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Honoris Causa Professor Dushan Fedorovich Lambl was a Man Truly Devoted to Science

Abstract

Vilém Dušan Lambl, better known in Russia as Dushan Fedorovich Lambl (1824-1895) — Czech and Russian anatomist, histologist, therapist and parasitologist, doctor of medicine, professor, head of the department of normal anatomy and pathological anatomy of the Imperial Kharkov University (from 1860 to 1871), head of the Faculty therapeutic Department of the Imperial University of Warsaw (from 1871 to 1895), Privy Councilor.

Professor Lambl D.F. the main works on parasitology (he was the first to describe in 1859 the simplest microorganism that parasitizes humans and now bears his name), pathological anatomy (his famous lithographs written on stone), normal anatomy, internal medicine, as well as natural science, ethnography, culture and linguistics of Slavs and others. He was a man with the broadest horizons, sharpness of thought, excellent erudition and incredible knowledge in the field of both fundamental and practical medicine.

Key words: *Dushan Fedorovich Lambl, Imperial Warsaw University, Imperial Kharkov University, giardiasis*

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The Path to Science

Dushan Fedorovich Lambl (Vilém Dušan Lambl) was born on December 5, 1824, in Bohemia (Czech Republic). In 1849, he graduated from the Faculty of Medicine of the University of Prague with a Doctor of Medicine degree. Lambl considered Professor Jan Dlaugi, who had taught him anatomic pathology, to be his teacher [1, 2].

As a young 21-year-old man, Lambl was a writer. His first works were devoted not to medicine, but rather to different branches of natural science, geography, ethnography, etc. They were primarily concerned with Slavic lands, their inhabitants, and natural wealth. These writings were based on personal observations and researches conducted during his travels to these lands.

Lambl published his first scientific work on anatomic pathology entitled *Ein Neues Querverengtes Becken* (translated from German as *The New Dollichopellic Pelvis*) in 1853. After that, without leaving his natural science studies, he wrote many valuable

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Figure 1. *Photo of Lambl V.D. in his younger years. The original document is stored in the Literary Archive of the Museum of Czech Literature (PNP), Prague, Czech Republic [11]*

articles on general and anatomic pathology, including those where he described circular fibers in the ciliary muscle he had previously discovered.

With a perfect command of German, French, Italian, English and Russian languages, Lambl dreamed of a journey whereby he could study and aggregate all those remarkable and rare materials on anatomic pathology that were scattered throughout various European museums. He made that journey across Europe in 1856. After visiting Germany, Holland, Belgium, England, France, Italy and Austria, and working hard in hospitals and museums in 26 cities, he published his wonderful *Reisebericht* (translated from German as *The Trip Report*), which included a lot of critically important and interesting pathological and analytical observations [3].

One of the most outstanding periods in Lambl's biography dates back to 1856–1860, when he was a privat-docent of the Anatomic Pathology Department at the University of Prague. There, he organized private courses in normal and pathological histology, which drew the attention of many doctors visiting Prague. During that period, many of the young Russian scientists (MDs) who were preparing for their academic careers worked under the supervision of Professor D.F. Lambl. Dushan Lambl soon established his school, whose students were treated with special warmth, love, and respect [4].

Professor Lambl's Work at the Imperial Kharkov University (1860–1871)

In September 1860, Dushan Lambl was appointed an extraordinary Professor of the Anatomy Department at the Imperial Kharkov University. He was notified of the appointment while abroad (in Prague), so he took up his duties in January 1861. Lambl acquired Russian citizenship. He gave lectures in impeccable Russian. Vladislav Skvortsov, the famous Kharkov scientist, claimed that "the period of Lambl's work at the Kharkov University was the golden age for the General and Anatomic pathology Departments. At that time, the teaching of general anatomy changed significantly, and it reached the level which existed only in some of the best universities abroad."

In Kharkov, Dushan Lambl met a young but promising lawyer, Anatoly Koni, who later became his close friend. Koni left wonderful memories of Dushan Lambl, thanks to which we can imagine (over 100 years on) Lambl as a vivid personality surrounded by students and colleagues and interacting with the university community. In his memoirs, Anatoly Koni writes: "...A student of the famous Joseph Hyrtl, an agile and energetic person with beautiful, lively, smart brown eyes on a lean face under a loosely-falling bush of iron-grey hair, Lambl made an impression of an outstanding man, and he was that man. A real master, he was not a narrow specialist. He responded to all kinds of spiritual demands of human nature. A lover and connoisseur of European literature, a subtle connoisseur of art, he could truly say about himself, "nihil humanum a me alienum puto" (translated from Latin as "nothing human is alien to me"). For example, he knew Dante's works in detail, and his explanations and remarks inspired me to like and take interest in artistic works of W. Hogarth." [5]

Dushan Lambl liked attending meetings of the Faculty and the Council of the Imperial Kharkov University. During his 11-year work in Kharkov, he was among the most active members of these meetings. He was interested not only in subjects directly related to his specialty and department but also in

most issues related to university life. His numerous opinions, reports and reviews were always substantial and based on facts and scientific evidence.

It is noteworthy that working at the Kharkov University, Dushan Lambl acted as an opponent during the defense of a doctoral thesis by still unknown doctor Nikolai Vasilievich Sklifosovsky, who later became a famous surgeon. He recommended the faculty to provide Doctor Mitrophan Popov with a university scholarship for studying anatomic pathology. Later on, Popov wrote an extensive and important scientific work titled *The Faculty of Medicine of the Kharkov University During the First Hundred Years of Its Existence. 1805-1905*. [6]

In 1862, Lambl was sent as an official adviser for medical subjects to the International Exhibition in London by the university. In the same year, he was elected an honorary member of the Czech Medical Society.

In 1865, Dushan Lambl was entrusted with managing a therapeutic hospital. Here, Professor also proved himself as an experienced organizer and manager. According to his contemporaries, "as a practitioner, he chuckled at the narrow specialization that was rapidly developing at that time. While trying to understand the nature of the disease, he relied on his creative thought, rather than recklessly following the ideas expressed in the latest foreign books, especially in different chemical and other studies. He cured not the theoretically understood disease, but each patient, individualizing his methods and instructions and giving special attention to psychological observation. He was often looked upon as an original, but this eccentric was famous for a lot of brilliant healings in cases when there was a certain identified serious disease, and in situations when it was just needed to put a person in a cheerful mood without attaching certain medical labels supported with a pre-determined treatment procedure and regime." [1]

As for the Anatomic Pathology Department at the Kharkov University, Lambl deserves great credit for its foundation and development. When he came to Kharkov, he took up a position as a Professor of the Anatomy Department. At a meeting held by the Faculty of Medicine on May 20, 1861, he was "offered to teach anatomic pathology," and he accepted that offer. [7, 8]

Lambl was a brilliant speaker. His lectures, which were attended not only by students and doctors, but also by professors from other departments, met the highest European standards and were distinguished by the breadth of the lecturer's horizon, clarity, and brilliance of thought. The Professors lectures were also interesting because he supported the explanation of material with pictures drawn with colored crayons on the board.

"... It was a real pleasure to listen to his vivid, strictly scientific speech full of experience and numerous examples," wrote Anatoly Koni. While preparing for his lectures, Professor conducted a detailed literature review of the latest medical achievements in the field and provided examples from his own research experience [5, 9].

Dr. Lambl was one of those scientists who had extensive knowledge and a gift for a clear, distinct, and consistent presentation of information. Professor Timofey Illinsky noted that the "constant presence of Lambl in a group of people working under his guidance, and his readiness to explain all arising questions make anatomic lessons in the hospital more valuable. Not only a specialist but also any educated physician will benefit from spending some time attending Lambl's classes, because, in addition to microscopic examinations, they will be able to use received knowledge in practice, at the patient's bedside" [17].

Dushan Lambl's student, Professor Popov, wrote: "At least two courses (around 300 people) attended Lambl's lectures. The first seats were taken with a fight a few hours before the lecture. Those sitting at the back had to use binoculars, and one-third of the audience usually listened to the lecture standing up... Lambl was very strict and demanding during the midterm exam in anatomy. He did not even think about excluding any topic from the exam. The student had to know all the sections of anatomy. I remember a very sad fact during the midterm exam in 1863 when twelve of my comrades got a "1" for failing to tell with sufficient accuracy the anatomical paths of blood circulation in a fetal body." [6]

Professor Lambl was a skillful anatomist. While working, he often liked to say "while medical practice failures make patients silent, anatomic pathology makes the dead talk." He performed autopsies professionally, carefully, quickly, and consistently; he noticed even the smallest pathological changes in all organs. In his opinion, autopsies had great educational and cognitive value, as well as the ethical impact on students and doctors, who always

filled the autopsy room. "I found him in front of a marble table surrounded by a group of students. He performed an autopsy... and did it very skillfully, with great precision and knowledge. Active and self-confident, fully involved in finding a solution to the pathological question, greedily smoking a little cigarette stub that miraculously did not burn his nose, he seemed a real high priest of science on his exceptional service," Koni admiringly wrote about his friend [5, 10].

Passion for his work and constant work with the audience sometimes distracted the Professor's attention. Twice — in 1867 and 1871 — it almost had dramatic consequences when Dushan Lambl came close to dying after getting an infection through a cut on the finger while dissecting a body that had died of pyemia. Both cases required lengthy treatment, but at that time, there were no antibiotics, and so it was a difficult task.

Lambl's knowledge was truly encyclopedic: he published over 100 works on various branches of natural science, ethnography, culture and linguistics of the Slavic community, general and anatomic pathology, histology, parasitology, and therapy. Lambl studied inflammation, ovarian tumors, purulent peritoneal inflammation, and congenital pathology of the skeletal system. Particularly worth mentioning is his outstanding scientific paper titled *Microscopic Examinations of Intestinal Discharges*. For the first time, he described an endamebas — a "single-celled infusoria" found in diarrheal discharges in case of bloody diarrhea in children. It was named "Cercomonas intestinalis". Later on, however, in France, the protozoan was renamed after the scientist who discovered it — "Lamblia intestinalis", and the disease caused by it became known as "giardiasis" [11].

In 1861-1865, Professor Lambl released the original version of anatomic pathology in the form of lapidary lithographic notes. He believed that "text engraving with drawings is more preferable than printing as it enables us to insert more original drawings in the text without wasting time and money for woodcut printing." Lambl's notes are the first and unique textbook on anatomic pathology in the Russian Empire. The first two issues were devoted to the bones and joints pathology, the third one (1862) — to tumors, and the fourth one (1865) — to skin diseases. However, later sections were not published and existed only as notes passed around students [6].

Lambl was a public figure who actively participated in the work of the Kharkov Medical Society. In 1861, he was given the high honor of heading the society, becoming its first president [12, 13].

On April 21, 1867, during a visit to the International Congress of Physicians held in Paris, Professor Lambl was elected Vice-President of the Congress, which emphasized his high authority in the European scientific and medical community.

In 1871, on the proposition of Professor Yakov Kremyansky, the Council of the Imperial Kharkov University gave Lambl the title of Doctor Honoris Causa (Honorary Doctor of Medicine) "in respect for his merits in the field of medical sciences".

In the same year, Dushan Lambl was transferred to the Ordinary Professor position at the Department of Intermediate-Level Therapy at the University of Warsaw. "Having moved to Warsaw, Lambl remained a man truly devoted to science, infinitely devoted to the throne and the Empire, a worthy representative of the Russian flag on the periphery", wrote P.I. Kovalevsky.

Professor Lambl's Work at the Imperial University of Warsaw (1871–1895)

His knowledge of anatomy, anatomic pathology, physiology and internal medicine made Professor Lambl a top-ranked multi-skilled specialist and scientist. His move to Warsaw, in particular, to the Department of Intermediate-Level Therapy at the Imperial University of Warsaw (IUW), was seen as a true godsend. A broad-minded person possessing ingenuity, excellent erudition, and incredible knowledge of both fundamental and applied medicine was vital to the department and the university, especially during the period of global reforms and transformations. Thanks to Dushan Lambl, the Department of Intermediate-Level Therapy began a new life. He constantly invented, developed and implemented something, tirelessly working in the hospital with his students and young doctors to bring the department's work to the required level. He completely changed the educational process, redid lecture courses, and improved approaches to the diagnosis and treatment of patients. Diverse hospital material allowed Lambl to share with his students the vast store of knowledge he possessed. Dushan Lambl continued to follow his principles,

working hard with students and medical residents at the patients' bedsides in the Holy Spirit Hospital [14]. Lambl was not always satisfied with the educational process at the Kharkov University, and it was the same in Warsaw. Anatoly Koni, who maintained a warm relationship with him after he left Kharkov, wrote: "Alien to all routine, putting working interests above everything, he experienced rough days (judging by his letters). A Czech by birth, he sincerely loved Russia and wished it true grandeur and involvement in cultural tasks of the West. Oftentimes the surrounding reality contradicted his wishes." [15, 16]

In the first years of leading the department, Professor Lambl prepared a crucial document, *The Project for Collecting Scientific Statistics in Warsaw Hospitals Exemplified by the Holy Spirit Hospital,* and presented it to the Faculty Medicine at the Imperial University of Warsaw.

Members of the Academic Council involved experienced Dushan Lambl in reviewing scientific articles, dissertations, and papers submitted for a competition to fill vacancies at the Faculty of Medicine. His detailed reviews were devoid of formalism and included important comments that helped papers look complete. Interestingly, in 1894, Professor Lambl received for review the works of Nikolai Mukhin, Doctor of Medicine, submitted "for the Department of Clinical Pathology and Therapy". Among other works, Dushal Lambl singled out the monograph *Toxic Spastic Paralysis*: "The book by privat-docent N.I. Mukhin ranks high thanks to its completeness and classified content, and it makes a useful contribution not only to Russian writing but to medical literature in general." Professor Mukhin headed and managed the department first in Warsaw and then, from 1915, in Rostov-on-Don [1, 3].

Professor Lambl tried to eradicate the long-standing tradition of evaluating dissertations, according to which the supervisor could be appointed as the censor. He criticized this practice when he was once appointed an official opponent to the dissertation of his student, physician Heinrich Patsanovsky: "Since this dissertation was written based on the material of the hospital under my jurisdiction, since I saw the work done with my own eyes, and therefore I am familiar with the content of the paper, I want to direct the attention of the Faculty of Medicine to the fact that, in my opinion, the evaluation of the dissertation's merits should be entrusted not to me,

Figure 1. *Photo of Lambl V.D. during his years in Warsaw. Original document is stored in the Literary Archive of the Museum of Czech Literature (PNP), Prague, Czech Republic [11]*

but rather to any other member of the faculty, to a Professor who could analyze the paper objectively as an outsider.

Therefore, it would be advisable for the Faculty of Medicine to engage teachers of relevant subjects to express their opinion based on the assessment, which should act as a scientific control. I neither intend to express my opinion nor wish to become an official opponent in a case involving my assistant." [1]

When Professor Lambl worked at IUW, such statements were often heard at the Faculty of Medicine; they always generated debates and helped reduce opportunism and formalism in the Council's work.

Professor Lambl devoted all his time to medicine. For him, the pleasures of social life were almost non-existent. He was married to Eugenia Edelberg, and they had a beautiful daughter Olga. Work and home were the only spheres he cherished. While Lambl "pursued science", said Professor P. Kovalevsky, "he was not alien to art. He painted, loved

music, and was no stranger to creativity in this field, he carved and drew on a stone, and being involved in these activities, he could not see another life."

It is worth noting that Dushan Lambl liked young people, placing great hopes on them, and often openly and selflessly defended young residents. There was a case at a meeting held by the Faculty of Medicine when he spoke in defense of residents, who he believed had been "unjustly slandered by the city council", and his speech generated a big debate. In 1881, Lambl was awarded the Order of Saint Stanislaus, first grade. Moreover, "on January 1, 1889, His Majesty the Emperor, having received the report of the Minister of Public Education, was most graciously pleased to promote Lambl to the rank of privy councilor." [1]

Professor Lambl spent 23 years at the University of Warsaw. This period was one of the most fruitful periods in the scientist's life. During that time, in addition to works published under his supervision, Lambl published many great articles on clinical medicine, filled with scientific interest. In 1893, Lambl received the P. Zagorsky award for his last great work *Selfdislocation of the Spine (Spondyl Olisthesis)*, which he had worked on for many years. The award given by the St. Petersburg Academy of Sciences came with a money reward amounting to 4,000 rubles, a huge sum which exceeded the professor's annual salary.

Dushan Lambl suffered from bronchial asthma for a long time, especially in his last years, when the attacks became more frequent and severe. On February 13, 1895, the acute disease suddenly cut the outstanding scientist's life short at 71. A few days later, Professor Lambl was buried in Warsaw's oldest necropolis in the Powazki Cemetery. "Professors, comrades of the deceased, students, many friends, and acquaintances led the coffin to the grave, which was covered with wreaths." [1, 10]

In his will, Professor Lambl, who loved young students dearly, ordered to donate "20,000 Austro-Hungarian forints to the Support Fund for Czech Students of the Faculty of Medicine at the University of Prague and the Technical University of Prague." [9]

He was a bright, energetic, intelligent, and clever man of his time, who valued life, loved art, raised more than one generation of doctors, and left a large footprint on the sands of time and Russian medical science.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Terentyev V.P. (ORCID ID: https://orcid.org/ 0000-0003-3607-5832): design, writing, editing and approval of the final version of the article

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Ambalov Yu.M.: editing and approval of the final version of the article

Batyushin M.M. (ORCID ID: https://orcid.org/0000-0002-2733-4524): editing and approval of the final version of the article

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Connective Tissue Growth Factor in Normal and Pathological Processes

Abstract

Modern concepts about the role of connective tissue growth factor in various physiological and pathological processes are described in the review. Connective tissue growth factor regulates a variety of cellular functions, including proliferation, migration, adhesion, differentiation and synthesis of extracellular matrix proteins in cells of different types. This factor is also involved in more complex biological processes of angiogenesis, chondrogenesis, wound healing, fibrosis and oncogenesis. Increased expression of connective tissue growth factor is observed in different cardiovascular and oncological diseases. Potential role of this growth factor in regulation of cellular senescence and aging processes is also discussed.

Key words: *connective tissue growth factor, fibrosis, nephrosclerosis, chondrogenesis, osteogenesis, aging*

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CHF — chronic heart failure, CTGF — connective tissue growth factor

Connective tissue growth factor (CTGF), also known as CCN2, is a small secreted protein of the CCN family, named after its three original proteins: Cysteine-rich 61 (Cyr61/CCN1), CTGF/CCN2, Nephroblastoma overexpressed (Nov/CCN3) [1, 2]. CTGF is a cysteine-rich extracellular matrix protein with four domains or modules. This protein, like other members of the CNN family, includes four different structural modules: aminoterminal insulin-like growth factor binding domain; cysteine-rich domain; thrombospondin type 1 repeat; carboxyl-terminal cysteine knot domain [3]. The synthesis of CTGF, which was discovered in 1991, stimulates such a profibrotic cytokine as transforming growth factor β [4].

Connective tissue growth factor regulates various cellular functions, including proliferation, migration, adhesion, differentiation and synthesis of extracellular matrix proteins in cells of different types; it is also involved in more complex biological processes of angiogenesis, chondrogenesis, osteogenesis, wound healing, fibrosis and oncogenesis [1]. Increased expression of CTGF is observed primarily in cases of pathological conditions associated with fibrosis [4].

As an extracellular matrix protein, connective tissue growth factor is thought to integrate different extracellular signals into complex biological reactions [2, 5]. CTGF respectively binds to various receptors on the cell surface (in particular, to integrin receptors and surface heparan sulfate proteoglycans), thus controlling the transmission of signals to cells, the recognition of the cell matrix and cell adhesion. This protein also binds growth factors (for example, bone

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morphogenetic protein 4, transforming growth factor β), vascular endothelial growth factor and extracellular matrix proteins [6].

CTGF expression is regulated by growth factors and cytokines, including angiotensin II, bone morphogenetic proteins, endothelin, as well as mechanical stimuli (including high blood pressure and tension in the vascular wall) [3]. Angiotensin II-induced arterial hypertension is an active inducer of CTGF expression in the vascular wall. Transforming growth factor β is the most important regulator of CTGF expression. CTGF expression correlates with the expression of transforming growth factor β in the vascular wall. Vascular endothelial growth factor also induces CTGF, which is of great clinical importance in case of dysregulation of angiogenesis, and, in particular, with underlying diabetic retinopathy [6].

Connective tissue growth factor is actively expressed during the development of the cardiovascular system, while embryos with a deficiency of this factor die soon after birth due to complex development defects. In adults, CTGF apparently plays a role in the development of certain pathological processes, including heart failure, cardiosclerosis and scar formation after myocardial infarction [2]. Increased expression of CTGF in vessels is associated with atherogenesis, apoptosis of smooth muscle cells and the formation of vascular aneurysms [7–9]. In an experiment, angiotensin II, high blood pressure and vascular wall tension enhanced CTGF expression, which contributed to changes in vascular smooth muscle cells [7]. As a result of such remodeling, the structural integrity of the vascular wall is disrupted, which apparently contributes to the formation of aneurysms and aortic dissection or rupture [9].

Stimulation of connective tissue growth factor may be involved in the development of cerebral microbleeding induced by hypertension due to the violation of vascular wall integrity [6]. CTGF is also expressed in atherosclerotic plaques. It is thought to play a role in the regulation of their stability and can also stimulate the migration of monocytes into atherosclerotic plaques [2]. It was demonstrated under experimental conditions that CTGF can play a role in atherogenesis. When mice were injected with a drug that blocks CTGF, the accumulation of macrophages in atheromas and the size of plaques decreased [10].

In cases of such a pathological process as hypertrophic cardiomyopathy (with interstitial fibrosis and excessive accumulation of extracellular matrix proteins), there was an increase in both tissue expression of CTGF and concentration of this factor circulating in the blood, starting from the earliest stages of this disease. It was demonstrated in experimental models that CTGF is a powerful stimulator of the expression of genes that encode extracellular matrix proteins [11]. According to some reports, the suppression of CTGF by transmitting signals of insulin-like growth factor-1 in cases of dilated cardiomyopathy also reduces myocardial fibrosis and improves cardiac function [12].

A significant correlation between the level of connective tissue growth factor in plasma and echocardiographic parameters of diastolic dysfunction was found in the Taiwan registry with 125 patients with diastolic heart failure. The severity of cardiac fibrosis assessed by magnetic resonance imaging also correlated with CTGF concentration in plasma [13].

Another study analyzed the expression of connective tissue growth factor in patients with heart failure who underwent heart transplantation. In the left ventricle tissue of patients with both ischemic and dilated cardiomyopathy, increased expression of CTGF was detected, along with overexpression of transforming growth factor β1, collagen and matrix metalloproteinases, which correlated with the severity of interstitial myocardial fibrosis [14].

In one of the largest clinical studies of connective tissue growth factor involving 1,227 patients with cardiovascular diseases, it was found that an elevated level of this factor in plasma increases the risk of new cardiovascular diseases. In this study, the high concentration of CTFG was associated with ischemic coronary events (1.4 times) and all-cause mortality but was not associated with ischemic stroke. CTGF level in plasma positively correlated with the level of total cholesterol and LDL-cholesterol and negatively correlated with glomerular filtration rate. CTGF concentration in cases of cerebrovascular pathology was significantly lower [15].

High levels of CTGF in the presence of type 2 diabetes mellitus can help to predict subsequent myocardial infarction or death from cardiovascular diseases. A study involving 952 patients with diabetes mellitus and high CTGF concentration showed a higher risk of myocardial infarction (2.4 times), and of cardiovascular mortality and mortality from all causes (2.7 times) compared with individuals with a low concentration of this factor [16]. CTGF is a powerful inducer of chemotaxis and formation of extracellular matrix, which contributes to the progression of inflammatory, proliferative and fibrotic changes with underlying cardiovascular disease [16].

Animal experimental models showed that the increased activity of CTGF in the myocardium after myocardial infarction is associated with the decreased remodeling of the left ventricle, which is achieved due to the inhibition of apoptosis and inflammation [17]. However, in a clinical study involving 988 patients with ST-elevation myocardial infarction, no significant relationship was found between CTGF level in blood and the size of the infarct area, left ventricular ejection fraction, end-systolic and end-diastolic volumes of the left ventricle, and the worsening the prognosis for patients and their mortality [18].

A recent study by Chi H. et al. (2019) involving 114 patients with diastolic heart failure showed that levels of connective tissue growth factor in this group of patients were significantly higher than in the control group and correlated with echocardiographic parameters of diastolic dysfunction [19]. Another study also demonstrated increased CTGF in 52 patients with chronic heart failure (CHF), and a significant correlation of this factor with the severity of CHF, concentration of cerebral natriuretic peptide and echocardiographic parameters of diastolic, but not systolic, dysfunction [20]. According to the authors of this study, the effect of CTGF on diastolic heart failure is a result of its profibrotic action. In a study of CTGF in patients with acute heart failure, maximum increase in CTGF was observed in patients with heart failure and reduced ejection fraction compared to the control group without heart failure or with heart failure and preserved ejection fraction [21].

A number of studies have demonstrated the important role of connective tissue growth factor in the development of atrial fibrosis and dilation and related fibrillation [22, 23]. Analysis of CTGF expression in atrial tissue removed during cardiac surgery revealed a higher content of CTGF in atrial fibroblasts in patients with atrial fibrillation compared with patients with sinus rhythm; moreover, the level of this growth factor positively correlated with the duration of atrial fibrillation and dilation [22]. Another study also revealed increased CTGF expression in fibroblasts and atrial myocytes removed during cardiac surgery; stimulation with angiotensin II further enhanced hyperexpression of this growth factor [23].

It was found that CTGF in atherosclerotic plaques removed during carotid endarterectomy is higher in patients with acute cerebrovascular events than in patients with transient ischemic attacks [24]. In this study, plaques with high CTGF had more collagen and smooth muscle cells. Therefore, the authors concluded that this growth factor is associated with a more stable phenotype of atherosclerotic plaques [24].

As shown in several studies, CTGF expression in the brain in cases of Alzheimer's disease correlates with the progression of the clinical signs of dementia and amyloid accumulation [6]. The experimental model of Alzheimer's disease showed that a diabetic diet leads to a significant increase in CTGF in the brain, along with the increased accumulation of amyloid, which is typical for this type of dementia [6]. Due to impaired maturation and regeneration of oligodendrocytes and inhibition of axon myelination, CTGF can also take part in the pathogenesis of neurodegenerative diseases where the processes of demyelination and axonal degeneration are important [25].

A number of studies have demonstrated the relationship between CTGF and adipose tissue. A recent study showed that CTGF expression was more significant in preadipocytes, not in adipocytes, and the level of this growth factor correlates with the level of adipose tissue and insulin sensitivity [26]. CTGF-positive cells were found primarily in the areas of fibrosis of subcutaneous adipose tissue of the anterior abdominal wall and decreased body weight led to a decrease in CTGF expression in adipose tissue. The authors of the study concluded that increased CTGF expression is associated with adipose tissue, adipose tissue fibrosis, and insulin resistance in obese individuals [26]. At the same time, experimental models showed that CTGF, which affects the differentiation of adipocytes, can play a role in the pathogenesis of obesity and the related insulin resistance [27].

The expression of connective tissue growth factor also increases in presence of many nephropathies. In an experiment, CTGF inhibition slowed disease progression with underlying diabetic nephropathy, unilateral ureteral obstruction and in mice that underwent nephrectomy. CTGF and degradation fragments thereof found in different biological fluids were advanced as risk biomarkers for several nephropathies [28, 29]. The carboxyl terminal module (specifically CCN2 IV) was of particular interest among the fragments. In cell culture, this fragment regulated cell migration and proliferation, increased the production of chemokines and the extracellular matrix, and participated in renal inflammation processes [3].

CTGF was shown to be a key factor in the development and progression of diabetic nephrosclerosis. In cases of experimental diabetic nephropathy, excessive expression of CTGF in glomeruli, tubules, and

interstitial tissue caused glomerulosclerosis, tubulointerstitial fibrosis, and albuminuria [29]. In cases of diabetic nephropathy in humans, overexpression of CTGF, detected by kidney biopsy, was also associated with tubulointerstitial fibrosis, proteinuria and impaired renal function, while CTGF levels in urine correlated with albuminuria [30]. CTGF level in blood can help to predict the onset of end-stage renal failure and death from diabetic nephropathy [31, 32]. The connective tissue growth factor can also be shown to play a certain role with underlying non-diabetic chronic kidney disease. CTGF in blood and urine was significantly high in patients with chronic kidney disease and proteinuria but without diabetes mellitus. The decrease in proteinuria as a result of the appropriate treatment was accompanied by a proportional stepwise decrease of CTGF concentration in urine but had no effect on the high level of this growth factor in the blood [29]. Increased CTGF in urine may be caused by the local synthesis of this protein in kidneys, for example, due to the activation of angiotensin II synthesis or excessive sodium intake. Local production of CTGF in the kidneys was observed in experimental conditions and based on the results of a kidney biopsy in humans [30, 32]. In addition to the local synthesis of CTGF in kidneys, enhanced ultrafiltration of CTGF and its impaired reabsorption in tubules can contribute to the increase in the negative effect of CTGF on the nephron, which further contributes to the stimulation of fibrosis in the kidneys [29].

The CTGF level in kidneys is low under normal conditions, but its expression increases with renal fibrosis. CTGF expression (in both the mesangium and extracapillary) increases with underlying glomerulonephritis. Besides being involved in fibrosis processes, CTGF induces the expression of inflammatory mediators and contributes to the increase in the number of macrophages and cell adhesion. Thus, CTGF can play an important role in the development of glomerulonephritis, causing an inflammatory process [33, 34].

A recent study involving 23 patients with IgA-nephropathy and hemorrhagic vasculitis showed that cytoplasmic expression of CTGF in the cells of renal tubules was significantly higher in these patients compared to the control group. However, there were no differences in CTGF expression in the glomeruli of kidneys. Subsequent follow-up revealed a direct correlation between the rate of nephropathy progression and CTGF expression in tubule cells. The authors of this work suggested that CTGF may be a new, early and sensitive marker of chronic kidney disease [35].

Four hundred and four patients on hemodialysis were enrolled in one of the largest studies of CTGF with underlying nephrological pathology. The results of this study indicate an inverse correlation between the concentration of this growth factor and the glomerular filtration rate. On the other hand, there was a direct relationship between CTGF and cardiovascular diseases, levels of interleukin-6 and β2-microglobulin, as well as the presence of polycystic kidney disease and tubulointerstitial nephritis. Patients with the highest CTGF concentrations were at a higher risk of mortality than patients with the lowest CTGF. It should be noted that the level of this growth factor decreased with hemodiafiltration [36].

Special studies demonstrated that connective tissue growth factor is an important mediator of fibrosis in renal transplant; moreover, CTGF levels in urine correlate with the development of interstitial transplant fibrosis. In a study involving 160 patients with a kidney transplant, tissue expression of CTGF and the level of this protein in urine were predictors of interstitial fibrosis and tubular atrophy. Even in patients with good histology results in the early post-transplant period, significant expression of CTGF was often detected, which could be a predictor of damage development [37].

A number of studies demonstrated the important role of CTGF in lung pathologies. One of the most interesting studies concerning this problem examined CTGF expression in bronchial epithelial cells of human and experimental animals; the results showed that the expression of this growth factor in humans increased with the severity of chronic obstructive pulmonary disease and was associated with accelerated cell aging [38]. According to the authors of this paper, by accelerating the aging of lung epithelial cells, CTGF can inhibit the regeneration of these cells and lead to pulmonary emphysema [38]. Experimental studies showed that CTGF plays a certain role in the development and progression of pulmonary fibrosis due to the activation of type I collagen [5, 39].

A recent study, which results were published in January 2020, showed good efficacy and tolerability of treatment with monoclonal anti-CTGF antibodies in patients with idiopathic pulmonary fibrosis. In the group of patients treated with anti-CTGF antibodies, a significant slowdown in the rate of decrease of lung function was observed (as measured by forced

vital capacity), along with a significant decrease in the progression of pulmonary fibrosis (according to CT results). After 48 weeks, there were fewer patients with disease progression in the treatment group than in the control group. The authors of this work concluded that the treatment of idiopathic pulmonary fibrosis with monoclonal anti-CTGF antibodies could be promising in the treatment of this prognostically unfavorable disease [40].

According to other authors, connective tissue growth factor may be a new target for the management of bronchopulmonary dysplasia in premature infants. This conclusion was made based on the results of experimental studies showing that CTGF contributes to the development of bronchopulmonary dysplasia. CTGF overexpression in alveolar epithelial cells led to impaired alveolar formation and also caused vascular remodeling and pulmonary hypertension. At the same time, CTGF inhibition with monoclonal antibodies stimulated normal formation of alveoli, decreased vascular remodeling and decreased pulmonary artery pressure [41].

In a study involving 95 patients with acute respiratory distress syndrome who were on mechanical ventilation, a direct correlation was revealed between the CTGF level and subsequent pulmonary fibrosis. The authors of this work suggested that connective tissue growth factor and transforming growth factor β1 may be of great prognostic value for assessing the risk of pulmonary fibrosis in patients with acute respiratory distress syndrome [42].

Inflammatory bowel disease is a relatively new area in studying the role of CTGF. A study involving 93 patients with ulcerative colitis showed increased CTGF expression in intestinal mucosa; the concentration of this factor correlated with the severity of colitis. Results of the experimental part of this study showed that CTGF inhibition contributed to a decrease in the severity of inflammatory process in the intestines and the normalization of intestinal microbiota [43].

At the same time, the results of recent experimental and clinical studies demonstrated the high activity of connective tissue growth factor in almost three dozen tumors. It was found that CTGF regulates the proliferation of tumor cells, their migration and metastasis, as well as angiogenesis and drug resistance, which ultimately lead to poor prognosis for a large number of cancer diseases [44]. It was established, for example, that CTGF overexpression predisposes to the progression of endometrial cancer, and this factor itself can be a new prognostic biomarker for this neoplasm [45]. Similar results were also obtained in cases of ovarian cancer when CTGF contributed to the metastasis of tumor cells and resistance to chemotherapy [46].

It was also established that CTGF is an important regulator of skeletogenesis. Studies demonstrated that CTGF is important for the condensation of mesenchymal cells in areas of future bones and the regulation of the proliferation and differentiation of chondrocytes and osteoblasts. Proper regulation of CTGF expression is required for the normal course of mesenchymal condensation, chondrogenesis and osteogenesis [1]. The ability of CTGF to interact with other skeletal growth factors and modulate their effects also plays a critical role for this growth factor in the regulation of skeletal development. The physiological significance of CTGF for normal skeletogenesis was confirmed in experimental mice models without this growth factor (the laboratory animals had craniofacial, axial and appendicular skeleton defects) [1].

In addition, connective tissue growth factor is actively involved in cartilage formation. CTGF significantly increases the production of cartilage matrix proteins, such as type II collagen and aggrecan, and also stimulates the proliferation of chondrocytes and differentiation and maturation of chondrocytes in physiological conditions [47, 48]. At the same time, CTGF increases the adhesion of chondrocytes to fibronectin, as well as angiogenesis by enhancing adhesion and migration of endothelial cells [49]. Skeletal muscle pathology was observed in mice with CTGF deficiency as a result of impaired chondrocyte proliferation and extracellular matrix composition, which indicates that CTGF is a key regulator of the formation of the extracellular cartilage matrix. Moreover, the implantation of CTGF-containing gelatin hydrogel into rat articular cartilage defects accelerated cartilage restoration, and cartilage-specific CTGF overexpression during development and growth reduced age-related changes in articular cartilage [49]. Another study showed that the decrease in the content of CTGF leads to cartilage thickening and has a protective effect against osteoarthritis [50]. Experimental studies showed that CTGF expressed and secreted by osteoblasts during proliferation, differentiation, bone formation and healing of fractures also regulates osteogenesis in osteoblasts [51]. Based on the results of these experiments, it was suggested that the pathological expression of connective tissue growth factor might be a new mechanism for the development of senile osteoporosis by suppressing the function of osteoblasts [51].

As for the regulation of the expression of connective tissue growth factor in skeleton cells, the primary inducer of CTGF in chondrocytes and osteoblasts, as in many other cells, is transforming growth factor β1. Also, glucocorticoids, retinoids, and taurine can stimulate CTGF induction with chondrocytes, while endothelin and cortisol were shown to regulate this growth factor in osteoblasts [1].

Another disease whose pathogenesis can involve CTGF is rheumatoid arthritis. It was established that in this disease, CTGF is secreted by fibroblast-like synoviocytes and stimulates the proliferation of these cells with the formation of pannus and cartilage destruction. In experimental studies, CTGF expression on the synoviocytes of patients with rheumatoid arthritis was shown to be more significant compared with the control group. Results of a clinical study revealed that CTGF serum concentration in patients with rheumatoid arthritis was significantly higher than in the control group [52].

Increased CTGF concentration in blood was also found in a group of 87 patients with Behçet's disease. The CTGF level in this study was much higher with the involvement of internal organs in the pathological process, as well as with severe ophthalmic manifestations. It should be noted that in patients who received treatment with glucocorticosteroids and cytostatics, CTGF concentration was significantly lower [53].

The CTGF level increases significantly with numerous underlying pathological conditions accompanied by fibrosis when excessive production of collagen is thought to be stimulated. CTGF is expressed in normal human dermis, which suggests that this protein is a physiological regulator of collagen expression. It was shown that the CTGF level is significantly lower in dermal fibroblasts, the primary collagen-producing cells, in the skin of individuals older than 80 years. On the other hand, CTGF overexpression stimulated the synthesis of type I procollagen [54].

Experimental data demonstrate that aging is associated with increased expression of connective tissue growth factor in vessels and the heart, which can contribute to the age-related remodeling of the extracellular matrix [6]. CTGF is involved in age-related changes in cardiomyocytes and vascular wall cells by lowering the expression of certain types of micro-RNA [6, 55]. Increased CTGF expression was also found in «aging» fibroblasts. [56]. In connection with these data, CTGF is considered as a possible marker of aging processes.

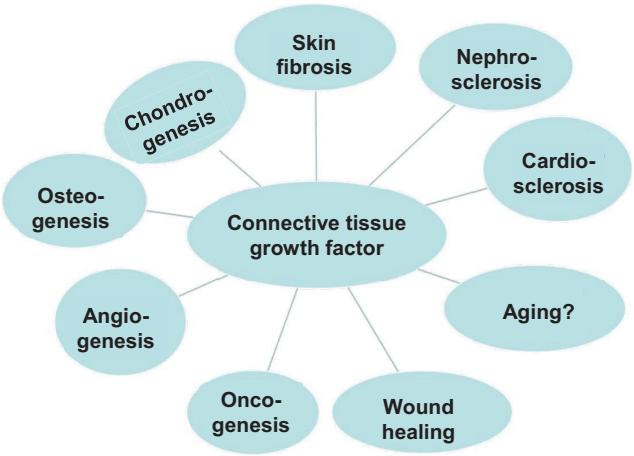

Figure 1. *The effect of connective tissue growth factor on various pathological and physiological processes*

Thus, connective tissue growth factor is a key mediator that modulates the effects of many other growth factors and regulates the formation and remodeling of the extracellular matrix. As a result, this growth factor significantly contributes to various pathological and physiological processes (Figure 1).

There is increasingly more evidence that CTGF is activated with aging. Further studies of this growth factor are required in order to better understand its clinical significance both in aging processes and in various pathological conditions and age-associated diseases.

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Metabolic Systemic Effects Triiodothyronine

Abstract

Triiodothyronine (T_3, 3,5,3'-L-triiodothyronine) is a thyroid hormone (thyroid), the secretion of which is carried out directly both by the gland (to a lesser extent) and outside it (the main amount; as a result of peripheral deiodination of thyroxine (T_4)). Getting into the nuclei of cells, T_3 interacts with specific nuclear receptors of target tissues, which determines its biological activity. This interaction leads to the activation of transcription of a number of genes.

In the pituitary gland and peripheral tissues, the action of thyroid hormones is modulated by local deiodinases, which convert T_4 to more active T_3, the molecular effects of which in individual tissues depend on subtypes of T_3 receptors and their interaction with other ligands, coactivators and corepressors, as well as on the activation or repression of specific genes. The reason for the lack of T_3 production is primarily a deficiency of iodine in the diet, less often, a defect in the genes encoding the proteins that are involved in T_3 biosynthesis. As a result of the low intake of iodide in the body, the so-called adaptive mechanism is activated, which consists in increasing the proportion of synthesized T_3, which increases the metabolic efficiency of thyroid homones. With a deficiency in the diet of such a trace element as selenium, the conversion of T_4 to T_3 is reduced.

Thyroid hormones play a vital role in the regulation of homeostasis and the metabolic rate of cells and tissues of humans and mammals. They are necessary for physical and mental development. Their insufficient production at the stage of formation of the internal organs of the fetus and in childhood can lead to various pathologies, primarily to pathology of the central nervous system, and as a result, growth retardation and mental retardation. In adulthood, hypothyroidism leads to a decrease in metabolism, memory impairment, depressive disorders, impaired fertility. Many discussions and ambiguous conclusions have been obtained regarding combination drugs (sodium levothyroxine + lyothironon) for the treatment of hypothyroidism. This article will examine the metabolic effects of T_3, the thyroid hormone with the highest activity.

Key words: *thyroid gland, triiodothyronine, triiodothyronine isoforms*

Conflict of interests

The authors declare no conflict of interests

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D_I, D_{II}, D_{III} — deiodinases, DIT — diiodothyrosine, ESS — euthyroid sick syndrome, GPX — glutathione peroxidase, HF — heart failure, LT_3 — liothyronine, MIT — monoiodothyrosine, NCOR1 — nuclear receptor coregulator 1, rT_3 — reverse T_3, T_3 — triiodothyronine, T_4 — thyroxine, TBG — thyroxine-binding globulin, TG — thyroid gland, THrα — thyroid hormone receptor alpha, THrß — thyroid hormone receptor beta

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The history of the discovery of thyroid hormones

In the 20th century, important discoveries were made in the field of the biochemistry of thyroid hormones. In 1915, E. C. Kendall, an American biochemist, isolated a hormone called thyroxine (T_4) from the thyroid gland (TG). A little later, in 1927, C. R. Harington and G. Barger synthesized the hormone. Another great event was the isolation and synthesis of triiodothyronine (T_3) by J. Gross and R. Pitt-Rivers in 1953. In 1955, R. Pitt-Rivers and her colleagues suggested that T_3 is produced in vivo through T_4 conversion, but this theory remained unproven for a long time.

In 1970, L. E. Braverman et al. demonstrated the conversion of T_4 to T_3 in individuals with no TG, and Anne Fausto-Sterling et al. revealed the same in healthy subjects. Over the next decade, T_4 detection methods were improved; specially-developed radioimmune analysis allowed to determine the level of reverse T_3 (rT_3, inactive) and to understand its physiological role. In 1975, D. Chopra et al. found mutual changes in T_3 and rT_3 levels in the presence of systemic diseases — somatic non-thyroid disease leads to decreased T_3 and increased rT_3. In 1977, K. D. Burman et al. developed a radioimmunoassay for rT_3 detection, which confirmed its presence in the blood serum of healthy individuals. It was also found that rT_3 level is lower in patients with hypothyroidism who take minimal daily doses of levothyroxine sodium. Conversely, rT_3 level was high in patients with hyperthyroidism who received large doses of levothyroxine sodium. The late 70s were marked by a surge in interest in T_3 metabolites, including the development of radioimmunoassay for 3,3′-diiodothyronine (3–3′T_2) [1].

T_3 biosynthesis and metabolism

T_3 is formed as a result of the combination of diiodothyrosine (DIT) and monoiodothyrosine (MIT) molecules. It then accumulates inside the follicle in the form of a colloid. T_3 is secreted with colloid resorption with the help of proteolytic enzymes. MIT, DIT, and T_3, which enters the bloodstream [2], are formed as a result. TG produces no more than 20% of T_3 circulating in the human body. The rest of it and rT_3 (95–98%) result from the peripheral conversion of T_4 by deiodination [1]. The effect of T_3 is about five times higher than that of T_4. T_3 half-life ($T_{1/2}$) is 1–2 days. If T_4 conversion to T_3 is impaired, rT_3 level increases [2].

About 40% of T_4 metabolizes to form T_3 and rT_3 (Fig. 1) [2].

The T_4 molecule has four iodine atoms; the loss of one atom leads to the formation of T_3 or rT_3 depending on which atom is lost. Iodine removal from position 5′ on the outer ring leads to the formation of T_3 — the most active thyroid hormone, which is produced at a rate of 30–40 μg per day. Conversely, when T_4 loses the iodine atom from position 5 on the inner ring, rT_3 is formed at a rate slightly lower than that of T_3, i.e., from 28 to 40 μg per day. rT_3 is inactive. Both T_3 and rT_3 can give up more iodine atoms to form various isomers of T_2, T_1, and, ultimately, T_0. Other pathways of thyroid hormone metabolism include glucuronidation, sulfation, oxidative deamination and cleavage of the ether bond [1].

Reactions of T_3 formation are catalyzed by three types of enzymes (deiodinases):

***D*$_I$** — participates in the deiodination of inner and outer T4 rings, supplies T3 to peripheral tissues. This enzyme provides the formation of most of T3 in plasma by converting T4 into active T3, and also deactivates it; it is localized mainly in the liver, kidneys, TG, and pituitary gland, and in a smaller amount — in the central nervous system [1, 3].

***D*$_{II}$** — catalyzes the conversion of T_4 into T_3, having effect exclusively on the outer ring of thyroid hormones, and falls in the category of

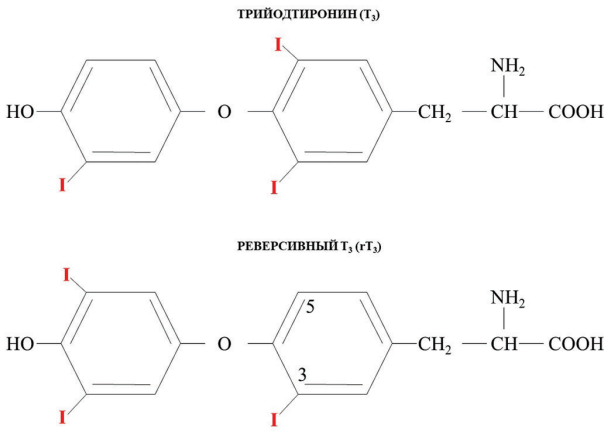

Figure 1. *Forms of triiodothyronine (Adapted from Troshina E.A., 2012)*

essential enzymes. D_{II} provides constant concentration of intracellular T_3; it is synthesized in the central nervous system, pituitary gland, brown adipose tissue, TG, placenta, skeletal muscles and the heart [1, 4].

D_{III} — is responsible for the transformation of T_4 into rT_3, deactivates T_3 and T_4 by catalyzing iodine removal from the inner ring. An inactive form — 3,3-diiodothyronine — is produced as a result. D_{III} synthesis takes place in the central nervous system, skin, hemangiomas, fetal liver, placenta, and fetal tissues [1, 3].

Free T_3 concentration in plasma is relatively constant; however, its concentration in tissues varies depending on the amount of hormone transferred and the activity of local deiodinases. The effect of T_3 depends on the duration of its binding to the nuclear receptor and the number of receptors. Under these conditions, deiodinases play an important role in maintaining concentrations of thyroid hormones in tissues and cells; signal transduction here can vary regardless of their serum concentrations. For example, T_3 production in the central nervous system by local D_{II} is crucial for maintaining T_3 homeostasis. According to Maia A. L. et al. (2011), D_{II}, which is expressed in human TG, plays an important role in maintaining T_3 level in plasma [1–3]. There is a theory that increased expression of D_{II} in enlarged TG leads to a relatively high level of circulating T_3 with certain underlying thyroid diseases, such as McCune—Albright syndrome, T_3-thyrotoxicosis in Graves' disease, abnormalities in thyroglobulin gene. Deiodinases also modulate tissue-specific T_3 concentrations in response to iodine deficiency and changes in thyroid function. In a developing brain, D_{II} locally converts T_4 into T_3. Iodine deficiency and hypothyroidism lead to increased D_{II} activity in tissues, especially in brain tissue, and, as a result, T_4 conversion into T_3 is locally increased [1]. In cases of hyperthyroidism, excessive expression of D_I contributes to the relatively excessive production of T_3, while the activation of D_{III} in the brain protects the central nervous system from excessive amounts of thyroid hormones. D_{III} is the main physiological inactivator of thyroid hormones and plays a major role in protecting tissues from the excessive amount thereof. This mechanism is crucial for fetal development and explains the high expression of D_{III} in the placenta and human fetal tissue. D_{II} and D_{III} regulate T_3 availability in the course of brain development. In tissues of adult individuals, the importance of D_{III} in the regulation of thyroid hormone homeostasis becomes apparent in the cases of certain pathophysiological conditions, such as non-thyroid diseases and malnutrition. Whenever a decrease in metabolism is "homeostatically desirable", for example, in intensive care unit patients or during fasting, T_3 formation decreases and rT_3 formation increases [1, 4, 5]. D_{III} is also activated during fetal hypoxia during delivery [6].

T_3 transport

Most of circulating T_3 is bound to plasma proteins (total T_3), and only 0.4% is free (unbound) T_3 that enters target cells [7].

Thyroxin-binding globulin (TBG), transthyretin and albumin are the main transport proteins.

In case of TBG deficiency, total T_3 concentration decreases. However, the level of free T_3 remains normal. The following may cause impaired hormone binding to protein: congenital TBG synthesis defects, taking medications (androgens, glucocorticoids, danazol, L-asparaginase), certain physiological and pathological conditions (most systemic diseases).

Excessive TBG can be caused by congenital malformations, pregnancy, estrogen-producing tumors, treatment with estrogens, 5-fluoro-uracil. TBG concentration in plasma and total T_3 level increase in this case.

In comparison with T_4, transthyretin has less affinity for T_3.

Among the abovementioned proteins, albumin has the lowest power of binding to T_3, but due to its high concentration, it binds about 15% of the hormone in plasma. Due to the rapid dissociation of the protein-hormone complex, albumin is the main source of free T_3. In cases of renal failure or liver cirrhosis accompanied by hypoalbuminemia, total T_3 level is lower, but free T_3 level remains normal [7].

Diagnostic value of total T_3 and free T_3

Total T3 has diagnostic value only in cases when the binding ability of proteins remains constant. Said constancy changes when taking certain

medications, and during severe general (non-thyroid) diseases. Therefore the determination of free T3 is more significant.

Total T_3 level correlates with total T_4 in most clinical cases. The determination of total T_3 is most reasonable with underlying thyrotoxicosis, since in some cases the total T_4 level shows no significant changes, and total T_3 concentration in serum increases sharply, which allows considering the latter as a more appropriate and objective parameter. In particular, in the absence of TBG binding disorder and with normal total T_3, thyrotoxicosis can be almost excluded.

Patients with myeloma, which produces a large amount of immunoglobulin G, or with severe liver diseases demonstrate falsely high total T_3. Total T_3 may decrease after different surgical interventions, in cases of chronic and acute somatic diseases (for example, diabetes mellitus; HIV infection; myocardial infarction; cirrhosis; anorexia; sepsis; nephrotic syndrome, etc.).

When diagnosing possible thyroid dysfunction, the determination of total T_3 is not enough, especially in patients with hypothyroidism, when in some cases, total T_3 level remains within reference values. The following are indications for determining total and free T_3: differential diagnosis of T_3 thyrotoxicosis; initial thyroid hyperfunction, in particular, in the presence of functional autonomy; relapse of thyrotoxicosis, symptomatically increased T_3; drug-induced thyrotoxicosis [8].

Reverse T_3

Reverse triiodothyronine (3,3',5'-triiodothyronine, reverse T_3, or rT_3) is a T_3 isomer. However, due to its inability to bind nuclear receptors of thyroid hormones, it is usually considered biologically inactive. Reverse T_3 suppresses the effect of nuclear T_3. This is a result of its ability to reduce T_4 conversion into T_3 in D_{II}-expressing tissues, such as the brain. According to Rastogi L. et al. (2018), rT_3 has a neuroprotective effect during ischemic reperfusion injury in vivo and in vitro [9]. In cases of severe general diseases, rT_3 level can increase rapidly, which can also be observed in newborns, with underlying liver failure, after taking certain drugs (beta-blockers, corticosteroids, antiarrhythmic drugs) [8]. Oxidative stress, apoptosis and inflammation are the primary mediators of tissue damage in stroke. It was noted that rT_3 reduces the induction of oxidative stress and apoptosis signaling after ischemic stroke [9]. According to Salazar P. et al. (2019), patients with Alzheimer's disease have high rT_3 and a high rT_3 to T_4 ratio in cerebrospinal fluid with a normal level of thyroid hormones in serum. T_3 inhibits the transcriptional activity of the β-amyloid precursor protein (APP) gene, which is an important risk factor for Alzheimer's disease [10].

Overall, the determination of rT_3 in serum had no clinical significance for the diagnosis of hypothyroidism in patients with systemic diseases. A retrospective study by L. A. Burmeister (1995) demonstrated that somatic non-thyroid pathology complicates the interpretation of thyroid function tests, and measuring rT_3 in serum does not allow to reliably distinguish a patient with hypothyroidism from a patient with euthyroidism. According to L. A. Burmeister, diagnosis requires the evaluation of clinical symptoms, determination of levels of free T_4 and thyroid-stimulating hormone (TSH), and patient monitoring [1]. rT_3 measurement is required only in some clinical situations. Its determination can be performed for differential diagnosis between hypothyroidism and euthyroid sick syndrome: rT_3 should always be considered in combination with TSH, free T_3 and free T_4, taking into account clinical evidence. Table 1 shows changes in the levels of thyroid hormones depending on the severity of systemic disease (as non-thyroid pathology progresses, more significant changes in thyroid function are registered; disease severity is defined conventionally; ultimately, everything depends on the initial and underlying disease).

The utility of determining rT_3 in an outpatient setting is debatable. Sometimes it is difficult to make a differential diagnosis between hypothyroidism and non-thyroidal illness syndrome in intensive care units. rT_3 can be low, normal or high regardless of thyroid function. Also, endogenous changes in the hypothalamus — pituitary — thyroid axis can be exacerbated by drugs commonly used in intensive care units, such as dopamine and glucocorticoids. Changes in thyroid function should be evaluated based on clinical evidence. However, regardless of the T_3 level, thyroid hormone replacement therapy should not be prescribed without taking into account the general clinical status of the patient; controlled

Table 1. *Changes in the levels of thyroid hormones depending on the severity of systemic disease*

Severity of disease	TSH	Total T_3	Free T_4	Reverse T_3	Probable cause
mild	no changes	slightly decreased	no changes	slightly increased	D_I, D_{II} slightly decreased
moderate	no changes or slightly decreased	decreased	no changes or moderately increased or decreased	increased	D_I, D_{II} decreased; slightly increased D_{III} is possible
severe	decreased	significantly decreased	slightly decreased	slightly increased	D_I, D_{II} decreased; slightly increased D_{III} is possible
recovery	slightly increased	slightly decreased	slightly decreased	slightly increased	unknown

Note: TSH — thyroid-stimulating hormone; total T_3 — total triiodothyronine; free T_4 — free thyroxine; reverse T_3 — reverse triiodothyronine

studies showed no evidence that such therapy is beneficial [1]. In cases of mild non-thyroidal somatic diseases, concentrations of free T_3 and TSH may be low. Patients often have abnormal rT_3 levels in blood serum even though TSH is within reference values. Therefore, it makes no sense to determine rT_3. The only relevant test for initiating or adjusting treatment with levothyroxine sodium is the measurement of the TSH level. If the decision to prescribe replacement therapy is based on rT_3 only, always consider the possibility of drug overdose, which can lead to subclinical or even clinical thyrotoxicosis. The discovery of molecular mechanisms that lead to D_{III} reactivation in cases of different diseases, such as HIV infection, chronic heart failure (CHF), and anorexia, is an important field of research today [1].

Effect of thyroid hormones on the development of the central nervous system

Physiological concentrations of thyroid hormones in brain tissue are crucial for pre- and postnatal development and for the regulation of the most important cellular mechanisms. Hypothyroidism in pregnant women significantly increases the risk of autism in the child, and low perinatal levels of thyroid hormones are associated with persistent cognitive impairment and attention deficit. Biosynthesis of T_4, its conversion to T_3 and activation of thyroid hormone receptors are vital processes for normal brain development.

In the developing fetal brain, D_{II} locally converts T_4 to T_3. D_{III} is responsible for the decrease in the cellular level of T_3.

There are two types of thyroid hormone receptors: THrα and THrß. THrα is widely expressed in the brain, THrß — mainly in subcortical areas. Alternative splicing leads to the formation of two variants of THrα — α1 and α2. T_3-dependent transcription is mediated by THrα1. THrα2 does not bind to T_3 and suppresses T_3-dependent transcription. The effect of thyroid hormones at the brain formation and development stages can be increased or decreased by changing the expression levels of THrα1 and THrα2.

Transcriptional coregulators (activators/repressors) can adjust T_3-dependent transcription. Nuclear receptor coregulator 1 (NCOR1) is particularly important for regulating the action of thyroid hormones in vivo. Coactivator MED1 (mediator of RNA polymerase II transcription subunit 1) induces T_3-dependent transcription, which can enhance the effect of thyroid hormones and counteract NCOR1. Local activation of thyroid hormone signaling is achieved at the early stage of development and during brain formation by increasing the activity of D_{II}, THαr1 and MED1. The activation of D_{III}, THrα2 and NCOR1 at the final stage of brain formation can inhibit the action of thyroid hormones and changes in gene expression.

TG dysfunction at an early age can significantly impact cerebellar-mediated motor function. Hypothyroidism leads to functional and structural changes within the cerebellum, hippocampus, cortex, and subcortical nuclei. Abnormal formation of cerebellar-cortical connections leads to autism. Normal TG function in the perinatal period is vital for the development of various behavior in vertebrates. According to Törel Ergür

A. (2012), perinatal levels of thyroid hormones are the basis for the development of various behavior in humans, rodents, birds, and fish [10, 11].

Children with congenital hypothyroidism are characterized by cognitive disorders, impaired speech and motor function. According to the study performed by Törel Ergür A. (2012), subclinical hypothyroidism in children and adolescents correlates with attention deficit. The study performed by Resch U. (2002) demonstrated that low levels of thyroid hormones in patients with manifest and subclinical hypothyroidism are associated with the development of oxidative stress [10]. Mechanisms underlying it have not been studied yet [10].

T_3 and iodine deficiency disorders

Iodine is an essential trace element for the synthesis of thyroid hormones that regulate metabolic processes in most cells and play a key role in the growth and development of the human body.

Iodine deficiency disorders (IDD) are a global public health problem. Their prevention is primarily associated with the prevention of brain formation disorders at the embryonic development stage. Additional intake of iodine preparations in early pregnancy and lactation allows eliminating the adverse effects of iodine deficiency [11].

Severe, prolonged iodine deficiency results in impaired synthesis and secretion of thyroid hormones. Iodine deficiency and decreased production of thyroid hormones lead to the increase of the MIT/DIT ratio in thyroglobulin and to the increase of T_3 secreted by TG. The hypothalamic-pituitary system responds by increasing TSH levels, which is accompanied by an increase in TG size. Due to this compensatory mechanism, hypothyroidism is briefly compensated. It is extremely important to note that thyroid hormone deficiency in newborns and infants leads to irreversible damage to the nervous system and other systems [11].

In addition to the formation of the central nervous system (CNS), other vital functions of T_3 should be noted:

- T_3 regulates the development of bone zones of fetal development and linear bone growth, and is also responsible for endochondral ossification and maturation of epiphyseal centers of ossification after birth. In adults, T_3 participates in bone remodeling and ensures the degradation of mucopolysaccharides and fibronectin in extracellular connective tissue.
- T_3 stimulates the breathing rate at rest and minute pulmonary ventilation, thereby normalizing oxygen concentration in arterial blood as compensation for the increase in oxidation rate. T_3 also contributes to the delivery of oxygen to tissues, simulating the production of erythropoietin and hemoglobin. It also facilitates the absorption of folate and cobalamin in the gastrointestinal tract [12].

Euthyroid sick syndrome or hypothyroidism?

Some patients with several pathologies, such as coronary heart disease (CHD), liver disease (decompensated cirrhosis), chronic kidney disease (CKD), sepsis, mental illness (including food deprivation), trauma, HIV infection, etc., and with no thyroid pathology, demonstrated low T_3, low or normal T_4, and normal TSH. These abnormalities are classified as the so-called euthyroid sick syndrome (ESS, low T_3 syndrome, non-thyroidal illness syndrome, thyroid pseudodysfunction syndrome). The first reports about it emerged around 1976 when methods for determining rT_3 were not widely available. However, some researchers associated this syndrome with high rT_3. In 1982, L. Wartofsky and K.D. Burman analyzed thyroid dysfunction in patients with severe systemic diseases and found a number of factors that can cause changes in thyroid function, such as age, stress, and various drugs [1].

Many somatic diseases are characterized by changes in thyroid hormones, but there are no clinical signs of thyroid dysfunction in such cases. Thyroid hormone levels are restored as the underlying disease is treated. The severity of changes in the thyroid hormone levels depends on the severity of the nonthyroidal disease. These abnormalities are the adaptive response to the pathological mechanisms of the underlying disease. ESS is associated with the impaired deiodination of T_4 in the liver, increased or decreased binding of thyroid hormones to plasma proteins, and impaired TSH production.

The role of D_{III} in the development of ESS was considered relatively recently. D_{III}, which is usually

undetectable in mature tissues, is reactivated in different types of cells in response to damage and is responsible for the decrease of T_3 in serum. Hypoxia induces the activity of D_{III} and messenger RNA in vitro and in vivo. The study by Wajner S. M. et al. (2011) discussed the role of cytokines in ESS. Interleukin-6 lowers the activity of D_I and D_{II} and increases activity of D_{III} in vitro [1].

Differential diagnosis of ESS with true thyroid pathology is important in clinical practice. Routine determination of thyroid function is not recommended for patients in the early postoperative period, as well as for patients who are in intensive care or trauma unit [1, 2, 13, 14].

Chronic heart failure and low T_3 syndrome

The supposed relationship between cardiovascular diseases and thyropathies was for the first time established more than 200 years ago by C. H. Parry, an English physician, who described a patient with goiter and palpitations.

Many patients with cardiac pathology have thyroid dysfunction (hypothyroidism, thyrotoxicosis), but these conditions are often underestimated and are not taken into account by clinicians.

Many direct and indirect effects of thyroid hormones on the heart and blood vessels are described. There is no conversion of T_4 to T_3 in the heart muscle. Therefore, only T_3 in serum has an effect on the myocardium. The primary transporters of thyroid hormones (mainly T_3) into myocytes are monocarboxylate transporters: MCT8 and MCT10. T_3 is an important regulator of the expression of cardiac genes, such as genes that encode contractile proteins, α-myosin heavy chain (MHC) and β-MHC, sodium-calcium exchanger (NCX1), sarcoplasmic reticulum calcium ATPase (SERCA2), β-adrenergic receptor. These mechanisms control changes in the contractile function of the heart, the calcium cycle, and diastolic relaxation of the myocardium. T_3 increases contractility and reduces systemic vascular resistance due to the dilation of peripheral resistance arterioles. Thus, T_3 has a direct effect on the heart and vasculature and an indirect effect on cardiovascular hemodynamics. Figure 2 shows the mechanisms of T_3 action at the level of heart muscle cells (cardiomyocytes).

There are several mechanisms of impairment of T_4 conversion to T_3. One of them is decreased D_I activity and increased expression and activity of D_{III}. Increased D_{III} gene expression may result from hypoxia and inflammation. There is a theory that the dysfunction of deiodinases may be associated with oxidative stress and selenium (Se) deficiency, which is also observed in cases of heart failure (HF). Glutathione peroxidase (GPX) is a marker of protection against oxidative stress. Se levels correlate with GPX enzyme activity. Deiodinases and GPX are selenium-containing proteins competing for Se uptake. In this case, Se deficiency can lead to both GPX deficiency and decreased T_4 conversion to T_3. According to some literature sources, these patterns can be caused by severe HF. On the other hand, oxidative stress and the so-called low T_3 syndrome can contribute to the progression of HF [15].

Low T_3 syndrome, which accompanies HF, can cause many disorders. Thyroid hormone deficiency may result in decreased expression of the α-myosin heavy chain (α-MHC) gene (MYH6), which leads to the deterioration of heart systolic function. Thyroid hormone deficiency contributes to the lowering of the sarcoplasmic/endoplasmic reticulum of calcium ATPase2 (SERCa2) due to the suppression of the ATP2A2 gene. Thyroid hormones activate phosphatidylinositol-3-kinase (PI3K) and serine/threonine protein kinase (AKT) signaling pathways through non-genomic action, inducing the production of endothelial nitric oxide. Also, both hormones (especially T_3) have a direct vasodilating effect that depends on their concentration. Low hormone levels can affect the function of ion channels, which leads to arrhythmia. Thyroid hormone deficiency affects the biogenesis of cardiac muscle mitochondria [15]. According to several studies, low T_3 syndrome is a predictor of death in patients with heart diseases. At the same time, low levels of T_3 are associated with HF severity (they are more often observed with underlying III–IV functional class, according to the New York Heart Association (NYHA) classification). Low concentration of free T_3 may have the same prognostic value as the N-terminal pro-B-type natriuretic peptide (NT-proBNP) in chronic and acute HF. The coincidence of low T_3 syndrome and Se deficiency in patients with HF is also interesting. In a recent study performed by Fraczek-Jucha M. et al. (2019), it was demonstrated that low

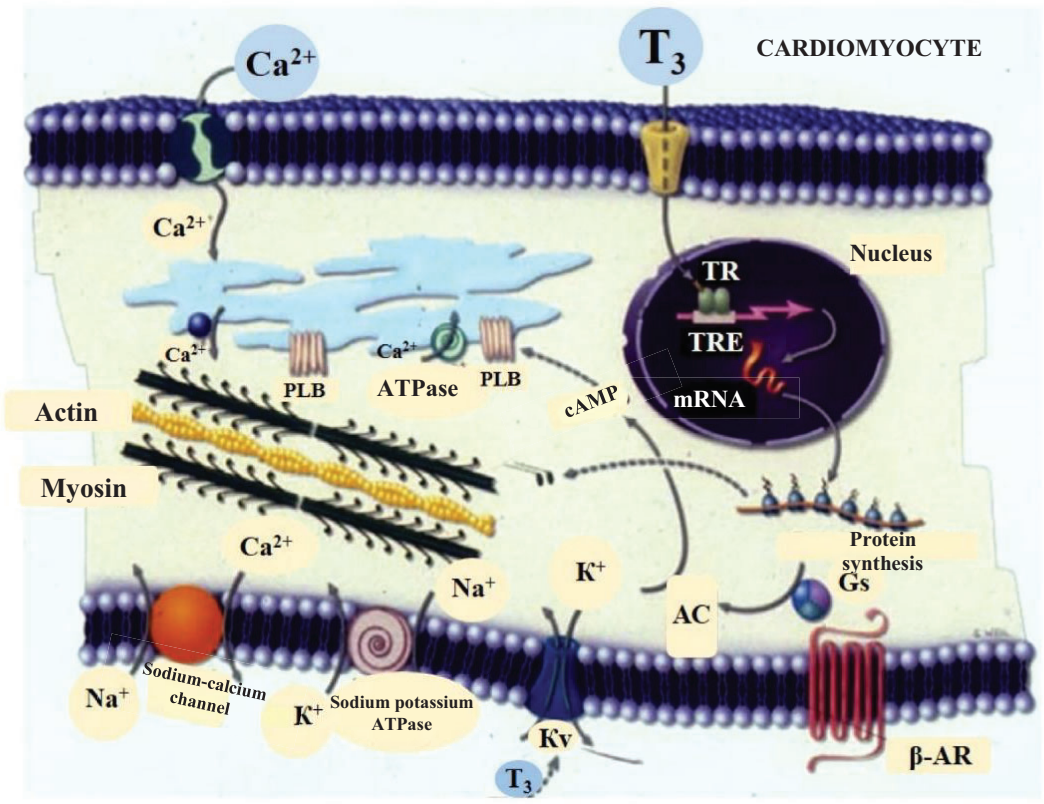

Figure 2. *Mechanisms of T3 action on the cardiomyocyte. T3 is involved in both genomic and non-genomic processes in the cell. Genomic mechanisms include the binding of T3 to thyroid hormone receptors in the heart muscle, which regulate the transcription of certain heart genes. Non-genomic processes are associated with continuous modulation of membrane ion channels.*

Note: TR — thyroid receptors; TRE — thyroid response element; Gs — guanine nucleotide binding protein; β-AR — beta-adrenergic receptor; Kv — voltage-dependent potassium channels; AC — adenylate cyclase; PLB — hydrophobic phosphoprotein of the sarcoplasmic reticulum of heart muscle (Adapted from Danzi S. et al., 2020 [17])

concentration of free T_3 is often found in patients with severe HF (15.3%). The same study revealed a significant number of cases of Se deficiency (74.6%). However, the correlation between Se concentration and free T_3 level was not proven [16].

The study by Pingitore A. et al. (2016) noted that parenteral intravenous administration of T_3 led to a lower heart rate, increased diastolic volume of the left ventricle and stroke volume, as well as improvement of the neurohormonal profile: decreased noradrenaline level in plasma, NT-proBNP and aldosterone [15, 16].

Synthetic triiodothyronine

Synthetic T_3 (liothyronine, LT_3) is classified as a thyroid hormone preparation and is used in replacement therapy for various forms of hypothyroidism solely as an experimental method of treatment. In contrast to sodium levothyroxine, the administration of liothyronine leads to short-term drug-induced thyrotoxicosis due to a sharp increase of T_3 in blood. According to several studies [17, 18], combination drugs (sodium levothyroxine + liothyronine) contribute to the improvement of clinical symptoms of hypothyroidism and patients' quality of life. However, this issue is still debatable. Many studies [14, 19–25] demonstrated that combination therapy (sodium levothyroxine + liothyronine) has no advantage over monotherapy with levothyroxine sodium. According to European Thyroid Association, "combination therapy should be considered solely as an experimental treatment modality" [26]. General limitations for LT_3 are its short half-life, risk of cardiovascular complications and mineral and bone metabolism disorders in the presence of hyperthyroidism or drug overdose [27–30].

According to Hoermann R. et al. (2019), there is a prolonged form of LT_3 (with slow release) with better pharmacological characteristics compared

with conventional LT_3. However, at present, this new formulated product is not available due to the lack of convincing evidence of drug efficacy. Large-scale randomized controlled clinical trials are required for the further recommendation of the use of this drug in clinical practice [31–32].

Conclusion

T_3 is a biologically active thyroid hormone. It is primarily formed by the conversion of T_4 to T_3 in extrathyroid peripheral tissues. Today, there are several known mechanisms of impairment of the conversion of T_4 to T_3, which in most cases are associated with iodine deficiency in the diet, as well as, possibly, with the deficiency of other trace elements, such as selenium, etc. These disorders can also be caused by severe somatic non-thyroidal diseases that require differential diagnosis with true thyroid pathology. Maintaining the physiological concentration of T_3 is extremely important for preventing the development and progression of HF, the formation of antimicrobial and antitumor immunity, and limiting autoimmune inflammation. Combination therapy for hypothyroidism — sodium levothyroxine + liothyronine — is still of great interest. Most of the studies performed revealed no advantages of this therapy compared with monotherapy with levothyroxine sodium. However, according to some studies, combination therapy has significant efficacy in the form of improved neurocognitive function and quality of life in general. Combination therapy may be preferable for certain categories of patients. However, high-quality, large-scale clinical trials are required for the substantiation of these conclusions and the creation of the corresponding evidence base.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication.

Troshina E.A. (ORCID ID: https://orcid.org/0000-0002-8520-8702): development of the concept and design of the study

Senyushkina E.S. (ORCID ID: https://orcid.org/0000-0001-7960-8315): data collection, analysis and interpretation; substantiation and writing of the manuscript

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Cholesterol Atheroembolism Syndrome: Current State of the Problem

Abstract

Cholesterol atheroembolism syndrome is a systemic pathological process caused by the embolization of small arteries with cholesterol crystals, which can develop spontaneously, and it is the result of intravascular surgery and / or the use of anticoagulants. Embolization cholesterol crystals leads to ischemic and inflammatory organ damage. The clinical picture is variable, various organs can be targets, but skin and kidneys are mainly affected. Specific clinical and laboratory signs aren't. Tissue biopsy is the gold standard for diagnosis cholesterol atheroembolism syndrome. The treatment is based on the correction of classical cardiovascular risk factors, the use of statins. In terms of benefit and risk failure from anticoagulants and thrombolytics should be considered. Studies on the use of corticosteroids, cytostatic, and colchicine have conflicting results. The use of monoclonal antibodies of IL-1 antagonists is a perspective direction.

Key words: *cholesterol-embolization, atherosclerosis, cardiac surgery, acute kidney injury, biopsies, statins*

Conflict of interests

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AKI — acute kidney injury, ARD — atheroembolic renal disease, CC — cholesterol crystals, CES — cholesterol embolization syndrome, CRP — C-reactive protein, GCS — glucocorticosteroids, IL-1ß — interleukin-1ß

Cholesterol embolization syndrome (CES) is a systemic pathological process caused by embolization of small arterial vessels in the skin, kidneys, retina, gastrointestinal tract and brain with microcrystals of cholesterol atherosclerotic plaques in the aorta and other major arteries, which results in ischemic and inflammatory damage to corresponding organs [1–3].

Previously, CES was detected primarily by pathologists based on autopsy results, and its incidence varied from 0.31 to 8.2% [4]. The incidence of CES was significantly higher (12–77%) in the autopsy of elderly patients who died after aortic surgery or aortography [2]. Clinically significant CES has an incidence of 0.09–2.9% due to widespread intravascular surgeries and the use of anticoagulants and thrombolytic agents. However, in most cases, CES can be easily overlooked. Therefore, its actual incidence is probably much higher [2].

Risk Factors

Atherosclerosis is the most important risk factor for CES. Factors contributing to CES include the causes

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of atherosclerotic plaque instability: inflammation, hemodynamic stress, smoking, dyslipidemia, old age, lack of statin treatment; the effect of anticoagulants and thrombolytics is also under consideration. According to Agrawal A. et al. (2018), who analyzed 24 studies, about 78% of patients with CES had arterial hypertension, 18% had diabetes mellitus, 31% had hyperlipidemia, 60% — coronary heart disease, 58% were smokers, and 79.8% were men [5]. The risk of CES increases in the presence of ulceration in atherosclerotic plaque, thrombus mobility and plaque thickness ≥4 mm [3].

In more than 70% of cases, CES arises after interventional procedures (coronary angiography and aortography), or cardiovascular surgery, about 20% of cases arise spontaneously [6, 7].

Inflammation is an equally important risk factor for CES. The level of C-reactive protein (CRP) in plasma of patients with CES was significantly higher than in patients without CES (2.4 and 0.7 mg/dl, respectively; p = 0.01). Multivariate analysis showed that a high CRP level was an independent predictor of CES (RR 4.6, p = 0.01) [8].

CES was previously associated with anticoagulants and fibrinolytics. It is believed that these drugs lead to the rupture of atherosclerotic plaques, causing internal hemorrhage and breakdown of fibrous capsules [9]. However, without intravascular interventions or surgeries, these drugs rarely lead to CES [10, 11].

According to Agrawal A. et al. (2018), 9 out of 23 studies referred to the use of warfarin, heparin, urokinase, or other fibrinolytic drugs that could be associated with CES [5].

In patients with atrial fibrillation and documented atherosclerotic plaque during treatment with warfarin, CES was detected with an incidence of 0.7–1.0% per year [10]. There are cases of cholesterol embolization with inadequate use of anticoagulants (warfarin, Coumadin) [12] and with adequate anticoagulation [11, 13]. At the same time, there are studies showing that oral anticoagulant therapy has no effect on the risk of CES [14].

Currently, there is not enough data indicating a reliable causal relationship between anticoagulants, fibrinolytics and CES, and the available results are quite contradictory.

Diagnostic (catheterization of the left heart) or therapeutic interventions on the aorta and its large branches (coronary artery bypass grafting, carotid endarterectomy, mitral valve prosthetics, aortic-iliac and aortic-femoral bypass grafting) [5, 12, 15, 16] play a crucial role in the cause of CES. Therefore, most cholesterol embolizations are iatrogenic.

It was previously believed that femoral access for angiography is associated with a higher risk of CES than radial access. Later, no significant difference was found in the incidence of CES depending on vascular access. However, the risk of acute kidney injury (AKI), acute kidney disease (first 90 days after AKI) and chronic kidney disease as a manifestation of CES was significantly lower when using radial access compared to femoral access [17–19].

As the skills of personnel performing diagnostic and therapeutic interventions on the aorta and its branches improved, there a decrease in the risk of rupture of atherosclerotic plaque with the release of cholesterol emboli. At the same time, a higher incidence of CES is predicted due to the increasing number of diagnostic and therapeutic interventions on the heart and blood vessels [12].

There is also information on the possible development of CES in the case of damage to the atherosclerotic plaque fibrous cap with blunt abdominal trauma or falling [12].

Pathogenesis

Spontaneous plaque rupture due to anticoagulants and/or thrombolytics, or its direct trauma by a probe tip (catheter) during intravascular surgery results in the contents of the plaque core (cholesterol crystals (CC)) entering the bloodstream and the subsequent transportation to distal vessels (small arteries, arterioles and capillaries). Damage of plaques in the ascending aorta results in the embolization of the retina and brain; if plaques are located in descending and abdominal parts, the vessels of the gastrointestinal tract, kidneys, skin, and lower limbs are embolized [1–3].

Initially, CC embolization causes ischemic damage, and subsequent inflammatory reaction exacerbates the process. Activation of complement, renin — angiotensin — aldosterone system, oxidative stress, leukocyte aggregation and release of leukocyte enzymes lead to endothelial damage.

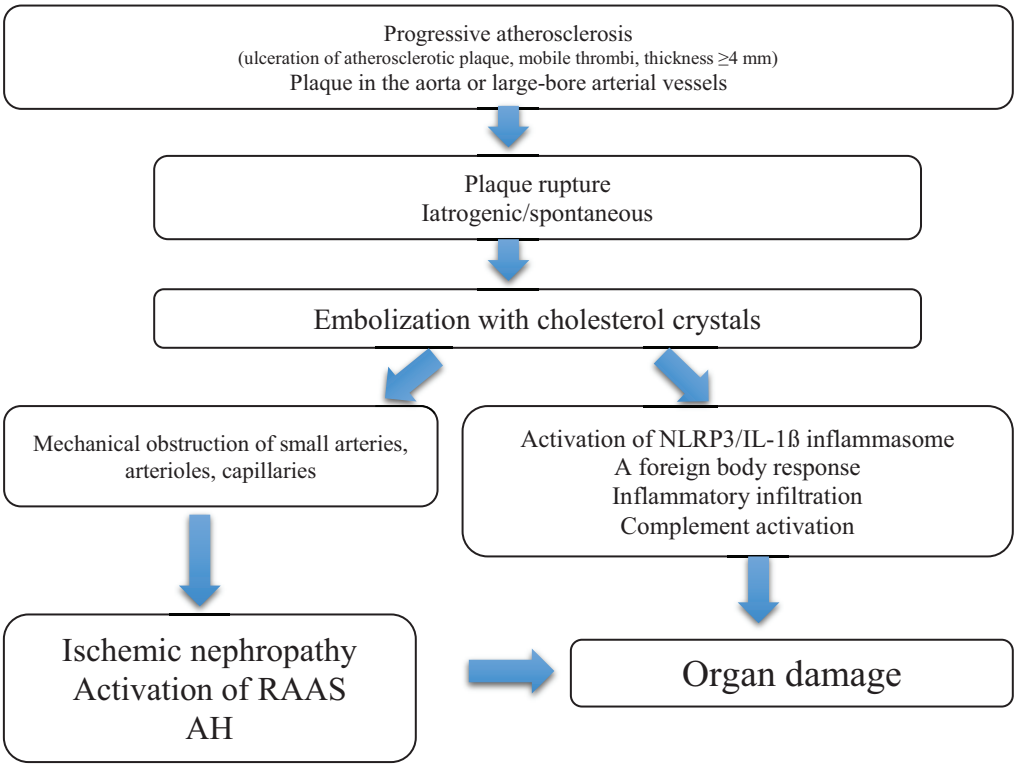

Figure 1. *Pathophysiological mechanisms of cholesterol embolization syndrome. (Adapted from A. Ozkok, 2019) [2]*

Note: AH — arterial hypertension; RAAS — renin-angiotensin-aldosterone system. IL-1ß — interleukin — 1ß

NLRP3 inflammasome is activated, resulting in the production of interleukin-1ß (IL-1ß) [20], and the secretion of other pro-inflammatory cytokines (tumor necrosis factor, macrophage inflammatory protein) is also induced [21]. Inflammation usually leads to intravascular thrombosis, followed by endothelial proliferation and fibrosis. The final result of CC embolization is the partial or complete occlusion of "target" arteries, which leads to tissue ischemia. Some episodes of microembolism may have no significant consequences, but massive embolism leads to systemic damage with the involvement of internal organs and the skin [1, 2]. Pathophysiological mechanisms of the development of CES are shown in Figure 1 [2].

Clinical Course

CES is characterized by a relatively long prodromal period between the triggering event and the onset of symptoms. Analysis of CES cases showed that skin findings were detected more than a month after the triggering event. However, if a fibrin thrombus can undergo recanalization (with partial restoration of vascular patency), cholesterol deposits do not undergo regression (cholesterol is insoluble in bodily fluids, and its crystals are not susceptible to phagocytosis by macrophages) [1, 2].

Clinical signs of CES vary. They reflect the systemic nature of the pathological process and are a combination of a systemic inflammatory response and symptoms that are typical for damage to the "end" organs. Inflammatory response often manifests in the form of fever, anorexia, weight loss, fatigue and myalgia, as well as several laboratory changes. The most common signs of CES are cutaneous and renal [14, 16, 22].

According to some authors, the skin and toes are affected in 75–96% of cases. Skin involvement is characterized by reticular asphyxia (livedo reticularis) in the form of a net-like reddish-blue pattern on lower limbs (lower leg, thigh) and sometimes on the body (lateral surfaces of the abdomen) [16, 23, 24]. Brown nodules that rise above the skin surface, i.e., palpable purpura, are a rarer skin lesion. The presence of palpable purpura indicates the development of leukocytoclastic vasculitis of small vessels as the body's response to the deposition of cholesterol microcrystals in said vessels. Along with a skin lesion, a symptom typical for CES may

appear — a cyanotic and cold big toe, with pulsation in the dorsal artery of the foot (the dorsalis pedis artery). Areas of soft tissue necrosis of the big toe may then appear and, in some cases, its gangrene, that requires amputation [11, 25, 26].

The incidence of kidney damage with CES is 92.2% [5, 26] and is defined as atheroembolic renal disease (ARD) that may be acute, subacute, or chronic [27, 28].

Massive embolization with CC may cause acute ARD during the first week after the trigger event. The course of AKI is progressive and is usually manifested by a sharp decrease in urine amount down to anuria; lower back pain resembling renal colic may arise. In period before anuria, urine color can resemble that typical for gross hematuria; blood clots may also be found in the urine. A sharp and difficult-to-manage rise in blood pressure (BP) is typical, sometimes accompanied by severe hypertensive encephalopathy and acute left ventricular failure [12].

ARD often has a subacute course with progressive renal dysfunction for several weeks [14, 16]. Chronic ARD is characterized by a slow and progressive decrease in renal filtration function. The chronic form is difficult to diagnose; it is often underestimated due to the absence of clinical (marked decrease in urine output) and extrarenal signs. Changes in urine sediment in the case of the chronic form are minimal: hematuria is rarely detected; cylindruria is usually absent [27].

The predominant lesion of renal tubulointerstitium may be accompanied by nocturia with a gradual decrease in urine specific gravity, which is confirmed by the results of the Zimnitsky test. Mild or moderate proteinuria is usually observed in cases of ARD. More rarely, in the case of induced focal segmental glomerulosclerosis, nephrotic proteinuria is detected [1, 5].

Lately, more and more publications on the development of CES after kidney transplant are emerging. CES of donor origin usually arises early after transplant and more often leads to allograft rejection. CES from the recipient's arteries becomes evident years after transplant, causing chronic allograft dysfunction, and is characterized by better organ survival. The prevalence of CES in this category of patients is expected to increase as the age of donors and recipients increases, and the donation criteria expand [29, 30].

Renal outcomes of ARD vary. Dialysis is necessary for 28–61% of patients (peritoneal dialysis may be advantageous since no anticoagulants are used), and 20–30% of patients have partially restored kidney function after several sessions [1, 5, 7, 11, 25]. Gastrointestinal symptoms (in 20–45% of patients) include abdominal pain, diarrhea, and bleeding. Cholesterol embolization of the visceral branches of the abdominal aorta may cause ischemia and fatal bowel infarction. Cases of necrotizing pancreatitis, focal necrosis of liver cells, and acalculous necrotizing cholecystitis were also described. In some cases, surgical interventions are necessary [31, 32].

Damage to the central nervous system can manifest as confusion, headache, dizziness, paraparesis, mononeuropathy. Cerebral artery embolization can occur with the development of transient ischemic attack, stroke, or spinal cord infarction. In such cases, CES usually results in diffuse brain damage with clinical signs of confusion and memory loss, rather than focal neurological signs and symptoms. Thromboembolism, in turn, is characterized by acute focal neurological symptoms [1, 33].

The pathognomonic symptom of CES is the presence of changes in the fundus in the form of specific Hollenhorst plaques (spots) that are associated with CC deposits. Embolization of retinal arteries (usually found in bifurcations of retinal vessels) leads to a sudden loss of visual fields (anopsia) with subsequent possible complete blindness. Hollenhorst plaques appear in the form of shiny and orange spots, with uneven contours, often with hemorrhage foci (Fig. 2). The most common source of these plaques is the carotid artery. This symptom is registered in almost 25% of patients. However, the presence of Hollenhorst plaques does not definitively confirm that the severity of the clinical picture is due to CES since these plaques can persist for more than a year and represent a previous attack of CES [34, 35, 36].

Myocardial and/or splenic infarctions, adrenal insufficiency, penile necrosis, tongue necrosis, and myositis were also described in cases of CES [37]. Damage to lungs during CES is rare [33].

Eosinophilia and/or hypocomplementemia can be registered simultaneously with skin lesions and general inflammatory reaction symptoms; they reflect the body's immune response to CC deposition in tissues. Blood eosinophilia is registered

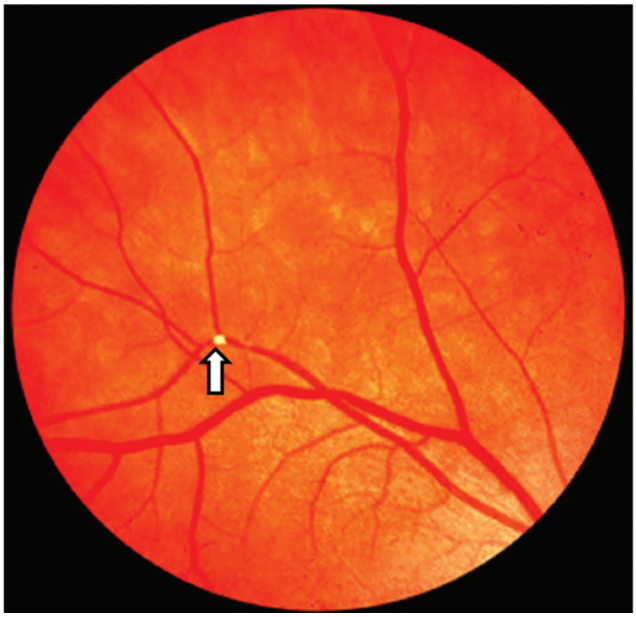

Figure 2. *Hollenhorst plaque when examining the fundus (Adapted from A.B. Dunlap, 2007) [36]*

in more than 60–80% of patients but is often observed only in the first few days [16, 22, 33]; the incidence of eosinophilia in tissues with underlying CES is unknown. The duration of eosinophil circulation in the blood is about 15 hours; in tissues (skin, gastrointestinal tract, connective tissues), it is 2–3 times longer, while eosinophils may return into the bloodstream. Eosinophils stop allergic reactions and inflammation (remove fibrin formed during inflammatory processes) and do not induce them. The severity of eosinophilia does not always correspond to the severity of the disease [38]. Less specific changes in laboratory parameters are: leukocytosis, anemia, thrombocytopenia, increased erythrocyte sedimentation rate, CRP, fibrinogen [22].

Diagnosis

The gold standard for CES diagnosis is tissue biopsy, which tissue can be obtained from the skin, muscles, kidneys, bone marrow, gastric mucosa and/or colon. Different stages of CES can be observed in the same biopsy specimen since CC-embolization can occur at different time intervals. Skin biopsy is a relatively non-invasive method, especially for taking material from the feet and legs; the method has a sensitivity of up to 92% [16].

The histological pathognomonic symptom of CES is the presence of biconvex and acicular CC or "cholesterol gaps" inside arterioles that appear due to CC dissolution during fixation of the biopsy sample [12, 16].

Kidney biopsy allows diagnosing CES in more than 75% of cases. Kidney biopsy helps to establish the diagnosis in more than 80% of cases of AKI but is very difficult in severely ill patients [26]. Since poor healing is observed at the sampling site, biopsy with underlying CES should be performed with caution. CES diagnosis can be established in practice in the presence of a combination of risk factors (triggering factors) and characteristic clinical symptoms. For example, clinical diagnosis of CES can be established after angiography, with delayed onset of AKI in combination with skin manifestations (livedo reticularis, lesion of the big toe). Tissue biopsy may not be required if CC are found in retinal vessels (Hollenhorst plaques) [1, 34, 35].

Differential Diagnosis

Since clinical signs of CES vary, with nonspecific features, the list of differential diagnoses is quite long, which allows considering CES one of the "great imitators":

- arterial thromboembolism;
- contrast-induced nephropathy;
- ischemic and/or drug-induced tubulointerstitial nephritis;
- endocarditis;
- aortic dissection (acute aortic syndrome);
- myxoma of left atrium;
- tuberculosis;
- pheochromocytoma;
- Raynaud's phenomenon;
- systemic diseases of connective tissue (rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis), vasculitis (polyarteritis nodosa), thromboangiitis obliterans;
- cryoglobulinemia;
- antiphospholipid syndrome;
- true polycythemia;
- thrombotic thrombocytopenic purpura and others [2]

First of all, CES should be differentiated from arterial thromboembolism, which usually causes acute ischemia and infarction of a "distal" organ. Although these two conditions have a common risk factor — progressive atherosclerosis, differential diagnosis is important since the prognosis and

treatment methods for these conditions are different. Thromboembolism usually starts suddenly and usually causes acute organ dysfunction due to ischemia and infarction. Clinical signs in cases of CES are usually subacute or chronic; dysfunction of the target organ is slow. Thromboembolism management should be started in a timely manner with anticoagulants, thrombolytics or interventional procedures. If optimal treatment is started earlier, then a good effect can be expected. CES management is more complex, the prognosis is usually worse, and anticoagulants, thrombolytics and invasive procedures can be more harmful than beneficial [1, 2].

During differential diagnosis, one should take into account the possibility of developing contrast-induced nephropathy (history of angiographic procedure), when AKI usually occurs within 48–72 hours after the procedure, and kidney function improves within 4–7 days. In contrast, renal dysfunction due to ARD usually has a subacute course with a gradual increase in serum creatinine within 1–2 months. Systemic manifestations of CES, including symptoms of damage to the skin, gastrointestinal tract, and central nervous system, may be useful in differential diagnosis [26].

Differential diagnosis with drug-induced acute tubulointerstitial nephritis can be difficult, especially in the presence of eosinophilia. The cause of eosinophilia in this case is considered to be allergic reactions (the so-called "glow of allergic fire") [38]. It should also be differentiated from acute and rapidly progressive nephritic syndromes.

Differential diagnosis of livedo reticularis varies greatly and includes a wide range of connective tissue diseases (Table.) [22, 33].

Prevention

CES is a manifestation of progressive atherosclerosis. Therefore secondary prevention of cardiovascular diseases is of primary importance in such patients. Secondary prevention measures are described in detail in the relevant domestic and foreign recommendations [39].

Patients with CES should avoid invasive interventional procedures whenever possible. An important preventive measure is a more rigorous and substantiated selection of patients for intravascular surgery. Radial access may be preferable in several aspects. Indirect angiography methods (contrast-enhanced magnetic resonance imaging, computed coronary angiography, etc.) should be used for diagnostic purposes in groups of patients at high risk. When performing the intravascular intervention procedure itself, much depends on the operator. The no-touch technique is recommended for manipulating guidewires and catheters through vessels, and also more thorough intraoperative removal of atheromatous contents using special devices. Although there is no proven relationship between anticoagulants, thrombolytics and CES, these drugs should not be used, only if they have no other indications (atrial fibrillation, valve replacement, etc.) [1, 2].

Treatment

There are no conventional protocols for the treatment of CES. Antiplatelet drugs are prescribed for secondary prophylaxis of cardiovascular diseases despite that there is no evidence for this as treatment of CES [15]. Statins can have three main positive effects for the treatment of CES: lowering the level of low-density lipoproteins; stabilizing atherosclerotic plaques; having pleiotropic anti-inflammatory effects (ability to directly reduce inflammation severity by blocking the expression of pro-inflammatory transcription factor (NF–B) and associated chemokines). Statins in patients with CES resulted in improved renal function and decreased skin manifestations [15, 40].

Inflammation is one of the main pathophysiological mechanisms in CES, and it was expected that anti-inflammatory drugs would be the main agents in pathogenetic therapy [40]. A clinical study including 51 patients with an established diagnosis of cholesterol embolism of intrarenal arteries assessed the effect of glucocorticosteroids (GCS) on short- and long-term renal outcomes. Patients of one subgroup (n = 32) received GCS with an initial dosage of 10–20 mg/day. The glomerular filtration rate was estimated at baseline, after 4 weeks and at the last follow-up stage (after a year). After 4 weeks, the glomerular filtration rate in patients taking GCS increased by 24% compared with the baseline, and in individuals not taking these drugs, it increased by only 5% (p = 0.03). However, this treatment had

no beneficial effect on the functional state of kidneys in the long-term [41]. Renal function significantly improved (serum creatinine level decreased from 7.5 to 1.6 mg/dl) with the administration of prednisolone per os and at a higher dose (1 mg/kg) [42]. Earlier, data were obtained on the decreased severity of renal failure and skin signs of cholesterol embolization due to the combination of GCS and cyclophosphamide [43]. Most studies suggest that GCS are preferable for CES [6, 42], although, in some cases, GCS had no proven efficacy and may have had a negative effect [7].

The ability of colchicine to block auto-inflammatory pathways (including NLRP3 and IL-1b) [44] and also reduce the risk of cardiovascular events was recently discovered [45]. There were reports of clinical cases of successful use of colchicine with corticosteroids in patients with CES and skin damage symptoms. [46].

The use of monoclonal antibodies of IL-1 antagonistsb (canakinumab) in atherosclerotic diseases [47] is a promising area with very promising results; it can certainly be considered for patients with CES. It was revealed that apheresis of low density lipoproteins reduced the need for dialysis for 6 months [48], and also improved symptoms in patients with CES [3].

Literature sources describe a case of improvement of skin lesion symptoms (blue toe syndrome) and prevention of toe amputation after lumbar sympathectomy [49].

Therapeutic measures for CES are limited, which is also associated with the lack of randomized controlled trials evaluating said methods of treating CES.

In general, the prognosis for CES is unfavorable, and mortality ranges from 15 to 30% during the first year of life [7].

Thus, CES is a multisystem condition and is often underestimated. Initially, CC embolization can have an asymptomatic course without visible signs, which significantly complicates the diagnosis. Also, due to a rather variable clinical picture, a practitioner of any specialty may encounter CES: cardiologist, nephrologist, dermatologist, gastroenterologist, neurologist, ophthalmologist, rheumatologist, surgeon. In clinical practice, physicians require constant clinical suspicion based on the knowledge of the risk factors, pathophysiological mechanisms and clinical picture of this pathology in order to establish the correct diagnosis and to determine optimal management tactics.

CES remains one of the least studied issues in cardionephrology. Therefore, further studies are necessary to determine optimal therapeutic strategies.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication.

Mikhailova Z.D. (ORCID ID: https://orcid.org/0000-0002-0926-6038): development of the concept and design of the review, writing and editing text

Klimkin P.F. (ORCID ID: https://orcid.org/0000-0001-0002-0231-5909): collection and analysis of manuscript materials, writing and editing text

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New Approaches to Studying Prevalence Gallstone Disease

Abstract

The aim — predicting the growth of gallstone disease based on the study of the dynamics of the incidence of the liver. **Materials and methods.** In clinical conditions, 98 patients (62 women and 36 men, average age 43.4 ± 3.3 years (21-60)) with various chronic liver diseases were examined. Anamnesis, clinical and laboratory data were used to verify the diagnosis. In portions "B" and "C" of bile obtained by multifractional duodenal sounding, the total concentration of bile acids, cholesterol and phospholipids was determined, and lithogenicity indices of bile were calculated: cholate-cholesterol and phospholipid-cholesterol coefficients. The results were analyzed using Microsoft Excel 2010 and PSPP statistical processing programs. The next stage of the work was the analysis of statistical indicators of the general and primary liver morbidity in the Udmurt Republic over the past 10 years (2008-2018). The study applied statistical forecasting methods. Models were built in the Microsoft Excel 2010 program in a polynomial trend. **Results.** In 52 (53,1%) examined patients, ultrasound examination of the gallbladder were signs of biliary sludge. Microscopic examination of bile 71 (72,6%) patients had crystals of cholesterol and calcium bilirubinate, which is evidence of stage I gallstone disease. In all patients with biliary sludge, a violation of the biochemical composition of bile was noted — a decrease in the concentration of bile acids and phospholipids, an increase in the concentration of cholesterol, a decrease in cholesterol and phospholipid-cholesterol coefficients. When studying statistical indicators over the past 10 years, a higher general and primary incidence of liver diseases in the Udmurt Republic was noted than in the Russian Federation as a whole. Based on the results of trend modeling, a significant increase in the total and primary liver morbidity is predicted both in the Udmurt Republic and in the Russian Federation. **Conclusion.** Summarizing the data obtained, it can be noted that over the past 10 years (from 2008 to 2018) among the adult population of Udmurt Republic, a clear tendency has been revealed for an increase in the general and primary incidence of the liver. As the results of trend forecasting showed, an increase in the incidence of the liver will continue in the coming years. With liver pathology, bile secretory function suffers, as a result of metabolic processes, bile produces supersaturated cholesterol, which is the basis for stone formation in the gall bladder. A study of the dynamics of liver disease allows predicting an increase in cholelithiasis in the coming years. Despite the fact that the asymptomatic course of cholelithiasis is often quite observed, if this disease is not diagnosed and the preventive treatment of stone formation is not carried out in a timely manner, this leads to the development of serious complications.

Key words: *gallstone disease, chronic liver disease, prognosis, trend modeling*

Conflict of interests

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BA — bile acids, BS — biliary sludge, CCC — cholate-cholesterol coefficient, CL — cholelithiasis, CS — cholesterol, PCR — phospholipid-cholesterol ratio, PL — phospholipids, UR — the Udmurt Republic, US — ultrasound

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In recent years, there has been a steady increase in the incidence of cholelithiasis (CL) — approximately twofold every ten years in all countries. More than 10% of the world population already has CL [1, 2]. It should also be noted that the incidence of CL varies greatly and depends on a number of contributing factors (place of residence, ethnic and national peculiarities, etc.) [3, 4].

The incidence of CL among residents of the Udmurt Republic (UR) from 2010 to 2015 was as follows: in men — on average 97.3 per 100 thousand of the adult population, in women — 333.8 per 100 thousand of the adult population. Thus, CL occurs in one in every 13–15 men and one in every 3–4 women. The ratio by gender in UR is 5:1, predominantly in women [2, 4]. Mortality from CL stands at 1.9 per 100 thousand adults.

CL can be considered a socially significant disease due to its high prevalence, steadily rising incidence in all developed countries of the world, and its effect on the working-age population [5]. Nevertheless, it is difficult to identify actual parameters of CL prevalence for several reasons. First of all, CL is often asymptomatic: 15–20% of the adult population have gallstones, while only 4 to 20% of them exhibit clinical signs of the disease [3, 6–9]. Also, cholelithiasis is diagnosed as a nosological form only at stages II and III (stage of lithogenesis and stage of recurrent episodes of chronic calculous cholecystitis).

It is known that bile does not become lithogenic in the gallbladder. It is excreted like that from the liver. Liver diseases often lead to the abnormal composition of bile and to the development of biliary sludge (BS), which is a sign of the early (pre-stone) stage of cholelithiasis [1]. In connection with the abovementioned facts, studying the prevalence of liver diseases will help to determine the prognosis for CL incidence.

The objective of our work was to predict the rise in the incidence of CL based on studying changes in the incidence of liver disease.

Materials and Methods

Ninety-eight patients (62 women and 36 men, average age 43.4 ± 3.3 years (21–60)) with different chronic liver diseases were examined in a clinical setting.

This study was performed at the Department of Internal Medicine of the Federal State Budgetary Educational Institution of Higher Education "Izhevsk State Medical Academy" (ISMA) of the Ministry of Health of the Russian Federation at the Budgetary Educational Institution of the Udmurt Republic "I. B. Odnopozov City Clinical Hospital No. 8 of the Ministry of Health of the Udmurt Republic" and the Budgetary Educational Institution of the Udmurt Republic "City Clinic No. 1 of the Ministry of Health of the Udmurt Republic" from 2016 to 2020. The patients were examined after obtaining voluntary informed consent and approval by the Committee on Biomedical Ethics of the Federal State Budgetary Educational Institution of Higher Education ISMA of the Ministry of Health of Russia (minutes of the Meeting No. 529 of January 24, 2017).

Inclusion criteria:

1. Male and female patients aged 20 to 60 years with liver disease.
2. Signed informed consent.

Exclusion criteria:

1. Pregnancy and lactation.
2. Chronic diseases in decompensation stage.
3. Oncological diseases.
4. Mental disorders.

Clinical and instrumental examinations and laboratory tests were used to verify liver diseases. All patients underwent tests for bilirubin and blood transaminases (Cormay Livia ACCENT 300 analyzer, Poland), markers of viral and autoimmune hepatitis (enzyme-linked immunosorbent assay); ultrasound of the hepatobiliary system was performed using the Mindray DC-60 Exp device (China). Seventy-six (77.6%) patients underwent liver elastography using the AIXPLORER analyzer (Supersonic Imaging SA, Aixen-Provence, France), 58 (59.2%) patients underwent the FibroMax test to exclude liver fibrosis and cirrhosis. Abdomen CT for 43 (43.9%) patients was performed using General Electric LightSpeed VCT XT 64 (China) device. Multifractional duodenal sounding with subsequent macroscopic and microscopic examination of bile, determination of its physical and colloidal properties and biochemical composition was performed for all of the examined patients.

Cystic and hepatic portions of bile obtained by multifractional duodenal sounding were analyzed for total content of bile acids (BA), cholesterol (CS) and phospholipids (PL); bile lithogenicity factors were calculated: cholate-cholesterol coefficient (CCC) and phospholipid-cholesterol coefficient (FCC) [10].

Verification of chronic hepatitis was carried out using ultrasound of the hepatobiliary system, increase in blood transaminase activity, positive markers of viral and autoimmune hepatitis, and alcohol consumption in hepatotoxic doses (in history). Diagnosis of liver cirrhosis was established based on cirrhotic changes in the liver according to the results of ultrasound, grade 4 fibrosis (F4) based on elastography results according to the Metavir scoring scale, typical changes in blood biochemistry.

All of the results were compared with those of the control group; the latter included 52 healthy individuals aged 21 to 60 years (36 women and 16 men, average age 40.1 ± 4.6 years).

The results were analyzed using statistical software Microsoft Excel 2010 and 2010 and Pspp 1.0.1. Distribution normality was checked using Kolmogorov—Smirnov and Shapiro—Wilk tests. The study used parametric statistical methods since the distribution was close to normal. The results are presented as M ± SD. Student's test (T) was used for assessing the statistical significance of differences (p) and comparing quantitative parameters in the two groups. Differences between the groups were deemed statistically significant at $p < 0.05$.

Clinical examination of patients was followed by studying statistical parameters of general and primary liver disease incidence in the UR over the past 10 years (2008–2018). To this end, the analysis of official statistical data was carried out, including parameters taken from the informational and analytical collection "Basic parameters of public health in the Udmurt Republic" prepared by the Budgetary Educational Institution of the UR "Republican Medical Information and Analytical Center of the Ministry of Health of the Udmurt Republic" [11].

This study used statistical forecasting (trend modeling). Models were built using Microsoft Excel 2010 in a polynomial trend. Model adequacy was checked using the *R criterion:*

$$R^2 = 1 - \frac{SSE}{SST},$$

where

$$SSE = \sum \left(Y_i - \hat{Y}_i\right)^2$$

and

$$SST = \left(\sum Y_i^2\right) - \frac{\left(\sum Y_i\right)^2}{n}$$

where Y_i — actual value of the studied parameter, $\hat{Y}_i$ — value of a model, $i = 1 \ldots n$

Results

Thirty-six of the examined patients (36.7%) were diagnosed with hepatic steatosis, 42 (42.9%) had chronic hepatitis (of alimentary (57.1%), viral (31%) and autoimmune (11.9%) etiologies), 20 (20.4%) had cirrhosis in the compensation stage. The predominant disease of the digestive system was the pathology of the small intestines and pancreas (Table 1).

This is due to the close anatomical and morphological position of the organs in the hepatobiliary and pancreatic regions, which leads to common pathogenetic mechanisms of diseases. Among concomitant pathologies of other organs and systems, cardiovascular diseases were the most common — in 38 (39.2%) patients. This can be attributed to

Table 1. *The frequency of concomitant diseases in examined patients with cholelithiasis*

Concomitant diseases	The absolute number	%
Gastroesophageal Reflux Disease	57	14,4
Chronic gastritis (including erosive)	48	12,1
Reflux gastritis	88	22,2
Chronic duodenitis (including erosive)	67	16,9
Duodenal ulcer	108	27,3
Chronic pancreatitis	113	28,5
Irritable bowel syndrome	63	15,9
Hypertonic disease	28	28,6
Coronary heart disease	15	15,3

atherogenic dyslipidemia, one of the common pathogenetic mechanisms of cardiovascular pathology, liver diseases and CL [12].

Gallbladder ultrasound in 52 (53.1%) of the examined patients revealed signs of biliary sludge (BS) (microlithiasis, putty-like bile). Microscopic examination of bile revealed crystals of cholesterol (CS) and calcium bilirubinate in 71 (72.6%) patients, which is a sign of CL stage I (pre-stone).

Also, all patients with BS demonstrated abnormal physical and chemical properties of B and C bile portions, as shown in Table 2.

Levels of bile acids (BA) and phospholipids (PL), which are stabilizers of bile colloidal properties, were low in comparison with the control group, while the CS level was high. Low bile lithogenicity coefficients, CCC and FCC, were also observed in the examined patients compared with the control group. Therefore, 52 (53.1%) patients with liver diseases and CL stage I (pre-stone) had a very high probability of gallstone formation.

The past ten years have seen a rapid increase in the incidence of liver diseases: in the Udmurt Republic, the incidence of liver diseases increased

Table 2. *Indicators of the biochemical study of bile in examined patients with biliary sludge*

Indicators	Group of control (n=52) M±SD	Patients with liver diseases (n=98) M±SD	Generally accepted norms	
Cholesterol (mmol/l)				
Portion B	7,56±0,07	26,34±0,65	5,2-15,6	0,00082
Portion C	3,63±0,06	16,21±0,52	1,3-2,8	0,00013
Bile acids (mmol/l)				
Portion B	54,33±0,14	30,22±0,47	57,2-184,6	0,004
Portion C	20,76±0,20	15,24±0,58	17,4-52,9	0,006
Phospholipids (mmol/l)				
Portion B	3,90±0,03	1,98±0,05	3,2-4,1	0,00035
Portion C	0,39±0,003	0,23±0,02	0,35-0,4	0,00042
Cholato-cholesterol coefficient (units)				
Portion B	7,15±0,07	1,52±0,10	8,5-7,8	0,00031
Portion C	6,14±0,10	1,13±0,05	7,1-6,3	0,00063
Phospholipid-cholesterol coefficient (units)				
Portion B	0,51±0,01	0,10±0,01	0,61-0,26	0,00052
Portion C	0,11±0,001	0,04±0,003	0,26-0,14	0,00012

Note: n — number of observations; ρ — the significance of differences between the indicators in the control group and in patients with liver diseases

Table 3. *Dynamics of the general incidence rate per 100 thousand population for liver diseases*

Class of diseases	Observed Dates (year)										
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Liver Diseases in the Udmurt Republic	461,6	444,5	429,0	464,7	480,1	479,0	623,4	641,9	774,7	821,1	783,6
Liver diseases in the Russian Federation	349,0	357,1	360,9	365,3	370,0	379,5	391,9	398,6	416,1	428,0	441,4

Table 4. *Dynamics of the primary incidence rate per 100 thousand population for liver diseases*

Class of diseases	Observed Dates (year)										
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Liver Diseases in the Udmurt Republic	57,2	46,8	44,4	48,3	38,1	43,4	78,3	149,1	113,2	144,6	125,5
Liver diseases in the Russian Federation	53,5	54,9	55,5	56,6	56,5	58,6	63,0	69,9	72,8	73,6	71,4

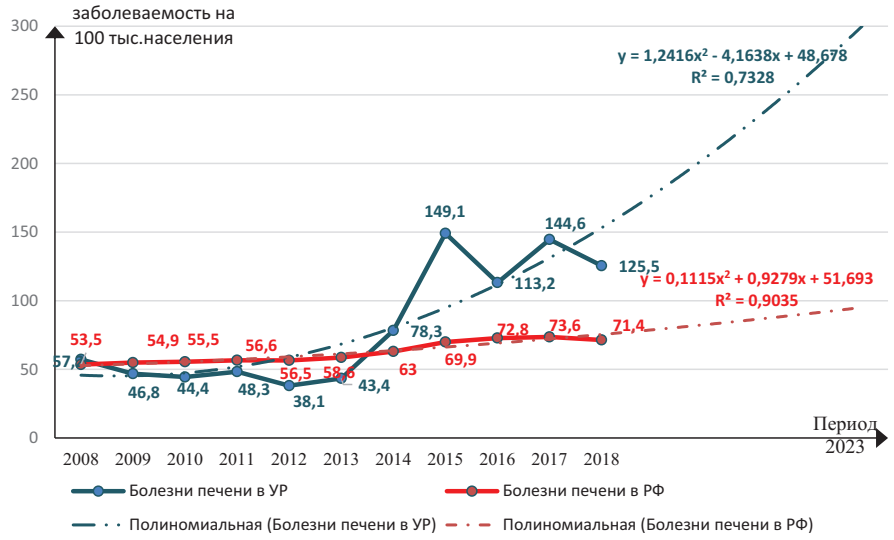

Figure 1. *Prediction for 5 years of the dynamics of the primary incidence of liver diseases in the Udmurt Republic and the Russian Federation (per 100 thousand population)*

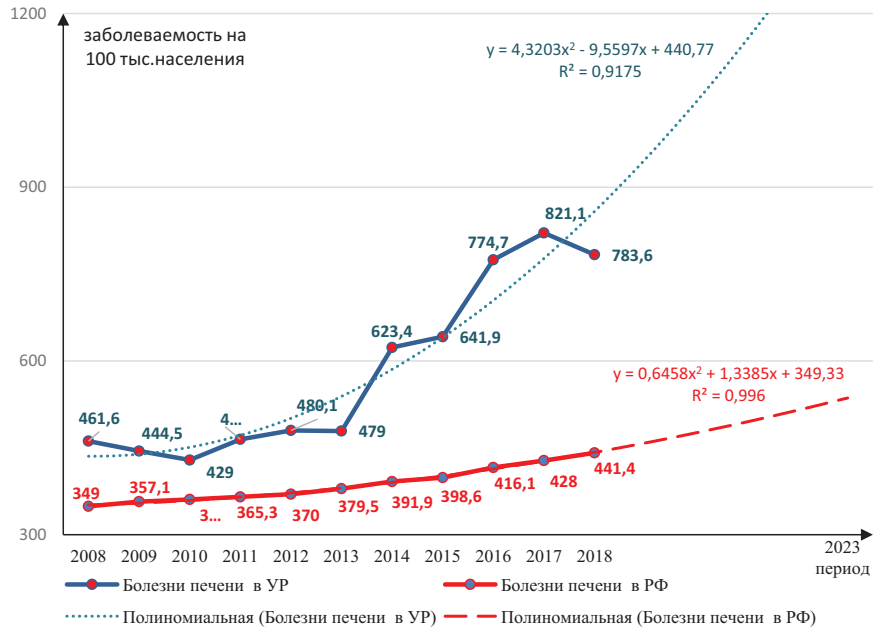

Figure 2. *Prediction for 5 years of the dynamics of the general incidence of liver diseases in the Udmurt Republic and the Russian Federation (per 100 thousand population)*

by 1.69 times, and in the Russian Federation by 1.26 times. The annual increase in the overall incidence of liver diseases in the Udmurt Republic ranged from 3.2% to 23.16%, in the Russian Federation — from 1.05% to 4.2%. During this period, the primary incidence of liver diseases in the Udmurt Republic increased at an even faster pace: by 2.19 times; in the Russian Federation, this figure was no more than 1.33 (Table. 3, 4). From 2008 to 2018, the primary incidence of liver diseases accounted for 7.9% to 23.2% of the overall incidence in the Udmurt Republic.

The trend modeling method shows that in the near future, a significant increase in the overall and primary incidence of liver diseases is expected both in the Udmurt Republic and the Russian Federation as a whole (Fig. 1, 2).

Discussion

The higher incidence of liver diseases in the Udmurt Republic in comparison with the Russian Federation as a whole can be explained by the worse economic situation compared to neighboring regions and, as a result, a greater number of risk factors for hepatic steatosis (diet dominated by flour-based food and sausages, stress-induced eating and alcohol consumption) [12]. The resulting high burden of liver diseases is accompanied by an increase in the incidence of CL since the impaired bile formation function of hepatocytes and the production of lithogenic (supersaturated cholesterol) bile are considered among the primary links involved in the pathogenesis of cholesterol cholelithiasis [13, 14]. The oversaturation of bile with cholesterol

is a consequence of abnormal complex metabolic processes in the liver; primary among said processes are the increased activation of hydroxymethyl-glutaryl-coenzyme-A reductase, which contributes to increased formation of cholesterol, or decreased activity of cholesterol-7-alpha-hydroxylase, which leads to reduced bile acid synthesis [15].

The results are consistent with published data [5]: the level of BA and PL, which are the stabilizers of bile colloidal properties, was low in the hepatic portion of bile, while the CS level was high. As a result, there was a decrease in bile lithogenicity coefficients (CCC and FCC).

Already oversaturated bile is transported to the gallbladder from the liver as a part of micelles and vesicles. The role of the gallbladder in the stone formation processes is undeniable: with a good functional state of the gallbladder and as a result of its contraction, all agglomerated vesicles and micelles enter the duodenum with bile flow; when gallbladder contractility decreases, subsequent crystal growth and BS formation begin [7]. Nevertheless, factors associated with functional disorders of hepatocytes and leading to bile oversaturation are fundamental in triggering lithogenesis [16].

Consequently, the results of studying the incidence of liver disease can be the determining parameters in predicting the incidence of CL. We think that all patients with liver disease require early detection of CL (ultrasound, multifraction duodenal sounding with subsequent macroscopic, microscopic examination of bile, determination of its physical and colloidal properties and biochemical composition), which is often neglected today.

Conclusion

In brief, the past ten years (2008–2018) have seen a clear trend of rising overall and primary incidence of liver diseases among the adult population of the Udmurt Republic. Results of trend forecasting showed that the increase in the incidence of liver diseases will continue in the coming years. Liver disorder affects the bile secretory function: bile oversaturated with cholesterol is produced as a result of disruption of metabolic processes, which is the cause of stone formation in the gallbladder.

Studying changes in the incidence of liver diseases enables to predict the increase in the incidence of cholelithiasis in the coming years. Although asymptomatic CL is very common, if stone formation is not diagnosed and preventive treatment is not carried out on time, it can lead to serious, often life-threatening, complications.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication.

Khokhlacheva N.A. (ORCID ID: https://orcid.org/0000-0003-4634-2658): development of the concept and design of the study; verification of critical intellectual content; final approval of the manuscript for publication.

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Clinical Features and Insulin Resistance in Men with a Metabolically Unhealthy Obesity Phenotype

Abstract

Purpose of the study: The aim of study was to analyze the characteristics of hormonal-metabolic parameters in men with a metabolically unhealthy obesity phenotype; identify the value of special indicators for diagnosis of insulin resistance. **Materials and methods:** The examination included 108 patients with body mass index ≥25 kg/m^2, which were hospitalized. According to the current national guidelines for the diagnosis and treatment of obesity, all examined patients were divided into 2 groups: 1 — with metabolically healthy obesity phenotype, 2 — with metabolically unhealthy obesity phenotype. The study presents the results of comparative simultaneous nonrandomized study of two groups with using of different methods of examination (anthropometric indicators, laboratory tests for inspection of the hormonal profile, biochemistry parameters, and calculation of TyG index for diagnosis of insulin resistance). **Results and discussion:** The study found that patients of working age with metabolically unhealthy obesity phenotype are characterized by unfavorable anthropometric and hormonal-metabolic parameters and more severe polymorbid pathology (first of all cardiovascular diseases). The results of study revealed the value of special indicators for the diagnosis of insulin resistance (visceral obesity index >1,85; TyG >3,98; fat mass >30,1). **Conclusion:** timely detection of insulin resistance indicators has great importance and practical application due to simplicity and accessibility.

Key words: *obesity, insulin resistance, adiponectin, fat mass*

Conflict of interests

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AF — atrial fibrillation, AI — atherogenic index, AH — arterial hypertension, ALAT — alanine aminotransferase, ASAT — aspartate aminotransferase, BMI — body mass index, BP — blood pressure, CHD — coronary heart disease, CHF — chronic heart failure, CS — cholesterol, DM — diabetes mellitus, ES — extrasystole, GIT — gastrointestinal

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tract, HC — hip circumference, HDL — high-density lipoproteins, HOMA-IR — insulin resistance index, IR — insulin resistance, LDL — low-density lipoproteins, MHP — metabolically healthy phenotype, MUP — metabolically unhealthy phenotype, NAFLD — non-alcoholic fatty liver disease, TG — triglycerides, TyG — triglycerides/glucose index, VAI — visceral adiposity index, WC– waist circumference

Introduction

Today, obesity (OB) is regarded as one of the most critical public health problems, which leads to early disability and high mortality [1, 2]. This is primarily due to the development of insulin resistance (IR), which, in turn, is the main risk factor for the development of severe hormonal and metabolic changes [3, 4]. The number of overweight and obese patients is growing steadily [5, 6, 7]; in light of this, the development and implementation of available methods for the diagnosis of the main IR parameters seem to be a significant aspect for real clinical practice.

Patients with the so-called metabolically unhealthy obese phenotype (MUP) are considered as a special group [8, 9]. In this study, we analyzed the features of hormonal and metabolic parameters in this category of patients; ROC analysis helped to determine the TyG index (logarithmic ratio of fasting triglycerides and plasma glucose), visceral adiposity index (VAI), and the percentage of adipose tissue for IR diagnosis in clinical practice.

Study Objective

To analyze clinical, hormonal and metabolic features in patients with metabolically unhealthy obese phenotype, to evaluate the possibilities of assessing insulin resistance (IR) at the primary care stage.

Materials and Methods

This study included 108 working-age men with body mass index (BMI) ≥ 25 kg/m^2, hospitalized in cardiac and therapeutic hospitals of the Federal Government Healthcare Institution "Primary Healthcare Unit of the Ministry of Internal Affairs of Russia in the Nizhny Novgorod Region". The patients were hospitalized for exacerbation of visceral diseases, annual routine medical examination, or adjustment of current treatment.

All of the examined patients were divided into two groups according to the criteria set by the National Clinical Recommendations for the Diagnosis, Treatment and Prevention of Obesity and Associated Conditions [8]: group 1 — group with metabolically healthy phenotype (MHP) and group 2 — group with metabolically unhealthy phenotype (MUP). Patients with no more than one additional associated pathological condition and normal tissue sensitivity to insulin were assigned to the MHP group (metabolically healthy obesity). Individuals with metabolically healthy phenotype were identified according to the National Clinical Recommendations for the Diagnosis, Treatment and Prevention of Obesity and Associated Conditions (2017).

In this group of patients, a slight increase in visceral adiposity index (VAI) and/or fat mass was permissible.

This paper is a cross-sectional comparative non-randomized descriptive study of two groups of patients. We used methods for assessing anthropometric parameters, including the calculation of visceral adiposity index (VAI) and the percentage of adipose tissue using the Deurenberg equation [10]. Laboratory tests, i.e., CBC, blood biochemistry and hormonal tests (immunoreactive insulin and adiponectin) were also performed. A detailed assessment of insulin resistance included, in addition to conventional parameters (HOMA-IR, Caro index), the analysis of TyG index, determined by the following formula:

$$\mathbf{[TG\ (mg/dL) \times glucose\ (mg/dL)]},$$

where TG is the level of triglycerides

Statistical processing of the material was carried out using Statistica 6.0 software package and Microsoft Excel 7.0 for Windows XP with nonparametric methods; ROC analysis was also used. Statistical processing of the results was performed using nonparametric methods of variation statistics (median and percentiles) and Mann—Whitney test to compare independent samples. Statistical significance of differences was evaluated with the probability of null hypothesis less than 0.05 ($p < 0.05$).

Data in the text and tables are presented as M ± m (where M is the arithmetic mean, and m is the arithmetic mean error). The correlation of quantitative features was analyzed using correlation and regression analysis methods and supplemented by a nonparametric method, i.e., calculation of Spearman's rank correlation coefficient, which reduces the effect of random outliers. Qualitative data were compared using the chi-square test (χ^2) (depending on the number of cases of comparison — Fisher's exact test or χ^2 test with Yates's correction for continuity). Cluster analysis was used for analyzing the compound effect of factors on the quality of life of men of working age.

Results

As a result, group 1 included 45 patients, group 2 — 63 patients. Table 1 presents the clinical features of the groups.

As the table shows, the average age of patients in the groups was approximately the same, while patients with MUP were characterized by a higher BMI. This group also had a significantly higher VAI and percentage of fat mass.

Comparative analysis of the incidence and nature of associated diseases in the two groups of patients revealed a number of differences in polymorbidity parameters (Table 2).

Table 1. *Clinical characteristics of groups of patients with metabolically healthy and metabolically unhealthy obesity phenotype*

Parameter	MHP n = 45	MUP n = 63	p-value
Age, years	43.9 ± 1.1	47.2 ± 0.9	0.06
BMI, kg/m^2	29.6 ± 0.4	36.1 ± 0.8	<0.001
WC, cm	96.5 ± 1.2	117.1 ± 1.9	<0.001
HC, cm	103.1 ± 1.1	112.3 ± 1.3	<0.001
WC/HC	0.91 ± 0.006	1.04 ± 0.008	<0.001
Fat mass, %	29.2 ± 0.6	38 ± 0.9	<0.001
VAI	1.5 ± 0.1	3.1 ± 0.4	<0.001

Note: BMI — body mass index, WC — waist circumference, HC — hip circumference, VAI — visceral adiposity index, MHP — metabolically healthy phenotype, MUP — metabolically unhealthy phenotype

Table 2. *Comorbid pathology in groups of patients with a metabolically healthy and metabolically unhealthy obesity phenotype*

Parameter, % (individuals)	MHP, n = 45	MUP, n = 63	p, χ^2
AH stage I	40.2 (19)	14.5 (9)	p = 0.002, χ^2 = 7.31
AH stage II	48.9 (26)	48.3 (30)	p = 0.9, χ^2= 2.04
AH stage III	0	38.7 (24)	p = 0.01, χ^2= 5.8
CHD: stable angina FC I-II	0	32.8 (20)	p = 0.002, χ^2 = 8.8
Atrial fibrillation: permanent form (n = 3) paroxysmal form (n = 2)	0	8.2 (5)	p = 0.053, χ^2= 3.74
CHF stage IIA (FC I-II)	0	32.8 (20)	p = 0.002, χ^2= 8.8
NAFLD (grade 1)	10.9 (5)	25.8 (16)	p = 0.04, χ^2= 9.45
Chronic pancreatitis	17 (8)	14.7 (9)	p = 0.7, χ^2= 15.8
Chronic cholecystitis	34 (16)	42.6 (26)	p = 0.3, χ^2= 7.55
Chronic gastroduodenitis	12.8 (6)	16.4 (10)	p = 0.4, χ^2= 16
Osteoarthritis (knee/hip joints)	0	31.6 (20)	p = 0.02, χ^2= 17.5

Notes: AH — arterial hypertension, CHD — coronary heart disease, FC — functional class, AF — atrial fibrillation, CHF – chronic heart failure, NAFLD — non-alcoholic fatty liver disease

Table 3. *Blood biochemical parameters in patients with a metabolically healthy and metabolically unhealthy obesity phenotype*

Parameter	MHP, n = 45	MUP, n = 63	p-value
ASAT, U/L	26 ± 1.1	27.4 ± 2.1	0.4
ALAT, U/L	32.1 ± 2.8	44.2 ± 4.9	0.8
Total bilirubin, μmol/L	10.6 ± 1.1	12.7 ± 1.4	0.1
Urea, mmol/L	6 ± 0.3	6.6 ± 0.3	0.1
Creatinine, μmol/L	94.4 ± 2.8	96.5 ± 2.6	0.5
Glucose, mmol/L	5.2 ± 0.09	5.8 ± 0.2	0.04

Patients with MUP were characterized by a more severe cardiovascular pathology: AH stage III, presence of CHD and CHF. AH without damage to target organs and associated clinical conditions was more often observed in group 1 (MHP) ($p = 0.002$ $\chi^2 = 7.31$).

The pathology of the gastrointestinal tract (GIT) was primarily characterized by fatty liver and chronic cholecystitis. Patients with MUP more often had non-alcoholic fatty liver disease (NAFLD) of grade 1 compared to the MHP group ($p = 0.04$, $\chi^2 = 9.45$). NAFLD grade 1 is characterized by symptoms of steatohepatosis without signs of inflammation and liver fibrosis, as well as without significant increase in hepatic transaminases (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT)) [8].

A comparative analysis of laboratory biochemical parameters in the two groups of patients is presented in Table 3.

Lipid profile of patients with different phenotypes of obesity is shown in Figure 1.

Compared with the MHP group, patients with MUP demonstrated more unfavorable values of carbohydrate and lipid metabolism, which was expressed in high levels of fasting glycemia ($p = 0.04$) and triglycerides ($p < 0.001$). Patients with MHP had a higher level of high density lipoprotein cholesterol (HDL cholesterol) ($p < 0.001$). There were no statistically significant differences in other biochemical parameters.

Table 4 presents the insulin resistance parameters of the patients.

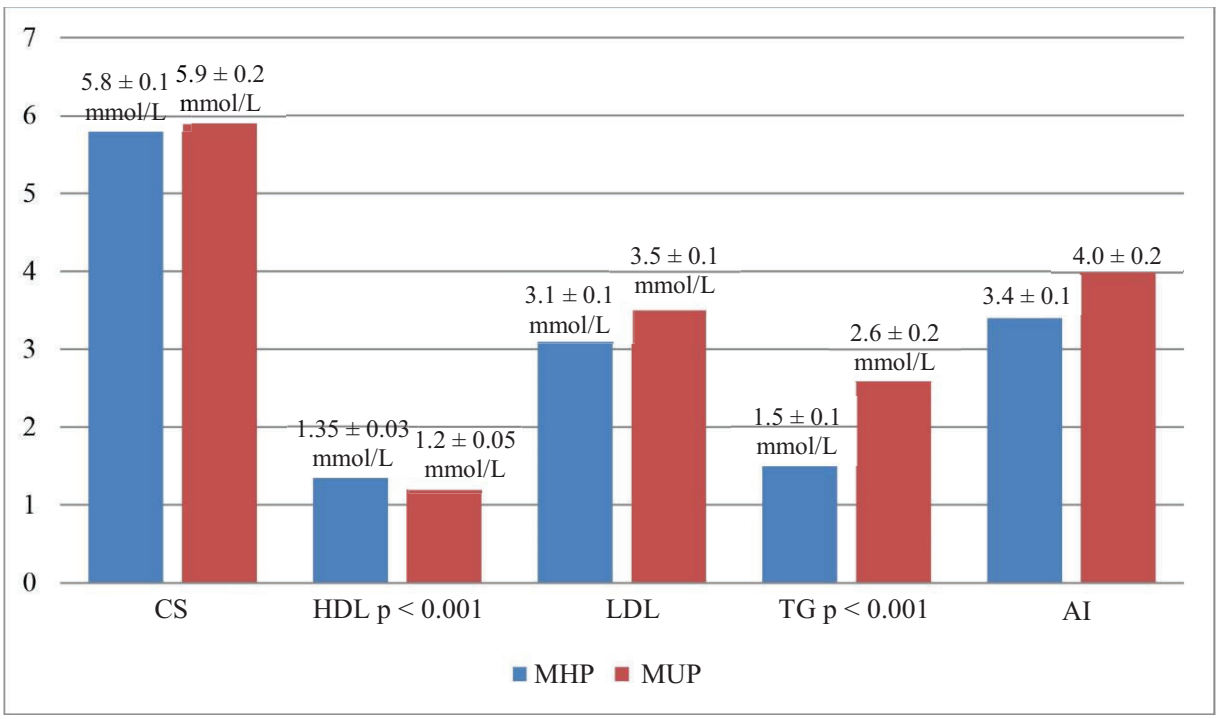

Figure 1. *Lipid profile in patients with different obesity phenotypes*

Note: CS — total cholesterol, HDL — high density lipoprotein cholesterol, LDL — low density lipoprotein cholesterol, TG — triglycerides, AI — atherogenic index

Table 4. *Indicators of insulin resistance in patients with a metabolically healthy and metabolically unhealthy obesity phenotype*

Index	Metabolically healthy phenotype, n=45	Metabolically unhealthy phenotype, n=63	P
Caro	0,85±0,11	0,39±0,05	<0,001
HOMA-IR [11]	2,32±0,26	5,99±0,7	<0,001
TyG	3,87±0,03	4,13±0,04	<0,001

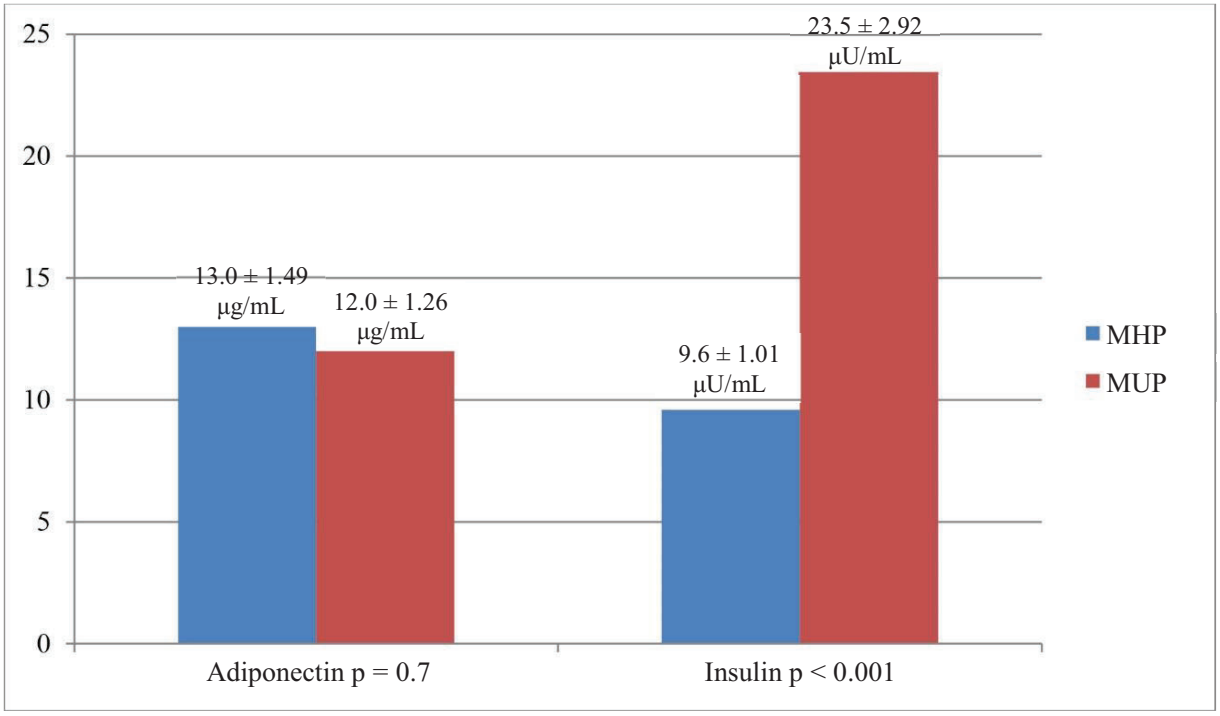

Figure 2. *Hormonal parameters in patients with metabolically healthy (MHP) and metabolically unhealthy obese phenotype (MUP)*

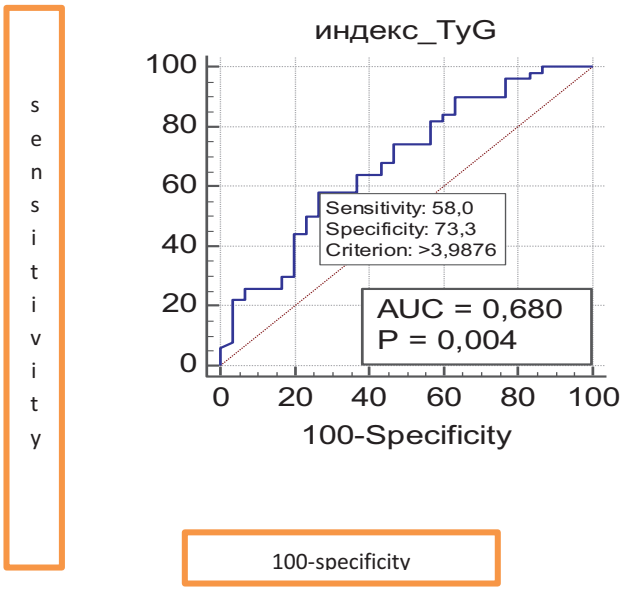

Figure 3. *Determining TyG index for IR diagnosis in men of working age*

Hormonal parameters in patients with different obesity phenotypes are presented in Figure 2.

As can be seen from results, patients with MUP showed more unfavorable values of hormonal profile expressed as hyperinsulinemia with signs of IR ($p < 0.001$). TyG index (logarithmic ratio of fasting plasma triglycerides and glucose) seems to be particularly significant; it allows detecting IR [12] and is considered a predictor of type 2 diabetes mellitus (2 type DM). Calculation of the TyG index can be simple and cost-effective at the primary health care stage since it requires just the determination of blood glucose and triglycerides, without assessing insulinemia. Analysis of the significance of the TyG index for IR diagnosis was performed using ROC analysis (Figure 3).

This analysis showed that a TyG index higher than 3.98 suggests the presence of IR. The predictive

Table 5. *Correlative relationships of adiponectin with anthropometric, metabolic parameters and insulin resistance in patients with a metabolically unhealthy obesity phenotype*

Parameter	Index (M ± m)	Adiponectin (M ± m)	Spearman's coef.	p-value
WC/HC	1.03 ± 0.008	12.2 ± 1.2	–0.3	0.03
VAI	3.12 ± 0.34	12.2 ± 1.2	–0.3	0.01
TG (mmol/L)	2.56 ± 0.25	12.2 ± 1.2	–0.3	0.03
CS (mmol/L)	5.9 ± 0.23	12.2 ± 1.2	–0.3	0.02
LDL (mmol/L)	3.5 ± 0.18	12.2 ± 1.2	–0.3	0.04
AI	4.0 ± 0.24	12.2 ± 1.2	–0.5	<0.001
ALAT (mmol/L)	44.4 ± 4.8	12.2 ± 1.2	–0.3	0.03
TyG	4.12 ± 0.04	12.2 ± 1.2	–0.3	0.01

Note: WC/HC — ratio of waist circumference to hip circumference; VAI — visceral adiposity index, TG — triglycerides; CS — total cholesterol; LDL — low density lipoproteins cholesterol, AI — atherogenic index; ALAT — alanine aminotransferase, TyG — triglycerides/glucose index

sensitivity of the TyG index was 58% (95% CI 43.2–71.8), the specificity of this method was 73.33%, the positive likelihood ratio was 2.17, and the negative likelihood ratio was 0.57.

Today, many authors have demonstrated that the accumulation of adipose tissue around internal organs (visceral OB) plays a key role in the development of different circulatory and metabolic disorders due to the dysregulation of adipocytokine secretion, including adiponectin [13, 14]. Low adiponectin concentrations are associated with IR and 2 type DM [15].

Angioprotective and antiatherogenic properties of adiponectin are known. In patients with severe visceral OB, adiponectin synthesis decreases, causing carbohydrate and lipid metabolism disorders [16]. These relationships were observed in our study (Table 5).

In order to determine the threshold value of VAI that will indicate the presence of IR, ROC analysis was carried out; its results are presented in Figure 4.

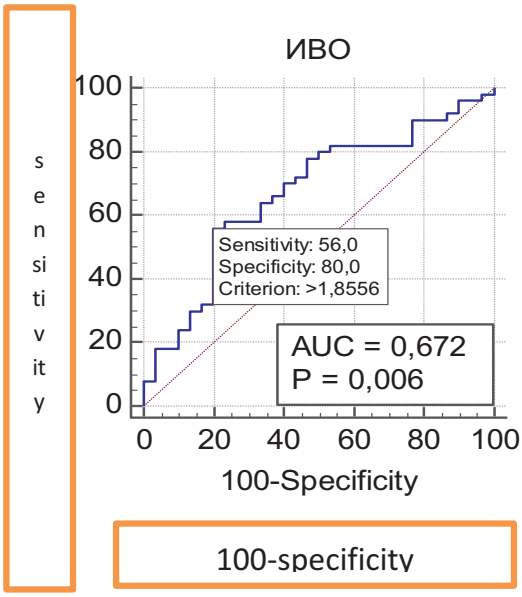

Figure 4. *Determination of VAI for predicting insulin resistance*

It was found that VAI > 1.85 is associated with the presence of IR, while VAI ≤ 1.85 is associated mostly with normal tissue sensitivity to insulin. The prognostic sensitivity of VAI was 56% (95 CI 41.3–70), specificity — 80% (95% CI 61.4–92.3), positive likelihood ratio was 2.8, and negative likelihood ratio was 0.55.

According to clinical recommendations, there is another parameter for the diagnosis of visceral obesity — calculation of the percentage of fat mass [8, 9]. In men, this figure should not exceed 25% [8]. Fat mass of more than 25% is a marker of visceral OB, as it indicates the predominance of visceral fat over subcutaneous fat. However, a moderate increase in this value is possible for MHP obesity parameters [8]. The examined patients with MHP showed an average fat mass of 29.2 ± 0.6%, the group with MUP — 38 ± 0.9%, p < 0.001. To determine the threshold value for fat mass in men of working age and its relationship with the severity of IR, ROC analysis was performed. Results are shown in Figure 5.

It was shown that fat mass of more than 30.1% is associated with IR. The predictive sensitivity of this parameter was 84% (95% CI 64–88.5),

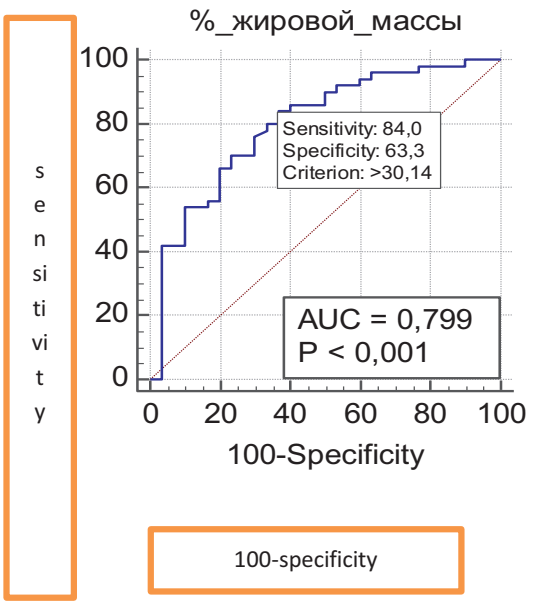

Figure 5. *Determination of the percentage of fat mass in men of working age for detecting insulin resistance*

specificity — 63.3% (95% CI 47.2–82.7), diagnostic efficacy — 73.65%, positive likelihood ratio — 2.34, negative likelihood ratio — 0.33.

This analysis can also be used in real clinical practice: calculation of fat mass percentage requires just the BMI and the age of subject.

The determination of IR factors, therefore, is an important stage in the assessment of the cardiometabolic and vascular risk in obese individuals. The development and use of examination procedures that are available in real practice for this category of patients seem to be the most significant issue. From this perspective, fat tissue percentage and VAI are parameters that are accessible at the primary health care stage and are informative in terms of assessing cardiovascular risks. This corresponds to the data obtained by other authors [17], although so far, there is no consensus on the diagnostic and prognostic significance of VAI [18].

Conclusions

1. Patients of working age with a metabolically unhealthy phenotype (MUP) of obesity are characterized by unfavorable anthropometric parameters and comorbid pathologies, primarily cardiovascular pathologies.
2. The special feature of patients with MUP is the presence of unfavorable hormonal and metabolic parameters and insulin resistance.
3. In terms of cost-effectiveness, TyG index > 3.98; VAI > 1.85 and fat mass > 30.1% are preferable as signs of insulin resistance at the primary health care stage, considering their simplicity, accessibility and no need to conduct additional (hormonal) studies.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication.

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The Influence of Non-Alcoholic Fatty Liver Disease on Indicators of Arterial Stiffness and Risk of Cardiovascular Complications in Patients with Arterial Hypertension

Abstract

Aim. To determine the value of concomitant non-alcoholic fatty liver disease in patients with arterial hypertension in the progression of rigidity of the main arteries and in increase of risk of cardiovascular complications. **Material and methods.** A cross-sectional comparative study was conducted. Group 1 (n=50, 35(70%) women, average age 57,4±6,9 years) included patients with arterial hypertension and non-alcoholic fatty liver disease, group 2 (n=50, 40(80%) women, average age 56,5±7,0 years) included patients with arterial hypertension only. The groups were comparable in the main clinical and demographic indicators (p>0,05). A comparative analysis of pulse wave velocity, central aortic pressure, vascular age and a common 10-year risk of developing cardiovascular complications in both groups was performed. **Results.** As a result of the study, it was found that the metabolic index is significantly higher in patients of the main group compared with patients in the control group (p=0,0489) and there is a statistically larger number of patients with metabolic index>7,0 (58,0% vs 28,0%, p=0,0019). It was also established that systolic (121,9±10,9 mm Hg vs 115,9±8,9 mm Hg) and diastolic (82,5±9,3 mm Hg vs 77,4±8,9 mm Hg) aortic pressure, as well as the augmentation index (26,5±8,5% vs 18,6±4,2%), were significantly higher in patients with arterial hypertension and non-alcoholic fatty liver disease than in patients with isolated arterial hypertension. In the 1st group, a statistically significant increased pulse wave velocity was found both in muscular (12,0±3,1 m/s vs 10,6±1,8 m/s) and elastic (10,4±2,8 m/s vs 9,1±1,7 m/s) vessels, which indicates an increase in arterial stiffness. In addition, there was an increase in post-occlusal pulse wave velocity in this category of patients (11,0±3,3 m/s vs 9,4±1,9 m/s, p=0,0037). A significant increase in vascular age in relation to the passport age (60,4 [56,0:68,0] years vs 58,0 [53,0:60,0] years) and an increase in the 5-year risk of developing cardiovascular complications (2,4 [1,8:4,0] points vs 1,8 [0,8:4,0] points, p=0,0390) were also revealed in patients with arterial hypertension and non-alcoholic fatty liver disease compared to patients with isolated arterial hypertension. In group 1, there were fewer patients with a low risk of CVD (3 (6,0%) vs 10 (20,0%), p = 0,037) and more patients with a high risk of cardiovascular complications (22 (44,0%) vs 12 (24,0%), p = 0,034) than in the group with AH without NAFLD. **Conclusions.** Arterial stiffness was significantly higher in patients with arterial hypertension and non-alcoholic fatty liver disease than in patients with isolated arterial hypertension, which is confirmed by a statistically significant increase in pulse wave velocity and central aortic pressure. Comorbid patients have pronounced endothelial dysfunction, which is confirmed by a significant increase in the post-occlusion rate of the pulse wave. An increase in vascular age in relation

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to the passport age indicates earlier aging of blood vessels in the 1st group compared with the 2nd group. Patients in the main group have a higher incidence of a high 10-year risk of developing cardiovascular events compared with patients in the control group.

Key words: *arterial hypertension, non-alcoholic fatty liver disease, arterial stiffness, central aortic pressure, pulse wave velocity, vascular age, cardiovascular complications*

Conflict of interests

The authors declare no conflict of interests

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AH — arterial hypertension, BMI — body mass index, CAP — central aortic pressure, CVC — cardiovascular complications, DBP — diastolic blood pressure, MI — metabolic index, NAFLD — non-alcoholic fatty liver disease, PWV — pulse wave velocity, SBP — systolic blood pressure

Introduction

According to modern research, non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in Russia and abroad, diagnosed in 20-37% of the population [1, 2]. Many studies have proved that NAFLD is an independent predictor of undesirable cardiovascular events (myocardial infarction, stroke, rhythm disturbances, etc.), which is associated not only with heart remodeling, but also with changes in the architectonics and functional capabilities of the vascular wall [4].

The progression of NAFLD is closely related to the activation of atherogenesis. Against the background of steatosis transformation of the liver, the concentration of pro-atherogenic blood components (pro-inflammatory, prothrombotic and oxidative-stress substances) increases, the degree of atherogenic dyslipidemia and insulin resistance increase [3]. In turn, some authors attribute the development of insulin resistance to increased arterial stiffness [3, 5]. On the example of patients with chronic hyperglycemia and hyperinsulinemia, an increase in the activity of the renin-angiotensin-aldosterone system and the expression of receptors for angiotensin I were demonstrated, which led to hypertrophy of the vascular wall and fibrosis. In these works, to determine vascular stiffness, the researchers used pulse wave velocity (PWV) in the vessels of muscle (PWVm) and elastic type (PWVe), which closely correlated with the risk of cardiovascular events and mortality [3, 5].

At the same time, a change in aortic stiffness directly affects the indicators of central aortic pressure (CAP), which, according to some authors, are more informative than indicators of arterial pressure on the brachial artery [6, 8].

A significant number of papers on the effect of NAFLD on the development of atherosclerosis, the risk of cardiovascular diseases and complications, has been published [1, 5]. But at the same time, we did not find studies in accessible sources that would perform a comparative study of changes in aortic stiffness and blood pressure parameters in patients with arterial hypertension (AH) and NAFLD and patients with isolated AH.

Thus, the aim of our study was to determine the value of concomitant NAFLD in patients with AH in the progression of stiffness of the main arteries and an increased risk of cardiovascular complications (CVC).

Material and methods

At the planning stage of the study, aims and inclusion/exclusion criteria were determined. Results were considered clinically significant when a comparative analysis of groups of 50 patients showed a statistically significant difference in the studied parameters. **Inclusion criteria**: 45-65-year-old

patients of both sexes with AH stage I and II (with or without NAFLD). **Exclusion criteria**: patients with secondary hypertension, other liver diseases (except for NAFLD), diabetes mellitus (type 1 and 2), chronic kidney disease (CKD), obesity class II and III, and oncological or hereditary diseases.

Between September 2019 and March 2020, a cross-sectional comparative study was conducted, which included 100 patients (75 (75,0%) women, average age 57,0±7,0 years). Group 1 (n=50, 35(70%) women, average age 57,4±6,9 years) included patients with arterial hypertension and non-alcoholic fatty liver disease, group 2 (n=50, 40(80%) women, average age 56,5±7,0 years) included patients with arterial hypertension only.

The study was guided by the ethical principles of the Helsinki Declaration of the World Medical Association (2008), the Good Clinical Practice Agreement (ICH GCP). All patients signed informed consent to participate in the study and publish the results. The study was approved by the local ethics committee of Volgograd State Medical University of the Ministry of Health of the Russian Federation (protocol No. 001-2019, expert opinion No. 001/5).

Diagnosis of hypertension and the volume of antihypertensive therapy (Table 1) were carried out in accordance with the clinical recommendations of the Russian Medical Society for Arterial Hypertension [16]. At the initial examination, a history of life and disease, anthropometric data on height and weight were collected with the calculation of body mass index (BMI), waist and hips. "Office" blood pressure indicators and heart rate (HR) were recorded on both hands using OMRON M1 Compact semi-automatic blood pressure monitor (Japan). We recorded the values of «office» blood pressure on both hands and heart rate (HR) according to the standard method. Using the method of analysis of changes in bioelectric impedance (using Omron BF508 52, Japan), an analysis of body composition was carried out with an assessment of the visceral fat percentage.

Based on Russian clinical recommendations for the diagnosis and treatment of NAFLD, the comorbid pathology, NAFLD, was diagnosed in patients with hypertension [12]. For this purpose, an anamnesis of the disease, an ultrasound examination of the liver on a Siemens Sonoline G50 scanner (Germany) were analyzed, with an assessment of the echogenicity of the liver parenchyma, vascular pattern, degree of the echo signal attenuation, as well as a biochemical blood test, with an assessment of the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), -glutamyltransferase (GGT), the level of total bilirubin in blood serum on a Liasys-2 biochemical analyzer (Analyzer Medical System S.r.l, Italy). For dynamic monitoring of the state of lipid metabolism, the levels of total cholesterol and its fractions (LDL cholesterol, HDL cholesterol) and triglycerides (TG) were determined by the enzymatic method using ASSEL kits (Italy) on a Liasys-2 biochemical analyzer (AMS, Italy).

To determine insulin resistance, the metabolic index was calculated:

MI = [TG on an empty stomach (mmol/L) Glucose on an empty stomach (mmol/L)] / LDL cholesterol^2 on an empty stomach (mmol/L).

Based on the obtained indicator, the presence of IR is determined when the MI index is equal to or more than 7.0 [7].

The organization of the study of PWV and CAP was carried out taking into account the recommendations of the Consensus of Russian experts on the assessment of arterial stiffness in clinical practice (2016) [9]. We analyzed the wall stiffness of the main vessels of muscle and elastic types in terms of pulse wave velocity using the PolySpektr 8/E apparatus with a PWV module (Neurosoft, Russia). To determine PWVe, sphygmography was performed on the carotid-femoral segment of the arterial bed, and PWVm — on the carotid-radial. Also, the PWVm/PWVe ration was calculated, and a 3-minute compression test with reactive hyperemia (PWV test) was performed to determine the functional reserve of the muscle segment.

Daily monitoring of CAP indices was carried out using the BPLab multifunctional complex and Vasotens 24 software (Petr Telegin LLC, Russia). The following parameters were determined: average daily, daytime and nighttime values of systolic (SAPao), diastolic (DAPao), pulse aortic pressure (PAPao) and augmentation index (AI). The study included CAP protocols with a validity of more than 70% (at least 20 valid measurements in the daytime and at least 7 in the nighttime).

To calculate vascular age and the 5-year risk of cardiovascular complications, the «ADVANT

AGE» calculator (Les Laboratoires Servier, France) was used, in which, according to the method of D'Agostino R.B. et al. (2008), gender, age, smoking factor, systolic blood pressure, total cholesterol (and its fractions) and glycemia were taken into account [10]. The SCORE score was used to assess total cardiovascular risk and 10-year fatal risk [16].

For statistical processing of the obtained data, we used parametric (Student t-test for unrelated groups, mean value (M) with standard deviation (σ)) and non-parametric criteria (Mann-Whitney U-test, Fisher exact test) calculated using the statistical software package Statistica 10 (StatSoft Inc., USA). All groups of variables were checked for compliance with the law of normal distribution using the Shapiro-Wilk test. When determining the correspondence of the normality of distribution among the variables, the data are presented as M ± SD, where M is the arithmetic mean, SD is the standard deviation; in case of deviation from normality — Me (IQR), where Me is the median, IQR is the interquartile range: 25 percentile — 75 percentile. When comparing the quantitative data, we used the Student t-test for unrelated groups and the Mann-Whitney U test (for a distribution other than normal). To compare groups on a binary (qualitative) basis, a four-field table was analyzed using Fisher's exact test. The difference in group indices of more than 95% (p <0.05) was considered statistically significant.

Results and discussion

A comparison of patients of the first and second groups was made according to the main clinical and demographic indicators (Table 1). It was found that comparison groups by age and gender, quality of therapy and hypertension duration, «office» indicators of central hemodynamics were comparable (p>0,05). Reliably higher subcutaneous and visceral fat indices (p=0,0000), as well as body mass index (p=0,0048) were quite expectedly obtained in patients of the 1st group.

In the main group, there were statistically significantly more patients with class I obesity (74 % versus 6,0% (p=0,0000)), but fewer patients with overweight (20,0% against 72,0% (p=0,0000)), than in the control group. In the 1st and 2nd groups, normal weight-growth indices were determined in 6,0% and 20,0% of patients, respectively.

When assessing the lipid spectrum (Table 2), no significant differences between the groups were established. The metabolic index is significantly higher in patients of the first group compared with patients of the 2nd group (p=0,0489). Also, in the main group there was a statistically greater number of patients with MI>7,0 (58,0 % vs 28,0 %, p=0,0019), which indicates a higher incidence of insulin resistance in this category of patients.

To assess arterial stiffness, PWV was measured. Table 3 shows the obtained PWV indicators in patients of the two groups.

In patients with AH and NAFLD, compared with patients with isolated AH, a significantly higher PWV was found vessels of both muscle type (12.0 ± 3.1 vs 10.6 ± 1.8 m/s, p = 0.0029) and elastic type (10.4 ± 2.8 vs 9.1 ± 1.7 m/s, p = 0.0220). In group 1, there were significantly more patients with exceeding the threshold level (more than 10 m/s) in vessels of elastic type (p = 0.0213) and/or muscle type (p = 0.0428), compared with patients of the 2nd group [9]. In addition, comorbid patients showed signs of endothelial dysfunction, as indicated by significantly higher values of PWV after a compression test (11.0 ± 3.3 vs 9.4 ± 1.9 m/s, p = 0.0037) [15].

In order to draw conclusions about changes in arterial stiffness, it is not enough just the indicators of PWV. For this purpose, many authors recommend to study the indicators of central aortic pressure [9]. A statistically significant increase in daytime, nighttime and daily average SBPao (p = 0.0018, p = 0.0079 and p = 0.0041, respectively), DBPao (p = 0.0053, p = 0.0178 and p = 0.0083, respectively) and AIx (p = 0.0013, p = 0.0022 and p = 0.0002, respectively) was revealed in patients of the main group compared with the control group (Table 4).

Also, in patients with AH and NAFLD vs isolated AH, a comparative analysis of the vascular age and 5-year risk of CVC was performed according to the methods described above. A significant increase in vascular age relative to the passport age was found in patients of the 1st group (60,4 [56,0:68,0] vs 58,0 [53,0: 60,0], p=0,0399). In patients of group 2, there was no significant increase in vascular age relative to the passport age (59,5 [52,0: 66,0] vs 58,0 [50,0: 64,0], p=0,3516).

Table 1. *Clinical and demographic indicators of patients included in the study*

Variable	Group 1 (patients with arterial hypertension and non-alcoholic fatty liver disease)(n=50)	Group 2 (patients with arterial hypertension without non-alcoholic fatty liver disease)(n=50)	p
Age:			
Average age, years, M±SD	57,4±6,9	56,5±7,0	0,5597
45-55 years, n (%)	19 (38,0)	22 (44,0)	0,6845
56-65 years, n (%)	31 (62,0)	28 (56,0)	
Gender:			
Women, n (%)	35 (70,0)	40 (80,0)	0,3558
Men, n (%)	15 (30,0)	10 (20,0)	
Smoking, n (%)	12 (24,0)	14 (28,0)	0,8200
BMI, kg/m^2, Me (IQR)	31,6 [30,0:33,6]	27,5 [25,0:29,1]	0,0000*
Subcutaneous fat, %, Me(IQR)	42,4 [30,2:46,9]	33,0 [21,4:41,6]	0,0000*
Visceral fat, %, Me (IQR)	12,0 [11,0:15,0]	9,0 [6,0:10,0]	0,0000*
AH duration, years, M±SD	9,1±3,5	8,7±3,2	0,2759
AH stage I, n (%)	8 (16,0)	15 (30,0)	0,0765
AH stage II, n (%)	42 (84,0)	35 (70,0)	0,0630
AH level 1, n (%)	18 (36,0)	21 (42,0)	0,3410
AH level 2, n (%)	32 (64,0)	29 (58,0)	0,3410
Total cardiovascular risk, n (%):			
Low	3(6,0)	4(8,0)	0,5000
Medium	2 (4,0)	17 (34,0)	0,0001*
High	45 (90,0)	29 (58,0)	0,0002*
Target blood pressure, n (%)	36 (72,0)	31 (62,0)	0,1976
Total risk of death from cardiovascular disease in the next 10 years , n (%):			
Low	6 (12,0)	10 (20,0)	0,2070
Medium	31 (62,0)	35 (70,0)	0,2634
High	13 (26,0)	5 (10,0)	0,0332*
10 year fatal risk, %, Me (IQR)	2,15(1,42-4,63)	1,05(0,52-2,82)	0,0043*
AH therapy, n (%)	39 (78,0)	41 (82,0)	0,8031
ACEI, n (%)	21 (42,0)	18 (36,0)	0,6820
BB, n (%)	15 (30,0)	17 (34,0)	0,8305
Diuretic, n (%)	5 (10,0)	8 (16,0)	0,5536
CCB, n (%)	18 (36,0)	23 (46,0)	0,4162
ARB, n (%)	6 (12,0)	4 (8,0)	0,7407
Concomitant pathology, n (%):			
Cholelithiasis	10 (20,0)	4 (8,0)	0,2623
Obesity I degree	37 (74,0)	3 (6,0)	0,0000*
SBP, mm Hg, M±SD	137,9±8,7	136,2±13,4	0,4362
DBP, mm Hg, M±SD	90,2±6,3	87,6±7,2	0,0532
PBP, mm Hg, M±SD	48,0±8,2	48,6±7,6	0,7146
HR, min^{-1}, M±SD	73,1±8,0	71,3±8,9	0,2984

Note: * — p<0,05; BMI — body mass index; ACEI — angiotensin-converting enzyme inhibitor; BB — β-blocker; CCB — calcium channel blocker; ARB — angiotensin II receptor blocker; SBP — systolic blood pressure; DBP — diastolic blood pressure; PBP — pulse blood pressure; HR — heart rate

Table 2. *Indicators of lipid, carbohydrate metabolism and metabolic index*

Index		Main group (n=50) M±σ	Control group (n=50) M±σ	ρ
Lipid spectrum	TC, mmol /L	5,4±1,3	5,6±1,1	0,4506
	HDL cholesterol, mmol /L	1,4±0,3	1,5±0,4	0,3050
	LDL cholesterol, mmol /L	3,1±1,2	3,1±1,1	0,7495
	Triglycerides, mmol/L	2,2±0,6	2,1±0,7	0,4409
	Atherogenic index	3,0±1,1	2,9±1,1	0,9040
MI		7,2±4,4	6,4±4,6	0,0489*
MI >7,0 n(%)		29(58,0)	14(28,0)	0,0019*
Glucose, mmol /L		5,7+1,1	5,3+0,8	0,1233

Note: * — ρ<0,05; TC — total cholesterol; HDL-C — high density lipoprotein cholesterol; LDL-C — low density lipoprotein cholesterol; MI — metabolic index

Table 3. *Indicators of the speed of the pulse wave in patients of comparison groups*

Index	Group 1 (n=50) M±σ	Group 2 (n=50) M±σ	ρ
PWVe, m/s	10,4±2,8	9,1±1,7	0,0220*
PWVm, m/s	12,0±3,1	10,6±1,8	0,0029*
PWV sample, m/s	11,0±3,3	9,4±1,9	0,0037*
PWVm / PWVe	1,2±0,4	1,1±0,2	0,6137
PWV more than 10 m/s:			
PWVe, n (%)	24 (48,0)	12 (24,0)	0,0213*
PWVm, n (%)	34 (68,0)	23 (46,0)	0,0428*

Note: * — ρ<0,05; PWV — pulse wave velocity (m — muscle type arteries, e — elastic type arteries, sample — muscle type arteries after compression test)

Table 4. *Indicators of central aortic pressure in patients of comparison groups*

Index	Main group(n=50) M±σ	Control group(n=50) M±σ	ρ*
Average daily:			
SBPao (mm Hg)	120,9±10,9	114,9±8,9	0,0041*
DBPao (mm Hg)	81,5±9,3	76,4±8,9	0,0083*
PBPao (mm Hg)	39,4±5,8	38,6±4,3	0,4401
AIx (%)	25,5±8,5	19,6±4,2	0,0002*
Daytime:			
SBPao (mm Hg)	123,5±11,0	117,0±9,0	0,0018*
DBPao (mm Hg)	83,7±9,5	78,4±8,8	0,0053*
PBPao (mm Hg)	39,8±6,4	38,6±4,2	0,2715
AIx (%)	23,4±9,3	18,2±9,4	0,0013*
Nighttime:			
SBPao (mm Hg)	114,0±12,5	107,5±10,4	0,0079*
DBPao (mm Hg)	73,6±9,4	68,8±9,5	0,0178*
PBPao (mm Hg)	40,5±7,2	38,8±5,7	0,2120
AIx (%)	27,3±10,1	23,5±9,4	0,0022*

Note: * — ρ<0,05; SBPao — systolic aortic pressure; DBPao — diastolic aortic pressure; PBPao — pulse aortic pressure; AIx — augmentation index

As shown in table 1, patients with hypertension and NAFLD, compared with patients with isolated hypertension, a statistically significant increase in 10-year fatal risk was found (2.15 [1.42: 4.63] and 1.05 [0.52: 2.82] %, p = 0.0043). In addition, in the 1st group, slightly fewer patients were observed with low (6 (12.0%) vs 10 (20.0%), p = 0.2070) and moderate (31 (62.0%) vs 35 (70.0%), p = 0.2634), but significantly more patients with high (13 (26.0%) vs 5 (10.0%), p = 0.0332) overall cardiovascular risk than in 2nd group.

Mitchell GF, et al. (2016) in their publication noted an increase in the stiffness of the main arteries in patients with NAFLD, based on changes in the parameters of CAP and PWV. Changes in vascular stiffness in patients of this category were associated primarily with an increase in the level of atherogenic agents, activation of the renin-angiotensin-aldosterone system, and dishormonal disorders. In our study, no significant difference in lipid metabolism were established between groups. It was found that patients of the main group had a statistically more significant increase in the metabolic index and a greater frequency of insulin resistance compared with patients in the control group. Our study found that comorbid patients have a statistically significant increase in stiffness of the main arteries, both in the vessels of the muscular and elastic types. In the main group, a significantly larger number of patients was noted with PWV values exceeding the threshold level of 10 m/s, which indicates a high risk of developing cardiovascular complications in this category of patients. Chou C. Y., et al. (2015) note that these changes are present in patients without AH, regardless of the presence or absence of clinical signs of metabolic syndrome and/or diabetes mellitus. In addition, in patients of the first group, daytime, nighttime, and daily average systolic aortic pressure, diastolic aortic pressure, and the augmentation index were significantly increased, which also indicates an increase in the rigidity of the main arteries in this category of patients.

Also, in patients with AH and NAFLD, in comparison with patients with isolated AH, a more pronounced violation of endothelial function is noted, which is confirmed by an increase in post-occlusal PWV. Gurfinkel, Yu.I. et al. (2009) indicate that endothelial cells are highly sensitive to the speed of blood flow and an increase in blood flow causes deformation of endothelial cells, which increases the production of NO.

A significant increase in the indicators of vascular age relative to the passport age in comorbid patients is noted, which is associated with a greater severity of atherosclerosis and arteriosclerosis in this category of patients. Protasov K.V. et al. (2011) indicate that this indicator can be used to assess the risk of development and timely diagnosis of complications of arterial hypertension. In addition, the researchers found that the vascular age, compared with the passport age, is more closely correlated with damage to the heart and blood vessels. A significantly higher 5-year risk of developing cardiovascular complications confirms the greater severity of the condition of patients with hypertension and NAFLD compared with patients with isolated hypertension.

Conclusions

1. Arterial stiffness was significantly higher in patients with AH and NAFLD than in patients with isolated AH, which is confirmed by a statistically significant increase in the parameters of PWV and central aortic pressure.
2. Comorbid patients have signs of endothelial dysfunction, which is confirmed by a statistically significant increase in post-occlusion PWV.
3. An increase in vascular age in relation to the passport age indicates earlier vascular aging in the main group compared with the control group.
4. Patients with hypertension and NAFLD have a higher incidence of a high 10-year risk of developing cardiovascular events compared with patients with isolated hypertension.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

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The Frequency of Hepatitis Virus Infection Markers Among Highly Qualified Sportsmen

Abstract

Study Objective is to evaluate prevalence of hepatitis A, B, C, E, and TT virus infection markers in highly qualified sportsmen. **Study Design:** multicenter open single-site clinical study. **Materials and Methods:** 100 blood serum samples of sportsmen (game, complex coordination, technical, etc.) were studied. Biological material (blood serum) was obtained from 54 men and 46 women aged 16 to 45 years during an in-depth medical examination. All sportsmen filled out a questionnaire, including demographic data, description of the sport, information about infection risk factors, information about the presence of acute viral hepatitis and vaccination. Anti-HAV IgG, HBsAg, anti-HBcore, anti-HCV, anti-HEV IgG, anti-HEV IgM were determined in the blood serum by enzyme immunoassay; using polymerase chain reaction — DNA Anelloviridae (TTV, TTMDV, TTMV). **Study Results:** Anti-HAV IgG was detected in 57/66 (86,4%) sportsmen, women (91,2%) predominated, among them a third were engaged in synchronized swimming. 7/57 (12,3%) of the examined had indications of vaccination against hepatitis A. The frequency of anti-HEV IgG did not exceed 3% (2/66). anti-HEV IgM were not detected in any case. Also, none of the examined sportsmen in the blood serum was not determined HBsAg. However, anti-HBcore (marker of latent HBV infection) was detected in 13% (13/100) of the samples. The detection rate of anti-HCV was low, combined with the presence of anti-HBcore was 2% (2/100). In addition, DNA TTV, TTMDV and TTMV, respectively, were found in serum samples from 66/100 (86%), 79/100 (79%), 71/100 of sportsmen. **Conclusion:** The high frequency of hepatitis virus markers was found (HAV — 74,1%, TTV/TTMDV /TTMV — 71-86%), HBV — 13%, HEV — 3%, HCV — 2%). All patients denied a history of acute viral hepatitis. Vaccination against hepatitis A and B is a modern strategy that prevents infection and the development of acute viral hepatitis. Its mandatory holding should become part of the targeted preparation of sportsmen to achieve the highest sports results.

Key word: *highly qualified sportsmen, markers of hepatitis viruses A, B, C, E, TT*

Conflict of interests

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AHB — acute hepatitis B, AHC — acute hepatitis C, anti-HAV IgG — antibodies to hepatitis A virus, class G immunoglobulins, anti-HBcore IgG/IgM — antibodies to hepatitis B virus capsid antigen, class G/M immunoglobulins, anti-HCV — antibodies to hepatitis C virus, anti-HEV IgG/IgM — antibodies to hepatitis E virus, class G/M immunoglobulins, HA — hepatitis A, HB — hepatitis B, bp — base pair, CHB — chronic hepatitis B, CHC — chronic hepatitis C, DME — detailed medical examinations, FMBA — Federal Medical and Biological Agency, HBsAg — hepatitis B surface antigen, HBV DNA — hepatitis B deoxyribonucleic acid, HC — hepatitis C, HCV RNA — hepatitis C ribonucleic acid, HE — hepatitis E, PCR — polymerase chain reaction, TTMV — Torque teno mini virus, TTMDV — Torque teno midi virus, TTV — Torque teno virus

Introduction

Physical training is a means of preventing hypodynamia and promoting health. It has a many-sided effect on the human body and increases the body's resistance to adverse ambient effects. Sport is one part of physical training, and high-performance sport is an activity aimed at achieving high results. It requires the mobilization of the emotional state and all functional capabilities of the body.

Achieving high sports results is based on the research and methodological support of athletes' training system during different periods of the training and competitive process. Biomedical support is the study of the competitive activity of athletes, as well as comprehensive, routine and detailed medical examinations (DME) that are carried out according to custom-tailored programs developed at the medical institutions of the Federal Biomedical Agency (FMBA) of Russia [1].

Medical monitoring is performed to assess the health, biological age, physical fitness, level of training, and special abilities of the athlete, and identify overstrain. It includes morphophysiological, ergometric, biochemical, psychophysiological, and sports and pedagogical study methods [2].

The hepatobiliary system is one of the most critical systems that provide an adequate response of the body to intense physical activity. Maladaptation of the hepatobiliary system results in different types of pain in the right hypochondrium at rest and during physical exertion. It is also often accompanied by dyspeptic symptoms (bitter or metallic taste in the mouth, heartburn, intolerance to fatty and fried foods). On palpation, there is tenderness in the liver and gall bladder area; the liver may be enlarged. Signs of liver maladaptation and overstrain include increased activity of aminotransferases (1.5–2 times higher than the upper limit of normal).

Clinical and laboratory methods, ultrasound of the abdomen, test breakfasts, and test loads are used to identify hepatobiliary disorders.

In the last few years, the number of athletes with hepatobiliary diseases has risen due to increased physical activity, nutrition problems, uncontrolled use of pharmacological agents, etc.

However, among etiological factors leading to hepatic diseases, hepatitis viruses remain the most significant. According to the State Report "On the sanitary and epidemiological well-being of the population in the Russian Federation in 2018", the incidence of acute hepatitis B (AHB) per 100 thousand people in 2018 was 0.67; that of chronic hepatitis B (CHB) — 9.27; acute hepatitis C (AHC) — 1.1; chronic hepatitis C (CHC) — 32.72; hepatitis A (HA) — 2.84; hepatitis E (HE) — 0.11 [3]. Infections caused by the hepatitis delta virus and Torque teno virus (TTV) are not officially recorded in our country.

Viral hepatitis can be asymptomatic for a long time and, therefore, can escape detection and control. According to epidemiologists, the prevalence of viral hepatitis is associated with the deterioration of living conditions, which contributes to infection routes.

It should be noted that the epidemic process of viral hepatitis depends on the level of herd immunity, and its main characteristic is the prevalence of infection markers in the population. Therefore, only the screening of representative populations yields objective data on the viral load in the population. The optimal strategy for this kind of assays is the detection of markers in blood serum [4].

The objective of our study was to assess the detection rate of serological markers of hepatitis A (HAV), B (HBV), C (HCV), E (HEV), TT in professional athletes.

Materials and Methods

A multicenter, open, cross-sectional clinical trial was conducted at Clinic No. 5 of the Federal Clinical Hospital No. 85 of the Federal Medical and Biological Agency (Federal State Budgetary Healthcare Institution Clinical Hospital No. 85 of the Federal Medical and Biological Agency, Center for Diagnosis and Management of Chronic Viral Hepatitis) and the Federal Clinical Center for Sports Medicine and Rehabilitation of the Federal Medical and Biological Agency (Federal State Budgetary Institution Federal Clinical Center for Sports Medicine and Rehabilitation of the Federal Medical and Biological Agency of Russia). The clinical trial protocol (No. 157 of September 19, 2018) was approved by the Local Ethics Committee of Clinical Hospital No. 85 of the Federal Medical and Biological Agency of Russia.

During DME, blood samples were taken from 340 professional athletes aged 16 to 45 years. All individuals enrolled in this study signed informed consent for blood sampling, test for hepatitis virus markers, and the publishing of the results.

The athletes also filled a questionnaire drawn up by the researchers.

Blood samples were transported to the Chumakov Federal Scientific Center for Research and Development of Immunobiological Products of the RAS (Federal State Budgetary Institution Chumakov Federal Scientific Center for Research and Development of Immunobiological Products of the Russian Academy of Sciences) where hepatitis virus markers were determined in the Laboratory for the Simulation of Immunobiological Processes with experimental models on marmoset monkeys.

Serological markers of HAV, HEV, HBV and HCV (anti-HAV Ig, anti-HEV IgG/IgM, HBsAg, anti-HBcore, anti-HCV) were detected by enzyme immunoassay using the following test systems in accordance with manufacturer instructions: Monolisa Total Anti-HAV (Bio-Rad, France); DS-EIA-ANTI-HEV-G; DS-EIA-ANTI-HEV-M; DS-EIA-HBsAg-0.01; DS-EIA-HBsAg-0.01-confirming; DS-EIA-HBs; DS-EIA-HCV, DS-EIA-ANTI-HCV-SPECTR-GM (Diagnostic Systems Research and Manufacturing Association, Nizhny Novgorod). Sixty-six out of 100 athletes were tested for anti-HAV Ig and anti-HEV IgG/IgM.

All blood serum samples were tested for deoxyribonucleic acid (DNA) of the viruses of the Anneloviridae family using polymerase chain reaction (PCR). Nucleic acids were isolated from serum samples using "Kit for the isolation of deoxyribonucleic acid / ribonucleic acid (DNA/RNA) from serum or plasma using MP@SiO_2 magnetic particles" — the reagent kit manufactured by ZAO Sileks, according to the manufacturer's instructions.

Blood serum samples were analyzed using PCR with nested primers proposed by M. Ninomiya et al. [5] to detect DNA of the viruses of the Anneloviridae family. This allowed differentiating TTV, Torque teno midi virus (TTMDV) and Torque teno mini virus (TTMV) based on the size of the amplified fragment. The amplification products had the following sizes: 112–117 nt for TTV, 88 nt for TTMDV, and 70–72 nt for TTMV. The obtained product with 207 base pairs (bp) was determined by electrophoresis in 2% agarose gel in TBE buffer (Tris-borate-EDTA).

Questionnaire of a highly qualified athlete

Full name	
Age	
Gender	Male / Female
Birthplace	
Type of sport	
Sports qualification	
A history of acute viral hepatitis	A, B, C, E (when?)
HAV vaccination	yes, no (when?)
HBV vaccination	yes, no (when?)
Surgery	yes, no (when?)
Blood transfusions	yes, no (when?)
Dental care	yes, no (when?)
Tattoos, piercings	yes, no (when?)
Acupuncture	yes, no (when?)
Departure to foreign countries (indicate which)	
Contact with patients with viral hepatitis	
Blood sampling date	

Statistical data analysis was performed using EXCEL 2010 and GraphPad Prism 4 statistical data processing software. Data are presented as M ± SD, where M is the arithmetic mean, and SD is the standard deviation.

Results and Discussion

This paper analyzes preliminary findings. The study sample of athletes whose blood samples were included in the study (100 out of 340) was formed by random sampling. The athletes were involved in various sports (team, technical, with precise coordinated movements, etc.) (Table 1).

The questionnaire survey enabled to obtain a sociodemographic description of athletes and data on risk factors for hepatitis viruses, as well as information on the history of acute viral hepatitis (A, B, C, E) and vaccination against hepatitis A (HA) and hepatitis B (HB). Among the respondents, 35% (35/100) of athletes were Candidates for Master of Sports, 39% (39/100) — Masters of Sports, 14% (14/100%) — Masters of Sports of International Class (according to Russian classification). Their athletic activities averaged 11 ± 1.55 years.

Table 1. *Characteristics of athletes by gender and type of sport*

Type of sport Sport	Male	Female
Ping-pong	1	3
Universal fight	5	4
Tennis	1	1
Golf	-	6
Athletics	1	-
Swimming	1	-
Ski jumping	2	-
Pentathlon	-	3
Synchronized swimming	-	12
Ski cross	1	1
Slopestyle	1	-
Snowboard	1	-
Gymnastics	14	6
Triathlon	-	1
Fencing	1	-
Freestyle	9	1
Hockey	-	2
Field hockey	16	6
Total	**54**	**46**

Risk Factors for Viral Hepatitis

Viral hepatitis is a polyethiological group of diseases. Therefore, the epidemiological role of the source of infection, as well as pathogen transmission mechanisms, are very different and are determined by social, natural, and biological factors.

Results of the analysis of personal data enabled to identify the following main risk factors: surgical operations, tattooing, dental treatment, acupuncture, and injuries (Fig. 1).

The prevailing risk factors for viral hepatitis, both in men and women, were traumatic injuries and dental treatment. In addition, athletes from the analyzed sample visited most countries of Western Europe, the Baltic States, North America, Egypt and China. Therefore, possible infection with HAV and HEV cannot be excluded. All respondents said they had not undergone blood transfusion and had no history of acute viral hepatitis and contacts with infected patients.

Serological Markers of HAV

The problem of HA as a socially significant infection remains urgent for health care in our country. The socio-economic status of the region is essential in understanding the epidemic process of HA. The most vulnerable part of the population includes people living in rural areas and using water from unprotected sources. At the same time, urban residents who use water from centralized water pipelines are no exception since the lack of proper sanitary and epidemiological control over the state of the sewage system can result in an outbreak of HA. Vaccination remains the most effective preventative measure against HA [3].

In 57/66 (86.4%) athletes, IgG antibodies to hepatitis A virus (anti-HAV IgG) were found; predominantly in women — 31/34 (91.2%); one third of whom were engaged in synchronized swimming. Among male athletes, the rate of detection of anti-HAV IgG was 81.3% (26/32). According to questionnaire data, 7 (12.3%) athletes who tested positive for anti-HAV IgG were vaccinated against HA. Anti-HAV IgG were primarily found

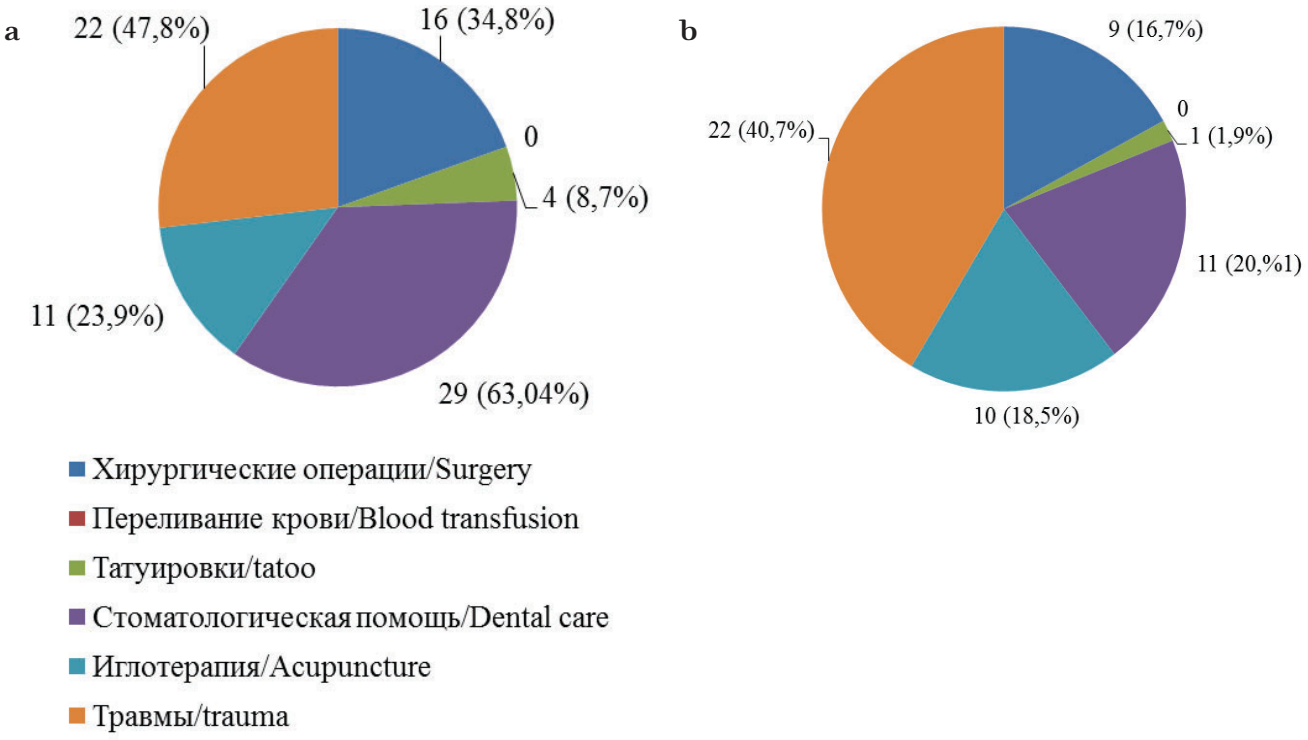

Figure 1. *Risk factors for infection with hepatitis viruses among athletes – women (a) and men (b)*

in the serum of athletes engaged in synchronized swimming and field hockey (average age 16.3 ± 3.8 years).

Relatively high detection rates of anti-HAV IgG 57/66 (86.4%) indicate persistent intensive circulation of HAV and, consequently, a high risk of infection in the population. Information obtained on the immunological structure of HA indicates the need to vaccinate of athletes. This will contribute to the formation of a group immune to HAV, thereby reducing HA prevalence among athletes.

Serological Markers of HEV

The epidemiology of hepatitis E (HE) has undergone several significant changes in recent decades: revealing areas where this infection is endemic (Germany, France, the United States of America, Canada) and registration of local cases there; confirmation of the anthropozoonotic nature of HE; high mortality among pregnant women in the third trimester; chronic forms with prolonged persistence of the virus (more than 6 months) in immunosuppressive individuals [6, 7]. The Russian Federation (RF) has both sporadic (indigenous, local) and outbreak cases. In Russia, the probability of importation of HE is determined by the high level of migration processes, a large influx of tourists from hyperendemic regions with a tropical and subtropical climate [8]. The Russian Federation has kept an official record of HE since 2013.

In our study, the rate of detection IgG antibodies to hepatitis E virus (anti-HEV IgG) in the examined athletes was 3% (2/66) — a man, 19 years old, engaged in freestyle skiing, and a woman, 21 years old, who played golf.

To confirm the latent form of HE, 66 out of 100 athletes were tested for the marker of current infection — IgM antibodies to hepatitis E virus (anti-HEV IgM); all tests were negative.

Due to the lack of vaccination against HE, it is recommended to raise the level of medical and hygienic knowledge and awareness of intestinal infections (including HE) among athletes traveling outside the Russian Federation for training and competitions, especially in countries with a tropical and subtropical climate.

Serological Markers of HBV

No hepatitis B virus surface antigen (HBsAg) was found in any of the 100 blood samples of the examined athletes. The detection rate of IgG antibodies to the HB-core hepatitis B virus antigen (anti-HBcore) in the general group was 13% (13/100) and was slightly higher in women (8/46 (17.4%) and 5/54 (9.1%), respectively). This observation suggests that female athletes have a higher risk of HBV. However, the final conclusion can be made after analyzing the whole group (n = 340).

When analyzing personal data, a group of individuals with a positive history was identified. The following risk factors prevailed in athletes with anti-HBcore: dental treatment — in 5 (38.4%), injuries — in 5 (38.4%), surgical operations — in 4 (30.8%), acupuncture — in 2 (15.4%), tattooing — in 2 (15.4%). There were no differences in risk factors between men and women. The average age of athletes with anti-HBcore was 16.1 ± 2.5 years.

Anti-HBcore was found in blood samples from four athletes engaged in gymnastics, three engaged in freestyle, two — in pentathlon, and one case each among athletes engaged in golf, fencing, field hockey and synchronized swimming.

During DME, none of the examined athletes showed abnormal blood biochemistry.

Only 19 (19%) athletes enrolled in this study were vaccinated (three doses) against HB. None of them had markers of HBV infection (HBsAg, anti-HBcore). The protective level of total antibodies to the HBV surface antigen (anti-HBs) was not assessed at this stage of the study. Therefore, the frequency of vaccination of athletes against HB cannot be assessed too.

The persistence of only anti-HBcore was previously considered as evidence of prior infection with the elimination of virus and disease remission [2]. However, serum HBV DNA levels in anti-HBcore positive patients were usually minimal or found in liver tissue only. Today, antibodies to HBV capsid protein (anti-HBcore) with no HBsAg are considered as a surrogate marker for latent HBV infection. However, no detected HBV DNA in serum cannot exclude latent infection. It should be noted that the diagnostic search for hepatitis etiology limited by the study of HBsAg is ineffective. Therefore, patients with anti-HBcore fall in the group of patients with hepatitis of unknown etiology [9]. Also, such patients are most likely to transmit this infection to others.

The final conclusion on the status of athletes will be made after more complete tests for serological markers of HBV (anti-HBs, anti-HBe), re-study of HBsAg, HBV DNA and fibroelastometry in order to identify and/or assess the stage of liver fibrosis.

Serological Markers of HCV

Hepatitis C (HC) is one of the most important medical and social health problems in many countries, including the Russian Federation. This is a result of significant socio-economic damage, ubiquitousness, severity and active involvement of individuals of reproductive and working age in the epidemic process [10].

According to estimates made by the World Health Organization, there are 71 million people infected with HCV in the world [11]. However, the registered incidence rates for acute and chronic HC do not fully reflect the HCV load on the population. HC may be asymptomatic for decades.

According to our study, only 2% (2/100) of athletes had total antibodies to hepatitis C virus (anti-HCV), which is not higher than the conditional average detection rate (3.5%) of these antibodies in Eastern Europe [11].

Anti-HCV antibodies were found in combination with anti-HBcore in two athletes. In one case, a female athlete, aged 16, was engaged in synchronized swimming, and in another case, — a man, aged 19, was engaged in freestyle. Biochemical parameters of the functional state of the liver (in particular, aminotransferases, bilirubin) did not exceed the upper limit of normal. Ultrasound examination of abdominal organs revealed no pathology.

A test for replication markers, HBV DNA and HCV RNA, was scheduled to exclude latent viral infection.

HBV co-infection with different hepatotropic and non-hepatotropic viruses may be the most significant cause of latent forms of chronic hepatitis [9].

Serological Markers of TTV, TTMDV, TTMV

From 1997 and over the following decade, Japanese virologists (H. Okamoto, T. Nishizawa, M. Ninomiya et al.) discovered viruses whose main characteristic is a gene with a ring structure of a single-stranded DNA molecule [5, 12]. It was only in 2009 that these viruses were registered as a new family of Anelloviridae. Even then, they were known to be extremely prevalent (nearly 100%), not only in humans but also in chimpanzees and African monkeys. This prevalence of Anelloviridae is due to the properties of both parenterally transmitted and enteric viruses. Their infection is thought to be asymptomatic. These viruses are represented by many genera and genotypes (in particular, TTV (genus *Alphatorquevirus*) — 29 genotypes, TTMV (genus *Betatorquevirus*) — 12, TTMDV (genus *Gammatorquevirus*) — 15) [13]. According to taxonomy, several viruses can coexist in a human body. They can cause damage to different organs and systems, but not all of them are related to liver pathology.

Over the past ten years, researchers have not only established the extremely high prevalence of these viruses in many countries but have also confirmed the hepatotropic and hepatopathogenic properties of their several genotypes [14–16].

The nature of chronic liver pathology caused by the viruses of this group is described; electron microscopic images of TTV, TTMDV, TTMV were obtained [16].

However, some researchers are still convinced of the absence of the pathogenicity of the *Anelloviridae* family for humans, believing that their existence in the human body is the result of a long (centuries-old) period of virus and host co-evolution.

According to this study, TTV was found in 86% (86/100) of athletes, TTMDV — in 79% (79/100), and TTMV — in 71% (71/100) (Table 2). The combination of TTV + TTMDV + TTMV was also found in 62% (62/100) of athletes. No abnormal blood biochemistry parameters were revealed.

Analysis of the personal data of athletes in whose serum samples anti-HBcore, anti-HCV and TTV/TTMD/TTMV were detected showed that they were not previously aware of their positive status.

Conclusion

This article presents preliminary results of a study of 100 serum samples obtained during DME from 340 professional athletes engaged in different

Table 2. *Hepatitis Virus Infection Markers among athletes*

Infection markers	Male, n (%)	Female, n (%)
anti-HAV IgG	26/32 (81,35%)	31/34 (91,2%)
Of them:		
HAV vaccinated	3/32 (8,6%)	4/34 (11,8%)
anti-HEV IgG	1/32 (3,1%)	1/34 (2,9%)
anti-HBcore IgG	5/54 (9,2%)	8/46 (17,4%)
Of them:	4/54 (7,4%)	7/8 (87,5%)
anti -HBcore IgG + TTV	1/54 (1,8%)	
anti -HBcore IgG + anti -HCV	+TTV + TTMDV (%)	1/8 (12,5%)
mono TTV	1/54 (1,8%)	3/46 (6,5%)
mono TTMDV	1/54 (1,8%)	1/46 (2,2%)
mono TTMV	2/54 (3,7%)	1/46 (2,2%)
TTV+ TTMDV	2/54 (3,7%)	6/46 (13,0%)
TTMDV + TTMV	4/54 (7,4%)	2/46 (4,3%)
TTV + TTMDV + TTMV	37/54 (6,9%)	25/54 (4,6%)

Note: HA — hepatitis A, anti-HAV IgG — antibodies to hepatitis A virus of class G immunoglobulins, anti-HBcore IgG — antibodies to capsid antigen of hepatitis B virus of class G immunoglobulins, anti-HCV — antibodies to hepatitis C virus, anti-HEV IgG — antibodies to hepatitis E virus of class G immunoglobulins, TTMDV — Torque teno midi virus, TTMV — Torque teno mini virus, TTV — Torque teno virus

sports. A high frequency of detection of hepatitis virus markers was established: HAV in 86.4%, TTV/TTMD/TTMV in 71–86%, HBV in 13%, HEV in 3%, HCV in 2%. Only 7 (12.3%) of 57 athletes were vaccinated against HA.

However, given the positive serological markers and risk factors for infection with hepatitis viruses specified in the questionnaires (dental treatment, surgical operations, injuries, tattoos), there were no reasons for diagnosing previous acute viral hepatitis and information on contacts with patients with hepatotropic infections.

During DME, none of the examined athletes showed abnormal blood biochemistry. However, considering the possible development of latent viral infections in the presence of anti-HBcore and anti-HCV, a molecular genetic test for HBV and HCV replication markers — HBV DNA and hepatitis C ribonucleic acid (HCV RNA) — should be added to the DME protocol.

Due to the high prevalence of viral hepatitis, tests for HBsAg, anti-HBcore, and anti-HCV, as well as information on vaccination, are required when taking children to sports clubs and schools.

Barring athletes from training and competing because of infection with hepatitis viruses is a very difficult decision that often triggers a backlash from the interested federation, relatives and people who have invested enormous moral and material resources in training the athlete.

Vaccine prophylaxis against hepatitis A and hepatitis B is a part of the modern-day strategy for preventing infection and the development of acute viral hepatitis. Its implementation should become a part of the targeted training of athletes to achieve the highest levels in sports.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Ilchenko L.Yu. (ORCID ID: https://orcid.org/0000-0001-6029-1864): development of research design, analysis of the obtained data, writing and editing text

Morozov I.A. (ORCID ID: https://orcid.org/0000-0002-3864-2848): research design, text editing

Kozhanova T.V.: determination of hepatitis virus infection markers; literary search

Soboleva N.V.: determination of markers of hepatitis virus infection; statistical data processing text writing

Melnikova L.I.: collection of material, analysis of the data

Kruglova I.V.: collecting material; analysis of the results

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The Case of Acute Steroid-Induced Myopathy in the Patient with Autoimmune Thrombocytopenia

Abstract

The article concerns one of the common adverse effects during treatment — steroid myopathy. The information about pathogenic specifics of myopathy development in administration of glucocorticoids, the most typical clinical manifestations are described, and results diagnostic methods with estimation of a role of enzyme level evaluation, electromyography, ultrasound study of the muscle tissue, computer and magnetic resonance tomography. There is description of muscle weakness development in 49-year old woman who has been receiving methylprednisolone 88 mg/day due to revealed thrombocytopenia. One week after the treatment was started the patient experienced onset and progression of muscle weakness limiting her motion and self-maintenance. After performing of investigation including electromyography steroid genesis of myopathy was suggested. The patient's condition began to improve after discontinuation of glucocorticoids and administration of calcium supplements, vitamin D, and anabolics, and the patient was discharged.

Key words: *thrombocytopenia, methylprednisolone, glucocorticoids, adverse effects, steroid myopathy, treatment*

Conflict of interests

The authors declare no conflict of interests

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ALT — alanine aminotransferase, AST — aspartate aminotransferase, BP — blood pressure, CCR — cell-color ratio, CPK — creatine phosphokinase, CRP — C-reactive protein, dsDNA — double-stranded DNA, ENMG — electroneuromyography, ESR — erythrocyte sedimentation rate, GCs — glucocorticoids, LC3-I — protein I light chain 3, MP — methylprednisolone, MRC — Medical Research Council Weakness Scale, MRI — magnetic resonance imaging, mRNA — matrix ribonucleic acid, MU — motor unit, PMU — potential of motor units, UPS — ubiquitin-proteasome system

Introduction

Harvey Williams Cushing was the first to describe glucocorticoid-induced myopathy in 1932 as one of the manifestations of Cushing's syndrome [1]. It is a quite common adverse effect of glucocorticoids (GCs) in Cushing's syndrome: its incidence varies from 42% to 83% of cases [2]. Although long-term

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use of any GC can cause myopathy, it is most often caused by fluorinated GCs, such as dexamethasone, triamcinolone and betamethasone [3]. There are also reports on steroid-induced myopathy when using non-fluorinated GCs (prednisolone, methylprednisolone (MP), hydrocortisone) [4]. Myopathy in Cushing's syndrome significantly affects proximal parts of lower limbs; its treatment can take from several months to several years. It should be noted that myopathy is somewhat more common in ectopic than in adrenal Cushing's syndrome, and is significantly more prevalent in men than in women [2].

Pathogenesis

An excessive amount of GCs affects the structure and function of skeletal muscles in different ways. GCs cause atrophy of type II muscle fibers through both anti-anabolic and catabolic effects [5]. Firstly, steroids inhibit the transportation of amino acids into muscle cells [6], thus limiting intracellular protein synthesis [3]. Secondly, GCs block the stimulating effect of insulin, insulin-like growth factor I and amino acids (in particular, leucine) on the phosphorylation of eIF4E-binding protein 1 and ribosomal protein S6 kinase 1, two factors that play a key role in the anabolic mechanism by controlling the initiation of translation of matrix ribonucleic acid (mRNA) [7].

The stimulating effect of GCs on muscle proteolysis is due to the activation of major proteolytic systems in cells [8]: ubiquitin-proteasome system (UPS), lysosomal system (autophagy), and calcium-dependent system of proteinases (calpains). The increased excretion of 3-methylhistidine and extracellular matrix proteins of muscle tissue indicates that protein destruction caused by GCs primarily affects its myofibrillar types [9]. GCs trigger this mechanism by stimulating the secretion of several UPS components that have an impact on the attachment of ubiquitin to target protein [10] or are directly responsible for the degradation of proteasome protein [11]. This activation of gene transcription is associated with the increased frequency of protein ubiquitination and increased proteolytic activity of the proteasome itself [12]. The blocking of different proteolytic pathways demonstrated that GCs stimulate not only UPS-dependent proteolysis but also calcium-dependent and lysosomal proteolysis [8]. The role of the lysosomal system, also called autophagy, in the atrophic effect of GCs is confirmed by the increased expression of cathepsin L [13] and enhanced transformation of microtubule-associated protein I light chain 3 (LC3-I) into LC3-II, which is the indicator of autophagy [14] in the muscles of animals treated with GCs. Since proteasomes do not destroy intact myofibrils, it is assumed that actin and myosin should separate from myofibrils prior to their possible destruction by UPS. Thus, the activation of caspase-3 is required for the transformation of actomyosin and protein myofibrils into substrates destroyed by UPS [15].

Another adverse effect of GCs is hypokalemia, which develops as a result of potassium excretion by kidneys; it can contribute to the development of muscle weakness, and, when used for a long time, to skeletal muscle atrophy [16].

Skeletal muscle atrophy is characterized by a decrease in the size of muscle fibers. It was found that GCs cause atrophy of fast-twitch type II muscle fibers (in particular, IIx and IIb) with less or minor effect on type I fibers [17]. Consequently, glycolytic muscles (e.g. anterior tibial muscle) are more sensitive to GCs than oxidative muscles (e.g. soleus muscle). In mixed-type muscles, type II muscle fibers become atrophied to a greater extent. The mechanism of such muscle specificity can be realized through a higher expression of GC receptors in the anterior tibial muscle than in the soleus muscle [18].

Clinical Picture

GC-induced myopathy can occur in acute or chronic form. In chronic myopathy, muscle weakness develops gradually and usually without pain [19, 20]. Weakness develops mainly in proximal parts of limbs; muscles of lower limbs are affected more than these of upper limbs; muscles of the pelvic girdle are affected much more often than these of the thoracic girdle; muscles innervated by cranial nerves and sphincters are usually not involved [19]. Respiratory muscles can be involved in pathological process. Chronic myopathy can lead to muscle atrophy, which slowly develops for up to several weeks and even months after the withdrawal of GCs [4]. Patients taking steroids for less than four weeks rarely suffer from said complication, since its development usually correlates with

the dose and duration of treatment with GCs. Prednisolone or an equivalent drug at a dose of less than 10 mg/day rarely leads to steroid myopathy. Higher doses of GCs induce rapidly developing clinical signs of muscle weakness, which can be observed within two weeks after the beginning of treatment [21]. Clinical management of such patients is complicated by the fact that there are almost no methods for accurate determination of the onset of the myopathy before its clinical presentation [22, 23].

Diagnosis

Direct quantitative measurement of isometric muscle strength can be an alternative approach to the semi-quantitative assessment of muscle strength [24]. This measurement is a simple, accurate, and reliable method that has a strong predictive relationship with the functional capabilities of the muscular system. When measuring isometric strength, the values obtained are assessed in comparison with normal values for a certain gender, age and physical activity [25]. It should be noted that the widely used hand dynamometers enable to assess the muscle strength of hands and arms, which may not be informative in primary myopathy when proximal muscle groups are primarily affected. Despite that not only muscle but also neural mechanisms may cause muscle weakness in patients with steroid myopathy [26], it is thought that the regular use of state-of-the-art dynamometers in routine examinations of patients can be of practical value for the diagnosis and monitoring of myopathy [23].

Needle biopsy most often reveals atrophy of type II muscle fibers and the apparent absence of signs of necrosis and regeneration [23]. Atrophy of oxidative (type I) muscle fibers can also be present, but to a lesser extent. Aggravation or correction of excessive GCs accordingly change biopsy results: actually, in severe steroid myopathy, there is a decrease in the size and content of lipid droplets in type I muscle fibers, while after the correction of hormonal disorders or withdrawal of GCs, muscle fibers are restored. However, it should be noted that said changes can also be found in other conditions characterized by predominant atrophy of type II muscle fibers, such as senile involution of muscle tissue, neuropathy, muscle atrophy in chronic diseases. Therefore, this method has very high sensitivity but low specificity.

Most patients with steroid myopathy can demonstrate no changes during needle electroneuromyography (ENMG); some patients may show only a slight decrease in amplitude of the potential of motor units (PMU) [3, 23].

The first motor unit (MU) that takes part in voluntary contraction consists of type I muscle fibers (slow MUs). Since these muscle fibers are not affected as much as type II fibers, slight electrophysiological dysfunction is observed. If the patient is asked to increase muscle contraction in order to engage MUs consisting of type II fibers (fast MUs), some abnormalities occur, although they may not be noticed. When fast MUs are engaged, too many slow MUs are activated at the same time, creating an overlap of PMU, thereby causing loss of information. However, when the disease becomes more pronounced and type I muscle fibers are affected, disorders appear that can be observed even with low contraction force [23]. It should be noted that such electrophysiological disorders can also be found in patients with some other physiological (long-term immobilization) or pathological conditions (aging, neuromuscular disorders, drug-induced myopathy), which are accompanied by a decrease in the volume of muscle fibers. That is why this study has high sensitivity but low specificity.

Khaleeli A. A. et al. (1983) revealed a high ratio of 3-methylhistidine to creatinine in urine and decreased activity of creatine phosphokinase (CPK) in plasma of patients with Cushing's syndrome [27]. A possible explanation for these phenomena may be increased muscle protein breakdown (high ratio of 3-methylhistidine to creatinine) and decreased muscle protein synthesis (low levels of CPK and myoglobin in blood serum). However, at present, there is no reliable biomarker (blood or urine) for the verification of steroid myopathy in clinical practice, as well as for monitoring changes and response to therapeutic measures [23].

Magnetic Resonance Imaging (MRI) is a relatively new and informative method for the diagnosis of inflammatory myopathies and monitoring changes in their course [19, 28]. For example, MRI can be used for diagnosing the severity of sarcopenia, since not only muscle size is estimated, but also infiltration of the muscle with fat [29, 30]. Unfortunately, no studies have been conducted to determine the effectiveness of MRI in the diagnosis and

monitoring of steroid myopathy [23]. Ultrasound is an alternative method for measuring muscle size. Despite its simplicity, accuracy, reliability [31, 32] and the fact that this method was previously used for the quantitative assessment of changes in muscle size in different physiological and pathological conditions [33, 34], there are only a few studies of GCs-induced muscle tissue disorders performed with the help of ultrasound [35].

The diagnosis of steroid myopathy is a challenging task. The onset of muscle weakness while taking GCs is the main reason to exclude this complication of steroid therapy in patients who have no history of neurological or neuromuscular diseases, even if this weakness appears very early (during the first 1–2 weeks, or even days of treatment). Medical history, results of laboratory tests and instrumental examinations are of importance in the diagnosis of steroid myopathy, although the role of latter more auxiliary than diagnostic. We present our clinical observation as a demonstration of some features of the diagnosis of steroid-induced myopathy.

Case Report

In October 2018, a patient, 49 years old, suffered an uncomplicated hypertensive crisis after emotional stress, with blood pressure (BP) 165/95 mm Hg, headache, tinnitus, palpitations and nosebleed. She had never experienced an increase in BP in the past. When managing the hypertensive crisis in the Therapeutic Department at her place of residence, the patient underwent general examination; CBC results revealed thrombocytopenia (55×10^9/L). After the crisis management, she was referred to the Department of Hematology for further examination and treatment. At the time of transfer to the Department of Hematology (October 22, 2018), platelet count was 46 x 10^9/L. The diagnosis was the following: Principal — «Primary immune thrombocytopenia, newly diagnosed»; secondary — «Hypertensive disease, grade 2, stage II, moderate risk (according to the Russian Hypertension Classification)». The treatment included hemostatic drugs (dicinone, ascorbic acid); then therapy with GCs was started (MP at a dose of 1 mg/kg/day, i.e. 88 mg/day). Daily dose was divided as follows: 32 mg in the morning, 32 mg in the afternoon and 24 mg in the evening.

It should also be noted that shortly before hospitalization at the Hematology Department, according to the patient, her body weight decreased sharply, by about 10 kg, for no obvious reason. After a week of treatment with MP, the patient started feeling muscle weakness. According to CBC results, platelet count reached normal values (205×10^9/L). The MP dose was reduced according as follows: by 8 mg once every 5 days, although this caused a decrease in platelet count ($<100 \times 10^9$/L); then the initial dose (88 mg/day) was used again for two months. Muscle weakness increased; hand tremor appeared, and memory and attention worsened.

On December 18, 2018, after two months of taking GC at a dose of 88 mg/day, the patient was hospitalized at the V. K. Gusak Institute of Emergency and Reconstructive Surgery with complaints of the inability to perform self-care activities due to severe muscle weakness. She also noted a significant decrease in the volume of legs and frequent constipations. Objective examination revealed hyperemia of the face and collar zone. There were no signs of hemorrhagic syndrome or lymphadenopathy. No cardiovascular or respiratory abnormalities were found. Electrocardiogram and echocardiography findings were within normal. BP was 154/96 mm Hg on both arms. Neurological status (December 21, 2018): the patient is awake, fully oriented. No oculomotor disorders found. Function of bulbar and expression muscles is not impaired. No weakness of neck or axial muscles. No respiratory muscle function disorders. Muscle strength in hands — 5 of 5 points (MRC — Medical Research Council Weakness Scale), in the proximal parts of lower limbs — 2 of 5 points, in the distal part of lower limbs --3 of 5 points. Hypotrophy in thigh muscles. Muscle tone is decreased. Deep reflexes are decreased. No myalgic syndrome. Bowel and bladder functions within normal.

Given the scarce information about the patient's condition before admission to the hospital, several theories about the causes of thrombocytopenia, increasing weakness and decreased muscle strength were proposed. As a result, the patient underwent tests aimed at excluding diseases of the hematopoietic system (repeatedly — CBC, myelogram), autoimmune diseases (systemic lupus erythematosus, systemic scleroderma, dermatomyositis/ polymyositis, antiphospholipid syndrome), and infectious

diseases, including viral infections. A systemic autoimmune disease was the most probable variant at the beginning of observation, taking into account thrombocytopenia and weakness that developed shortly after detection of decreased platelet count. Newly diagnosed immune thrombocytopenia remained the working diagnosis based on repeated CBC results with decreased platelet count less than 100 x 10^9/L and the absence of other obvious initiating and/ or underlying causes of thrombocytopenia [36].

Complete blood count on December 19, 2018: Hb — 160 g/L, RBC — 4.4 × 10^{12}/L cell-color ratio (CCR) — 1.0, WBC — 8.4 g/L, erythrocyte sedimentation rate (ESR) — 3 mm/h, myelocytes — 2%, stab neutrophils — 1%, segmented neutrophils — 84%, monocytes — 2%, lymphocytes — 11%, hematocrit — 43%, platelets — 136 g/L, reticulocytes — 0.9%. Blood biochemistry on December 19, 2018: total bilirubin — 15.47 mol/L (direct — 3.86, indirect — 11.61 mol/L), aspartate aminotransferase (AST) — 21 U/L, alanine aminotransferase (ALT) — 34 U/L, total protein — 75.0 g/L, urea — 8.32 mmol/L, creatinine — 89.64 mmol/L, rheumatoid factor — 9.49 U/ml, C-reactive protein (CRP) — 1.3 mg, K^+ — 4.3 mmol/L, Na^+ — 138 mmol/L, Cl^- — 97 mmol/L, Ca^{2+} — 1.23 mmol/L. Urinalysis on December 19, 2018: reaction — acid, no protein, no glucose, RBC — 1 in preparation, WBC — 2–4 per field of vision, squamous epithelium — small amount, transitional epithelium — 0–3 per field of vision. In addition, glycemic profile test revealed hyperglycemia (January 10, 2019: fasting blood glucose 08:00 a.m. — 5.9 mmol/L, 12:00 p.m. — 9.3 mmol/L, 04:00 p.m. — 14.08 mmol/L; January 15, 2019: fasting blood glucose — 6.2 mmol/L; January 18, 2019 — 6.45 mmol/L; January 23, 2019 — 6.3 mmol/L). Glycated hemoglobin (HbA1c) — 6.6%. Lactate dehydrogenase — 696 U/L (reference values 135–214 U/L), CPK — 5.5 U/L (<167 U/L).

Sternal puncture was performed. Bone marrow cell differential count: bone marrow is cellular, there are single mitotic figures, and focal hematopoiesis in erythroid lineage was registered, megakaryocytic lineage remained unchanged. Profile of antinuclear antibodies on December 20, 2018: no antinuclear antibodies found. No antibodies against double-stranded DNA (dsDNA), antinuclear antibodies (anti-Sm, Scl-70, Jo-1, PCNA, against nucleosomes, histones, ribosomal proteins) found. Antibodies against cardiolipin IgG — 3.2 U/ml, IgM — 1.6 U/ml, lupus anticoagulants (screening) — negative (SD = 0.98). No antibodies against HIV, viral hepatitis, herpes simplex virus type I and II (IgM) and cytomegalovirus (IgM) found.

The patient was examined by an endocrinologist and diagnosed with steroid-induced diabetes mellitus. According to the patient, the glycemia level at the prehospital stage was within the normal range, although there were no data confirming euglycemia. During ENMG, there were no signs of lesions of the neural or synaptic level (parameters of M-response and sensory potential of peripheral nerves in lower limbs were within normal; there was no decrement/ increment of M-response). Results of needle ENMG suggested a myogenic lesion: spontaneous activity in the form of fibrillation potentials (+++) was registered in the muscles of the peroneal group; in minimal contraction state, PMU duration decreased by 36.6%, with a 52% pathological decrease in PMU amplitude. Fibrillation (spontaneous) potentials (+++) were registered in the muscles of the medial thigh group; during dosed muscle contraction, PMU duration decreased by 47.9%, with a 56.1% abnormal decrease in PMU amplitude (Fig. 1).

Constipation and painful bowel movements during further examination (examination by a surgeon, fibrocolonoscopy) were attributed to coprostasis associated with long-term uncontrolled use of steroids that resulted in paresis of intestinal muscles. Intestinal motility improved in the course of symptomatic therapy (diet, micro-enema).

The patient was diagnosed with «Newly diagnosed primary immune thrombocytopenia; complications: steroid myopathy, steroid-induced diabetes mellitus, intestinal hypotension with coprostasis; secondary: hypertensive disease stage II, AH grade 2, risk 2 (according to the Russian Hypertension Classification)».

Gradual tapering of the MP dose was conducted (4 mg/day for 3 days) with platelet control simultaneously with the examination. Also, potassium ororate, calcium and vitamin D, an anabolic drug (nandrolone decanoate, injections), antihypertensive treatment (ramipril, bisoprolol), and a proton pump inhibitor (omeprazole) were prescribed. The patient went through movement therapy and physiotherapy.

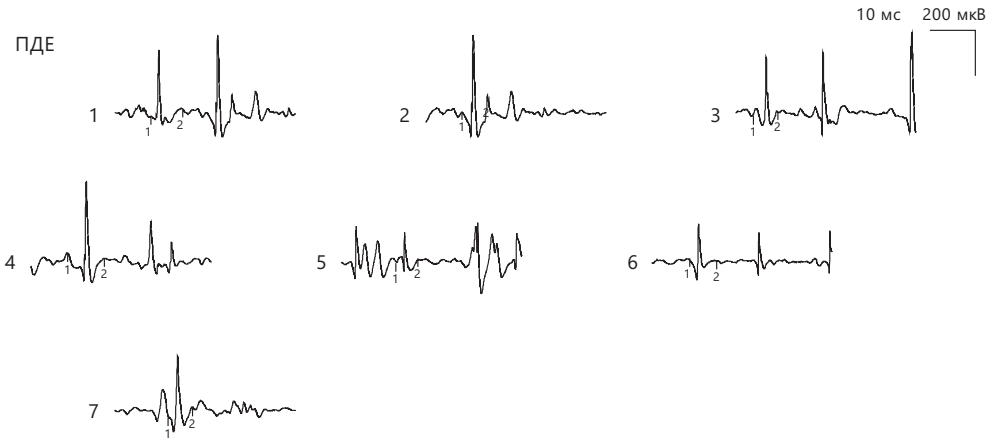

Figure 1. *Quantitative electroneuromyography*

left, Tibialis anterior, Peroneus, L4 L5 s1
Note: MUP — motor unit action potential.
Description. The illustration of myogenic restructuring of motor unit potentials (MUP) according to data of needle electroneuromyography indicating decrease in duration and amplitude of potentials in the observed patient

The patient's condition gradually improved, and after bowel function recovery and diet stabilization, the patient recovered quickly. At a daily MP dose of 8 mg, there was a sharp decrease in platelet count (to 39 × 10^9/L) without signs of hemorrhagic syndrome. It was decided to further reduce the GC dose with daily monitoring of complete blood count. However, platelet count later started increasing gradually: February 4, 2019 — 85 × 10^9/L, February 6, 2019 — 97 × 10^9/L, and February 7, 2019 — 155 × 10^9/L. Fasting blood glucose — 5.6 mmol/L, BP — 138/90 mm Hg on both arms. The patient was discharged from the hospital in satisfactory condition with the following recommendations: platelet control; with decreased platelet count less than 30–50 × 10^9/L without hemorrhagic syndrome — vessel-strengthening therapy; with decreased platelet count less than 30–50 × 10^9/L and hemorrhagic syndrome — immunoglobulin i.v. (first-line therapy); second line therapy — splenectomy; Romiplostim, Eltrombopag; third-line therapy — Rituximab, immunosuppressive drugs [37].

Discussion and Conclusion

The case described above is interesting in several aspects. Despite that the MP prescribed for thrombocytopenia is not a fluorinated GC, its administration at a dose of 88 mg/day or less (with dose tapering) led to clinically significant steroid myopathy. In literature, there are descriptions of GC-induced myopathy as a complication of treatment with MP [16]. A case of acute myopathy after two-day oral administration of MP was described: 24 mg on the first day of treatment, and 20 mg on the second day. Treatment was discontinued due to myalgia and severe drowsiness [38]. There is even a case of steroid myopathy after a single dose of prednisolone (40 mg) prescribed for exacerbation of chronic obstructive pulmonary disease [39]. In our case, the patient noticed the first signs of myopathy (muscle weakness) after a week of treatment with MP. Based on the analysis of the obtained data and early signs of steroid myopathy cited in literature (less than 14 days) [40], we can conclude that treatment with GCs of any duration, including single doses per day and possibly even several hours, can result in acute GC-induced myopathy.

In regard to the verification of the diagnosis, we think that muscle symptoms were associated with the prescribed GC. Although during hospitalization of the patient, versions of secondary immune thrombocytopenia were discussed as a syndrome in connective tissue systemic disease [41], the absence of any additional signs, along with negative results of immunological tests, cast doubts over the secondary nature of thrombocytopenia. Most important of all, the onset of myopathy coincided with the start of GC therapy, and complaints of muscular symptoms completely disappeared at the time of GC withdrawal and subsequent treatment. When discussing the cause of muscle symptoms, taking

into account long-term treatment with MP, we also considered hypokalemia as a common complication of treatment with GCs [42]. However, the normal potassium level in the period of maximum severe muscle weakness excluded hypokalemia from the reasons discussed.

Thus, this case once again demonstrates steroid myopathy as a typical complication of treatment with GCs. In such cases, accurate diagnosis requires the close attention of the general practitioner, therapist, hematologist and rheumatologist. The following is required: thorough analysis of medical history and examination, which enables the objective assessment of the functional state of the muscular system for justified and timely adjustment of management tactics. The first step here will be the withdrawal of GCs and, if necessary, a switch to another immunosuppressive treatment.

Contribution of Authors:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Vatutin N.T. (ORCID ID: https://orcid.org/0000-0003-4307-1522): generation of the idea and general manuscript presentation, case management, the final approval of the manuscript

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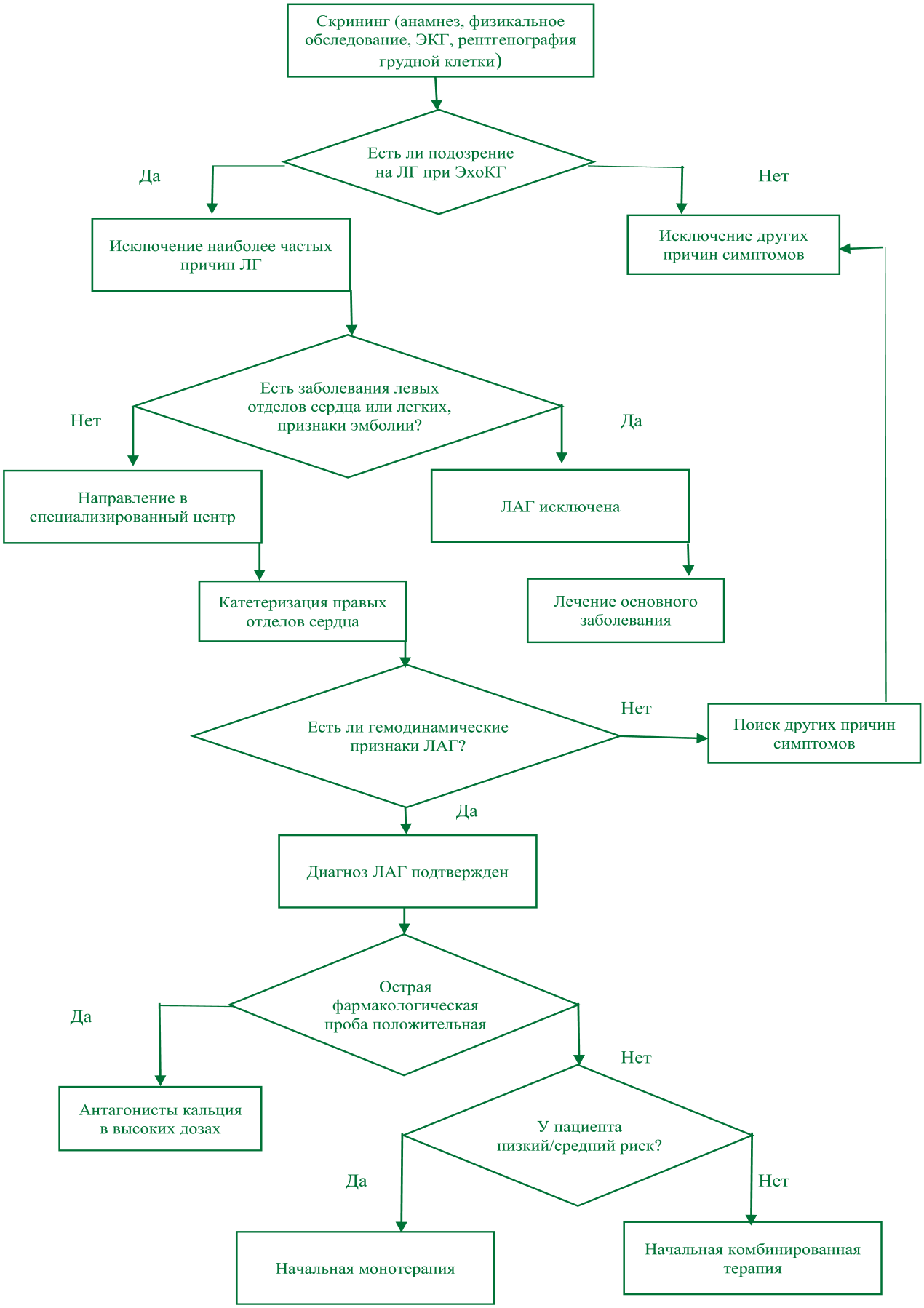
Алгоритм ведения пациента с подозрением на легочную гипертензию



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