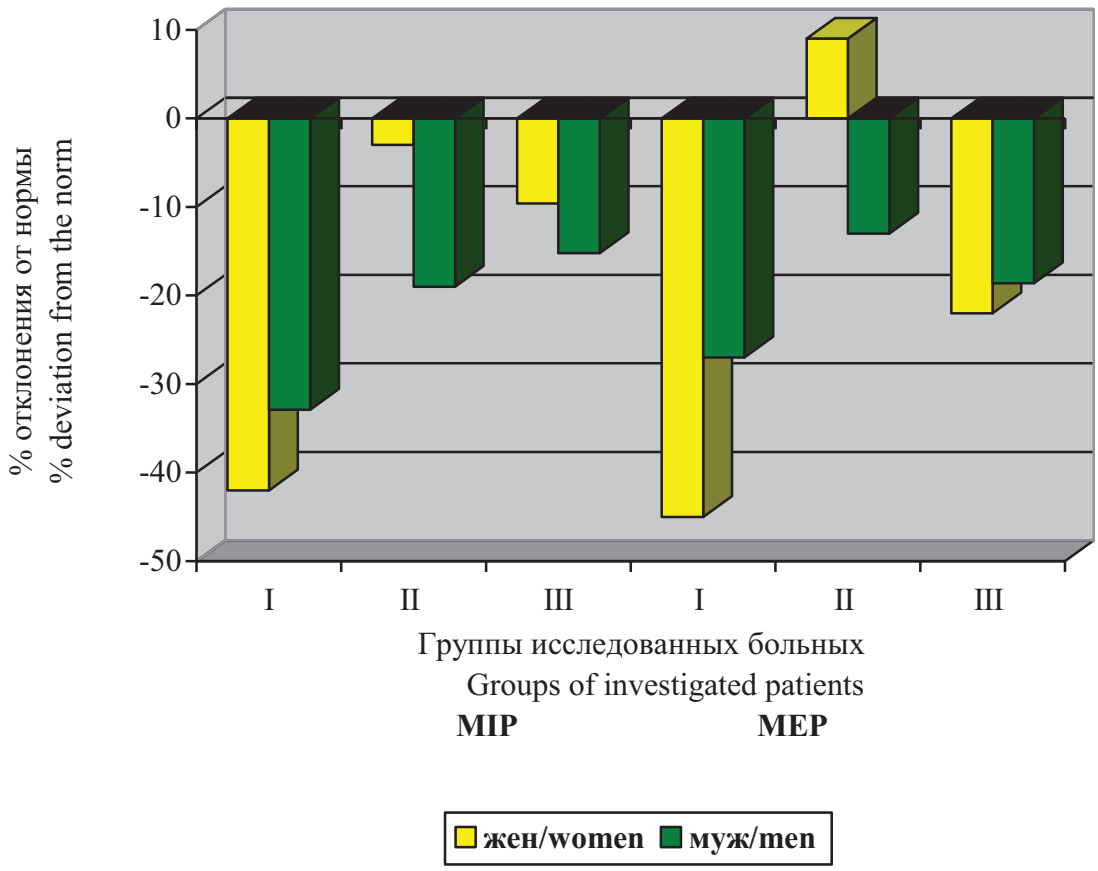

Figure 1. *Deviation from the norm of indices of the respiratory muscles strength in men and women in studied groups*

It is obvious that the most significant decrease relative to the normal values of the RM strength is observed on inspiration in women and in men in the group of patients with DFS. On expiration, the RM strength is reduced more significantly in the first group in women.

A significant decrease of the RM strength at rest is thus typical for patients with DM complicated by DFS, which confirms the RM weakness. It can be assumed that the causes of these changes are multifactorial, associated with the RM dysfunction because of the metabolic and degenerative processes, with a disorder of neuromuscular transmission, the impairment of circulation, morphofunctional characteristics of airways, and respiratory failure [11]. When DM is present before the development of late diabetic complications, it is necessary to monitor the RM strength to identify weakness and to implement the medical rehabilitation measures.

Conclusion:

1. Respiratory muscle weakness together with a decrease in inspiratory and expiratory muscle strength are observable in patients with diabetes mellitus complicated by the diabetic foot syndrome.

2. A more significant RM strength decrease in expiratory effort is observed in women with the diabetic foot syndrome compared to men with this pathology.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 15.01.2018
Accepted for publication on 11.05.2018

UDC 616.833-007.271-02

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THE ROLE OF MYOFASCIAL SYNDROME IN THE GENESIS OF NOCTURNAL PAINFUL PARESTHESIAS

Abstract

The **objective** of our study was to investigate clinical and neurophysiological features of nocturnal painful paresthesias in the upper limbs.

Material and methods. The article presents the results of the study of 107 patients with pain and nocturnal paresthesias in their hands. It was revealed that the syndrome of nocturnal painful paresthesias is mixed in etiology and has myofascial pain syndrome as an initial part. The clinical symptoms of nocturnal pains and paresthesias in the hands of patients with myofascial pain syndrome of the shoulder girdle and upper limbs were described.

Results. It is shown that active myofascial trigger points are the key link in the clinical pattern formation of the syndrome of nocturnal painful paresthesias in patients with myofascial pain syndrome. In the study of short-latency somatosensory evoked potentials from the upper extremities, the pathological peak Px in the CVII-Fpz lead is described, which is the marker for the presence of a pathologically enhanced excitation generator in the suprasegmental structures.

Conclusions. The syndrome of nocturnal painful paresthesias is mixed in etiology and has, as an initial link, myofascial pain syndrome. Detection of a pathologically enhanced excitation generator in the suprasegmental sections of the sensitive pathway in the registration of short-latency somatosensory evoked potentials is an adequate method for diagnosing painful paresthesias.

Key words: *nocturnal painful paresthesias, myofascial pain, somatosensory evoked potentials*

For citation: Mindubaeba L. G., Alekseeva O. A., Karimova G. M., Karimova D. J., Abashev A. R. THE ROLE OF MYOFASCIAL SYNDROME IN THE GENESIS OF NOCTURNAL PAINFUL PARESTHESIAS. The Russian Archives of Internal Medicine. 2018; 8(3): 209-214. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-209-214

DOI: 10.20514/2226-6704-2018-8-3-209-214

MPS — myofascial pain syndrome, VAS — visual analogue scale, MTP — myofascial trigger point, NCV — nerve conduction velocity, AP — action potential, SSEP — somatosensory evoked potentials

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Introduction

Hand paresthesia is a common symptom of somatic, neurological, and mental disorders. To manage this symptom, patients seek help from various healthcare specialists [1]. According to S. Marshall, C. Murray, 7.4 to 45% of adult population report pain and nocturnal paresthesias in their hands [2]. Medical literature assigns a major role in the development of nocturnal paresthesias to myofascial pain phenomena [3, 4, 5]. This is due to the fact that the most important signs of myofascial pain syndrome (MPS) are: pain, and psychovegetative, dissomnic and motor disorders [6, 7].

Dysfunction of afferent systems of the brain and spinal cord plays an important role in the pathogenesis of MPS. At the same time, an analysis of somatosensory evoked potentials (SSEP) allows us to clarify the proportions of central and peripheral mechanisms in the genesis of MPS. Numerous publications describe nocturnal painful paresthesias; however, there has yet to be a classification of their clinical and physiological mechanisms. The role of MPS in the pathogenesis of this disease has not been studied. The state of afferent systems of the brain and spinal cord in nocturnal painful paresthesia syndrome has not been evaluated.

The **goal** of our study was to investigate clinical and neurophysiological features of nocturnal painful paresthesias in the upper limbs.

The **objectives** of the study were:

1) To investigate clinical symptoms of nocturnal pains and paresthesias in the hands.
2) To investigate the role of myofascial syndrome in the development of nocturnal painful paresthesias in distal and proximal parts of the upper limbs.
3) To provide neurophysiological assessment of the functional status of peripheral and central regions of the somatosensory analyzer in patients with nocturnal painful paresthesias.

Materials and Methods

The study was based on the clinical and neurophysiological examinations of 107 patients reporting nocturnal painful paresthesias in their hands, aged 17 to 63. Duration of paresthesias (the reported time since the appearance of first clinical signs) varied from between several days to 12 years. Patients were selected based on a comprehensive examination of subjects seeking the assistance of a neurologist for nocturnal paresthesias in their hands.

The study had the following inclusion criteria:

- Crawling feelings in the upper limbs, mainly occurring at night.
- Active myofascial trigger points with marked tenderness of the shoulder girdle and upper limb muscles.

The study had the following exclusion criteria:

- Severe comorbidity (diabetes, alcohol abuse, kidney failure, endocrine, systemic and blood diseases).
- Organic lesions of central nervous system.
- Inflammation in the upper limb joints.
- Mental disorders and mental retardation.

The patients underwent clinical neurological examination of the spine and manual diagnosis [7, 8, 9, 10]. A visual analogue scale (VAS) was used to assess the severity of paresthesias and pains. Somatosensory evoked potentials were studied to assess the functional state of peripheral and central segments of the somatosensory analyzer. A multifunctional Neuro-MVP computer complex (NeuroSoft, Russia) was used in the study. To evaluate the integrity of peripheral nerves and to confirm neuropathy, we studied the action potential of a sensory nerve in response to electric stimulation.

Based on the results of clinical and neurophysiological examinations, all patients were divided into three groups: Group 1 (n = 40) — patients with impairment of the peripheral nervous system (tunnel mononeuropathies of the upper limbs); Group 2 (n = 37) — patients with impairment of an intervertebral disc accompanied by radiculopathy, cervicalgia, cervicobrachialgia, and clinical symptoms of fibromyalgia, MPS, active myofascial trigger points in the shoulder girdle and upper limb muscles; Group 3 (n = 20) — patients with pronounced clinical symptoms of fibromyalgia and MPS of the shoulder girdle and upper limb muscles.

Table 1. *Distribution of patients by gender*

Groups	Women		Men	
	Abs.	%	Abs.	%
1	32	80.0	8	20.0
2	32	86.5	5	13.5
3	20	100.0	0	0.0
4	5	50.0	5	50.0
Generally	89	83.3	18	16.8

Group 4 was a control group (n = 10) that included patients with peripheral nerve injuries who had permanent paresthesias in their hands. Patient distribution by gender is presented in Table 1.

The study group consisted mainly of women: 83.2% (men comprised 16.8%).

Twelve patients without upper limb disorders (healthy volunteers who did not report any crawling feelings [comparison group]) were examined in addition to the study patients (with disorders) to analyze and compare SSEP.

Student's t-test (for the comparison of quantitative variables between two groups), Newman–Keuls test (for the comparison of quantitative variables between three and more groups), and Student's t-test for proportions (for qualitative variables) were used to perform a statistical analysis of the clinical and neurophysiological findings. All calculations were made in the Biostatistica software.

Results

The analysis revealed that 99.0% of patients had reported nocturnal paresthesias in the upper limbs. All patients in Groups 1, 2, and the control group (100%) complained about nocturnal paresthesias. These patients comprised 97.3% of those in Group 3. Steady symptoms were more commonly reported (57.8%) than symptoms that increased (33.3%) or decreased (8.8%) in severity.

The localization of the paresthesia was an important factor. A total of 69.6% of patients reported hand paresthesias: Group 1 accounted for 62.5% of cases ($P_{I-III} < 0.05$), whereas Group 2 accounted for 64.9% ($P_{II-III} < 0.05$) and Group 3 accounted for 90.0%. Fingers (one or several) were affected in 51.0% of cases: Group 1 accounted for 52.5% of cases, whereas Group 2 accounted for 62.2% of cases and Group 3 accounted for 25.0% of cases. Therefore, when a peripheral nerve was damaged, the distribution of paresthesias was more distal, while the presence of myofascial trigger points (MTPs) led to paresthesias in hands and to a lesser degree in fingers. Active MTPs predominantly resulted in proximal paresthesias and distal pains. The distribution of paresthesias and pains was almost identical in Groups 1 and 2. This may indicate a key role of nerve tissue damage rather than MTPs in the development of pain and paresthesias.

The duration of the disease in Group 1 was 2.6 ± 0.3 years ($P_{I-III} < 0.05$), in Group 2 — 4.0 ± 0.3 years ($P_{I-II} < 0.05$, $P_{II-III} < 0.001$, $P_{II-IV} < 0.01$), and in Group 3 — 1.3 ± 0.2 years. The longest duration was reported in Group 2. This was due to a combination of peripheral nerve damage and MTPs. The shortest duration was reported in Group 1 (without active MTPs), which allowed estimating time required for MTP development after peripheral nerve damage.

83.8% of patients in Group 2 reported sleep disorders. Severity of nocturnal paresthesias: patients from Group 2 had severe paresthesias in 56.8% of cases; no patients from Group 3 had severe paresthesias. Severity of nocturnal paresthesias according to the VAS scale: Group 1 — 7.7 ± 0.3 ($P < 0.05$), Group 2 — 9.2 ± 0.4 ($P < 0.05$), and Group 3 — 4.2 ± 0.1 ($P < 0.05$).

According to our results, MTPs in Groups 2 and 3 were most common in the trapezius muscle as well as in the greater and smaller pectoral muscles. Group 3 included more patients with MTPs in the

Table 2. *Results of NCV study in patients with nocturnal painful paresthesia m/s (M ± SD)*

Groups	Side	
	Affected	Healthy
First (n = 12)	33.93 ± 3.14	52.06 ± 6.54
Second (n = 14)	40.02 ± 3.84	55.81 ± 5.66
Third (n = 10)	49.33 ± 4.18	53.12 ± 6.21

brachioradial and pronator teres muscles. MTPs in the brachioradial muscle induced pains and paresthesias in the wrist and the first web space. MTPs in the pronator teres led to the development of reflected pains in the forearm and deep in the palm of hand. The incidence of MTPs in Groups 2 and 3 was almost equal, which put into question the role of peripheral nerve damage in their development.

A study of sensory fibers conductivity in the upper limbs by electric stimulation allowed us to confirm nerve damage in patients from Groups 1 and 2. Nerve conduction velocity (NCV) in these fibers slowed down in distal regions, and the amplitude of the action potential decreased. NCV delay in sensory fibers dominated over amplitude decrease, which indicated predominant nerve demyelination typical for tunnel syndromes. In Group 3, no sensory fiber damage was found, which allowed us to classify deficiency symptoms as signs of myofascial pain syndrome.

Table 2 presents the results of NCV measurement in sensory fibers for patients from the study groups.

The analysis of the results indicated a relative decrease in the response amplitude in the affected side of patients from Groups 1 and 2. The amplitude of the sensory potential in the affected side of patients from Group 3 exceeded values obtained for Groups 1 and 2 ($P_{I-III} < 0.05$, $P_{II-III} < 0.05$). Therefore, the study of sensory fibers conductivity in the upper limbs provided a neurophysiological proof of nerve damage in patients from Groups 1 and 2. Patients from Group 3 had no neurophysiological signs of sensory fibers disorder in the upper limbs, both in the affected and healthy sides.

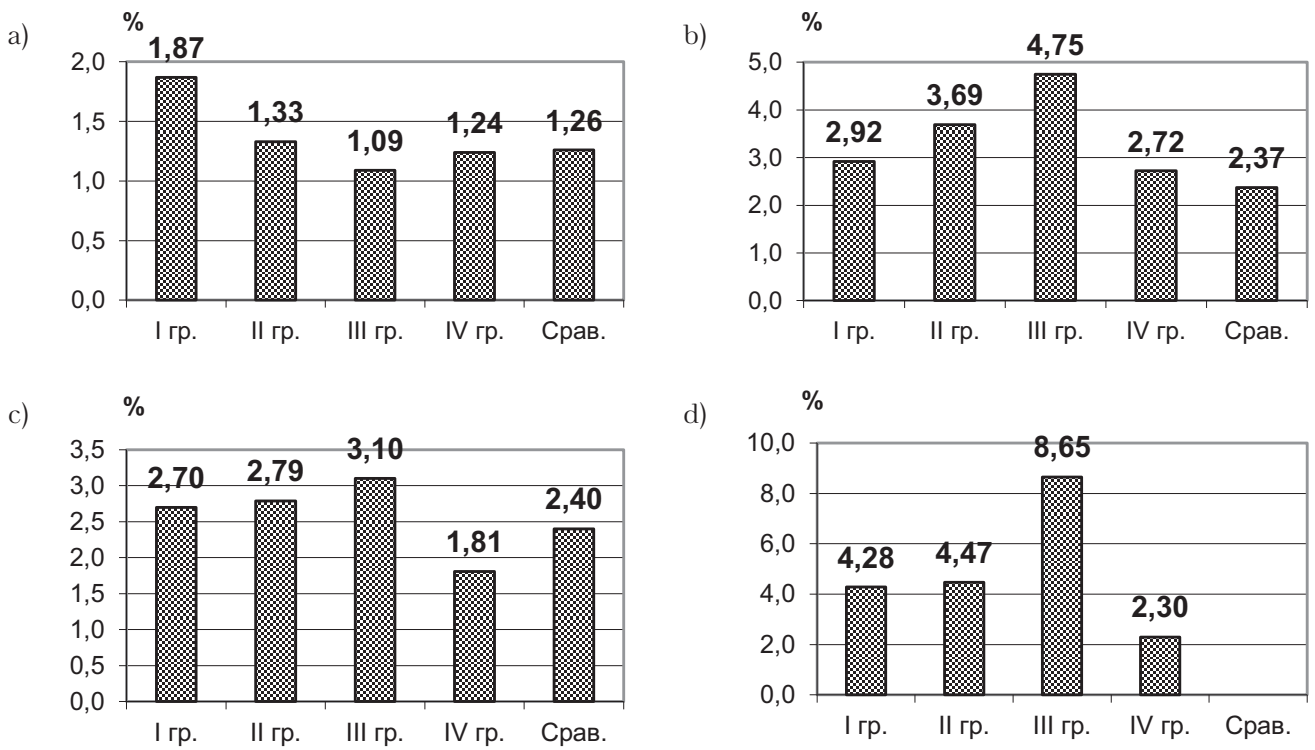

Figure 1. *Peak amplitudes of short-latency somatosensory evoked potentials, ms: a – peak N13, b – peak P17, c – peak N20, d – peak Px*

A study of short-latency somatosensory evoked potentials (SSEPs) allowed us to determine the preserved conductivity of central sections of deep sensitivity pathways in patients from Groups 1, 2, and 3.

Amplitude characteristics of SSEP peaks reflected both the conditions of impulse conduction and the excitability of structures generating these peaks. The amplitude of N20 peak was higher in Groups 1, 2, and 3 than in the comparison group (healthy volunteers). The amplitude of N20 peak in Group 1 was 2.70 ± 0.36 μV, in Group 2 — 2.79 ± 0.40 μV, in Group 3 — 3.1 ± 0.37 μV. In the control group, the amplitude of N20 peak was 1.81 ± 0.44 μs, and in the comparison group — 2.40 ± 0.38 μV (Fig. 1c). Increased amplitude of cortical peaks has been described in clinical studies and experiments as a result of acute and chronic pain.

The amplitude of the thalamic peak P17 was increased in Group 3 in comparison with the other groups (Fig. 1b). The amplitude of P17 peak in Group 1 was 2.92 ± 0.24 μV, in Group 2 — 3.69 ± 0.23 μV, in Group 3 — 4.75 ± 0.57 μV.

In the control group, the amplitude of P17 peak was 2.72 ± 0.26 μV — significantly lower than in Groups 2 and 3 and not different from the value obtained for Group 1. In the comparison group, the mean amplitude of P17 was 2.37 ± 0.26 μV. In patients from Group 3, the amplitude of P17 exceeded values obtained for other groups. The obtained data allowed us to hypothesize that pains and paresthesias induced by tunnel and traumatic neuropathy differed in their pathogenesis and the locus of the development of a pathologically enhanced excitation generator.

The analysis of SSEP peaks in patients from Groups 1, 2, 3, and 4 revealed Px peak in the CVI-Fpz lead with a latency of 21 to 35 ms (Fig. 2).

The analysis of SSEP in healthy volunteers showed no such peak despite identical experimental conditions. The analysis of Px peak parameters revealed the following.

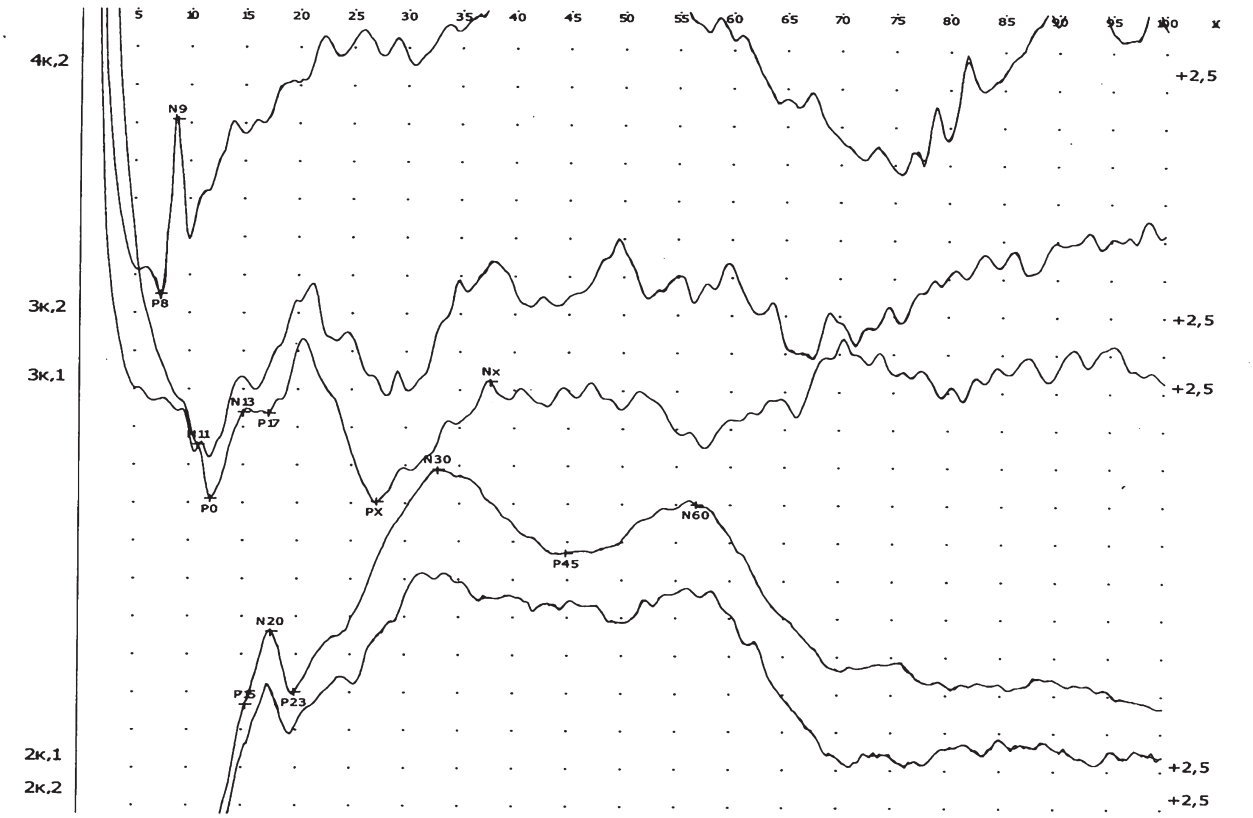

Figure 2. *Short-latency somatosensory evoked potentials with stimulation of the right median nerve in a patient with carpal tunnel syndrome and active MCPF in the brachial muscle and pronator teres*

The amplitude of Px peak in Group 1 was 4.28 ± 0.56 μV, in Group 2 — 4.47 ± 0.68 μV, in Group 3 — 8.65 ± 1.54 μV, in the control group — 2.30 ± 0.85 μV. In the comparison group, no Px peaks of SSEP were observed. It can be seen that the amplitude of Px peak in Group 3 was higher than in other groups ($P_{I-III} < 0.01$, $P_{II-III} < 0.05$, $P_{III-IV} < 0.01$).

The diagram shows that Px peak with a latency of 26.8 ms was registered at Channel 3 (CVII-Fpz). The latency of Px peak in patients from the comparison group had the following values: Group 1 — 21.98 ± 1.31 ms, in Group 2 — 27.46 ± 1.04 ms, in Group 3 — 26.49 ± 1.14 ms, in the control group — 22.63 ± 1.49 ms. The obtained data show that the latency of Px peak in patients from Groups 2 and 3 was higher than in patients from Group 1 and from the control group ($P_{I-II} < 0.01$, $P_{I-IV} < 0.05$, $P_{I-III} < 0.01$).

It was impossible to find its source; however, CVI-Fpz lead and the peak's positivity indicated that it was thalamic in nature. It is known that thalamic damage due to a hemorrhage or ischemia can result in excruciating burning pains (causalgia) in a certain part of the body. This indicated the possibility that the origin of the pain was in the thalamus.

Appearance of a previously undescribed peak may mean the development of a pathologically enhanced excitation generator that induces painful paresthesias. This peak may be connected both to the pain syndrome and to paresthesias observed in the examined patients.

Conclusions

Active myofascial trigger points were the root cause of nocturnal painful paresthesias in patients with myofascial pain syndrome of the shoulder girdle and upper limbs. In patients with painful hand paresthesias induced by tunnel mononeuropathies of the upper limbs, myofascial trigger points had no effect on the clinical course. The intensity of painful paresthesias was higher in patients with tunnel neuropathies than in patients with myofascial pain syndrome.

Therefore, the syndrome of nocturnal painful paresthesias was mixed in etiology and had, as an initial link, myofascial pain syndrome. The detection of a pathologically enhanced excitation generator in the suprasegmental sections of the sensitive pathway when short-latency somatosensory evoked potentials are recorded provided an adequate method for diagnosing painful paresthesias.

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 03.04.2018
Accepted for publication on 11.05.2018

UDC 616.147.3-007.64-08

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TEN-YEAR RESULTS OF VARICOSE VEINS TREATMENT

Abstract

The objective: To study the quality of life of patients with varicose veins of the lower extremities treated between 2007 and 2016 by surgical methods, including the RFO method.

Materials and methods. Our study is based on the medical records of the patients of the surgical department, summary data from the department of statistics at the NGHCI branch hospital, Tyumen Station, OAO Russian Railways, and data on the venous duplex ultrasound of lower extremities. We used the following research methods: statistical methods, expert clinical analysis, and the systematic approach. The degree of chronic venous insufficiency of lower extremities was determined using the clinical section of the international classification of CEAP (1995).

Results and discussion. We analyzed the quality of life of patients with varicose veins who underwent surgical treatment between 2007 and 2016. It was found that the overwhelming majority of patients (83%) had a hereditary factor from the etiologic factors of the disease. Obesity was reported in 57% of cases, and patients reported long-term static loads in 44% and use of hormonal contraceptives in 5% of cases. We gave a subjective evaluation of the results of surgical treatment using the classical method (phlebectomy) and the method of radiofrequency obliteration of veins. The mean duration of temporary disability of railway workers after crossectomy and phlebectomy was (18 ± 2.5) days, while after the RFO the majority of patients (70.5%) returned to normal life on the day of surgery, 23.5% — 2 days after surgery, and 8.8% — 3 days after surgery. It is shown that when planned operations are conducted promptly before complications of varicose veins can develop certain aspects of the patient's quality of life significantly improve.

Conclusion: The process of rehabilitation of patients is reduced 3 times if surgical treatment is performed by the method of endovenous segmental radiofrequency obliteration of veins.

Keywords: *varicose veins, quality of life, radiofrequency obliteration of veins*

For citation: Komarova L. N., Ryakhin R. N., Aliev F. S., Zvezda S. A. TEN-YEAR RESULTS OF VARICOSE VEINS TREATMENT. The Russian Archives of Internal Medicine. 2018; 8(3): 215-218. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-215-218

DOI: 10.20514/2226-6704-2018-8-3-215-218

GSV — the great saphenous vein, RFO — radiofrequency obliteration, TD — trophic disorders, VU — venous ulcer, CVI — chronic venous insufficiency.

Relevance

More than 20% of the world's population suffers from chronic lower extremity venous disease. According to Hanevich M. D., 2003, in Russia there are more than 35 million of such patients, and up to 35% of them are members of the working-age population. More than 50% are elderly senior citizens. The prevalence of trophic disorders (TD) and venous ulcers (VU) in chronic venous insufficiency (CVI) is 5–8%, while the rate of venous trophic ulcers incidence increases

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with age and reaches its maximum after 60 years (Kirienko A. I., 2007). Surgery is considered to be curative and the most effective method of treatment of varicose veins (Losev R. Z., 2005). Today, a variety of surgical methods of treatment remain one of the most effective tools for combating varicose veins. Timely surgery is able not only to eliminate all of the manifestations of varicose veins, but also to prevent the development of dangerous complications of the disease. The surgical methods for treating varicose veins, which are used at the NGHCI branch hospital, Tyumen Station, OAO Russian Railways, are: combined phlebectomy, invaginated phlebectomy, ultrasound-guided sclerotherapy of great saphenous vein, and endovenous radiofrequency segmental obliteration (RFO). The latter method has been most actively used since 2016, and it has already established itself as an alternative to crossectomy and stripping. Radiofrequency vein obliteration is ultrasound-guided from the moment of vein puncture to the completion of the procedure. Since the varicose vein is affected by radio waves, it is heated; it collapses and is subsequently replaced by connective tissue. This procedure has many advantages: it is carried out under local anesthesia, it does not require a long stay in the hospital, it is not traumatic, and it has a good cosmetic outcome.

The objective of the study was to explore the life quality of patients with varicose veins, which was surgically treated between 2007 and 2016, including the RFO method of treatment.

Materials and Methods

The following research methods were used: statistical method, clinical and expert analysis, and systematic approach. The degree of chronic venous insufficiency of the lower extremities was determined using the clinical section of the international classification CEAP (1995). It should be noted that the vast majority (77.8%) of patients had II degree CVI (CEAP: C3, Ep, As, p, Pr, 2,18, LII); and 20% of patients had III degree CVI.

Results and Discussion

We conducted a retrospective analysis of the medical records of 861 patients (378 of them were railway workers and 483 were not). Railway employees accounted for 44.7% of all patients treated, and the vast majority of them were women (206 — 52.7%); the percentage of men was 47.3%. According to the professional status, the distribution of varicosis among male railway workers was as follows: in 20% of cases they were handymen, and 13% (51 people) were rolling stock repairman. The group of train drivers and their assistants accounted for 14.3% of all patients. Among female railway workers, the distribution based on socioprofessional status was as follows: 20% were train dispatchers and operators, 18% were service personnel on the trains (conductors of passenger trains, conductors of cargo escort and special cars, etc.), and the rest worked in the maintenance officer group.

The mean age of patients who underwent operations was 51.1 years old, where the youngest age was 22 and the oldest was 83. There were 605 operations on one lower extremity (70.3%) and 256 operations on both lower extremities (29.7%). Among the etiological factors, a hereditary factor was discovered in the vast majority of patients (83%). The remaining in frequency of occurrence were: obesity (57%), long-term static load (44%), and use of hormonal contraceptives (5%). The average duration of the disease was 11.8 years. According to vascular ultrasound imaging, the maximum diameter of the great saphenous vein (GSV) was 27 mm, and the minimum diameter of GSV was 12 mm.

The venous duplex ultrasound of lower extremities was the main instrumental diagnostic method, and it was performed in all cases to clarify the localization, nature and extent of the venous pathology. The number of concomitant diseases as well as their severity determined the patient's condition. Surgery and anesthesia risk was assessed according to the American Society of Anesthesiologists (ASA) physical status

classification system. Surgical treatment in 2/3 of patients was carried out under spinal anesthesia and under intravenous anesthesia. Endovenous segmental radiofrequency obliteration (ESRFO) of varicose veins of the lower extremities was performed in 68 patients mainly under local anesthesia (a "paravasal tumescent pillow" was created using special VNUS introducers and ultrasound guidance). Vein coagulation was carried out using a radiofrequency catheter, with a margin of 2 cm from the saphenofemoral and/or saphenopopliteal junction anastomosis. In 36% of cases RFO was supplemented using dissection of insolvent perforator veins from a short-scar incision. The extent of intervention was determined while taking into account the degree of chronic venous insufficiency of the affected lower extremity and the severity of patient's condition, as well as the severity of inflammatory infiltration in the area of thrombotic varicose veins. Surgery contraindication was extremely high surgery and anesthesia risk due to decompensated diabetes mellitus revealed in two elderly patients.

We would like to point out that crossectomy, invagination phlebectomy of the great saphenous vein on the hip with subsequent occlusion of the great saphenous vein on the lower leg; ligation of perforating veins from a short-scar incision according to Mueller, the ligature dissection of the saphenous and perforating lower extremity veins were prevailing surgical methods prior to the introduction of the ESRFO method into clinical practice. Since 2016 ESRFO combined with miniphlebectomy and sclerotherapy has been the most popular surgical procedure.

29 patients out of all those who underwent surgery in the near postoperative period from the intervention had postoperative inguinal hematoma. Longer after the operation 11% of patients noted tactile sensitivity disorder. The average duration of temporary disability of railway workers after surgical treatment of varicose veins was (18 ± 2.5) days, while after RFO the majority of patients (70.5%) returned to normal life on the day of surgery, 23.5% — 2 days after surgery, 8.8% — 3 days after surgery.

In order to assess the quality of life of patients after vein surgery was carried out, we used the CIVIQ questionnaire, which includes such criteria as the severity of pain, compromises to work or everyday life, sleep disorders, and impairment to physical and social functioning. It turned out that 17% of patients after classical phlebectomy experienced restrictions in their physical and social functioning, and 12% noted psycho-emotional distress. On the other hand, after RFO 53% of patients did not experience any restrictions in their physical and social functioning, and 73% claimed that "they did not even feel like they had underwent surgery", and there was absolutely no deterioration in the psycho-emotional background.

At the same time, all patients were asked to give their own assessment of the surgical treatment results, focusing on the success of the elimination of varicose and edematic syndromes, as well as such CVI symptoms as pain, heaviness, and fatigue in the legs, and night cramps. The vast majority of patients after ESRFO (65.0%) evaluated the effect of the operation as "excellent", noting the elimination of varicose syndrome, reduced swelling, night cramps, pain, and the disappearance (or reduction) of trophic disorders on lower leg. No patient assessed the results of repeated surgical treatment as unsatisfactory.

Conclusion

Lower extremity endovenous segmental radiofrequency obliteration (ESRFO) is thus the most advanced and minimally invasive method that significantly reduces perioperative stress during preoperative period and improves the life quality of the vast number of patients.

Conflict of Interest and Source of Funding
The article was prepared with the support of MEDTRONIK, which did not influence the data processing and final results of the work.

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Article received on 05.02.2018
Accepted for publication on 20.03.2018

UDC: 611.61:616.633.962.3

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MORPHOLOGICAL CHANGES OF STRUCTURES OF TUBULAR AND VASCULAR KIDNEY SYSTEMS ON PROTEIN LOAD

Abstract

Objective. Protein is an important component in the process of functioning of the body. But for those who have kidney disease, excessive consumption of protein leads to the opposite effect. Since the glomeruli cannot completely filter blood, toxic substances accumulate in the body. This leads to the disease of other organs. Therefore, the topic is important for research. The main objective of the is to study the morphological changes in the structures of the tubular and vascular systems of the kidneys on protein load. Regulation of protein homeostasis is provided by structural and functional systems and may be accompanied by proteinuria.

Materials and methods. Therefore, in order to study the structural bases of integration of functional kidney systems in the regulation of protein homeostasis, the author created a model of protein homeostasis disruption in rats. For the experiments, adult white outbred rats weighing 140–160 g were used, which were divided into three groups.

Results. On the first day after the experiment, dilation of the afferent and constriction of the efferent arterioles, and an increase in the percentage of glomeruli were seen. The structure of the proximal tubule cells did not change. On the third day, the degree of opening of the blood capillaries, surface and juxtamedullary nephrons exceeds the parameters of the control animals. As a result of the morphological study of the kidney, it was established that under different physiological conditions there are regular changes in the cells of JGA and capillaries of the glomeruli of superficial and juxtamedullary nephrons, which are aimed at increasing the functional reserve of the kidneys in regulating protein homeostasis.

Conclusion. It was established that a single protein load is accompanied by activation of the juxtaglomerular complex, and by changes of nephrons functioning.

Key words: *protein load, kidneys, glomerular filtration rate, arterioles, juxtamedullary nephrons*

For citation: Chinieva M. I. MORPHOLOGICAL CHANGES OF STRUCTURES OF TUBULAR AND VASCULAR KIDNEY SYSTEMS ON PROTEIN LOAD. The Russian Archives of Internal Medicine. 2018; 8(3): 219-222. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-219-222

DOI: 10.20514/2226-6704-2018-8-3-219-222

SG – secretory granules, COR – capillary opening rate, JGA – juxtaglomerular apparatus

Introduction

The life of an organism is a broad spectrum of genetically programmed constant reorganizations in response to different environmental and internal factors and changes in homeostasis arising from fluctuations of continuous metabolic processes. Adaptive reactions that have developed over the course of evolution are realized in ontogeny as genetically programmed reactions.

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They are highly diverse but can be divided into those realized relatively quickly (fractions of a second, seconds) and slowly (days, months, years) [1, p. 54].

The kidneys play role in homeostasis and, thus, are very sensitive to any changes in the diet. The kidneys perform not only excretory function but also a number of other most important functions, including ones related to metabolism and homeostasis. Despite their sensitivity to the slightest changes in the ratio of ingredients in the diet, the duration of the complete renal response to a change in any individual ingredient can vary. To decode mechanisms of renal homeostatic functions and integration of functional systems ensuring the performance of kidneys, a protein load model has been proposed for different age groups. However, the structural mechanism governing interactions between different functional systems of the kidneys in different physiological states remains understudied [2, p. 14].

The **objective** of this study was to define structural bases of integration of certain renal functional systems in the regulation of protein homeostasis.

Materials and Methods

The experiments were performed in adult white outbred rats with body weights of between 140 and 160 g. The first group (n = 15) received a protein load on their kidneys via single and multiple parenteral doses of albumin. The second group (n = 15) was subjected to protein deprivation, with ad libitum access to water. The third group (n = 15) was a control group.

In all experiments, the right kidney was dissected through the middle line from the convex surface to the portal area. A 1.5 mm slice was taken parallel to the dissection plane, and the cortex and medulla were separated. Then the cortex was cut into three equal parts: internal, intermediate, and superficial. Renal tissue corresponding to superficial and juxtamedullary nephrons was fixed in a 2.5% buffered glutaraldehyde solution. Sections of the tissue were prepared in an ultramicrotome according to the general technique used in electronic microscopy. The sections were mounted on slides, dried at room temperature and stained with methylene blue and basic fuchsin. Microscopic images were made using a light microscope equipped with a digital camera.

Renal tissue obtained on days 1, 3, and 7 was studied using morphometry and electron microscopy.

Results and Discussion

Results showed dilation of the afferent and constriction of the efferent arterioles, increase in the proportion of glomeruli with higher capillary opening rate (COR) and activation of cells in the juxtaglomerular apparatus (JGA) on the first day of protein load [3, pp. 79–80].

The cell structure of proximal tubules was unchanged. The cells are characterized by light and homogeneous cytoplasm with nuclei in the basal area. Mesangial matrix is scarce, with single mesangial cells (Fig. 1).

Three days later, when the JGA structure had normalized, COR of both superficial and juxtamedullary nephrons exceeded that observed indicators in the control animals.

Cells in the macula densa were cleared, and the length of basal and lateral parts of their membrane was increased. Juxtavascular cells were hypertrophic and contained secretory granules.

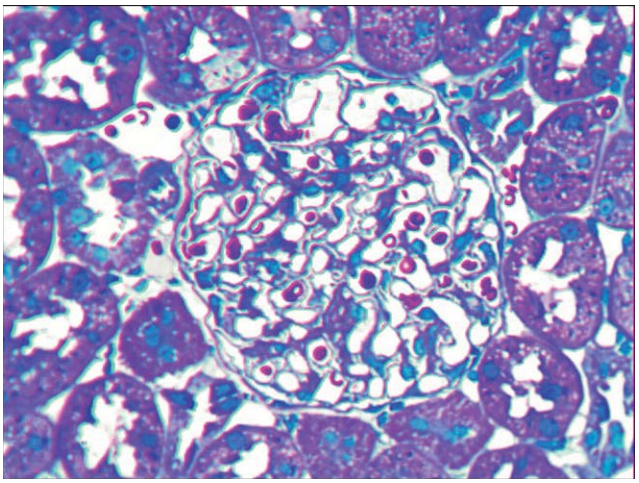

Figure 1. *Coloring: methylene blue. Inc. 40×10*

Mesangial cells grow in size and become irregular in shape under protein load (Fig. 2).

After three days of protein deprivation, COR is increased; however, no JGA activation is observed [4, p. 147].

7 days later, COR remains high in juxtamedullary nephrons only [5, p. 53].

Light microscopy results showed natural morphologic changes in different parts of the nephron. The size of glomeruli was increased, the urinary space of the Bowman's capsule was dilated, the mesangial matrix expanded and the number of mesangial cells increased, and capillary loops formed adhesions to capsule's walls and were compressed. In addition, focal sclerosis of the capsule and sclerosis of capillary loops were observed in single glomeruli. Significant changes were registered in proximal tubules. They included an increased number of secretory granules in cell cytoplasm, extrusion of the apical membrane of tubule cells into the tubular lumen, as well as nuclei in the apical or intermediate areas in significant number of cells (Fig. 3).

Therefore, different physiological states induce characteristic changes in JGA cells, glomeruli capillaries, and tubules of superficial and juxtamedullary nephrons. These changes aim to increase the functional reserve of the kidneys [6, pp. 54–55].

In control animals, juxtaglomerular cells of the afferent arteriole are the main renin-producing component of the juxtaglomerular apparatus [7, p. 84; 8, p. 102; 9, pp. 91–92]. They are polygonal in shape and contain numerous organelles: rough endoplasmic reticulum evenly distributed throughout cytoplasm and closely interacting with round-shaped moderate-size mitochondria; the Golgi apparatus is located close to the nucleus. Secretory granules (SG) are moderate in quantity, round-shaped, with high electron density, and are evenly distributed throughout cytoplasm. These data indicate their moderate functional activity [10, pp. 8–12; 11, pp. 8–82–85].

Juxtamedullary cells in the wall of the efferent arteriole are smaller and contain fewer SGs than those in the wall of the afferent arteriole. Cells in the macula densa are cylindrical, basal folds are single, low, do not contact with mitochondria, and are diffusely distributed in cytoplasm. The basement membrane is thin and non-continuous in the areas of cell membrane contact [12, p. 49].

Juxtavascular cells located between the afferent and efferent arterioles are irregular and elongated, have few organelles, numerous ribosomes and polyribosomes. Mesangial cells are located between capillaries in the glomerulus and are almost identical in structure to juxtavascular cells [13, p. 23].

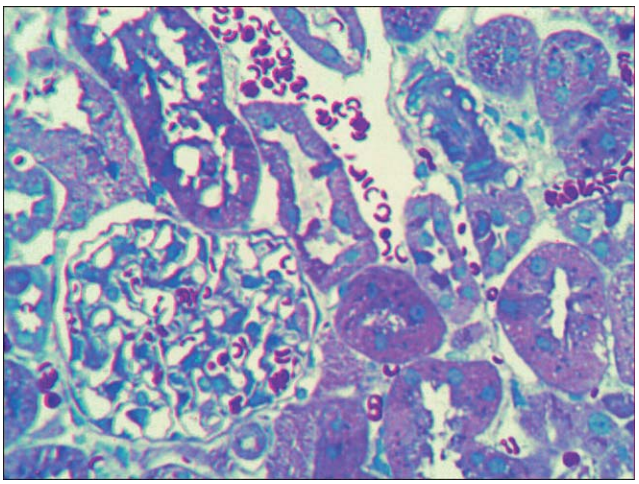

Figure 2. *Coloring: methylene blue. Inc. 40×10*

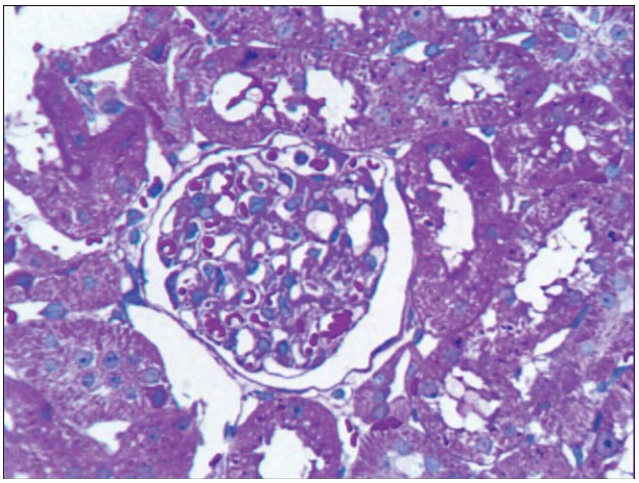

Figure 3. *Coloring: methylene blue. Inc. 40×10*

Conclusion

The obtained data indicate that single protein load is accompanied by activation of the juxtaglomerular complex and changes in nephron performance.

Morphological data characterize relatively early stages of experimentally-induced chronic renal dysfunction, since only the first signs of nephrosclerosis development were found together with clear morphological signs of changes in the glomerular hemodynamics and dystrophic changes in tubules.

The obtained data reveal new opportunities to study the role of the kidneys in protein metabolism in the event of the development of kidney failure, including tubular reabsorption of not only endogenous but also exogenous proteins. The data also point out the need to study the most important non-excretory renal functions and their effects in the analysis of nephropathies progression.

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 07.03.2018
Accepted for publication on 13.03.2018

UDC 616.441-006.6-085.849

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RADIATION-INDUCED MYELITIS AFTER RADIOIODINE THERAPY OF PAPILLARY THYROID CANCER: CLINICAL CASE

Abstract

Thyroid cancer incidence has been rising in most countries around the world in recent decades, and the most common form of thyroid cancer is papillary thyroid cancer. Application of radioiodine therapy for papillary thyroid cancer depends on the degree of postoperative risk of the disease recurrence. Radioactive iodine is recommended after radical thyroidectomy in case of intermediate or high risk of recurrence to reduce the probability of disease progression and to increase survival. The aim of radioiodine therapy is the ablation of thyroid tissue left after thyroidectomy and metastases, accumulating radioactive iodine. The recommended activity of the radiopharmaceutical is 30 mCi in the intermediate risk group and 30 to 150 mCi in a high risk group, but total doses and multiplicity of courses varies widely. The acute side effects of radioiodine therapy, the probability of which increases with a radioiodine dose of more than 100 mCi, include allergic reactions to iodine, radiation-induced parotitis and sialadenitis, gastritis, cystitis, pulmonitis (with lung metastases), myelodepression, transient amenorrhea and hypospermia. The listed violations are transient and last from several days to several months.
In the presented article the clinical case of papillary thyroid cancer with metastases in the neck lymph nodes was examined. The patient underwent thyroidectomy, central lymphadenectomy and radiotherapy. One year after the third course of radioiodine therapy the patient experienced the development of radiation-induced gastritis, myelodepression and myelitis, manifested by a severe pain syndrome in the cervical spine and sensory and motor disorders of hands and legs. Pulse therapy with glucocorticoids in combination with drugs that improve microcirculation, neuromuscular conduction and reduce the severity of neuropathic disorders allowed to manage radiation-induced complications.

Key words: *thyroid, papillary thyroid cancer, radioiodine therapy, radiation-induced complications*

For citation: Farkhutdinova L.M. RADIATION-INDUCED MYELITIS AFTER RADIOIODINE THERAPY OF PAPILLARY THYROID CANCER: CLINICAL CASE. The Russian Archives of Internal Medicine. 2018; 8(3): 223-230. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-223-230

DOI: 10.20514/2226-6704-2018-8-3-223-230

TgAb — Thyroglobulin autoantibodies, CT — computed tomography, RIT — radioiodine therapy, rhTSH — recombinant human TSH, WBS — whole body scintigraphy, Tg –thyroglobulin, TSH — thyroid-stimulating hormone, USS — ultrasound scan, CLND — central lymph node dissection

Thyroid cancer incidence has been rising in most countries around the world in recent decades. Over the course of 10 years this figure has almost doubled in the Russian Federation, affecting 6.1 persons per 100,000 residents. Each year about 8,000 primary cases are registered. However, thyroid cancer rarely is the cause of death, since in 90% of cases highly differentiated cancer (papillary, follicular) with a good clinical prognosis occurs, and the most common form of adenocarcinoma is papillary cancer (about 80%). Cells of highly differentiated thyroid adenocarcinomas can produce thyroglobulin (Tg), a specific protein of thyroid tissue, and concentrate iodine, the binding of which to the molecule of Tg ensures formation of thyroid hormones. This provides the basis for the use of the blood Tg level test for the monitoring of treatment effectiveness to detect residual tissue as well as to indicate when to apply radioiodine for diagnostic and therapeutic purposes [1–3, 10, 13].

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Principles of Treatment of Patients with Papillary Cancer

The extent of surgical intervention depends on the degree of risk of the disease. Hemithyroidectomy is thus considered to be an adequate operation for a tumor that is up to 4 cm in diameter without extrathyroidal extension, which is determined clinically or on the basis of the results of ultrasound scan examination (USS) and/or metastatic lymph nodes, a hereditary factor, and head and neck irradiation in past medical history. If the tumor size is more than 4 cm or there is a significant extrathyroidal extension, regional or distant metastases, thyroidectomy is performed with the maximum removal of tumor tissue. If metastases in central neck lymph nodes (anterior lymph nodes) are detected at the preoperative or intraoperative stage, a central neck lymph node dissection (CLND) is recommended. Advanced form of the disease (a tumor that is more than 4 cm in diameter within the thyroid tissue or that is any size with extrathyroidal extension) and preoperatively verified lateral neck nodal metastases are indications for preventive CLND.

The use of radioiodine therapy (RIT) for thyroid papillary cancer treatment is determined by the degree of postoperative risk of disease recurrence, which is classified according to the American Thyroid Association Guidelines 2009 as amended in 2015 [2, 7, 8, 12].

The following cases are classified in the ***group that is at low risk*** of papillary cancer recurrence*:

- If macroscopically all tumor tissue is removed, locoregional and distant metastases, extrathyroidal extension and vascular invasion are absent, and the first ^{131}I whole body scintigraphy (WBS) does not detect metastatic foci, the histological variant is nonaggressive.
- Metastases to regional lymph nodes are absent (cN0, c — clinical classification) or no more than five lymph nodes are affected (pN1, p — pathological classification), and the metastases do not exceed 0.2 cm in the largest dimension (micrometastases).
- The intrathyroidal papillary microcarcinoma (unifocal or multifocal); in these cases BRAFV600E** mutations are considered to be prognostically insignificant.

The ***intermediate risk group*** includes:

- Intrathyroidal papillary cancer that is less than 4 cm in diameter with a BRAFV600E mutation.
- Metastases of more than 5 lymph nodes (cN1 or pN1), and up to 3 cm in the largest dimension (macrometastases).
- Microscopic extrathyroidal extension.
- Papillary cancer with vascular invasion.
- Multifocal papillary microcarcinoma with microscopic extrathyroidal extension and BRAFV600E mutation.
- Metastatic foci in the neck, accumulating radioactive iodine according to data of the first ^{131}I WBS.

The following cases of papillary cancer recurrence are classified in the ***high risk group***:

- Residual tumor.
- A tumor in combination with a TERT***±BRAF mutation.
- Metastases of lymph nodes pN1 with any metastatic node more than 3 cm in the largest dimension.
- Macroscopic extrathyroidal extension.
- Proven distant metastases.
- High concentration of Tg, which is characteristic of distant metastases.

Radioactive iodine is used when there is an intermediate or high risk of thyroid cancer recurrence after radical thyroidectomy, which reduces the probability of disease progression and increases survival rate. At low risk, RIT is not appropriate, as it does not affect the mortality rate in this group of patients. The aim of RIT is the ablation of thyroid tissue left after thyroidectomy and metastases, which accumulate radioactive iodine. The first course of RIT is performed 3–6 weeks after thyroidectomy. If initially an organ-preserving operation was performed on the thyroid, classification of the patient in the group of intermediate or high risk of recurrence is an indication for radical thyroidectomy.

* This article proposes a classification that is specific to papillary thyroid cancer only.

** The BRAF gene mutation is associated with multifocal tumor growth, lymph node metastasis, and recurrence development. Therefore, it is considered as a marker of risk stratification.

*** The mutation of the TERT gene, which codes telomerase activity, determines the ability of unrestricted cell division.

The effectiveness of radioiodine ablation depends on the activity of ^{131}I absorption by thyroid cells, which in turn are determined by the level of thyroid stimulating hormone (TSH). It is recommended to carry out RIT before prescribing levothyroxine. In addition, the patient should follow a diet low in iodine over the course of 3–4 weeks before the RIT procedure. If patients are taking levothyroxine, then they should stop taking this medicine 4 weeks before RIT. The alternative is administration of recombinant human TSH (rhTSH)**, which makes it possible to examine and treat a patient using radioiodine without canceling levothyroxine sodium. The target level of TSH is considered to be more than 30 mIU/l. However, the optimal concentration of this hormone has not been determined.

To assess the accumulation of the radiopharmaceutical, ^{131}I WBS is performed at a dose ranging from 2–5 (to locate the residual tissue after thyroidectomy) to 10 mCi (to detect distant metastases). RIT is carried out in case of radiopharmaceutical high absorption using 30 mCi for patients in the intermediate risk group and from 30 to 150 mCi for patients in the high risk group. Distant metastases to the lungs respond to radioiodine treatment, although as metastatic foci increase in size the effectiveness of RIT is reduced.

Patient monitoring in dynamics is aimed at early detection of recurrence or progression of the disease (metastasis). Two to three months after initial treatment the thyroid status (TSH, free fractions of thyroxine and triiodothyronine) is analyzed to assess the adequacy of replacement therapy with levothyroxine sodium. To confirm remission, a conventional examination, USS, computed tomography (CT) at indications, WBS, stimulated Tg test*** (levothyroxine withdrawal 4 weeks before the analysis or administration of rhTSH) and antibodies to thyroglobulin (TgAb) are carried out after 6–12 months.

Postoperative management of the patient involves constant monitoring of the patient's current risk category. Four main groups are identified according to treatment results.

I. Biochemical remission:
- ✓ USS, WBS, CT do not reveal pathological foci.
- ✓ Unstimulated Tg: less than 0.2 ng/ml.
- ✓ Stimulated Tg: less than 1.0 ng/ml.

For this group of patients the probability of recurrence is 1–4%.

II. Biochemical recurrence:
- ✓ USS, WBS, CT do not reveal pathological foci.
- ✓ Unstimulated Tg: more than 1.0 ng/ml.
- ✓ Stimulated Tg: more than 10 ng/ml.
- ✓ Increase of antibodies to Tg (TgAb).

In approximately 30% of cases biochemical indicators spontaneously decrease for the patients in this group, in 20% of cases there is remission after additional RIT is observed, and in 20% of cases a structural recurrence is observed. In this regard maintenance of stable concentration of Tg or its decrease allows, in most cases, to limit the procedure to monitoring. When Tg or TgAb increase, an active examination and additional RIT are recommended. The mortality rate of thyroid cancer does not exceed 1%.

III. An unidentified tumor status:
- ✓ USS, WBS, CT do not reveal pathological foci or the results are nonspecific.
- ✓ Unstimulated Tg: from 0.2 to 1.0 ng/ml.
- ✓ Stimulated Tg: from 1.0 to 10 ng/ml.
- ✓ The TgAb titer is stable or decreases.

The probability of structural recurrence in this group is slightly lower than in the previous group: 15–20% (nonspecific changes can be stable or disappear). In most cases, examinations (visualization, Tg) and biopsy of suspicious changes are carried out. The mortality rate of thyroid cancer does not exceed 1%.

IV. Structural recurrence:
- ✓ Structural or functional signs of the tumor at any level of Tg or TgAb.

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* The medication rhTSH has been in use since 2005 in EU member countries and since 2007 in the USA. In Russia, the medication rhTSH is not covered by the patient management guidelines for differentiated thyroid cancer treatment. Therefore, if necessary, patients must purchase it independently at their own expense. The cost is about 1,200 euros.

** Highly sensitive methods for determining blood Tg (< 0.1 ng/ml) are considered an alternative to carrying out stimulating tests.

Approximately 50–60% of patients in this group have a persistent tumor, despite additional treatment. Mortality rate from thyroid cancer with regional metastases reaches 11%, and it reaches 50% when there are distant metastases.

The degree of risk of the disease determines the choice of treatment regimen with thyroid hormones:

- ✓ Replacement therapy aimed at correction of hypothyroidism. The target TSH level is 0.5–2.0 mIU/l.
- ✓ Suppressive therapy, inhibiting TSH-dependent growth of residual tumor cells, and TSH less than 0.1 mIU/l; free thyroxine does not exceed the upper limit of the norm.
- ✓ Mild suppression. TSH is within the range of 0.1–0.5 mIU/l.

Suppressive therapy is performed in a group of high-risk and structural recurrence (with the exception of patients with atrial fibrillation for whom mild suppression is recommended). In addition, in the intermediate risk group there are biochemical recurrence and an unidentified tumor status (except for cases of tachycardia and a menopause period when mild suppression is recommended, along with patients over 60 years old, patients with atrial fibrillation and osteoporosis, for whom replacement therapy is recommended).

The determination of Tg is considered to be the most sensitive method of dynamic observation, since this parameter is a specific marker of thyrocytes and cells of highly differentiated thyroid cancer (papillary and follicular). To carry out this study, methods with a sensitivity of at least 0.2 ng/ml were used. It is necessary to consider that the presence of TgAb in blood can produce a false negative result when the Tg determination method is used. However, Tg can be detected within a few months after the initial treatment. Therefore, it is not advisable to perform the test within the first three months after the last treatment stage.

Postoperative examination of Tg and TgAb during levothyroxine therapy is recommended every 6–12 months. For the high risk group the intervals may be shorter, and for those in biochemical remission it can be longer: from 12 to 24 months. Re-testing of stimulated Tg is carried out for the high risk, structural and biochemical recurrence, and unidentified status groups, while for the low risk and biochemical remission groups repeated Tg testing is not recommended. The level of TSH should be estimated at least once every 12 months. Performing of neck USS is recommended after 6–12 months, depending on the risk group and the results of Tg testing. If suspicious lymph nodes are detected with a maximum size of more than 0.8–1.0 cm, targeted fine-needle aspiration biopsy (FNAB) and Tg measurement in needle washout fluid are recommended. If the node is smaller, dynamic monitoring is allowable. A CT scan is justified in case of doubtful results of USS in relation to the spreading of disease, suspicion of extension in neck structures, as well as to reveal metastases to the lungs and mediastinal lymph nodes in the high risk group with elevated levels of Tg (usually more than 10 ng/ml) or increased TgAb, regardless of the WBS data. Abdominal CT or MRI, MRI of the brain and skeleton are recommended for the group of high risk patients with elevated Tg concentrations (as a rule, more than 10 ng/ml) in the presence of signs of metastatic affection of these organs as well as in the absence of metastases to the lungs, lymph nodes of the neck and mediastinum [2].

The majority of the recurrences occur within the first three years of diagnosis, while local recurrences and regional metastases do not worsen the prognosis. In rare cases, a recurrence may develop after 20 years.

According to A. R. Shaha, the survival rate for highly differentiated thyroid cancer in the low risk group is 99%; for the intermediate risk group it is 87%, and for the high risk one it is 57% [5, 6, 11, 14, 15].

Complications of Radioiodine Therapy

For both treatment and for diagnosis, radioactive ^{131}I with a half-life of 8.05 days is used. The penetrating power of β-particles, constituting 90% of the radiation, does not exceed 2.2 mm, thus avoiding damage to surrounding tissues.

The recommended activity of the medicine for radioablation amounts, as noted above, in the intermediate risk group 30 mCi, and in the high risk group it varies from 30 to 150 mCi, while the total

doses and the multiplicity of courses vary widely. The acute side effects of radioiodine therapy, the risk of which increases with a radioiodine dose of more than 100 mCi, include allergic reactions to iodine, post-radiation parotitis and sialadenitis, gastritis, cystitis, pulmonitis (with lung metastases), myelodepression, transient amenorrhea and hypospermia. The listed disorders are transient and last between several days and several months.

The long-term consequences of radioiodine application are cancers of other localizations, the risk of which increases when doses exceed 600–700 mCi [4].

The authors have not found information about the chance of developing myelitis as a result of radioiodine therapy in the available literature. In this connection, we present our own observation.

Clinical Case

On October 13, 2016, a 26-year-old patient was admitted to the Department of Neurology of G. G. Kuvatov Republican Clinical Hospital (RCH), Ufa. Complaints on admission: numbness in hands and feet, a decrease in sensitivity in hands, weakness in limbs, difficulty in fine motor skills, periodic pain in hands, feet and cervical spine, and pain in lumbar spine and legs when the head is tilted. Neurological examination diagnosed upper peripheral light distal paraparesis, lower limb pyramidal tract dysfunction, and distal paresthesia. The patient noted that his health deteriorated over the course of two months: at first there was intense pain in the cervical spine, then numbness, tingling, and weakness in the limbs. It became difficult for the patient to hold instruments (he works as a locksmith) and to write. He had difficulty walking, and subsequently numbness spread to the whole body.

According to the results of cervical spine MRI on October 5, 2016, a pathological zone of 1.8 cm in length intramedullary at the level of the Th1 vertebra was revealed, which was interpreted as a probable manifestation of transverse myelitis. The patient was urgently admitted to the Department of Neurology of the Central City Hospital (Uchaly), where he received pulse therapy (Metypred 1,000 mg three times for 1 week). During treatment, the severity of pain and weakness in the limbs decreased, after which the patient was transferred to the Neurological Department in Ufa.

The medical history: 5 years ago, in January 2012, during the preventive examination, thyroid cancer was found on the right. According to USS data, a focal mass that was 3 cm in diameter in the lower pole of the thyroid right lobe had exhibited changes that raised suspicions that it might be cancer: fuzzy uneven contours and increased intranodular blood flow. Papillary cancer, T3N0M0, was diagnosed on the basis of the results of FNAB. In February 2012, thyroidectomy was performed. Histological examination of the right lobe — papillary carcinoma with invasion of the thyroid capsule into half of its thickness, left lobe — macrofollicular nodular goiter in connection with autoimmune thyroiditis. Recommended observation by an endocrine surgeon, taking levothyroxine 100 μg/day, and a TSG test in 2 months. However, the patient for family reasons arrived for his control examination 14 months later, in April 2013. A focal mass that was 9 mm in diameter was revealed in a bed of the left thyroid lobe and bilateral increase of regional lymph nodes. The tumor of the left lobe was removed. Histological conclusion: toxic nodular goiter, reactive sinus histiocytosis. It was recommended to consult a radiologist to decide if RIT was necessary.

Re-examination after 3 months, in July 2013, did not detect pathological changes according to the USS of the thyroid, but the level of Tg was high: 81.4 ng/ml, and therefore on July 12, 2013, a course of RIT (81 mCi) was carried out and the dose of levothyroxine was increased to 275 μg/day. Whole body scintigraphy after 3 days revealed an increased accumulation of the radiopharmaceutical in the projection of the removed thyroid gland (10.2% of WBS), which along with a high concentration of Tg was the cause for planning the second course of radioiodine therapy after 6 months.

According to the results of the examination in February 2014, Tg was 66.87 ng/ml, and TgAb was less than 20 IU/ml (on levothyroxine withdrawal). The USS of the neck showed no pathology in the projection of the removed thyroid gland; however, in the region of the neck to the right in the middle third along the course of the vascular bundle, two

hypoechoic nodes, 14×7 and 9×7 mm, were visualized. Ultrasound-guided cervical node puncture was performed on the right. Cytological examination revealed a group of cells more similar to papillary thyroid cancer among lymphoid cells. The 2nd course of RIT (81 mCi) was carried out. Three days after treatment WBS showed the focus of increased accumulation of the radiopharmaceutical in the projection of the neck: 2.8% of WBS, and thyroid scintigraphy — the focus of increased accumulation in the projection of the neck lymph nodes, which, taking into account the results of USS, indicated metastasis to neck lymph nodes.
In the same month, the patient underwent an operation: CLND (levels I–V) on the right. Histological study confirmed metastases of thyroid papillary carcinoma in the lymph nodes (levels IIa–IV) with focal ingrowth in the capsule of lymph nodes, without proliferation into the surrounding soft tissues. The dose of levothyroxine was recommended to be increased to 300 μg per day.

After 3 months, in May 2014, the following was determined according to the results of the thyroid ultrasound examination: state after thyroidectomy on the left and resection of the right lobe and isthmus, the right part measuring 14×18×44 mm, 5.3 cm^3, left — 7×7×21 mm, 0.5 cm^3, total volume — 5.8 cm^3. Abdominal USS, chest X-ray also showed no pathology. In August 2014, the Tg level was 0.75 ng/ml, and the TgAb was less than 0.9 IU/ml (after levothyroxine withdrawal). In the same month, the 3rd course of RIT (81 mCi) was carried out. The examination, which was carried out 3 days later, revealed 2 foci of hyperfixation of the radiopharmaceutical in the neck region: 4% of WBS. The result of the neck USS: state after thyroidectomy.

In July 2015, thyroid USS did not visualize the left lobe and isthmus, there were no additional formations in their projection, and in the projection of the right lobe the following partially survived glandular tissue was determined: 13×7×26 mm, a volume of 1.2 cm^3, of heterogeneous structure due to two hypoechoic avascular formations in the lower pole up to 2.3 mm in diameter with clear, even contours. Chest X-ray and abdominal ultrasound did not reveal pathology. Tg was 0.29 ng/ml, and TgAb was less than 0.9 IU/ml (after levothyroxine withdrawal).

In August 2015, WBS and thyroid scintigraphy did not reveal hyperfixation foci of the radiopharmaceutical. Considering the low level of Tg and TgAb, the absence of pathological accumulation of the radiopharmaceutical according to scintigraphy data, remission of the disease was diagnosed. It is recommended to continue levothyroxine intake in the maximum tolerated dose as well as neck USS and unstimulated Tg measurements once every 6–9 months, chest X-ray and abdominal ultrasound once a year, and oncologist and endocrinologist follow-ups at the place of residence.

Starting in the autumn 2015, a year after the third course of radioiodine, the patient experienced periodic abdominal pain and weakness. According to the results of a clinical blood test, there was a fluctuating decrease in leukocytes, platelets, erythrocytes and hemoglobin, as well as relative lymphocytosis with neutropenia. To treat these symptoms, the patient received iron supplements, vitamin B12, and folic acid. However, the epigastric pain syndrome and weakness progressed. In June 2016, according to the examination data, pancytopenia in peripheral blood was detected: leukocytes were 1.5×10^9/l ($4.04–5.90\times10^9$/l, here and below, the reference interval is indicated in parentheses), platelets — 80×10^9/l ($142–424\times10^9$), erythrocytes — 2.24×10^{12}/l ($4.04–5.90\times10^{12}$), and hemoglobin — 68 g/l (120–170). In the leukogram an increase in the relative number of lymphocytes to 57% (19.0–37.0) along with the reduction in the number of segmented neutrophils to 37% (47–72) were observed. A biochemical analysis of the blood detected the cytolysis phenomena: an increase in the activity of creatine phosphokinase up to 417 IU/l (up to 190) and aspartate aminotransferase to 47 IU/l (5.0–38.0). Fibrogastroduodenoscopy revealed hemorrhagic gastritis, and the abdominal MRI noted an increased spleen to 139×64 mm.

The patient was admitted to a hospital, where he received treatment, including blood transfusions, proton pump blockers, gastric cytoprotectors, and enzyme supplements. As a result, the state of health was normalized, and blood parameters reached reference values. However, 2 weeks after discharge, neurological complaints, which were discussed above, appeared requiring emergency admission to

the clinic at the place of residence, Metypred pulse therapy administration and subsequent transfer to a specialized department in Ufa.
At the Department of Neurology of G. G. Kuvatov RCH, a differential diagnosis between paraneoplastic spinal cord injury, dysmetabolic changes in the nervous system and neurological pathology of inflammatory genesis was performed.

According to the results of clinical blood test, transient thrombocytopenia and relative lymphocytosis were identified. On October 14, 2016, the number of platelets was thus 80×10^9/l ($142–424\times10^9$), and 43% (19.0–37.0) were lymphocytes (in the leukogram). After 10 days, on October 25, 2016, platelets and lymphocytes were within the normal range: 241×10^9/l and 27%, respectively.

Sternal puncture data revealed bone marrow hypocellularity (the number of myelokaryocytes was 34×10^9/l [$50.0–150\times10^9$]) and an increase in the relative number of lymphocytes (17.3% [4.3–13.7]). According to the results of immunophenotyping of bone marrow cells, an increase in the relative amount of B-lymphocytes ($CD3^-$, $CD19^+$) up to 25% (7–17) and T-cytotoxic cells ($CD3^-$, $CD19^+$) up to 40% (19–35) was registered. Examination of cerebrospinal fluid revealed no pathological abnormalities.
Thyroid indicators corresponded to euthyroid status: TSH — 0.86 mIU/ml (0.23–3.4), T4 — 22.6 pmol/l (10.0–23.2). The low level of Tg — 0.07 ng/ml (1.4–74.0) along with the absence of pathological changes in thyroid USS and chest X-ray confirmed the remission of cancer. According to the results of abdominal USS, the size of the spleen was normalized (121×60 mm).
Stimulation electroneuromyography determined signs of dysfunction of conduction along the C6–C8, L5–S1 nerve roots and moderate myelopathy of the sensory fibers of the median and ulnar nerves on the left. MRI data from October 24, 2016, compared with data from October 5, 2016, reflected a positive course of improvement: the signal intensity in the myelopathic focal area decreased.
Based on the results of the examination, the paraneoplastic genesis of changes in the nervous system was excluded on the basis of remission of cancer. Dysmetabolic cause of the disease was also rejected because of the absence of risk factors (alcohol abuse, toxic substance abuse, etc.).

In addition, the gradual appearance and progression of the neurological symptoms, the reversal of most of the symptoms due to Metypred pulse therapy, including the positive developments according to MRI results, mostly corresponded to the inflammatory nature of the pathological process, apparently of an immune nature, associated with irradiation.

The activation of the immune system is indicated by relative lymphocytosis in the peripheral blood, an increased level of B-lymphocytes and cytotoxic cells according to immunophenotyping of bone marrow cells, and also a splenomegaly. Preemptive damage to the cervical spine is apparently due to its anatomical closeness to the thyroid gland, in the residual tissue of which the radioiodine concentrates. The probability of radiation-induced myelitis is also confirmed by a relatively high cumulative dose of radiation received by the patient: more than 200 mCi.

As is known, radiation exposure mechanism works on the basis of oxidative stress with the formation of a large number of chemically aggressive radicals that damage cells. Unlike high doses of radiation that cause massive cell death with the development of radiation sickness, the average doses cause the activation of autoimmune reactions, which in the present case is confirmed by the contingency of the revealed disorders with the activation of the lymphocytic segment of immune system and the effect of glucocorticoid therapy [9].

The diagnosis was formulated at the Department of Neurology of G. G. Kuvatov RCH as follows: Polyneuropathy secondary to somatic disorder (thyroid nodular goiter, state after surgical treatment and repeated courses of RIT, fluctuating reactive pancytopenia), upper peripheral light distal paraparesis, lower limb pyramidal tract dysfunction, and distal paresthesia. The treatment was aimed at improving microcirculation (pentoxifylline) and neuromuscular conduction (proserin) as well as at reducing the severity of neuropathic disorders (carbamazepine, amitriptyline, magnet therapy of the collar zone).

The patient was discharged with significant improvement. Follow-up examinations in 2016–2017 did not reveal pathological abnormalities.

In the presented case, radiotherapy was thus accompanied by the development of acute radiation-induced complications: gastritis, myelodepression, and myelitis. The predominant damage to the gastric mucosa is associated with oral administration of the medicine. Manifested disorders of blood system attributed to high sensitivity of blood cells to ionizing radiation and the revealed transient changes in the hemogram are explained by the lifespan of these cells and the periodic restoration of their number in the peripheral blood due to the formation of immature blood cells. The development of myelitis of the cervical region is apparently due to its anatomical proximity to the thyroid gland, and the possibility of this complication should be taken into account when performing radioiodine therapy for papillary cancer.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 19.03.2018
Accepted for publication on 07.05.2018

UDC 616-085.065-06:616-022.8

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LYELL'S SYNDROME: CLINICAL CASE

Abstract

In the article, the interdisciplinary problem of drug allergy is being analyzed, namely its systemic symptoms. The variants of systemic allergic reactions and the characteristics of the toxic epidermal necrolysis or Lyell's syndrome as one of the most serious and rare form of illness have been shown. The analysis of the clinical case of the Lyell's syndrome has been carried out with diagnostic difficulties in the initial phase of the disease and favorable outcome. The dynamics of clinical signs and their reversed development has been proved.

Key words: *drug allergy, Lyell's syndrome, toxic epidermal necrolysis*

For citation: Khokhlova Z. A., Gileva R. A., Konyakhina I. G., Tishkina A. P. LYELL'S SYNDROME: CLINICAL CASE. The Russian Archives of Internal Medicine. 2018; 8(3): 231-236. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-231-236

DOI: 10.20514/2226-6704-2018-8-3-231-236

BP — blood pressure, ALT — alanine aminotransferase, AST — aspartate aminotransferase, GGT — gamma-glutamyltransferase, IgG — immunoglobulin G, IgM — immunoglobulin M, DA — drug allergy, ESR — erythrocyte sedimentation rate, TEN — toxic epidermal necrolysis, T° — body temperature, RR — respiratory rate

Introduction

The incidence of drug allergies has become more frequent at the present time. This is believed to be related to the increase in the number of allergic and autoimmune diseases, the use of a large number of medicines for a single patient, the presence of concomitant diseases involving various organs, and the development and use of new medicines [1, 2]. A drug allergy (DA) is an immunologically mediated hypersensitivity to medications that develops with repeated exposure to them. It is one of the most severe allergic reactions with a variety of clinical symptoms that are difficult to treat [1–6]. A physician in any field of specialization can face this pathology. In Russia, adverse drug complications affect 2–3% of outpatients and 10–15% of in-patients [2, 6].

Background

There is DA with systemic clinical manifestations and DA that predominantly results in injury of certain organs [3].

Systemic clinical manifestations of DA are diverse. Anaphylaxis and the following acute severe generalized dermatoses may develop: erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis (Lyell's syndrome); serum sickness, systemic drug-induced vasculitis, drug-induced lupus erythematosus, drug fever, and drug-induced hypersensitivity syndrome (which is not fully understood) [3].

Clinical manifestations of DA that predominantly affect particular organs include lesions of the skin,

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respiratory system, hematopoietic system, cardiovascular system, gastrointestinal tract, hepatobiliary system, urinary system, and nervous system. Skin lesions are characterized by the following events: maculopapular exanthems, urticaria and angioedema, hypersensitivity vasculitis, allergic contact dermatitis, fixed drug eruption and other toxicodermias, erythema multiforme, photodermatitis, Arthus reaction, exfoliative erythroderma, erythema nodosum, and acute generalized exanthematous pustulosis [3].

Lyell's syndrome, one of the rare life-threatening conditions, is a systemic manifestation of DA. In particular, it is one of the acute severe generalized dermatoses (epidermolytic drug reactions). These conditions are characterized by extensive lesions of the skin and mucosa [1–3, 5–7]. Drugs that can cause these syndromes include sulfonamides, penicillins, and more rarely cephalosporins, fluoroquinolones, vancomycin, rifampicin, nonsteroidal anti-inflammatory drugs, and anticonvulsants [1–4]. According to different authors, morbidity ranges between 0.4 to 6 cases per million [1, 3, 4] and up to 0.3% of all drug allergy cases [2, 7]. It can occur at any age. Drug allergy risk is higher in HIV-positive persons (by a factor of 1,000 times) [4] as well as in patients with systemic lupus erythematosus and cancer [1, 4]. The period between administration and first clinical manifestation can be 2 to 8 weeks. It can take this amount of time to produce an immune response. The pathogenesis is associated with massive death of basal skin keratinocytes and mucosal epithelium caused by Fas-induced and perforin/granzyme-mediated cell apoptosis. Programed cell death results from immune-mediated inflammation, in which cytotoxic T-cells play an important role [2–4]. Stevens-Johnson syndrome with a lesion area of less than 10%, Lyell's syndrome with a lesion area of more than 30%, and an intermediate form with a lesion area of 10–30% have all been identified [4]. In fact, these are just the stages of a single process [1, 3, 4]. The disease prognosis depends on the patient's age, concomitant diseases, and the extent of the skin lesion. Mortality due to epidermolytic drug reactions is 5–12% [4], and it is 30–70%–100% due to Lyell's syndrome [2, 6].

Steven–Johnson syndrome is a severe form of exudative erythema multiforme, in which visceral lesions are observed along with skin and mucosal lesions. Extensive polymorphic rashes as well as the formation of bullae and ulcers of the mucous membranes (two or more) and skin are characteristic. Epidermal necrolysis covers less than 10% of the total skin surface. Severe fever and fatigue are observed [3, 6].

Lyell's syndrome (*toxic epidermal necrolysis, TEN)* is an acute and severe life-threatening disease that is characterized by extended bullous lesions of the skin and mucosa. Appearance of epidermal necrolysis (positive Nikolsky's sign) and skin exfoliation associated with severe intoxication and multiple organ dysfunction are typical [3, 6].

Usually, pathological processes in the skin and mucosa undergo stages from erythema multiforme to Stevens–Johnson syndrome and result in extensive toxic epidermal necrolysis of the skin with 30 to 100% coverage by lesions. The time interval that is needed for TEN to develop may vary from several hours to several days [1, 3, 4].

The disease may start as common urticaria that is resistant to therapy with histamine antagonists and calcium agents [4, 6]. This is succeeded by the following symptoms: nausea, vomiting, joint pain, and fever. Alternatively, fever, chills, weakness, headache, muscle and joint pain, sore throat, rhinitis and pharyngitis may develop initially, often leading to an initial diagnosis of ARVI [1, 2]. Soon, an erythematous edematous rash of various sizes, which is often confluent, develops on the face, torso and mucosae along with tenderness and burning of the skin. After a short time, multiple flabby bubbles are formed, which are confluent, and they form extensive erosions and massive exudation, which results in dehydration and deterioration in the patient's condition. Headache and lesions of the internal organs progress, and loss of consciousness is observed. TEN may take one of several possible courses: a hyperacute disease with fatal outcome, an acute disease with a toxic infectious syndrome with a potentially fatal outcome, and a benign disease with resolution by the 10th–15th day [2].

Fleck's leukocytes agglomeration test, specific immunoglobulin E serum testing, lymphocyte blastogenic response, Shelley's basophil degranulation test, etc., and challenge tests performed by an allergist-immunologist are recommended to perform a laboratory diagnosis of TEN at the present time [2, 3]. It is also necessary to perform a frozen section biopsy of skin lesions where perivascular lymphocytic and eosinophilic infiltrations are discovered [4].

Differential diagnosis of skin lesions is performed during the early stages with severe infectious diseases (chicken pox, measles, scarlet fever, meningococcemia, etc.), and it is performed at the late stages with generalized herpetic skin and mucosa lesions, systemic diseases, bullous pemphigoid, paraneoplastic pemphigus, pustular form of psoriasis, generalized staphylodermia and streptodermia, Duhring's herpetiformis dermatitis, etc. [1–3].

When an epidermolytic drug reaction is identified, the physician, regardless of his/her specialization, must provide emergency medical care to the patient and ensure his/her transportation to the burn center (department) or to the intensive care unit. Treatment of patients with TEN is similar to that of those with burn conditions.

Clinical Case

A 22-year-old female patient S. was admitted to the Infectious Disease Hospital on November 19, 2017, at 12:15 a.m., by ambulance with a diagnosis of "rash of unknown origin". Complaints: body rash, sore throat, eye redness, body temperature (T°) elevation to 38.2 °C.

Medical history: the patient fell ill on November 15, 2017, when she experienced weakness, neck pain, and enlargement of the left neck lymph nodes. Next day T° was 37.3–37.5 °C, and she experienced a burning sensation in the eyes. The patient took one Aspirin pill and one Ciprolet pill (the patient cannot remember the dosage). On November 17, body temperature was normal in the morning but elevated to 37.5–37.7 °C by the afternoon, and a burning sensation in the eyes persisted. The patient applied an ointment with cobra venom to reduce neck pain on the advice of her mother living in another city. The patient took Flemoxin (cannot remember the dosage) and Decaris pills (the patient had been scratched by a cat the day before and assumed that she could get infected with toxocariasis). On November 18, the 4th day of the disease, she experienced a minor cough and a sore throat, and small vesicles appeared in the mouth. A rash appeared on the chest in the evening, and T° was 37.7 °C. The patient called an ambulance and was admitted to an infectious diseases hospital.

It is known from the patient's medical history that she experienced rubella, chicken pox, parotitis in childhood. The patient experienced exacerbation of tonsillitis in mid-October 2017, was treated with amoxicillin 500 mg as an outpatient, and was discharged and allowed to return to work on October 26, 2017. The patient denies having an allergy. Epidemiological history: the patient lives alone in a comfortable apartment, and has had no contacts with infectious patients.

On admission, the patient's condition was assessed as of medium severity. T° was 37.7. Mental status was normal. The patient was active. A few elements of small-spotted skin rash localized on the chest and isolated elements on the back were discovered. The patient had pronounced conjunctival hyperemia and scleral congestion. Diffuse hyperemia was discovered at the oropharyngeal mucosa. The tonsils were not enlarged, mucosal granularity was advanced, and an enanthema was discovered on the hard palate. The anterior neck lymph nodes were enlarged to grade II, and they were not painful. Nasal breathing was not obstructed. Dyspnea was absent. Mild cough was observed. Breath sounds were vesicular. The heart sounds were clear. Pulse volume was normal, and the heart rate was 106 per minute. BP was 136/95 mmHg. The abdomen was soft and not painful. Bladder and bowel function was normal.

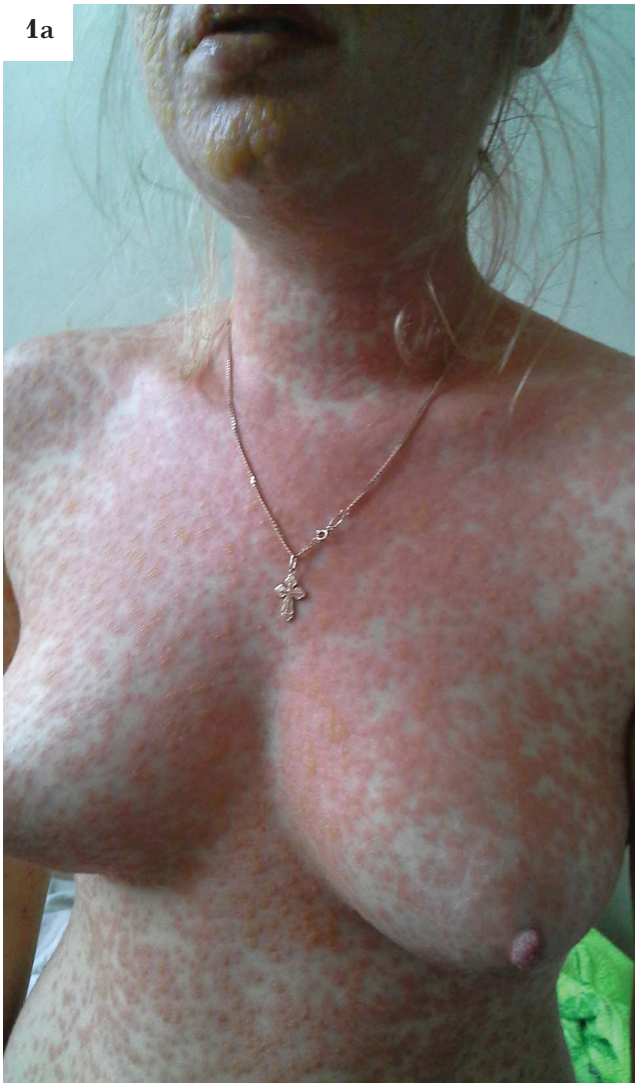
Taking into account that the disease began with enlargement of the lymph nodes followed by low-grade fever, sore throat, hyperemia of the oropharyngeal mucosa with pronounced granularity, as well as the absence of leukocytosis and tendency to leukopenia observed in the complete blood count (white blood cells $4.3*10^9$), a viral infection was suspected. Herpetic infections, ARVI, and

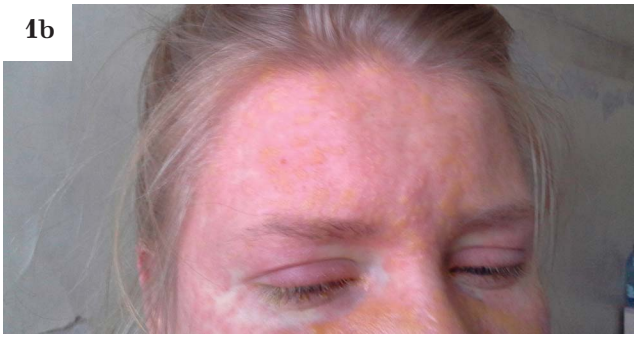
enteroviral infection were the choices that needed to be narrowed down using differential diagnosis. Antiviral treatment (Viferon, aciclovir) and topical antiseptics were prescribed.

In the morning of November 19, the patient presented the same complaints as on admission, but itchy eyes were reported in addition to burning. Her general condition had deteriorated. T° was 37.5. Heart rate was 74 per minute, and respiration rate (RR) was 18 per minute. The pattern of exanthema and its localization were changed. The rash started to resemble small- and medium-sized papules, and it extended to the face, torso, arms and hips; it was profuse, bright pink, and it did not itch. An enanthema on the soft palate, a buccal enanthema, conjunctival hyperemia, injection of scleral vessels, eyelid puffiness were also observed. Antiviral treatment was continued, and detoxication therapy and systematic desensitization were initiated.

November 20: T° was 37.3. Heart rate was 94 per minute, and RR was 20 per minute. BP was 110/70 mmHg. The patient's condition had worsened considerably. Sore throat and eye redness persisted. A bright, profuse rash in the form of medium-sized papules extended across the entire torso, and they became confluent with purulent content in some places, including the face and limbs (Fig. 1, a, b, c).

Swelling of the face, lips, eyelids, as well as pus discharge from the eyes (Fig. 2), and purulent plaques appeared at the mucous membrane of the hard palate and at the tongue. ENT specialist diagnosed necrotic stomatitis. Purulent discharge





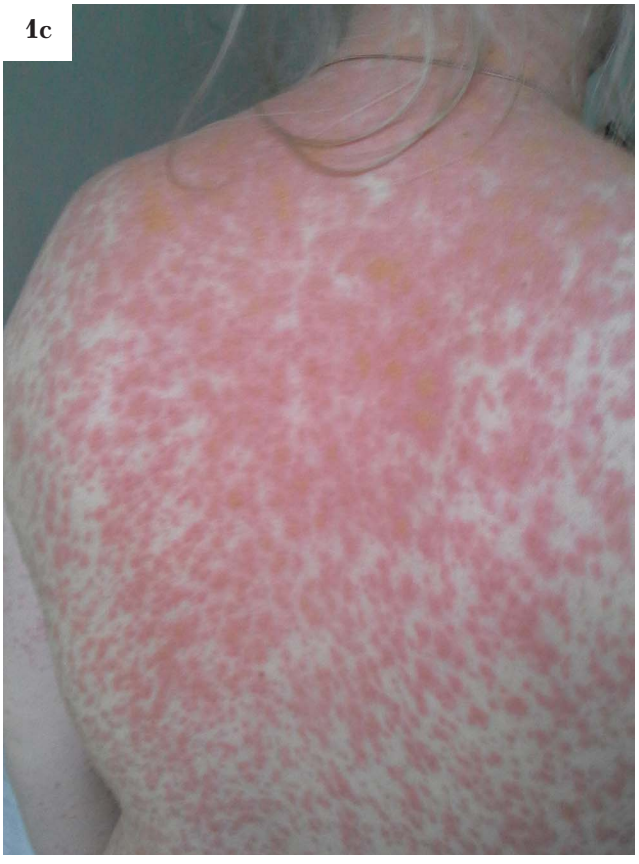


Figure 1 (a, b, c). *Skin and mucous membranes rashes, November 20, 2017*

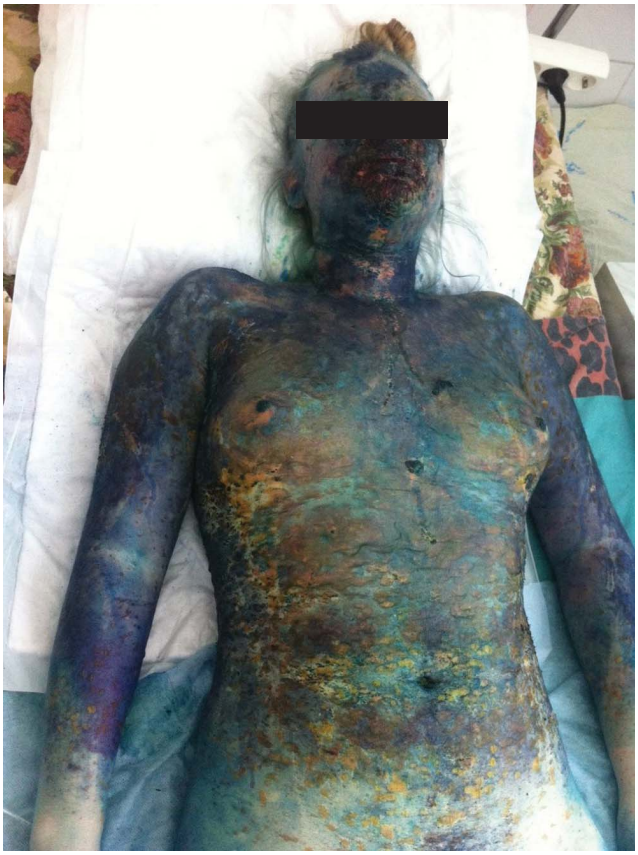


Figure 2. *December 3, 2017*

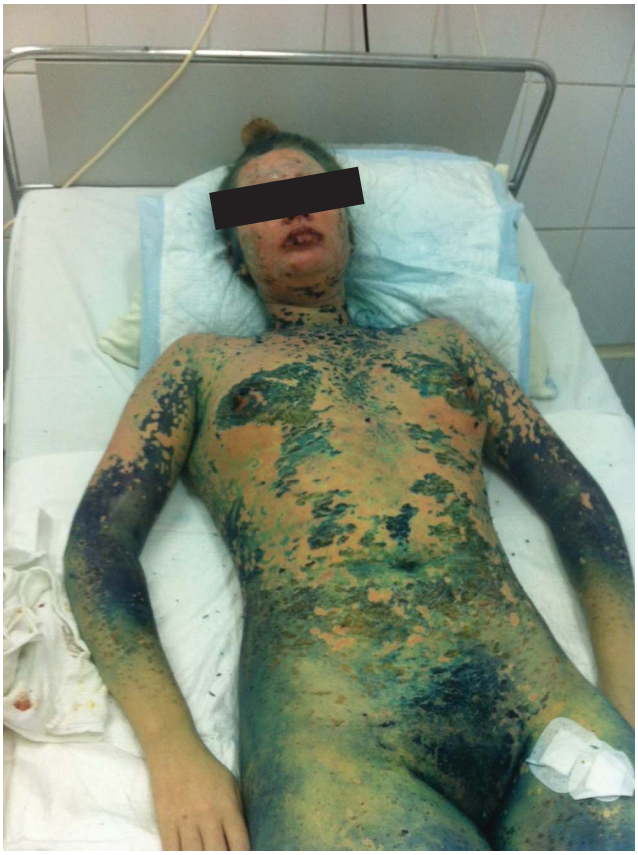


Figure 3. *December 13, 2017*

from the genitals was also observed. Signs of liver injury were discovered while taking into account blood chemistry parameters.

The nature of skin abnormalities in combination with the lesions of the oral and genital mucosa and development of reactive hepatitis syndrome were indicative of an allergic reaction that is probably associated with the use of medicines before the patient was hospitalized, and they provided evidence for diagnosing Lyell's syndrome.

The results of a laboratory examination were obtained.

A complete blood count was performed:

November 19, 2017: Hemoglobin: 144 g/l, ESR: 25 mm/h, WBC: $8.6*10^9$, RBC: $4.27*10^{12}$, PLT: 148, Eos. (eosinophils): 12, bands (banded neutrophils): 14, segs (segmented neutrophils): 53, lymph (lymphocytes): 8, mon (monocytes): 13.

November 20, 2017: Hemoglobin: 129 g/l, ESR: 17 mm/h, WBC: $4.3*10^9$, RBC: $4.74*10^{12}$, PLT: 154, Eos.: 0, bands: 34, segs: 53, lymph.: 11, mon.: 2.

Biochemical blood test, November 20, 2017: Total bilirubin 79.6 μmol/L, direct bilirubin 73.1 μmol/L, indirect bilirubin 6.5 μmol/L, AST 195 U/L, ALT 179 U/L, thymol test 4 U.

Urinalysis: unremarkable.

Serum procalcitonin concentration, November 20, 2017: 0.46 ng/ml (reference values – less than 0.5 ng/ml).

IgG antibodies against the capsid protein with an avidity index of 100% were discovered in a blood test for EBV infection.

A test for parasitic invasions was performed in accordance with the algorithm for examining patients with exanthema syndrome. IgM antibodies against Trichinella were discovered with titer of 1:100, and the degree of positiveness was discovered to be 1.6 (where the parameters do not match the diagnostic value). Total antibodies to lamblia antigen were discovered with a titer of 1:200; and total antibodies to Toxocara and Opisthorchis were not discovered.

The indirect hemagglutination test for pseudotuberculosis turned out negative.

A bacteriological examination of the oropharyngeal mucosa dated November 20, 2017, revealed a culture of pigmented Neisseria and Staphylococcus haemolyticus with heavy growth.

A bacteriological examination of the bullae content dated November 20, 2017, revealed a culture of Staphylococcus aureus.

On November 20, 2017, the patient was diagnosed with Lyell's syndrome and was transferred to the ICU at a multidisciplinary hospital due to the severity of the condition caused by the systemic disease, including extensive lesions of skin (Fig. 3), mucous membranes of the oral cavity and genitals. In addition, involvement of the liver (reactive hepatitis), bone marrow (decrease in RBC count to $3.21*10^{12}$, neutrophil left shift and WBC count to $3.6*10^{9}$, PLT count to $105*10^{9}$), and pancreas (reactive pancreatitis: amylase 1,269 U/L). No abnormalities of other organs and systems were discovered by comprehensive examination. At the same time, the patient had staphylococcal bacteremia (a culture of Staphylococcus aureus was isolated in the blood sterility tests dated November 30 and December 5; no growth was discovered in the test dated December 15), which indicated the need for the administration of an antibacterial treatment. Detoxication, desensitization (Medopred, prednisolone, Suprastin), hepatoprotective therapy, as well as topical treatment of the skin, mucous membranes, eyes (antiseptics, desensitization and wound healing agents), and specialized nutrition supplements were provided. The patient was kept at the ICU for treatment until December 19, 2017. Manifestations on the skin and mucous membranes have epithelized gradually (Fig. 2, 3), whereafter the patient was transferred to the gastroenterological department due to the development of cholestatic drug-induced hepatitis that was difficult to be resolved. (The viral etiology of hepatitis was excluded.) Gradual increases of the total bilirubin values (with maximum of 339.5 μmol/L), mainly due to the conjugated bilirubin fraction (with maximum of 287.1 μmol/L), of GGT (with maximum of 2,484 μkat/L), cholesterol (to 27.4 mmol/L), and alkaline phosphatase (to 28.7 μkat/L) were observed over time. An increase in hepatic transaminases values was less pronounced (maximum of ALT/AST: 25.55/12.57 μkat/L), followed by normalization in 1 month. Glucose and salt solutions for parenteral administration, liver protectors, gastrointestinal adsorbents, and glucocorticosteroids were used as part of a comprehensive treatment.

The patient was discharged from the hospital on January 11, 2018, in satisfactory condition, with persisting clinical and laboratory signs of a cholestatic syndrome. The patient was assigned to the follow-up care of a general practitioner, gastroenterologist, and dermatologist.

Conclusion

The case that we reviewed demonstrates the difficulties of diagnosing and performing a differential diagnosis of Lyell's syndrome at an early stage, when multiple organs are affected by the peak manifestation of the disease. The case history confirms that successful treatment is possible when the lesion area is large. In addition, this case demonstrates the severity and protracted course of reactive cholestatic hepatitis requiring long-term treatment and follow-up. Different types of specialists are required for the correct management of a patient with Lyell's syndrome.

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 14.03.2018
Accepted for publication on 29.03.2018

UDC: 616.5-002.525.2-07;616-06]-21.144

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DIFFICULTIES OF "COMPETITIVE" PROCESSES DIAGNOSIS: THE SYSTEMIC LUPUS ERYTHEMATOSUS IN PATIENT WITH MODERN COMORBIDITY. CLINICAL CASE

Abstract

The paper deals with the problem of recognizing systemic lupus erythematosus, which is getting a rather common disease. It presents a clinical case of a female patient with type 2 diabetes mellitus, hypertension, hyperuricemia, who developed systemic lupus erythematosus at the age of 52. The onset of a new disease in a patient with comorbidity is often concealed by the symptoms of the new disease. It troubles the well-timed diagnosis of the new disease. The diagnosis of systemic lupus erythematosus was established according to the accepted criteria in Federal Clinical Recommendations. The authors emphasize that according the Federal Clinical Recommendations a long-course of hydroxychloroquine added to the basic therapy proved to be effective in relieving clinical manifestations of systemic lupus erythematosus and preventing its exacerbations. Detailed differential diagnosis in the presented case is given in discussion. The considerable attention is paid to diagnosis and treatment of the fast-progressing lupus nephritis. The example of standard pulse-therapy is given at the fast-progressing lupus nephritis for an induction of remission and its effectiveness for reversal of a nephrotic syndrome on a concrete example is shown. The authors considered pathological processes which lead to fast progression of secondary nephrotic syndrome. Recently opened pathophysiological mechanisms of development of anemia of chronic disease are presented in the paper, which contribute to complete understanding of pathogenesis of these processes. Diagnostic search of the reason of not expressed joint syndrome with systemic manifestations was performed among probable autoimmune and metabolic diseases for people of the senior age group. Specific liver function tests indicated the diagnosis of lupus hepatitis. Probable mechanisms of liver injury in this clinical case are described. Work of authors has cross-disciplinary character and it can be useful to experts of theoretical and clinical medicine.

Key words: *systemic lupus erythematosus, nephrotic syndrome, glomerulonephritis, anemia of chronic disease, hepcidin.*

For citation: Kostenko D. Yu., Khelimskaya I. V. DIFFICULTIES OF "COMPETITIVE" PROCESSES DIAGNOSIS: THE SYSTEMIC LUPUS ERYTHEMATOSUS IN PATIENT WITH MODERN COMORBIDITY. CLINICAL CASE. The Russian Archives of Internal Medicine. 2018; 8(3): 237-242. [In Russian]. DOI: 10.20514/2226-6704-2018-8-3-237-242

DOI: 10.20514/2226-6704-2018-8-3-237-242

BP — blood pressure, ALT — alanine aminotransferase, AST — aspartate aminotransferase, AB — antibody, ACD — anemia of chronic disease, ALP — alkaline phosphatase, CHF — chronic heart failure, FOV — field of view, Hb — hemoglobin, gl — globulin, GGT — gamma-glutamyl transferase, Ig — immunoglobulin, ACEI — angiotensin converting enzyme inhibitor, WBC — white blood cells, LN — lymph node, CBC — complete blood count, CU — clinical urinalysis, PTI — prothrombin index, X-ray — roentgenography, RES — reticuloendothelial system,

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DM — diabetes mellitus, SCT — spiral computed tomography, ESR — erythrocyte sedimentation rate, SLE — systemic lupus erythematosus, Pl — platelets, T° — body temperature, US — ultrasound scan, CIC — circulating immune complex, HR — heart rate, RBC — red blood cells

Systemic lupus erythematosus (SLE) is a typical autoimmune disease that has not lost its relevance with the passage of time. This disease is currently incurable. However, SLE symptoms can be controlled with appropriate therapy, which gives the majority of diagnosed patients an opportunity to lead an active and healthy life. Today, successful pharmaceutical treatment is available. Nevertheless, despite obvious medical breakthroughs, some SLE-related issues remain unsolved; the most important of them is probably timely diagnosis. Symptoms of the disease are highly diverse, and they do not always manifest themselves in typical ways, which makes diagnosis difficult and confirms its name as "the great imitator" of other diseases [1]. SLE can be diagnosed at any age; however, the onset is more common between 15 and 45 years. SLE onset can occur in elderly patients, when comorbidities are present and differential diagnosis is especially problematic. To prove this, we provide a case report of SLE.

A 52-year-old female patient was admitted at the Internal Medicine Department of Khabarovsk Railway Hospital on November 30, 2015. The patient complained of hypertension, weakness, dyspnea on exertion, low appetite, morning nausea, daily fevers of up to 37.5 °C in the morning and to 38.5 °C in the evening (without chills), pain in shoulder joints, and leg swelling by night. The patient had a medical history of type 2 diabetes mellitus for 2 years and was treated with metformin (glycemia of 5.8–6.5 mmol/L). The patient has hypertension for about 5 years (BP up to 200 mm Hg), but she did not feel sick and took beta-blockers, ACEI and Arifon irregularly. Episodes of feet, legs, hands and face swelling were registered periodically for 3 years. Tenderness in shoulder joints was observed for several years. The patient attributed these symptoms to being overweight and doing sports at younger age. She had considered herself healthy until August 2015, when abundant red rash appeared in the vermilion zone, facial skin, shoulders, upper parts of the chest and back, sometimes accompanied by itching. The patient consulted with an allergy specialist. The event was considered to be a symptom of allergic dermatitis (due to the administration of Ascorutin, vitamins B and magnesium). When these drugs administration was discontinued, the signs of dermatitis decreased significantly. Blood tests had been showing leukopenia and ESR 30 mm/h since September, and blood count was within normal limits. After high levels of uric acid had been detected, the patient was referred to a rheumatologist. Rheumatoid arthritis and psoriasis were excluded. Outpatient examinations continued. Periodic fever and ESR elevations to 60 mm/h had been registered since the second half of September. The hemogram of November 21, 2015, showed anemia (RBC — $3.3*10^{12}$/L, Hb — 97 g/L). WBC and platelet counts were within normal limits. Blood biochemistry showed dysproteinemia with normal total protein. US results: no abnormalities were detected in the liver, spleen and lymph nodes. The patient consulted with a hematologist. Iliac crest bone biopsy was performed: no abnormalities were detected. Contrast-enhanced spiral computed tomography (SCT) of the head, neck, chest, and abdominal cavity organs revealed liver hemangioma, calcified foci in the spleen, small secondary renal cysts. No abnormalities of the lymph nodes were detected. Blood tests for HIV as well as for viral hepatitis B and C all produced negative results. Tumor markers were within normal limits. Fibrogastroduodenoscopy: reflux esophagitis, incompetence of the cardia, duodenogastric reflux. The patient's health worsened progressively, and she contacted the Railway Hospital for additional examination with the following referral diagnosis: fever of unknown origin.

Patient's state at admission was satisfactory. Obesity was diagnosed (grade 3). Faint hyperemia foci with slight desquamation were present in the upper part of her chest and back. Mild swelling of the face, eyelids, lower third of the calves and feet was observed. T° 37.6 °C, BP was 150/100 mm Hg, HR — without abnormalities.

Complete blood count: RBC — $3.2*10^{12}$/L, Hb — 91 g/L, Pl — $258*10^9$/L (N: 150–390), WBC — $3.8*10^9$/L, lymphocytes — 14% (18–40) / $0.5*10^9$/L (1.2–3.0), banded neutrophils — 7%, segmented neutrophils — 75%, ESR — 69 mm/h, reticulocytes — 6 per mille (N: 2–13); peripheral blood smear: RBC hypochromia (++), mixed anisocytosis (+), occasional megalocytes and hypochromic macrocytes; clinical urinalysis: color — yellow, clarity — cloudy, specific gravity — 1,010 (N: 1,012–1,024), reaction — acidic, protein — 4.6 g/L (N: 0–0.12), glucose — absent, squamous epithelium — 2–3 per FOV, granular, waxy and WBC casts — single casts per FOV, bacteria (+); blood biochemistry: glucose — 7 mmol/L (N: up to 5.5), total protein — 49 g/L (65–85), albumins — 43.6% (46.9–61.4), α_1-gl — 5.31% (2.2–4.2), α_2-gl — 15.8% (7.9–10.9), β-gl — 14.6% (10.2–18.3), γ-gl — 20.7% (17.6–25.4), total bilirubin — 9.7 μmol/L, direct bilirubin — 2.2 μmol/L, ALP — 157 U/L (up to 270), GGT — 61 U/L (up to 32), creatinine — 128 μmol/L (up to 106), urea — 11.6 mmol/L (up to 8.3), uric acid — 732 μmol/L (up to 310), serum iron — 4.9 μmol/L (6.3–30.1), PTI — 103% (80–110), ALT and AST — within normal limits; procalcitonin — 0.23 ng/mL (up to 0.5), and serum ferritin — 887 μg/L (15–150) were detected; blood test for syphilis (rapid plasma reagin) was negative.

Clinical urinalysis on day 3: specific gravity — 1,003, reaction — acidic, protein — 1.9 g/L (0–0.12), WBC — 45–55 per FOV, abnormal RBC — 10–15 per FOV, glucose — absent, squamous epithelium — single cells per FOV, waxy casts — single casts per FOV. ECG results: sinus rhythm with HR 93 bpm, moderate changes in the left ventricular myocardium. Cardiac ultrasound: grade 1 hypertrophy of left ventricular myocardium, ejection fraction — 69%. Cardiac chambers dilation. Grade 2 mitral regurgitation, grade 1 tricuspid regurgitation. Mild pulmonary hypertension. Increased pericardial effusion (pericardial cavity thickness 0.9 cm behind the posterior wall of the left ventricle). The patient continued complaining of fatigue, low appetite and nausea.

Based on the results of laboratory and instrumental testing, the patient was given a conservative treatment of urinary infection as a possible cause of fever (empiric antibacterial treatment — ceftriaxone + ciprofloxacin). Anemia, hypertension, edema, and diabetes mellitus were treated: allopurinol, insulin Actrapid, Maltofer, vitamin B_{12}, metoclopramide, omeprazole, Arifon. However, an acute worsening of dyspnea and swelling was registered on December 5 while on therapy. Edema increased and orthopnea appeared. Examination results: moderately severe condition, massive swelling of the lower extremities (up to the groin), upper extremities, anterior abdominal wall, and anterior neck surface; oliguria. Dyspnea at rest up to 22 respiratory movements per minute, BP was 180/110 mm Hg, tachycardia at rest — 100 bpm, ECG results unchanged compared to those of December 2, 2015. The therapy was adjusted: furosemide 80 mg, intravenous bolus; spironolactone 50 mg twice a day (in the morning and in the afternoon); bisoprolol 7.5 mg in the morning, HR-controlled; enalapril 10 mg twice a day (in the morning and in the evening).

Hands X-ray results: subchondral bone sclerosis and paraarticular osteoporosis of hand bones, single subperiosteal dystrophic cysts around the base of the middle phalanx and the head of the proximal phalanx of the fifth right finger; spinous syndesmophytes along the dorsal surface of the nail phalanx of the first right finger. Narrowing of the joint space in the phalangeal joints. Conclusion: dishormonal arthropathy, necessary to differentiate from rheumatoid arthritis. During hospital stay, changes in the body temperature were irregular, from 36.8 °C to 38.9 °C. The following tests were performed to determine the cause of inflammation: colonoscopy (histological results — moderate chronic enteritis), blood culture (no growth), repeated procalcitonin test less than 0.5 ng/mL. C-reactive protein — 18 mg/L (0–6), rheumatoid factor — 16 IU/mL (0–8). Serum cryoglobulins — positive. Clinical urinalysis: specific gravity — 1,010, protein — 4.1 g/L, WBC — the entire FOV, RBC — 40 per FOV. Urinary infection secondary to other disorders was predominant in the clinical pattern; however, the autoimmune disorder was becoming more and more probable.

Despite the therapy, edema, fatigue, dyspnea and nausea persisted. Blood biochemistry results on

December 8 (compared to December 1, 2015): normalized total protein — 55 g/L, and uric acid — 595 μmol/L, worsening of hypoalbuminemia — 21 g/L, increased creatinine — 350 μmol/L, and urea — 15.2 mmol/L. IgA — 5.07 g/L (1–6.5), IgM — 0.59 g/L (0.6–2.8), IgG — 19.15 g/L (9–20). Antinuclear antibodies were detected. The analysis of homeostasis showed a tendency towards hypercoagulation with signs of intravascular coagulation, positive lupus anticoagulant, CICs without abnormalities, daily protein loss — 7 g/day (0–0.141), with diuresis — 1,300 mL/day. TSH — 1.2 pg/mL. CBC showed that the anemia parameters remained almost the same compared to December 1, 2015: RBC — $3.3*10^9$/L, Hb — 89 g/L, leukocytosis — $11.8*10^9$/L, banded neutrophils — 2%, segmented neutrophils — 88%, lymphocytes — 6% / $0.7*10^9$/L, ESR — 66 mm/h, platelet count within normal limits; microscopy showed mixed anisocytosis (+) with hypochromia (+). Tests for antineutrophil cytoplasmic antibodies, anticardiolipin antibodies, and anti-DNA antibodies were in progress. Renal ultrasound detected no abnormalities. Chest X-ray: increased pulmonary vascularity due to vascular and interstitial components, bilateral pleural effusion, signs of heart enlargement due to enlarged left chambers.

Taking into account patient's condition and test results indicating the development of nephrotic syndrome, the therapy was adjusted after consultation with a nephrologist: albumin transfusion followed by stimulation with furosemide at 1 mg/kg/day (80–120 mg/day) was added to treatment. Continued antibacterial therapy was recommended based on the glomerular filtration rate: ceftriaxone 1.0 once a day together with ciprofloxacin 500 mg/day. Due to progressive azotemia, enalapril was replaced by moxonidine 200 μg twice a day. Treatment with spironolactone (potassium- and ECG-controlled) and allopurinol was continued. Laboratory tests showed signs of disseminated intravascular coagulation. Therefore, Clexane was added to the therapy.

On December 10, the primary diagnosis was determined considering medical history, clinical pattern of the disease, and additional tests: acute SLE with serositis (pleuritis, pericarditis), skin lesions (faint erythematous rash in the upper part of the chest and back), renal involvement (rapidly progressive lupus nephritis), hematologic (anemia, lymphopenia) and immunologic disorders (elevated antinuclear antibodies, anti-DNA antibodies); high SELENA-SLEDAI score (17), and high SLICC index (5) [2, 3]. Complications: acute kidney injury, RIFLE stage I. Secondary diagnoses: urinary infection; T2DM, HbA_1c < 7.5%; stage 3 essential hypertension. NYHA class III CHF; grade 3 obesity; hyperuricemia.

SLE diagnosis is based on ACR 1997 classification — 4 of 11 criteria should be present (rash, serositis, renal involvement, abnormal titer of anti-DNA antibodies were diagnosed), or is based on SLICC 2012 — 4 criteria should be present, one of them clinical and one immunologic (the following were confirmed: nephritis, serositis, lymphopenia < $1.0*10^9$/L, a more than twofold increase in anti-DNA antibodies titer) [2]. Daily protein loss was 7 g/day (0–0.141). The patient was examined by a rheumatologist and was transferred to a specialized Rheumatology Unit; prednisolone 45 mg/day was initiated. The patient reported slight improvement. Intravenous pulse therapy was prescribed according to therapeutic indications: methylprednisolone 1,000 mg once a day (for 3 days) + cyclophosphane 1,000 mg once a day (one day). Laboratory results of December 14, 2015, after initiation of therapy: anticardiolipin IgM antibodies — 3.0 (N less than 7), IgG — 6.1 (less than 10), anti-β_2-glycoprotein IgM antibodies — 4.1 (less than 7), IgG — 5.7 (less than 10), antiphospholipid IgM — 3.6 (less than 10), IgG — 7.9 (less than 10); *serum anti-DNA antibodies* — 586.2 (0–25); CIC — 56 RU (54.24 ± 2.0 RU), IgA level — 336 mg% (91–360), IgM — 78 mg% (61–160), IgG — 864 mg% (720–1,460). During the period from December 17 till January 14, 2016: RBC count increased from 2.8 to $3.6*10^{12}$/L, Hb — from 81 to 104 g/L, lymphocytes level — from 15 to 27%; WBC count decreased from 11 to $10*10^9$/L, ESR — from 38 to 32 mm/h. During the same period, urea level decreased from 32.7 to 18.1 mmol/L, creatinine — from 245 to 98 μmol/L, total bilirubin — from 11 to 8 μmol/L; cholesterol level decreased from 6.6 to 3.2 mmol/L, with reduced enzymatic activity: ALT — from 28 to 13 U/L,

AST — from 20 to 10 U/L, and ALP — to 97 U/L. Clinical urinalysis showed regression of proteinuria from 1.4 to 0.44 g/L, and it showed regression in daily protein loss from 7 to 0.28 g/day. Subjective condition of the patient also improved: fatigue and dyspnea decreased, nausea disappeared, and appetite increased.

The patient was discharged with improvement on January 18, 2016, with the following recommendations: a rheumatologist follow-up at the place of residence, continued hypotensive therapy as before, methylprednisolone 30 mg/day, omeprazole 20 mg in the morning, torasemide 5 mg in the morning, alphacalcidol 0.5 μg in the morning, and cyclophosphamide administration scheduled in one month.

The reported case was difficult to diagnose, since this systemic, rapidly progressing, highly active disease was developing in an elderly patient with several comorbidities. Each of these comorbidities could be accompanied by cardiovascular and kidney damage. Therefore, differential diagnostics included multiple diseases. Determining the cause of kidney danage was of special interest. The absence of stable hypotensive therapy could lead to hypertensive glomerulonephritis. The patient had type 2 DM; therefore, diabetic nephropathy could not be excluded: the glomerular capillary basement membrane becomes glycosylated, which leads to impaired nutrition and hypoxia of underlying tissues, including podocytes and juxtaglomerular apparatus. A reactive increase in BP accelerates the course of hypertensive glomerulonephritis, and podocytes cell death results in diabetic glomerulosclerosis. This could lead to kidney failure and nephrotic syndrome. Secondary cysts found in the kidneys confirmed this "expected" long-term nephropathy. At the same time, nephropathy secondary to hypertension and DM leads to excretory hyperuricemia. The accumulation of urates in renal interstitium induces abnormal tubular function and proteinuria leading to chronic kidney disease, hypertension and secondary gout. The patient was taking indapamide, which significantly increased the risk of this type of kidney damage. Existing DM predisposed the patient to recurrent renal infections with anemia, fever and nephrotic syndrome. Infectious endocarditis and oncological processes can induce nephrotic syndrome and fever, but they were excluded in the differential diagnosis. Nephrotic syndrome in the absence of marked systemic manifestations also could be explained by IgA-nephropathy (atypical Berger disease or IgA-nephropathy induced by other disorders: celiac disease, psoriasis, SLE) or renal type of Henoch-Schönlein purpura. Normal blood IgA levels did not exclude these diseases, and renal biopsy was considered as a reserve diagnostic measure. Hypothyroidism as the cause of edema was also excluded. Insignificant articular syndrome with systemic symptoms required differential diagnosis with rheumatoid arthritis, psoriatic arthropathy, gout, and osteoarthritis deformans, which are common in elderly patients. The key factor in the diagnosis of nephrotic syndrome, considering a peculiar clinical pattern, was detection of abnormal anti-DNA antibodies titer. Rapidly progressive lupus nephritis has the following symptoms: twofold increase in serum creatinine within 3 months, nephrotic syndrome, erythrocyturia, severe hypertension [3]. Clinical symptoms fully corresponded to this type of lupus nephritis.

According to current recommendations, renal biopsy is the gold standard of diagnostic methods of kidney damage in SLE [2, 3]. However, some patients are ineligible for biopsy due to various reasons (obesity, coagulopathy, diabetes mellitus, risk of secondary infection, etc.). The patient had rapidly progressing kidney damage secondary to SLE; therefore, pulse therapy was indicated. In Russian guidelines, mycophenolate mofetil and belimumab in lupus nephritis treatment have higher levels of evidence. However, "classical" medications for SLE treatment, such as prednisolone, cyclophosphan and azathioprine, remain relevant. The role of hydroxychloroquine in the comprehensive therapy of SLE should be noted. This product of a 4-aminoquinoline derivative is prescribed to all patients with SLE — class of recommendation A, level of evidence 1 [2]. In the present case hydroxychloroquine administration had been postponed until comorbidities were stabilized.

The hematologic status of the patient is also interesting. Based on the clinical urinalysis and blood biochemistry results, the following disorders were considered: iron deficiency anemia (given decreased serum iron level, hypochromic RBC, and mixed anisocytosis), hyperchromic anemia (presence of megalocytes), autoimmune hemolytic anemia with antilymphocyte antibodies (based on direct to total bilirubin ratio of 1:4.4, absolute lymphopenia, and positive test for serum cryoglobulins), hereditary hemochromatosis (ferritin level 887 μg/L, with N up to 150), and blood cancer as the cause of these changes. Anemia of chronic disease should be seriously considered as a disorder that can explain the majority of these changes. Recent studies of ACD have provided a detailed description of its etiology. Pro-inflammatory factors (IL-1, IL-6, TNF-α, etc.) inhibit production of erythropoietin, suppress proliferation and differentiation of erythroid progenitors, stimulate erythrophagocytosis by cells of RES [4] and ferritin production [5], increase the number of transferrin receptors on hepatocytes [6] and, thus, increase their iron intake, and activate production of an antimicrobial peptide with a role in iron regulation — hepcidin. Hepcidin blocks exit of iron from hepatocytes and RES cells [4, 7]. Considering the presence of ferritin receptors on hepatocytes, lymphocytes and erythroblasts [8], we can expect that a decrease in number of ferritin-consuming cells will lead to an increase in its serum concentration. This can explain hyperferritinemia with low serum iron.

Observation of the patient over time revealed an interesting tendency in liver function parameters, which do not exclude a diagnosis of lupus hepatitis, in our opinion, although liver function parameters were within normal limits at admission. The patient received intensive pharmaceutical treatment during the period from December 17, 2015, to January 14, 2016: prednisolone pulse therapy (followed by oral methylprednisolone 50 mg/day with dose tapering), and other medications, with simultaneous 2-week therapy with antibiotics. During this treatment, liver function markers (ALT, AST, ALP, bilirubin) decreased from the upper limit of normal to the lower limit. We can explain this by immune-mediated (antinuclear antibodies, rheumatoid factor, etc.) damage of hepatocytes and their overload with ferritin.

Therefore, the reported case demonstrated a multidisciplinary approach to differential diagnosis in this patient. Our observation, which highlights current explanations of pathogenesis of certain disorders, may be useful for primary care physicians and hospital physicians: it is a case of SLE in female patient over 50 years old with "not uncommon comorbidity".

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 15.01.2018
Accepted for publication on 23.04.2018